Benha University

Faculty of Veterinary Medicine

Department of pharmacology

Allicin and omega-3 as a therapeutic preference against hepato-renal toxicity induced by paracetamol

Thesis submitted to

Faculty of Veterinary Medicine - Benha University

For obtaining Master degree in Veterinary Medicine (Pharmacology)

Presented by

Moamen Hussien Elsayed Elsafty

Bachelor of Veterinary Medical Sciences, Faculty of Veterinary Medicine, Benha University (2019)

Under Supervision of

Prof. Dr.

Mohamed Hafez Aboubakr

Professor of Pharmacology, Faculty of Veterinary Medicine, Benha University.

Dr.

Ahmed Abdelhafez Abdeen

Assistant Professor of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine Benha University.

2022

Benha University

Faculty of Veterinary Medicine

Department of Pharmacology

Approval Sheet

This is to approve that the dissertation presented by: **Moamen Hussien Elsayed Elsafty** to Benha University entitled " Allicin and omega-3 as a therapeutic preference against hepato-renal toxicity induced by paracetamol" for obtaining Master degree in Veterinary Medicine (Pharmacology) has been approved on Tuesday: 25/10/2022 by the examining committee:

Examiner	Signature
1- Prof. Dr: Ashraf Abdel-Hakim Ahmed El-Komy Professor of Pharmacology Faculty of Veterinary Medicine Benha University	A. El-Komy
2- Prof. Dr: Waleed Fathy Khalil Mahmoud Professor and Head of Pharmacology Department Faculty of Veterinary Medicine Suez Canal University	
3- Prof. Dr: Mohamed Hafez Mohamed Aboubakr Professor of Pharmacology Faculty of Veterinary Medicine Benha University	Mohamed Hafez Aboubakr
4- Dr: Ahmed Abdelhafez Abdeen Assistant Professor of Forensic Medicine and Toxicology Faculty of Veterinary Medicine Benha University	

List of Contents

DECLARATION الإقرار

I declare that this thesis has been compiled by myself, and is the results of my own work. It has not been submitted for any other degree and all sources of information have been properly acknowledged.

Name: Moamen Hussein Elsayed Elsafty

Signature: Moamen Elsafty

Date: 25 / 10 / 2022

Acknowledgement

First of all, I would pray, thank and express my deepest gratitude and indebtedness to the Glorious ALLAH who gave me the ability and the strength to start and complete this work.

I would like to express my great, cardial and sincere thanks and deep appreciations to *Prof. Dr: Mohamed Hafez Aboubakr*, Professor of Pharmacology, Faculty of Veterinary Medicine, Benha University, for his supervision, continual encouragement, fruitful advices and valuable help.

My great, cardial and sincere thanks to *Dr: Ahmed Abdelhafez Abdeen*; Assistant Professors of Forensic Medicine and Toxiology, Faculty of Veterinary Medicine, Benha University, for their supervision and their continual help.

I am most grateful to *Dr. Mohamed Mahmoud Salem Ahmed Gaballa*, Lecturer of Pathology, Faculty of Veterinary Medicine, Benha University for his valuable role in preparation and processing of the different tissue specimens for for both histological and immunohistochemical examinations, capturing the photomicrographs, interpretation of the findings.

My great thanks to *Staff Members of Pharmacology Department*, Faculty of Veterinary Medicine, Benha University, for their help during my study. Appreciation is also extended for the facilities offered by the *Center of Excellence in Screening of Environmental Contaminants* (STDF grant no. 31290).

My heart-full thanks and all my love to *my family* for bearing all responsibilities and adapted the optimum environment to complete this work. Last but not the least, I thank all the individuals who have in any

way been associated with the complete of this work but have not been mentioned so far.

LIST OF ABBREVIATIONS

ABBREVIATIONS	FULL NAME
AC	Allicin
ALA	Alpha-lipoic acid
ALF	Acute liver failure
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
APAP	Acetaminophen (Paracetamol)
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
B.wt.	Body weight
BUN	Blood urea nitrogen
CAT	Catalase
CKD	Chronic Kidney Disease
Cox-2	cyclooxygenase-2
CP	cyclophosphamide
CT	Collecting tubules
CYP	Cytochrome P450
DCT	distal convoluted tubules
DHA	Docosahexaenoic acid
DILI	drug-induced liver injury
EPA	Eicosapentaenoic acid
FAs	Fatty acids
FTIR	Fourier transform infrared
Gm/g	Gram

GPx	Glutathione peroxidase
GSH	Glutathione
HSP70	The 70 kilodalton heat shock proteins
IL	Interleukin
LDH	Lactate dehydrogenase
LDL	Low density lipoproteins
LPO	Lipid peroxidation
MDA	Malonaldehyde
NAC	N-acetyl cysteine
NAG	Urinary N-Acetyl-β-d-Glucosaminidase
NAPQI	N-acetyl-p-benzoquinone imine
NLRP3	NOD-like" receptor (NLR) proteins
NMDA	N-Methyl-D-aspartate
NO	nitric oxide
NSAIDs	Non-steroidal anti-inflammatory drugs
OMG-3 FAs	Omega-3 fatty acids
PCT	Proximal convoluted tubules
PDE3B	Phosphodiesterase 3B
PO.	Per oral
PUFAs	polyunsaturated fatty acids
RBCs	Red blood cells
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TC	Total cholesterol
TG	Triglycerides
TNF-α	Tumor necrosis factor-α

List of Figures

	APAP-Omega3 group, **(G)** APAP-Allicin+Omega3 group. Immunostaining was performed using anti-Caspase-3 antibody and developed with DAB. Brown color indicates caspase-3 positivity.	
3-9	Bar graph of caspase-3 immunohistochemical expression in the different study groups. Area percent of immunoreactivity of caspase-3; data are presented as mean ± standard deviation, *: statistically significant relative to APAP group at **P > .005, ***P > .0005, ****P > .00005 using ANOVA, Bonferroni post hoc test.	**60**
3-10	Cross section in the kidney of Paracetamol induced nephrotoxicity model showing changes in renal HSP70 Immunohistochemical expression after treatment with Allicin and/or Omega3. **(A)** Control sham group, **(B)** Allicin alone group, **(C)** Omega3 alone group all showing no expression of HSP70 in the cortical regions of kidney, **(D)** Paracetamol-intoxicated group showing diffuse intense expression, **(E)** APAP-Allicin group, **(F)** APAP-Omega3 group both showing moderate immunopositivity, **(G)** APAP-Allicin+ Omega3 group showing partial inhibition of HSP70 expression as evidenced by weak immune staining in the cortical regions. Brown color indicates immunopositivity.	**61**

Abstract: المستخلص

Title: Protective effect of allicin and omega-3 fatty acids against hepato-renal toxicity induced by paracetamol in rats.

Student Name: Moamen Hussein Elsayed Elsafty

Nationality: Egyptian

Degree: For obtaining Master degree in Veterinary Medicine (Pharmacology).

Specialization: Pharmacology

Department: Pharmacology department, Faculty of Veterinary medicine, Benha University, Egypt.

Supervisors:

1. **Prof. Dr: Mohamed Hafez Mohamed Abubakr**
   Professor of Pharmacology, Faculty of Veterinary medicine, Benha university
2. **Dr: Ahmed Abdel Hafez Abdeen**
   Assistant professor of Forensic Medicine and Toxicology, Faculty of Veterinary medicine, Benha university

Key words: Paracetamol, Allicin, Omega3, oxidative stress, caspase 3, HSP70, apoptosis, rats.

Our experimental goal was to assess the protective effects of allicin (AC) and omega3 (OMG-3 FAs) against paracetamol (APAP) induced hepato-renal injury in rats. Seventy Wistar albino rats were assigned to 7 experimental groups, each was composed of 10 rats; 1st group received saline only (Control), 2nd group supplemented with allicin (AC) (10 mg/kg. b. wt. orally), 3rd group supplemented with omega-3 (OMG-3 FAs) (100 mg/kg b. wt. orally), 4th group paracetamol (APAP) toxic control group received saline orally once daily and a single 1 g/kg orally dose of APAP on the 27th day of the experiment. 5th group (AC + APAP), 6th group (OMG-3 + APAP), and 7th group (AC+ OMG-3+ APAP). rats in these groups have been received allicin, omega-3, and/or APAP as described above. Saline, allicin, and omega-3 were administered for 30 days. Paracetamol showed a significant increase in ALT, AST, ALP, urea, creatinine and MDA and a significant decrease in SOD, CAT and GSH levels. Also, The APAP intoxicated group showed a significant decrease in albumin and total protein and a marked increase in cholesterol and triglycerides when compared to the control group. Hematological parameters also investigated and indicated in result. Histopathological changes were also recorded and indicated in the results. Caspase-3 and HSP70 were substantially unregulated by APAP in the renal and hepatic tissues. Concurrent supplementation of AC and/or OMG-3 FAs with APAP resulted in a notable improvement in estimated parameters compared to the APAP group. Therefore, we anticipate that prescribing omega3 and allicin in patients undergo paracetamol regimen would be beneficial in reducing the adverse effect of paracetamol-induced hepatic and renal damage.

1- General Introduction

1.1 Paracetamol (Background):

Paracetamol (APAP) is a p-aminophenol derivative with analgesic and antipyretic activities. Although the exact mechanism through which acetaminophen exert its effects has yet to be fully determined, acetaminophen may inhibit the nitric oxide (NO) pathway mediated by a variety of neurotransmitter receptors including N-Methyl-D-aspartate (NMDA) and substance P, resulting in elevation of the pain threshold. The antipyretic activity may result from inhibition of prostaglandin synthesis and release in the central nervous system (CNS) and prostaglandin-mediated effects on the heat-regulating center in the anterior hypothalamus **(National Institute of Diabetes and Digestive and Kidney Diseases; 2012).**

Paracetamol is the most widely used non-prescription analgesic in the world. Paracetamol is commonly taken in overdose either deliberately or unintentionally. In high-income countries, paracetamol toxicity is a common cause of acute liver injury **(Chiew *et al*., 2018)**. It is safe when used at the recommended doses for adults (4 gm/day) and children (50–75 mg/kg/day) **(Mikhail *et al*., 2019, Moore *et al*., 2019).** Paracetamol (Acetaminophen, N-acetyl-p-aminophenol; APAP) has been regarded as a safer drug compared with other non-steroidal anti-inflammatory drugs (NSAIDs). **(Ishitsuka *et al*., 2020).**

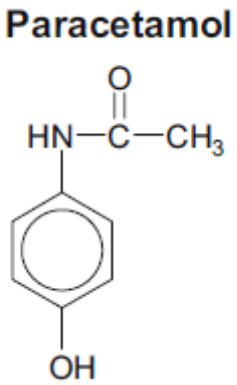

Fig (1-1): Chemical structure of paracetamol **(Bertolini *et al*., 2006).**

1.1.1. Hepatotoxicity of Paracetamol:

Liver diseases are considered as major reasons for deaths worldwide (**Wang *et al*., 2014)**. About two million people die per year because of liver illnesses such as chronic hepatitis B virus and hepatitis C virus infection, autoimmune liver disease,

and drug-induced liver injury (DILI) (**Ellahi *et al.*, 2014**). DILI is a growing reason for liver injury, which leads to hepatocellular death and acute liver failure (ALF) (**Yuan *et al.*, 2013**).

In 2018, the American Association of Poison Control Centers provided approximately 2.1 million tele-consults for the United States and associated territories, of these over 100 000 calls related to paracetamol exposure (**Gummin *et al.* 2019**). About 50% of acute liver failure cases admitted in USA is caused by APAP poisoning (**Larson *et al.*, 2005**). In England and Wales, UK, APAP poisoning claimed the lives of 284 people aged 12 years and over between 1993–1996 (**Hawton *et al.*, 2004**). APAP is metabolized in the liver and the hepatotoxic metabolites that represent about 10% of whole metabolites are rapidly inactivated by glutathione (GSH) to protect against hepatic cell death (**James *et al.*, 2003**).

Yayla *et al.*, (2014) examined the Protective effect of Et-1receptor antagonist bosentan on paracetamol induced acute liver toxicity in rats. Reporting that According to biochemical results, TNF-α, ALT and AST levels were statistically increased in the paracetamol group, these parameters Were improved in the bosentan groups. Paracetamol administration decreased SOD activity, GSH level and increased level of MDA in the liver, while bosentan administration significantly improved these parameters. In immunohistochemical staining ET-1receptor expression was excessively increased in paracetamol group, but not in bosentan groups when compared to healthy control. All these results suggest that bosentan exerted protective effects against experimentally induced paracetamol toxicity in liver.

Tittarelli *et al.*, (2017) investigated the hepatotoxicity of paracetamol and related fatalities and clarified that repeated supratherapeutic misuse, non-intentional misuse, and intentional ingestion may all result in hepatic toxicity, the main cause of acute liver failure (ALF) in the United States and Europe. Since paracetamol is responsible for nearly half of the cases in the US of acute liver failure and remains the leading cause of liver transplantation, continued awareness promotion, education and research should be constantly undertaken.

El-Boshy et al., (2019) investigated the protective effect of vitamin D against oxidative stress, inflammation and hepatorenal damage induced by acute paracetamol toxicity in rat and found that the APAP group showed significantly elevated serum ALP, ALT, and AST enzymes, creatinine, and urea together with marked declines in

serum total protein, albumin, 25-OH VD and Ca^{++} compared with the negative control group for all parameters. Prophylactic VD3 lowered the levels of liver and renal function parameters as well as augmented the levels of circulatory 25-OH VD and Ca^{++} significantly compared with the APAP group. The histological studies demonstrated that the APAP group displayed major pathological alterations in hepatic lobular morphology that was associated with massive leukocytic infiltration, especially around the portal tracts and central veins, in addition to widespread areas of cellular degeneration as indicated by the large numbers of apoptotic bodies and increased staining for cleaved Casp-3.

Samra *et al*., (2020) investigated the hepatoprotective effect of allicin against acetaminophen-induced liver injury and find that APAP significantly increased AST, ALT, and ALP, whereas allicin significantly decreased their levels. Also, APAP significantly decreased serum albumin while allicin significantly improved it. APAP produced changes in liver morphology, including inflammation and massive coagulative necrosis. Allicin protected the liver from APAP-induced necrosis, apoptosis, and hepatocellular degeneration via increasing Bcl-2 and Ki-67 levels. Paracetamol significantly increased the hepatic MDA, whereas allicin significantly prevented this increase. APAP markedly activated the NLRP3 inflammasome pathway and consequently increased the production of caspase-1 and IL-1β. Interestingly, he found that allicin significantly inhibited NLRP3 inflammasome activation, which resulted in decreased caspase-1 and IL-1β levels. So, allicin has a hepato-protective effect against APAP-induced liver injury via the decline of oxidative stress and inhibition of the inflammasome pathway and apoptosis.

Akgun *et al*., (2021) clarified the potential protective role of folic acid against acetaminophen-induced hepatotoxicity and nephrotoxicity in rats. And reported that the antioxidant parameters of the liver tissue were significantly higher and oxidant parameters were significantly lower in the control group compared to the APAP group. The antioxidant parameters were significantly increased, and MDA level was decreased in both APAP+FA and APAP+NAC groups compared to the APAP group. Whereas MPO activity was higher in the APAP group compared to the control group. Characteristics of cell damage and lesions in the liver and kidney tissues were scored on a semi quantitative scale.

Bouhlali *et al.*, (2021) investigated the Protective Effect of Phoenix dactylifera L. Seeds against paracetamol-induced hepatotoxicity in rats: in comparison with vitamin C and found that the APAP group revealed a significant increase in serum levels of AST, ALT, ALP, LDH, and direct and total bilirubin along with a notable reduction in the total protein when compared to the normal control. However, as compared to the APAP-treated group, the administration of different date seed varieties extracts significantly reduced in a dose-dependent manner the levels of AST, ALT, ALP, LDH, and direct and total bilirubin along with remarkable elevation in the total protein. the glutathione, SOD, and CAT activities in the APAP intoxicated group were significantly lower compared to the control., e administration of date seed extracts improved considerably and dose dependently the enzymatic (SOD, CAT, and GPx) and non-enzymatic GSH antioxidant levels compared to the APAP alone treated animals.

El-Gendy *et al.*, (2021) examined the protective effect of Omega-3 PUFAs against acute paracetamol induced hepatic injury confirmed by Fourier transform infrared (FTIR) and reported that acute overdose of paracetamol resulted in massive destruction to hepatocytes, leading to elevation of serum ALT, AST, total cholesterol, and triglycerides levels; in addition to a generalized hepatic oxidative stress status represented by accumulation of MDA as well as NOx and depletion of GSH. In addition, inflammatory response represented by elevated level of TNF-a was recorded.

Islam *et al.*, (2021) studied the Effects of nerol on paracetamol-induced liver damage in Wistar albino rats. His study resulted in that the APAP significantly increased serum ALT, AST, ALP, GGT and LDH levels in comparison to the vehicle group. APAP administration decreased total protein and albumin, while the increase in globulin and serum bilirubin levels with decrease in GSH, SOD and CAT levels when compared to the vehicle group. The paracetamol intoxicated liver shows infiltration of lymphocytes, the presence of hemorrhage and extensive coagulative necrosis of the perivenular, and midzonal region with periportal sparing. It also exhibits a coagulative-type necrosis in the perivenular zone.

Tripathi *et al.*, (2022) studied metformin ameliorating effect against acetaminophen-induced sub-acute toxicity via antioxidant property. His study results recorded a significant increase in APAP treated rats, total cholesterol, LDL, HDL,

Triglyceride, ALT, and AST levels. However, in the metformin treated rats, serum marker levels decreased significantly when compared with the respective control group. A significant increase in the SOD level, catalase and GST were found in the metformin treated group as compared to the control rats. After APAP treatment, there was a significant decrease in the level of SOD, catalase, GST, respectively, as compared to the control rats. However, SOD, catalase, GST activity were restored after supplementation of metformin and showed significantly increased level of SOD, catalase, and GST in comparison to APAP group. The level of lipid peroxidation in liver tissues was measured in terms of MDA. There was a significant increase in MDA level after APAP treatment whereas the MDA level in metformin supplemented APAP treated group was decreased significantly.

1.1.2. Nephrotoxicity of Paracetamol:

The main cause of nephrotoxicity in vivo system is exposure to drugs, toxins, or compounds such as carbon tetrachloride, sodium oxalate, ethylene glycol, and heavy metal (**Lakshmi *et al.*, 2012**). One of these drugs is acetaminophen or paracetamol (N-acetyl-p-aminophenol) (APAP) marketed as Panadol or Tylenol and other preparations which in over dosage result in nephrotoxicity (**Baleni *et al.*, 2015**). In therapeutic doses, APAP is excreted in the urine as glucuronide or sulfate metabolites (90%) or unchanged (2%), while the remaining is converted to N-acetyl-para-benzoquinone mine (NAPQI) via the hepatic cytochrome 450 enzymes and excreted in the urine following its detoxification mostly by hepatic glutathione (GSH) (**Hodgman and Garrard, 2012, McGill and Jaeschke, 2013**).

In contrast, in large doses of APAP, significant amounts of NAPQI are produced which overwhelm the cellular antioxidant system through depletion of GSH and GSH dependent enzymes enhancing excessive production of reactive oxygen species (ROS) accompanied by oxidative stress (**Abdel-Daim and Abdeen, 2018).**

Previous reports have noted that abuse and frequent use of APAP at therapeutic doses could cause renal injury beside its hepatotoxic effect (**Wang *et al.*, 2017**).

Madinah *et al.*, (2015) clarified the protective effects of aqueous extract of *Carica papaya* seeds in paracetamol induced nephrotoxicity in male wistar rats. The result of this study revealed that serum creatinine, uric acid, and urea concentrations were significantly increased in paracetamol treated group of animals compared to the normal animals indicating the induction of severe nephrotoxicity. Treatment with the

aqueous extract of *Carica papaya* showed significant decrease in these renal function markers compared to the paracetamol treated group. Histopathological examination showed symptoms of nephrotoxicity such as leukocyte infiltration, moderate necrosis and degeneration, proximal tubules show dilatation, and there is brush border in some of them.

Canayakin *et al*., (2016) studied the protective role of *Nigella sativa* on paracetamol-induced nephrotoxicity and oxidative stress in rats. His study resulted in paracetamol administration significantly increased serum urea and creatinine when compared with the sham (take Nigella sativa) group. However, serum urea and creatinine level were reduced with different doses of the extract, respectively. *Nigella sativa* administration increased superoxide dismutase and glutathione, and decreased malondialdehyde levels in the kidneys. Kidney histopathological examinations showed that NS administration antagonized paracetamol-induced kidney pathological damage.

Ko *et al*., (2017) investigated the protective effects of diallyl disulfide against acetaminophen-induced nephrotoxicity and the possible role of CYP2E1 and NF-κB and reported that APAP caused severe nephrotoxicity as evidenced by significant increases in renal tubular cell apoptosis, mitochondria-mediated apoptosis, and up-regulation of nuclear transcription factor Kappa-B (NF-κB), cyclooxygenase-2 (Cox-2), and tumor necrosis factor-α (TNF-α) in the kidney with histopathological alterations. After APAP administration, glutathione content and activities of catalase, superoxide dismutase, and glutathione reductase were significantly decreased whereas malondialdehyde content was significantly increased, indicating that APAP-induced kidney injury was mediated through oxidative stress. In contrast, DADS pretreatment significantly attenuated APAP-induced nephrotoxic effects.

El-Maddawy and El-Sayed, (2018) examined compared between the protective effects of curcumin and N-acetyl cysteine (CUR and NAC) against paracetamol-induced hepatic, renal, and testicular toxicity in Wistar rats and documented that a large single dose of APAP induced lipid peroxidation along with a significant decline in glutathione content and catalase activity in the liver, kidneys, and testicles. The apparent oxidative damage was associated with evident hepatic, renal, and testicular dysfunction, which was confirmed in histopathological lesions, and increased serum aspartate aminotransferase, alanine aminotransferase, and

alkaline phosphatase activities. APAP decreased serum total protein, albumin, and globulin contents. Increased bilirubin, urea, and creatinine contents; and induced hematotoxicity. Both CUR and NAC administration provided substantial organ protection with pronounced efficacy against APAP nephrotoxicity with CUR.

Abdeen *et al.*, (2019) investigated the protective effect of cinnamon against acetaminophen-mediated cellular damage and apoptosis in renal tissue and documented that APAP markedly increased serum levels of creatinine, BUN, and glucose and decreased levels of albumin and total protein. In addition, APAP could also exert severe alteration in the kidney histopathology along with upregulation of caspase-3 and PCNA. However, pre-treatment with cinnamon ameliorated the APAP-induced cellular alterations and apoptosis, possibly through its high content of antioxidants.

Koyuncuoğlu *et al.*, (2020) examined estrogen receptor agonists protective role against acetaminophen-induced hepatorenal toxicity in rats and documented that Compared to their control groups, levels of AST, ALT, BUN, creatinine, hepatic, and renal myeloperoxidase activity and chemiluminescence levels were increased, and hepatic glutathione level was decreased in acetaminophen-administered male groups, while ALT and hepatic chemiluminescence levels were not elevated in sham-OVX-rats. Both ER-agonists and E2 reduced BUN, creatinine and reversed all oxidative parameters in renal tissues of OVX-rats. Additionally, ERα-agonist reversed all hepatic injury parameters, while ERβ-agonist elevated hepatic glutathione level. He concluded his result that acetaminophen toxicity in female rats presented with a more preserved hepatic function, while renal toxicity was not influenced by sex or by the lack of ovarian hormones.

Wans *et al.*, (2021) clarified ameliorative effects of corn silk extract (CSME) on acetaminophen-induced renal toxicity in rats. The results of this study revealed that APAP caused a significant increase in serum urea, creatinine concentrations, and malondialdehyde (MDA) concentrations in renal tissues. In addition, APAP caused a significant decrease in superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities in renal tissues compared with the control group. Furthermore, APAP caused marked renal damage as revealed by alterations in histopathological architectures of kidney tissues. Paracetamol resulted in a marked expression of caspase 3 and nuclear factor κB (NFκβ) within the renal tubules. However, pre-

treatment with CSME showed renal protective effect against acetaminophen induced renal toxicity.

Dallak *et al*., (2022) investigated the suppression of glomerular damage and apoptosis and biomarkers of acute kidney injury induced by acetaminophen toxicity using a combination of resveratrol and quercetin. And found that APAP significantly increased blood levels of urea, creatinine, malondialdehyde (MDA), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-a), which were effectively reduced by resveratrol and quercetin. In addition, APAP overdose induced the tissue expression of the apoptotic biomarker, p53, and caused profound kidney damage as demonstrated by substantial alterations to the glomerular basement membrane, podocytes, endothelial cells, widening of Bowman's space, and vacuolation of the cells lining the parietal layer, which were substantially protected by resveratrol and quercetin. Furthermore, a significant positive correlation was observed between either glomerular basement membrane or podocyte foot processes and these parameters, urea, creatinine, MDA, and TNF-a. Thus, he concluded that APAP induces alterations to the glomerulus ultrastructure, which is protected by resveratrol plus quercetin, which also reduces blood levels of urea and creatinine, and biomarkers of oxidative stress and inflammation. Briefly, APAP caused severe nephrotoxicity as evidenced by significant increases in renal tubular cell apoptosis, mitochondria-mediated apoptosis, and up-regulation of nuclear transcription factors in the kidney with histopathological alterations.

1.2 Allicin:

Allicin (diallylthiosulfinate) is the sulphur-containing, bioactive compound derived from freshly chopped garlic when the phosphopyridoxal enzyme known as alliinase catalyses' the conversion of the nonproteinogenic amino acid, alliin (allyl cysteine sulphoxide), to form allyl sulphonic acid (**Bayan *et al*., 2014**). Allicin is a defense molecule from garlic (*Allium sativum L*.) with a broad range of biological activities. Allicin is produced upon tissue damage from the non-proteinogenic amino acid alliin (S-allyl cysteine sulfoxide) in a reaction that is catalyzed by the enzyme alliinase **(Borlinghaus *et al*., 2014)**.

Moreover, allicin exhibits many bioactive properties that span across various fields of studies including antimicrobial (**Dwivedi *et al*., 2019**), anti-inflammatory (**Alam *et al*., 2018; Metwally *et al*., 2018**), anti-cancer (**Shang *et al*., 2019**) and

immunomodulatory activities (**Arellano Buendía *et al*., 2018; Foroutan-Rad *et al*., 2017**). Two allyl sulphonic acid molecules will then condense spontaneously to form allicin through the removal of water (**Salehi *et al*., 2019**). Garlic (*Allium sativum L.*) is a well-known spice widely utilized for its medicinal properties. There is an extensive record of the many beneficial health effects of garlic which can be traced back to as early as the ancient Egyptian era. The antimicrobial activities exhibited by garlic were first reported to be due to allicin (**Choo *et al*., 2020**).

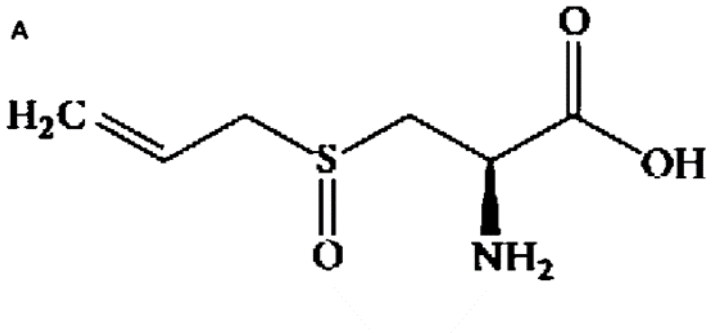

Fig (1-2): Chemical structure of Allicin (**Sarvizadeh *et al*., 2021**).

1.2.1 Hepatoprotective effect of Allicin:

Liver injury is associated with two types of liver cell death: necrosis and apoptosis. Apoptosis is considered as one of the main cellular activities that play a central role in balancing the physiological functions of the organs **(Hsu *et al*., 2004**). **Vimal and Devaki**, (**2004**) examined the hepatoprotective effect of allicin on tissue defense system in galactosamine endotoxin challenged rats and his study resulted in a significant decrease in the activities and levels of antiperoxidative enzymes (SOD, CAT, GPX and GST) in the liver and significant increase of lipid peroxidation were observed in d- galactosamine lipopolysaccharide-intoxicated rats as compared with the levels of normal control rats. His study proved that oral pre-treatment with allicin for 15 days Significantly, prevented these adverse effects and maintained the levels of evaluated parameters to near normality.

Zhang *et al*., (2012) investigated the Protective effect of allicin against acrylamide-induced hepatocyte damage in vitro and in vivo. the results of this study revealed that allicin significantly decreased the levels of maleic dialdehyde (MDA) and 8-hydroxy-desoxyguanosine (8-OHdG) both in vitro and in vivo study. Allicin also markedly increased the activity of total superoxide dismutase (SOD) and level of glutathione (GSH). The protective effects of allicin against AA-induced hepatocyte damage may be due to its ability to scavenge free radicals and its effective recovery of

the ant oxidative defense system, and its ability to block the epoxidation process of AA to GA by inhibiting P450 enzyme.

Suddek, (2014) investigated that allicin enhances chemotherapeutic response and ameliorates tamoxifen- induced liver injury in experimental animals and found that TAM-intoxication produced significant elevation of serum liver enzymes, AST, ALT, GGT, LDH, and ALP and total bilirubin compared with normal control rats. All these mentioned changes were significantly reduced as compared with TAM-treated rats upon administration of allicin to TAM-treated rats. Significant decrease in serum total protein level was observed in the TAM group, which was ameliorated by allicin administration his results proved the beneficial role of allicin as an adjuvant to TAM in cancer treatment by alleviating liver injury.

Wang *et al*., (2015) studied the Protective effect of allicin against glycidamide-induced toxicity in male and female mice and found that the given dose of glycidamide had more toxic effects and damage effects to the mice compared to the previous study of acrylamide. It could markedly increase the level of AST, ALT, LDH, BUN, ROS, MDA while decrease the SOD, GST and GSH. However, our data showed the oral administered allicin could significantly decrease the damage indexes of AST, ALT, LDH, BUN, ROS, MDA, and MPO, while increase the antioxidant indicators of SOD, GST and GSH. Thus, allicin could be used as an effective dietary supplement for the chemoprevention of glycidamide genotoxicity internally, and to prevent the tissue damage and toxicity induced by glycidamide.

Panyod *et al*., (2016) investigated diet supplementation with allicin protects against alcoholic fatty liver disease (AFLD) in mice by improving anti-Inflammation and ant oxidative Functions. His study demonstrated that allicin reduced fat accumulation, increased glutathione, and catalase levels, and decreased microsomal protein cytochrome P450 2E1 (CYP2E1) expression in the livers of the AFLD mice. Further, allicin supplementation significantly decreased the levels of proinflammatory tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6, and suppressed the expression of sterol regulatory element-binding protein-1 (SREBP-1). Collectively, these findings demonstrated that allicin attenuates liver oxidative stress and inflammation.

Samra *et al*., (2020) studied the protective effect of allicin on acetaminophen (APAP) - induced hepatotoxicity in mice and investigated the underlying mechanisms

of the anti-inflammatory and anti-apoptosis properties of allicin. And found that APAP significantly increased AST, ALT, and ALP, whereas allicin significantly decreased their levels. Also, APAP significantly decreased albumin and allicin significantly improved it. APAP produced changes in liver morphology, including inflammation and massive coagulative necrosis. Allicin protected the liver from APAP-induced necrosis, apoptosis, and hepatocellular degeneration via increasing Bcl-2 and Ki-67 levels. APAP significantly increased the hepatic MDA, whereas allicin significantly prevented this increase. APAP markedly activated the NLRP3 inflammasome pathway and consequently increased the production of caspase-1 and IL-1β. Allicin has a hepatoprotective effect against APAP-induced liver injury via the decline of oxidative stress and inhibition of the inflammasome pathway and apoptosis.

Saleh *et al.*, (2021) examined thioacetamide-induced acute hepatic encephalopathy (HE): central vs peripheral effect of Allicin and his result revealed that Induction of HE by a single dose of thioacetamide (TAA) was associated with a marked elevation in the serum levels of alanine aminotransferase, aspartate aminotransferase, bilirubin, albumin, total protein, blood urea nitrogen and serum ammonia besides reduction in the serum level of albumin. Moreover, it was accompanied with an increase in the hepatic and brain levels of inflammatory mediators; TNF-α and IL-1β as well as elevation of the hepatic and brain levels of oxidative stress biomarkers; reduced glutathione and lipid peroxidation evidenced by malondialdehyde. Oral administration of allicin for 6 days prior to TAA injection restored the serum liver function, hepatic, and brain levels of inflammatory mediators as well as oxidative stress biomarkers in a dose-dependent manner. From his results, it can be concluded that allicin has a protective effect on TAA-induced HE in rats in a dose-dependent manner due to its powerful antioxidant and anti-inflammatory properties.

Sun *et al.*, (2021) studied allicin mitigates hepatic injury following cyclophosphamide administration via activation of Nrf2/ARE pathways and through inhibition of inflammatory and apoptotic machinery and reported that administration of CP at a single dose was associated with liver injury, as is seen by the significant increase in the levels of ALT, AST, and ALP, as compared to the levels in the control group. Meanwhile, supplementation with allicin prior to administration of CP significantly ameliorated the levels of these physiological markers, as compared to the

CP-treated group. Also, CP led to a significant elevation in the levels of MDA, a lipid peroxidation marker which were accompanied by a significant decrease in GSH content in the hepatic tissue, as compared to the levels in the control group. Pretreatment with allicin significantly inhibited the elevation of oxidative and nitrogenous stress in the hepatic tissue after administration of CP, as evidenced by the decreased MDA along an increased GSH content. These finding reflected the hepatoprotective efficiency of allicin against CP-induced liver injury.

1.2.2 Nephroprotective effect of Allicin:

El-Kashef *et al*., (2015) investigated the Protective effect of allicin against gentamicin-induced nephrotoxicity in rats and clarified that gentamicin administration caused a severe nephrotoxicity as evidenced by an elevated serum creatinine, blood urea nitrogen (BUN), serum lactate dehydrogenase (LDH) and proteinuria with a reduction in serum albumin and creatinine clearance as compared with control group. In addition, a significant increase in renal contents of malondialdehyde (MDA), myeloperoxidase (MPO), nitric oxide (NOx) and tumor necrosis factor-alpha (TNF-α) concomitantly with a significant decrease in renal reduced glutathione (GSH) and superoxide dismutase (SOD) activities was detected upon gentamicin injection. Administration of allicin significantly decreased serum creatinine, LDH, renal MDA, MPO, NOx and TNF-α while it significantly increased creatinine clearance, renal GSH content and renal SOD activity when compared to gentamicin-treated group. his study indicated that allicin exerted protection against structural and functional damage induced by gentamicin possibly due to its antioxidant, anti-inflammatory, and immunomodulatory properties in addition to its ability to retaining nitric oxide level.

García *et al*., (2017) investigated The Beneficial Effects of Allicin in Chronic Kidney Disease Are Comparable to Losartan and found that after CKD induction, increased creatinine, and blood urea nitrogen (BUN) levels in serum, increased albuminuria, increased urinary excretion of N-acetyl-_-D-glucosaminidase (NAG), increased nephrin expression, and increased histological alterations in the cortex. The levels of angiotensin receptors and endothelial nitric oxide synthase (eNOS) were decreased in the renal cortex from the CKD group. Otherwise, lipid and protein oxidation were higher in the CKD group than in the control group. A disturbance was observed in the antioxidant enzymes catalase, superoxide dismutase, and heme oxygenase 1. Allicin or losartan treatments relieved renal dysfunction, hypertension,

and oxidative stress evidenced by decreasing renal biochemical marks. Allicin showed antihypertensive, antioxidant, and nephroprotective effects.

Abdel-Daim *et al*., (2019) examined nephroprotective effects of allicin and ascorbic acid against cisplatin-induced toxicity in rats and his study resulted in administration of CDDP induced marked body weight loss and renal damage, manifested by significant increases in serum creatinine, urea, and uric acid levels and significant reductions in serum Na, Ca, and phosphorus concentrations, in addition to severe alterations in serum and renal tissue levels of tumor necrosis factor-α in comparison with control rats. Moreover, CDDP-intoxicated rats exhibited significantly higher lipid peroxidation, as well as lower levels of reduced glutathione and activities of glutathione peroxidase, superoxide dismutase, and catalase enzymes in the renal tissue, compared with control rats. The administration of allicin or AA significantly reduced the CDDP-induced changes in all the afore mentioned parameters. Interestingly, allicin achieved comparable nephroprotection to AA in most assessed parameters; however, the restoration of normal serum and renal tissue concentrations of these parameters was more frequent in the CDDP-AA group. These results are probably mediated by their antioxidant and anti-inflammatory activities.

Orabi *et al*., (2020) studied Allicin modulates diclofenac sodium induced hepatonephro toxicity in rats via reducing oxidative stress and caspase-3 protein expression and reported that Diclofenac sodium significantly elevated activities of serum aspartate aminotransferase and alanine aminotransferase and the effects of diclofenac sodium and/or allicin on serum levels of urea and creatinine. Serum levels of urea and creatinine were significantly increased in rats administered diclofenac sodium compared to that of the control rats. However, the administration of rats with diclofenac sodium and allicin simultaneously prevented diclofenac sodium induced alteration of serum urea and creatinine levels and kept them at normal control values. In addition, it induced hyperglycemia, lipid peroxidation, pathological alteration, and caspase 3 protein expression in hepatic and renal tissues. However, it decreased reduced glutathione concentration and proliferating cell nuclear antigen protein expression in hepatic tissues. In contrast, allicin modulated the diclofenac sodium induced alteration in liver and kidney functions and structures dose dependently.

1.3 Omega3:

Omega-3 ($C_{60}H_{92}O_6$) polyunsaturated fatty acids (PUFAs) include α-linolenic acid (ALA; 18:3 ω-3), stearidonic acid (SDA; 18:4 ω-3), eicosatetraenoic acid (EPA; 20:5 ω-3), docosapentaenoic acid (DPA; 22:5 ω-3), and docosahexaenoic acid (DHA; 22:6 ω-3). In the past few decades, many epidemiological studies have been conducted on the myriad health benefits of omega-3 PUFAs. **(Shahidi and Ambigaipalan., 2018).**

ALA is a precursor of DHA and EPA, but its synthesis in our body is very limited **(SanGiovanni *et al.*, 2008).** Omega fatty acids are reported to show several beneficial effects including antioxidant, anti-inflammatory, immunomodulatory, hypolipidemic, anti-cancer as well as cardioprotective, neuroprotective and hepatoprotective activities (**Swanson *et al.*, 2012)**. Furthermore, OMG-3 fatty acids play a key role in maintaining optical function by protecting macular region from oxidative stress (lipid peroxidation), inflammation **(Christen *et al.*, 2011).**

Long-chain (LC) OMG-3FAs such as EPA and DHA occur in the body lipids of fatty fish, the liver of white lean fish, and the blubber of marine mammals. Fish oils are sold as OMG-3PUFA supplements or in a concentrated form as ethyl esters (EEs) or acylglycerols, whereas algal, fungal, and single-cell oils have recently become popular as novel and renewable sources of LC OMG-3FAs. Researchers have also incorporated OMG-3PUFAs into different oils such as borage oil and evening primrose oil to provide a better balance of PUFA components **(Hamam and Shahidi, 2006).**

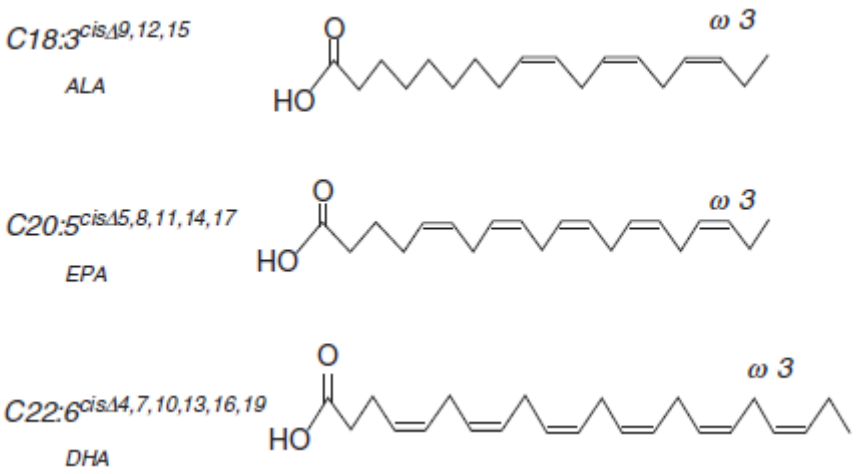

Fig (1-3): Chemical Structure of Omega-3 Fatty Acids (Chiu *et al.*, 2018).

The beneficial effect of OMG-3PUFAs in patients with myriad health conditions and diseases, such as cardiovascular disease (atrial fibrillation, atherosclerosis, thrombosis, inflammation, and sudden cardiac death, among others), diabetes, cancer, depression and various mental illnesses, age-related cognitive decline, periodontal disease, and rheumatoid arthritis, has been investigated (**Finley and Shahidi, 2001; Lopez *et al*., 2011**). Omega-3 fatty acids, one of the key building blocks of cell membranes **(Cholewski *et al*., 2018).**

1.3.1 Hepatoprotective effect of Omega3:

Mathews *et al*., (2014) studied the mitigation of hepatotoxic effects of arsenic trioxide through omega 3 fatty acid in rats and found a significant rise in lipid peroxidation with concomitant decline in reduced glutathione, glutathione dependent antioxidant enzymes and antiperoxidative enzymes in the liver tissue of rats treated with arsenic. The supplementation of omega-3 fatty acid with As2O3 offers ameliorative effect against hepatocellular toxicity. Omega-3 fatty acids maintained hepatic marker enzymes, antioxidant enzymes and decreased lipid peroxidation. The combination treatment clearly reduced the hepatic structural abnormalities such as hemorrhage, necrosis and cholangiofibrosis in the rats treated with arsenic. This study concludes that the omega- 3 fatty acid might be useful for the protection against As2O3-induced hepatotoxicity.

Adeyemi and Olayaki, (2017) investigated the protective effect of low dose of omega-3 fatty acids against diclofenac induced hepatotoxicity. the result showed that diclofenac significantly increased malondialdehyde, lactate dehydrogenase, and pro-inflammatory markers (total white blood cell count, uric acid, platelet/lymphocyte, and neutrophil/lymphocyte ratios). Moreover, DF significantly elevated the activities of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase, but significantly reduced the total antioxidant capacity and the activities of superoxide dismutase, catalase, and glutathione peroxidase. The histological results were parallel to the biochemical and hematological findings. Pre-treatment with OMG-3 FAs significantly prevented the manifestation of the abnormalities brought about by DF. Although there were indications of the dose-dependent effects of OMG-3 FAs, the low dose was found to be more effective.

Wang *et al*., (2017) indicated Omega-3 polyunsaturated fatty acids ameliorate ethanol-induced adipose hyper lipolysis: A mechanism for hepatoprotective effect

against alcoholic liver disease and his results demonstrated that endogenous and exogenous OMG-3 PUFA enrichment ameliorates ethanol stimulated adipose lipolysis by increasing PDE3B activity and reducing cAMP accumulation in adipocyte, which was associated with activation of GPR120 and regulation of Ca2+/CaMKKβ/AMPK signaling, resultantly blocking fatty acid trafficking from adipose tissue to the liver. His findings identify that endogenous and exogenous OMG-3 PUFAs enrichment ameliorated alcoholic liver injury by activation of GPR120 to suppress ethanol stimulated adipose lipolysis, which provides the new insight to the hepatoprotective effect of OMG-3 PUFAs against alcoholic liver disease.

Eraky and Abo El-Magd, (2020) examined Omega-3 fatty acids protective effect against acetaminophen-induced hepatic and renal toxicity in rats. His study resulted in pre-treatment with OMG-3fatty acids enhanced liver and kidney functions indicated by decreased serum aminotransferases activities and serum creatinine and urea concentrations. Moreover, OMG-3fatty acids showed antioxidant properties confirmed by decreased malondialdehyde level and increased total antioxidant capacity. Antioxidant Nrf2, its regulators (HO-1 and BACH1) and the anti-inflammatory cytokine (IL-10) were up-regulated by APAP administration as a compensatory mechanism, and they were normalized by OMG-3fatty acids. OMG-3fatty acids showed anti-inflammatory actions through down-regulating nuclear factor kappa B (NF-κB). These findings suggested the protecting actions of OMG-3fatty acids against APAP-induced hepatic and renal toxicity through regulation of antioxidant Nrf2 and inflammatory NF-κB pathways.

El-Gendy *et al.*, (2021) indicated the hepatoprotective effect of Omega-3 PUFAs against acute paracetamol induced hepatic injury confirmed by Fourier transform infrared **(FTIR).** FTIR results revealed that Omega-3 PUFAs limited the toxic effects of paracetamol by restoring the hepatic amide I to amide II ratio. In addition, biochemical analyses demonstrated that serum ALT, AST, Cholesterol, LDL-cholesterol, and Il-6 levels as well as hepatic TNF-a, MDA, NOx levels were decreased. Besides, serum HDL-cholesterol level and hepatic GSH level were increased. this study recommended to use Omega-3 PUFAs in low doses on daily bases as a hepatoprotective agent.

1.3.2 Nephroprotective effect of omega3:

Khan *et al.*, (2012) indicated protective effect of OMG-3 polyunsaturated fatty acids on L-arginine-induced nephrotoxicity and oxidative damage in rat kidney and found that ARG-induced nephrotoxicity was recorded by increased serum creatinine and blood urea nitrogen. ARG significantly altered the activities of metabolic and brush border membrane (BBM) enzymes. ARG caused significant imbalances in the antioxidant system. These alterations were associated with increased lipid peroxidation (LPO) and altered antioxidant enzyme activities. Feeding of FO and FXO with ARG ameliorated the changes in various parameters caused by ARG. Nephrotoxicity parameters lowered and enzyme activities of carbohydrate metabolism, BBM and inorganic phosphate (32Pi) transport were improved to near control values. ARG-induced LPO declined, and antioxidant defense mechanism was strengthened by both FO and FXO alike. The results of the present study suggest that OMG-3 PUFAs-enriched FO and FXO from seafoods and plant sources, respectively, are similarly effective in reducing ARG-induced nephrotoxicity and oxidative damage.

Naqshbandi *et al.*, (2012) studied the protective effect of dietary fish oil on cisplatin induced nephrotoxicity in rats and the result revealed that CP nephrotoxicity was recorded by increased serum creatinine and blood urea nitrogen. CP decreased the activities of metabolic enzymes, antioxidant defense system and BBM enzymes. In contrast, FO alone increased enzyme activities of carbohydrate metabolism and brush border membrane (BBM). FO feeding to CP treated rats markedly enhanced resistance to CP-elicited deleterious effects. Dietary FO supplementation ameliorated CP induced specific metabolic alterations and oxidative damage due to its intrinsic biochemical antioxidant properties.

Goksu *et al.*, (2013) investigated the protective effects of omega 3 fatty acids and sesame oil against cyclosporine A-induced nephrotoxicity and his investigation found that BUN was found to be increased in the CsA treated groups compared to control, whereas ALB was found to be decreased in the aforementioned groups, compared to control. Serum ALT level was found to be decreased compared to control. No difference was detected among groups for serum protein, AST, and GGT. So, his result concluded that OMG-3 FAs and SO have impressive nephroprotective and antiapoptotic effects on renal damage induced by CsA, with no difference in their protective effect, when compared to each other. However, the mechanisms underlying

the nephroprotection of OMG-3 FAs and SO treatment with lower doses should be further investigated.

Owumi *et al.*, (2020) documented that Cadmium and nickel co-exposure exacerbates genotoxicity and not oxido-inflammatory stress in liver and kidney of rats: Protective role of omega-3 fatty acid. The result of his study showed that renal functional indices (serum creatinine and urea levels) were significantly increased following exposure to Cd alone and Ni alone when compared with control. However, administration of OMG-3 FAs to rats co-exposed to Cd + Ni markedly decreased the serum creatinine, urea when compared with rats treated with Cd + Ni alone. Moreover, OMG-3 FAs markedly abrogated the reduction in the antioxidant enzyme activities, the increase in reactive oxygen and nitrogen species, and lipid peroxidation induced by Cd and Ni co-exposure. Additionally, OMG-3 FAs administration markedly suppressed the increase in hepatic and renal myeloperoxidase activity, nitric oxide, tumor necrosis factor alpha, and interleukin-1 β levels in the co-exposure group.

Saleh *et al.*, (2020) studied that omega-3 fatty acids can ameliorate doxorubicin-induced cardiorenal toxicity: In-vivo regulation of oxidative stress, apoptosis and renal Nox4, and in-vitro preservation of the cytotoxic efficacy. His study revealed that Induction of cardiac toxicity with DOX has been noticed by the marked elevation in the serum creatinine level as compared to the normal control group. Oral administration of OMG-3 FAs for 4 consecutive weeks to DOX-injected rats showed a suppression of the creatinine level, in a dose dependent manner. However, renal toxicity has been observed after DOX intraperitoneal injection by significance incrimination of serum levels of urea and creatinine, as compared to normal control group. Administration of OMG-3 FAs for 4 consecutive weeks succeed to alleviate the serum levels of urea and creatinine.

Xu *et al.*, (2021) examined Omega-3 polyunsaturated fatty acids alleviate adenine-induced chronic renal failure via regulating ROS production and TGF-β/SMAD pathway and found that Serum levels of Cr and BUN in OMG-3PUFAs group were remarkably decreased compared with those of adenine group. Higher contents of SOD, GSH, CAT and T-AOC were observed in OMG-3PUFAs group compared with those of adenine group. Besides, MAD content and ROS production were lower in OMG-3PUFAs group than those of adenine group. Pathological changes of kidneys were alleviated after OMG-3PUFAs treatment. Western blot

results demonstrated that OMG-3PUFAs treatment remarkably upregulates Nrf2, HO-1, NQO1, but downregulates relative genes in TGF-β/SMAD pathway. So, he concluded that OMG-3PUFAs alleviated adenine-induced chronic renal failure through enhancing antioxidant stress and inhibiting inflammatory response via regulating Nrf2 and TGF-β/SMAD pathway.

Aim of the study:

The aim of our study to assess the protective effect of Allicin in combination with Omega-3 on hepatorenal toxicity induced by APAP in rats through evaluating of these parameters:

1) Biochemical analysis of serum (ALT, AST, ALP, urea, creatinine, albumin, total protein, triglycerides and cholesterol).
2) Immunohistochemically testing (Caspase-3 and HSP70).
3) Antioxidant status of the kidney and liver (MDA, SOD, CAT, GSH).
4) Liver and kidney histopathological evaluation.

Protective effect of allicin and Omega-3 fatty acids against paracetamol-induced hepatic toxicity

Abstract

The most prominent over-the-counter antipyretic-analgesic drug is paracetamol (n-acetyl-para-amino-phenol, APAP). This study attempted to examine whether allicin (AC) and/or Omega-3 fatty acids (OMG-3FA) could protect rats from the liver damage induced by APAP. Seventy rats were randomly distributed into seven groups (n=10): Control (saline), AC group received allicin (10 mg/kg, PO), OMG-3FA group given omega-3 (100 mg/kg, PO), APAP group given paracetamol (1000 mg/kg, PO single dose on the 27th day), AC+APAP group received AC (10mg/kg) for 30 days and then given single dose of paracetamol on the 27th day (1000 mg/kg, PO), OMG-3FA+APAP group received OMG-3FA (100 mg/kg) for 30 days and then given single dose of paracetamol on the 27th day (1000 mg/kg, PO), and AC+OMG-3FA+APAP group received OMG-3FA (100 mg/kg) and AC (10 mg/kg) for 30 days and then given single dose of paracetamol on the 27th day (1000 mg/kg, PO). APAP had a significant negative impact on hematological and serum biochemical markers suggested that hepatic injury occurred in response to the APAP exposure. Antioxidant parameter were determined in liver tissues. Histopathological examination of liver sections confirmed this hepatic damage where hepatic degeneration and necrosis were evident after APAP treatment. Also, in APAP-induced hepatotoxicity, liver Caspase-3 and HSP70 expressions were considerably elevated. Allicin and/or omega 3 fatty acids treatment restore hepatic tissue architecture after treatment. Thus, pre-treatment with AC and OMG-3FA alone or in combination was effective to reduce hepatic injury in APAP-intoxicated rats.

Keywords: Paracetamol; Liver; Allicin; Omega-3 fatty acids; damage.

1. Introduction

Liver is the most important body organ which plays multi-biological functions during micro- and macromolecular metabolism, including drugs, carbohydrates, proteins, and lipids (**Islam *et al*., 2021**). Paracetamol (APAP) is a common analgesic and antipyretic drug which is regarded as a safe medication at therapeutic doses because it is mostly metabolized to pharmacologically inactive glucuronide and sulfate conjugates, with a minor fraction (5–10%) being oxidized to a reactive metabolite known as N-acetyl-p-benzoquinone imine (NAPQI) (**Li *et al*., 2016**). The

non-toxic glutathione-NAPQI then easily excreted out from the body **(Hussain *et al.*, 2020**).

NAPQI, generated by hepatic cytochrome P-450 isoforms (CYP2E1 and CYP2A6) which are mainly responsible for APAP-induced hepatotoxicity **(Wang *et al.*, 2017).** After an overdose of APAP, NAPQI level gets elevated which diminishes the cellular level of glutathione (GSH), affects mitochondrial proteins, induces mitochondrial oxidative stress, and subsequently cause overproduction of reactive oxygen species (ROS) such as superoxide anions (**Woolbright and Jaeschke 2018; Ebada, 2018**). Excess levels of ROS can bind biological molecules such as DNA, protein, and phospholipids, which leads to lipid peroxidation, depletion of antioxidant enzymes and the loss of calcium homeostasis following oxidative stress and cell death (**Ozdatli *et al.*, 2015**).

Several studies recorded the hepatic toxicity of paracetamol **(Elshal and Abdelmageed, 2022; Sinaga *et al.*, 2021; Sreevallabhan *et al.*, 2021; Jaeschke *et al.*, 2020; Gong *et al.*, 2018).** More and more people are using herbal medications made from plant extracts that have natural antioxidant and pharmacological properties to counteract the potentially toxic effects of chemical agents like paracetamol (**Wu *et al.*, 2017**).

Allicin, as a diallyl thiosulfinate, is the main biologically active compound derived from garlic **(Wang *et al.*, 2015).** Once garlic is damaged, alliinase catalyzes the conversion of S-allylcysteine sulfoxide into diallylthiosulfinate or allicin (**Abdel-Daim *et al.*, 2019; Borlinghaus *et al.*, 2014**). Allicin is a thiosulfinate compound with numerous biological and pharmacological activities, including antioxidant (**Saleh *et al.*, 2021),** antimicrobial (**Reiter *et al.*, 2017**), hepatoprotective (**Yang *et al.*, 2017**), nephroprotective (**Abdel-Daim *et al.*, 2019**), neuroprotective (**Kong *et al.*, 2017**).

Allicin is a natural antioxidant, that not only scavenges oxygen free radicals and hydroxyl radicals, but also prevents the lipid peroxidation of liver homogenates induced by hydroxyl radicals (**Chung *et al.*, 2013; Zhang *et al.*, 2012**). Allicin has been demonstrated to have hepatoprotective effects against paracetamol-induced hepatic damage (**Samra *et al.*, 2020**) by inhibiting apoptosis, lowering the inflammasome pathway and reducing oxidative stress. Consequently, allicin may be a

cutting-edge strategy in order to resist the development of APAP-caused hepatotoxicity **(Samra *et al.*, 2020).**

Long-chain fatty acids called omega-3 polyunsaturated fatty acids are distinguished by the presence of a double bond at the third carbon atom of the hydrocarboxylic chain counted from the methyl end **(Calder, 2018)**. The highest sources of docosahexanoic acid (DHA) and eicosatetranoic acid (EPA) are fish (particularly oily fish) and other seafood, despite the fact that these very long chain fatty acids can be found in a wide variety of foods **(Scorletti and Byrne, 2018)**.

Omega-3FAs, particularly the biochemically active downstream fatty acids eicosatetraenoic acid (EPA) and docosahexaenoic acid (DHA) are metabolized to anti-inflammatory and proresolving mediators (**Serhan and Petasis, 2011; Calder, 2012).** They have a variety of proposed mechanisms of action; the most significant of which would be modulating cell proliferation, regulating fatty acid metabolism, inhibiting lipogenesis as well as suppressing inflammation and oxidative stress (**Bellenger *et al.*, 2011; Huang *et al.*, 2015; Wang *et al.*, 2017**). Therefore, the current study attempts to elucidate the preventive role of AC and/or OMG-3FAs in preventing liver damage caused by APAP.

2. Materials and methods

2.1. Chemicals:

Paracetamol (APAP, 1 g) was bought as Panadol® from GlaxoSmithKline Pharmaceuticals Company (Brentford, United Kingdom). Allicin, was bought as pure powder (35% Conc.) from Delta Vet Center (Cairo, Egypt). Omega-3 fatty acids, was bought as pure fish oil (Conc.100%) from Sigma Pharmaceutical Industries (Cairo, Egypt). The used kits were bought from Bio-diagnostic Company (Giza, Egypt).

2.2. Experimental animals:

Seventy male albino Wister rats, 2 months age weighing 160-200 gm were obtained from the Center of Laboratory Animals, Faculty of Veterinary Medicine, Benha University, Egypt. Prior to the experiment, the rats were left for acclimatization for 14 days (temperature 25°C) and were fed ideal laboratory commercial diet and water *ad libitum*. Ethical approval from Animals Care and Use Committee Research Ethics Board was obtained from Faculty of Veterinary Medicine, Benha University (BUFVTM 07–03-22).

2.3. Experimental design:

Rats were divided into 7 equal groups (10 rats in each group). Group 1 (Control); had been given distilled water. Group (2); AC (10 mg/kg b. wt, orally). Group (3); OMG-3FAs (100 mg/kg b. wt, orally). These doses of AC according to **Samra *et al*., (2020)** and for OMG-3FA **(El-Gendy *et al*., 2021)**. Group (4); APAP toxic control group that received saline, orally once daily and a single dose of APAP 1 g/kg b. wt orally on the 27^{th} day of the experiment. Group (5); (AC+APAP). Group (6); (OMG-3FA+APAP). Group (7); (AC+OMG-3FA+APAP). rats in these groups have been received allicin, omega-3 and APAP as described before. Saline, allicin, and omega-3 were administered for 30 days.

2.4. Sampling:

Rats were anaesthetized at 31^{st} day of the experiment, blood samples were collected from Retro-bulbar venous plexus. Then all rats were euthanized and liver tissues were taken out and cut into two parts, one for histopathological investigation; the other part was preserved at -80°C for biochemical analysis.

2.5. Hematological analysis:

The whole blood samples were used directly after collection on EDTA for estimation of hematological parameters including the red blood cells (RBCs) count, hemoglobin (Hb) concentration, white blood cells (WBCs) count, hematocrit value (APAPV%) and platelets (Plt) count. These parameters were estimated using automated hematology analyzer (Mindray BC-2800, China).

2.6. Serum biochemical studies:

The biochemical markers were AST and ALT **(Reitman and Frankel, 1957)**, ALP (**Tietz *et al*., 1983**), triglycerides (TG) and cholesterol (**Shah *et al*., 2011**), albumin (**Doumas *et al*., 1971**) and total protein (**Doumas and Biggs, 1975**). The previous biochemical tests were evaluated in accordance with data protocol provided by using commercial kits (Bio-Diagnostic Company, Giza, Egypt).

2.7. Tissue homogenate preparation for oxidative cascade evaluation:

The tissue was dissected and rinsed by a PBS solution (phosphate-buffered saline) consist of 0.16 mg/ml heparin to separate any RBCs and curd. Tissues were homogenized by sonicator homogenizer using 5 ml of 5-10 ml buffer (i.e., 50 mM potassium phosphate, pH 7.5 1 mM EDTA) added each gram of tissue. Tissue homogenates aliquots were centrifuged in a cooling centrifuge (4000 rpm for 20 min)

and then kept at − 80^{o}C. Next, oxidative status was done by determination of malondialdehyde (MDA) level **(Uchiyama and Mihara, 1978)**, catalase (CAT) activity **(Aebi, 1984)**, and reduced-glutathione (GSH) level **(Beutler, 1963)** utilizing specific diagnostic kits get from the Laboratory Biodiagnostic Company.

2.8. Histopathological alteration:

The liver tissue from each rat was fixed quickly in 10% neutral-buffered formalin for histopathology. The liver was progressively dehydrated, embedded in paraffin, cut into 5-µm sections, and stained with the hematoxylin and eosin (H&E) for histological inspection according to the method described by **Bancroft and Gamble (2008)**. Finally, light microscopy was used to examine liver tissue sections (Leica, Germany).

2.9. Immunohistochemical examination:

Liver tissue sections were heated at 60°C for 25 min in an oven (Venticell, MMM, Einrichtungen. Germany) and then deparaffinized in xylene and rehydrated using graded alcohol. The process of antigen retrieval was performed in 10 mM sodium citrate buffer boiled in a microwave. Immunohistochemistry staining steps were performed following the manufacturer's instructions (DakoCytomation, USA). In brief, endogenous peroxidase was blocked using 0.03% hydrogen peroxide sodium azide for 5 min. Tissue sections were washed gently with wash buffer and then incubated with HSP70 were applied at a dilution of 1:200 and of 1:250 respectively and polyclonal anti-caspase 3 antibodies (Invitrogen, Cat# PA5-77,887, dilution 1/100) for overnight at 4 °C followed by incubation with avidin–biotin complex (ABC kit, Vector Laboratories) at 37 °C for 45 min. Sections were gently washed with wash buffer and kept in the buffer bath in a humid chamber. A sufficient amount of streptavidin-HRP was then added and incubated for 15 min followed by washing. Diaminobenzidine-substrate chromagen was added to the sections and incubated for over 7 min followed by washing and counterstaining with hematoxylin for 5 sec. The sections were then dipped in weak ammonia (0.037 M/L) 10 times, washed and cover slipped. Positive antigens stained brown under light microscopy.

2.10. Statistical analysis:

Statistical analysis was carried out using SPSS (Version 20; SPSS Inc., Chicago, USA). The significant divergence through multiple groups comparisons

were analyzed by one-way ANOVA and Duncan test as a post hoc test was used. All values are expressed as mean ± SE, with significance considered at $P \leq 0.05$.

3. Results

3.1. Hematological examination:

Figure 1 depicts the haematological analysis results. When compared to the control group, exposure to APAP significantly reduced the values of RBC counts, Hb concentrations, plateletes counts (PL), and packed cell volumes (PCV), while increasing the values of WBC counts. Allicin and/or omega3 administration reduced the harmful effects of APAP by reversing these changes in haematological parameters to the values observed in control rats.

3.2. Biochemical analysis:

The increased serum levels of the liver biomarkers showed the induction of hepatotoxicity (**Figure 2**). When compared to the control rats, the effects of APAP toxicity significantly elevated the AST, ALT, and ALP activities as well as the cholesterol and triglycerides levels. Also, APAP decreases the concentrations of total protein and albumin levels in serum. Contrarily, the case is different where these parameters were significantly decreased in the APAP treated-rats with AC, OMG-3FA, or combination treatment (AC and OMG-3FAs) in comparison with the APAP group. When APAP-intoxicated rats were treated with both AC and OMG-3FAs, these parameters were restored almost to normal levels when compared to treatment with either AC or OMG-3FA alone. Thus, combination of AC and OMG-3FA showed better protection from liver damage caused by APAP than either alone.

3.3. Oxidative stress markers assay:

Results of APAP toxicity and pretreatment with allicin and/or omega-3 on oxidative parameters and lipid peroxidation in the liver have been shown in **(Figure 3)**. Remarkable reduction in CAT, SOD and GSH levels with Significant elevation in MDA levels in liver tissues in APAP-intoxicated rats compared to control rats. Besides that, the damaging effects of APAP on liver SOD, CAT, MDA and GSH were significally decreased by the administration of allicin and/or OMG-3.

3.4. Histopathological changes of liver:

Liver sections from control, AC and OMG-3FA treated rats exhibited normal hepatic histo-architecture. Hepatocytes organized in cords radiating from central veins and separated by regular sinusoids (**Figs. 4A, B, C**). Otherwise, APAP intoxicated

rats revealed several histological changes represented by severe congestion of the central veins and hydropic degeneration of the hepatocytes (**Fig.4D**), necrosis of some hepatocytes as well as inflammatory cells aggregation (Fig. 3E). Liver sections from paracetamol+allicin treated rats represented mild congestion in the central veins with few inflammatory cells infiltration (**Fig. 5A**). The examined liver of rats in paracetamol+OMG-3FA group revealed few inflammatory cellular infiltrations in addition to congestion of blood sinusoids (Fig. 4B). While the liver in paracetamol +AC+OMG-3FA group showed congestion of central veins and sinusoids with no inflammatory cellular infiltration (**Fig. 5C**).

3.5. The expression of HSP70 and Caspas-3 was associated with pathological changes of the liver:

The immunohistochemical staining of the liver (Cleaved caspase-3 expression& HSP70)

In liver sections of normal control rats, no caspase-3 immunoreactive hepatocytes were detected **(Fig. 6A)**. Neither Allicin nor Omega3 alone considerably changed the caspase-3 expression. APAP-intoxicated group, however, showed strong expression of cleaved caspase-3 **(Fig. 6D)**. As for livers treated with either APAP-Allicin or APAP-Omega3, they were moderately immune **(Fig. 6E, F)**. Both APAP-Allicin and APAP-Omega3 produced almost the same intensity of positive reactions.

On the other hand, there was a weak positive caspase-3 reaction in sections from the APAP-Allicin+ Omega3 group **(Fig. 6G).**

To elucidate the function of HSP70 more directly during hepatic APAP-injury, we employed HSP70 immunostaining. HSP70 semi quantitative analysis did not differ significantly between the groups in their response to allicin and or omega3 treatment **(Fig. 8)**. However, APAP+ Allicin + Omega3 group showing a smaller number of immune positive cells.

4. Discussion

The liver is an important organ for the detoxification and deposition of endogenous and exogenous substances. The goal of the current study was to establish a scientific basis for the use of allicin and omega-3 combination therapy in conventional medicine by examining its protective effects against APAP-induced hepatotoxicity in Wistar rats. One of the most commonly used experimental models for assessing a drug's hepatoprotective capabilities is APAP-induced liver damage. Indeed, APAP-treated rats showed higher serum levels of each of these indicators than did controls, which supported the existence of hepatic injury and demonstrated

the model's viability. Drug-induced hepatotoxicity is one of the major reasons for the mortality and morbidity of human beings across the world **(Bhawna & Kumar, 2009)**.

Paracetamol (acetaminophen) is used globally for its analgesic and antipyretic properties: however, it causes acute liver damage if administered in overdose **(Akhilraj *et al.*, 2021).** The toxic effect of paracetamol on the liver is not only from paracetamol, but also from its metabolite N-acetyl P benzo quinonimine (NAPQI) also known as N-acetyllimidoquimone. NAPQI depletes the liver's natural antioxidant glutathione and directly damages liver cells, leading to liver failure **(Akhilraj *et al.*, 2021).** APAP is also directly involved in the induction of oxidative stress resulting in lipid peroxidation, depletion in antioxidants, and ATP synthesis, and ultimately leading to liver damage **(Rabiul *et al.*, 2011)**.

The current investigation showed that a single acute overdose of APAP led to a significant change in some hematological and biochemical parameters.

Oyedeji *et al.*, (2013) stated that erythrocyte deformability is decreased and membrane permeability is increased in toxic APAP doses, which lowers erythrocyte survival. Our results recorded decreased values of RBCs, Hb, APAPV, and PL counts in APAP-intoxicated rats. Therefore, it was presumed that APAP increases the degradation rate of erythrocytes. The rise in WBCs seen in the APAP-treated rats is consistent with the result of **Matić et al. (2021)** and that may be an indicator to acute inflammation. The harmful effects were reduced by allicin and/or omega3 treatment, which returned changes in the hematological parameters to the recorded values in the control group.

Furthermore, APAP intoxication revealed significant elevations of serum ALT, AST, ALP, triglycerides (TG) and total cholesterol (TC). Both serum ALT and AST concentrations as a diagnostic for hepatic necrosis. Also, APAP caused reduction in serum levels of total protein and albumin. The reductive transfer of amino acids from alanine or aspartate, respectively, to alpha ketoglutarate to produce pyruvate or oxaloacetate, is carried out by both the ALT and AST enzymes. Hepatocytes that have been damaged discharge their contents, including ALT and AST, into the extracellular space **(Islam *et al.*, 2021).**

The hepatic cells are harmed by the NAPQI which created by an excess intake of paracetamol through lipid peroxidation, which damages cellular permeability and raises blood levels of ALT and AST **(Islam *et al*., 2021).**

Moreover, the elevated serum ALP level observed, in this study, could be attributable to defective hepatic excretion or increased ALP synthesis by hepatic parenchymal or duct cells in the presence of increasing biliary pressure as reported by Iyanda and Adeniyi **(Iyanda and Adeniyi, 2011).** The intoxication of APAP seems to cause impairment of metabolism of lipoprotein **(Kobashigawa and Kasiske, 1997).** leading to alteration of cholesterol metabolism. The availability of free acid, slower hepatic release of lipoprotein, and enhanced esterification of free acids may all contribute to the higher blood level of TG produced by APAP.

The reduction in total protein levels seen in APAP-treated rats suggests the destruction of many hepatic cells, which may result in a decrease in hepatic capacity to synthesis protein as most plasma proteins are synthesized by hepatocytes **(Chaphalkar *et al.*, 2017).** Oxidative stress is another possible indicator of liver damage and as reported before it is an important mechanism that has been proposed to have a role in the development of APAP toxicity **(Wang *et al.*, 2017).**

Oxidative stress induces the production of free oxygen radicals, an undesirable by-product. It is the main factor in APAP induced liver toxicity and can exacerbate free radical chain reactions **(Parikh *et al.*, 2015).** In the current study, we observed that the APAP treated animal group showed significant reduction in GSH, SOD, CAT and increased MDA level compared with the vehicle group as indicator of lipid peroxidation in the liver of rats. These changes in these biomarkers are due to depletion of the enzyme substrates and irreversible inactivation of enzyme proteins from increased ROS production **(Verma *et al.*, 2017).** Treatment with allicin and/or omega 3 significantly lessened the damaging consequences, reverting alterations in the biochemical and oxidative parameters to those that had been observed in the control group.

Allicin is the main active ingredient in freshly crashed garlic. It has been reported to have anti-inflammatory and antioxidant properties (**Shang *et al.*, 2019**). Allicin is a natural antioxidant, that not only scavenges oxygen free radicals and hydroxyl radicals, but also prevents the lipid peroxidation of liver homogenates induced by hydroxyl radicals (**Zhang *et al.*, 2012**). Our present study provides that

dietary allicin can partially offset the toxicity of APAP. The findings highlight the critical function of dietary antioxidants like garlic in the nutritional protection of oxidative stress-related pathologies.

Cytosolic aminotransferases and ALP are used as an index for hepatocellular membrane damage, as they leak out into the blood stream following exposure to chemicals, including drugs and toxic substances (**Al-Brakati *et al.*, 2019**). The APAP-treated group showed a considerable increase in these indicators. Serum liver functions, including ALT, AST, ALP, TG, and cholesterol, were significantly reduced. Additionally, allicin elevated total protein and albumin level when compared to toxic group. Remarkably, the elevated serum liver function markers that occurred after APAP administration were decreased by allicin supplementation. These findings suggest that allicin protects the liver from damage caused by APAP exposure by maintaining the hepatocyte membrane's shape and integrity.

Antioxidant enzymes such as CAT, MDA, GSH, SOD can protect cellular compounds against damage induced by free radicals. Therefore, the activities of these enzymes have been used to assess oxidative stress in cells (**Liu *et al.*, 2010**). Allicin can restore the activity of antioxidant enzymes and possibly reduce the generation of free radicals in vitro and in vivo. The antioxidant effect of allicin may be direct through scavenging of ROS or indirect by activating and increasing activity of endogenous cellular antioxidant defenses (**Kelsey *et al.*, 2010**).

Omega-3 long-chain polyunsaturated fatty acids (Omega-3 PUFAs) daily doses are nowadays recommended for their antioxidant and anti-inflammatory potentials (**El-Gendy *et al.*, 2021**). They have a variety of proposed mechanisms of action; the most significant of which would be modulating cell proliferation, regulating fatty acid metabolism, inhibiting lipogenesis as well as suppressing inflammation and oxidative stress (**Huang *et al.*, 2015; Wang *et al.*, 2017**). Omega-3 fatty acids have been shown to have hepatoprotective properties in several investigations (**Adeyemi and Olayaki, 2017**; **Eraky and Abo El-Magd, 2020**).

The mechanisms underlying the hepatoprotective effects of Omega-3 PUFAs includes its ability to increase GSH along with its capability to scavenge free radicals and consequently inhibit lipid peroxidation (**Shaaban *et al.*, 2014; Sohail *et al.*, 2019**).The APAP-induced hepatocellular injury was confirmed by a significant increase in serum ALT, AST, ALP activities, TG and cholesterol concentrations and

significant reduction in total protein and albumin levels compared with normal rats, indicating cell damage with cell membrane disruption, leading to cellular leakage and hepatic dysfunction. Pretreatment with OMG-3fatty acids ameliorated this increase in these activities and protected against hepatic dysfunction.

APAP toxicity is mainly associated with the over-production of ROS as well as deterioration of antioxidant capacity (**Hasanein and Sharifi, 2017**). ROS promotes membrane lipid peroxidation, fragmentation of polyunsaturated fatty acids, leading to cellular damage (**El-Ashmawy *et al.*, 2018**). This was demonstrated by the significant increase in hepatic MDA concentration as an indication of lipid peroxidation as well as a significant decrease in the hepatic GSH, SOD and CAT as an indication of oxidative stress. Antioxidant activity of OMG-3fatty acids was evidenced by the ability to decrease MDA concentration, inhibiting lipid peroxidation as well as increasing GSH, SOD and CAT.

Additionally, the results of histopathological examination of the liver confirmed the serum biochemical and oxidative findings which have been reported in other studies and showed that APAP hepatic damages tissue by causing oxidative damage **(Saritas *et al.*, 2014; Uysal *et al.*, 2016)**. The liver tissue was arranged in lobules according to the histological report of the normal control group. Significant and widespread necrosis with degenerative changes, central veins dilatation, were seen in the paracetamol group and these result compatible with previous study **(Akhilraj *et al.*, 2021)**. While the treated rats with allicin or omega 3 showed more hepatic protective evident and represented mild congested central veins with some inflammatory cells infiltration in addition to congestion of blood sinusoids in the combined treated rats, the examined liver showed congestion of central vein and sinusoids without inflammatory cells infiltration.

Besides that, a significant increase in the levels of apoptosis-related proteins such as caspase-3 was observed suggesting a vital role of apoptosis in paracetamol-induced hepatic injury as showed in the results. While our findings showed that allicin and/or OMG-3FAs downregulated the pro-apoptogenic proteins caspase 3.

Also, the HSP70 immunohistochemical staining of liver made to elucidate the function of HSP70 more directly during hepatic APAP-injury, we employed HSP70 immunostaining. HSP70 semiquantitative analysis did not differ significantly between the groups in their response to Allicin and or Omega3 treatment. However, APAP-

Allicin+ Omega3 group showing a smaller number of immune positive cells. Finally, our results reported that there was a direct link between allicin and/or OMG-3FAs pretreatment and suppression of apoptosis represented by a reduction in active caspase-3 and HSP70.

5. Conclusion

Pretreatment with allicin and/or omega-3 fatty acids had better protective effects on APAP-induced liver injury than either one alone. Therefore, combining allicin with omega-3 therapy could be a unique way to slow the progression of APAP-induced hepatotoxicity.

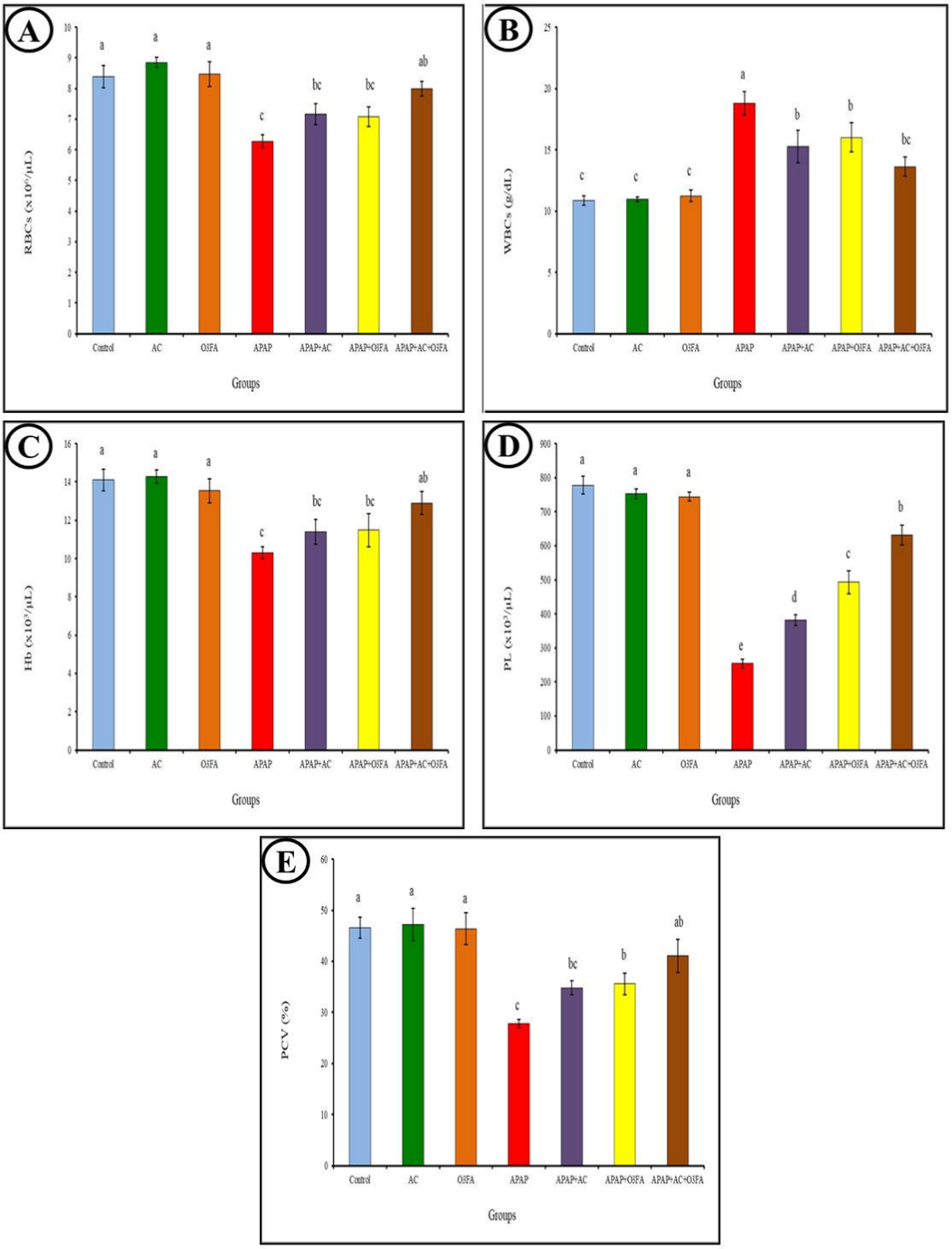

Fig. 2-1. Effect of allicin and/or omega3 and paracetamol on hematological parameters in rats

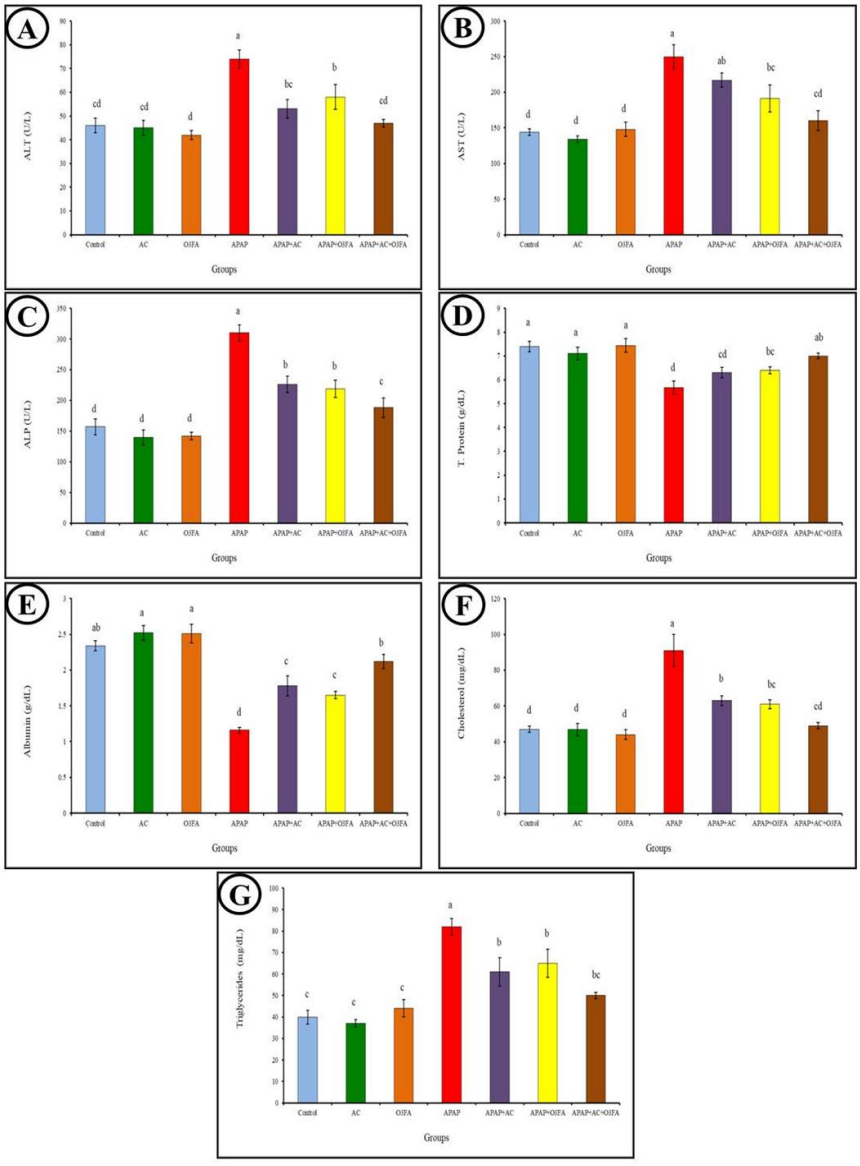

Fig. 2-2. Effect of allicin and/or omega3 on liver biomarkers in paracetamol-induced hepatotoxicity in rats including, ALT, AST, ALP, total protein, albumin, cholesterol and triglycerides.

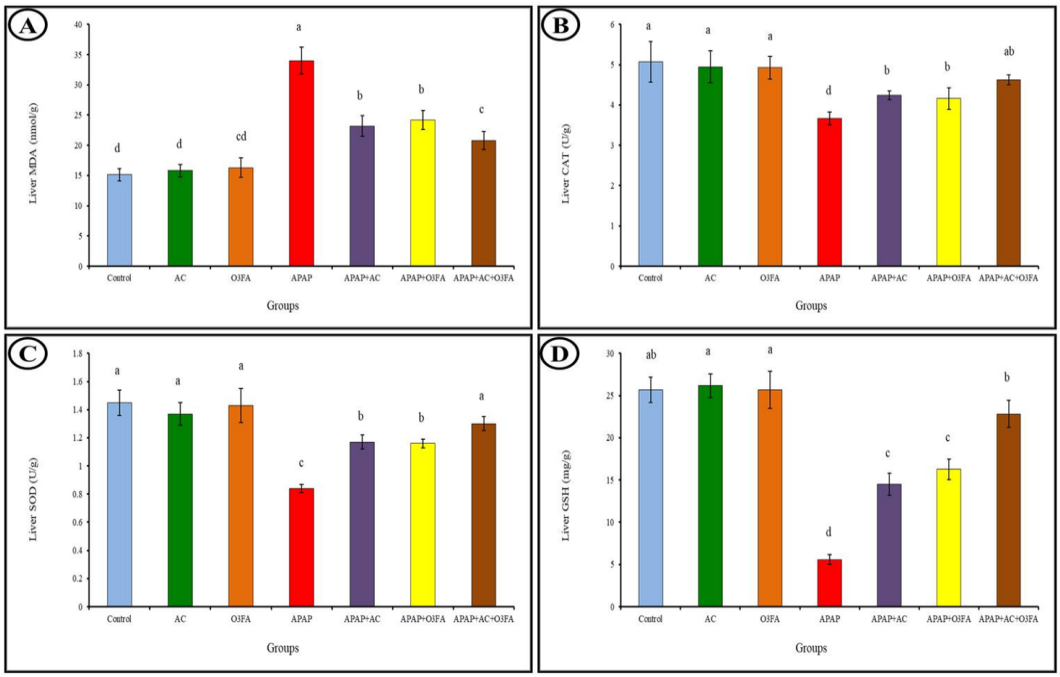

Fig. 2-3. Protective effect of allicin and/or OMG-3 on hepatic MDA, CAT, SOD and GSH level in rats with acute paracetamol (APAP) exposure. Values are Bars carrying different letters are significantly different at 0.05 propability.

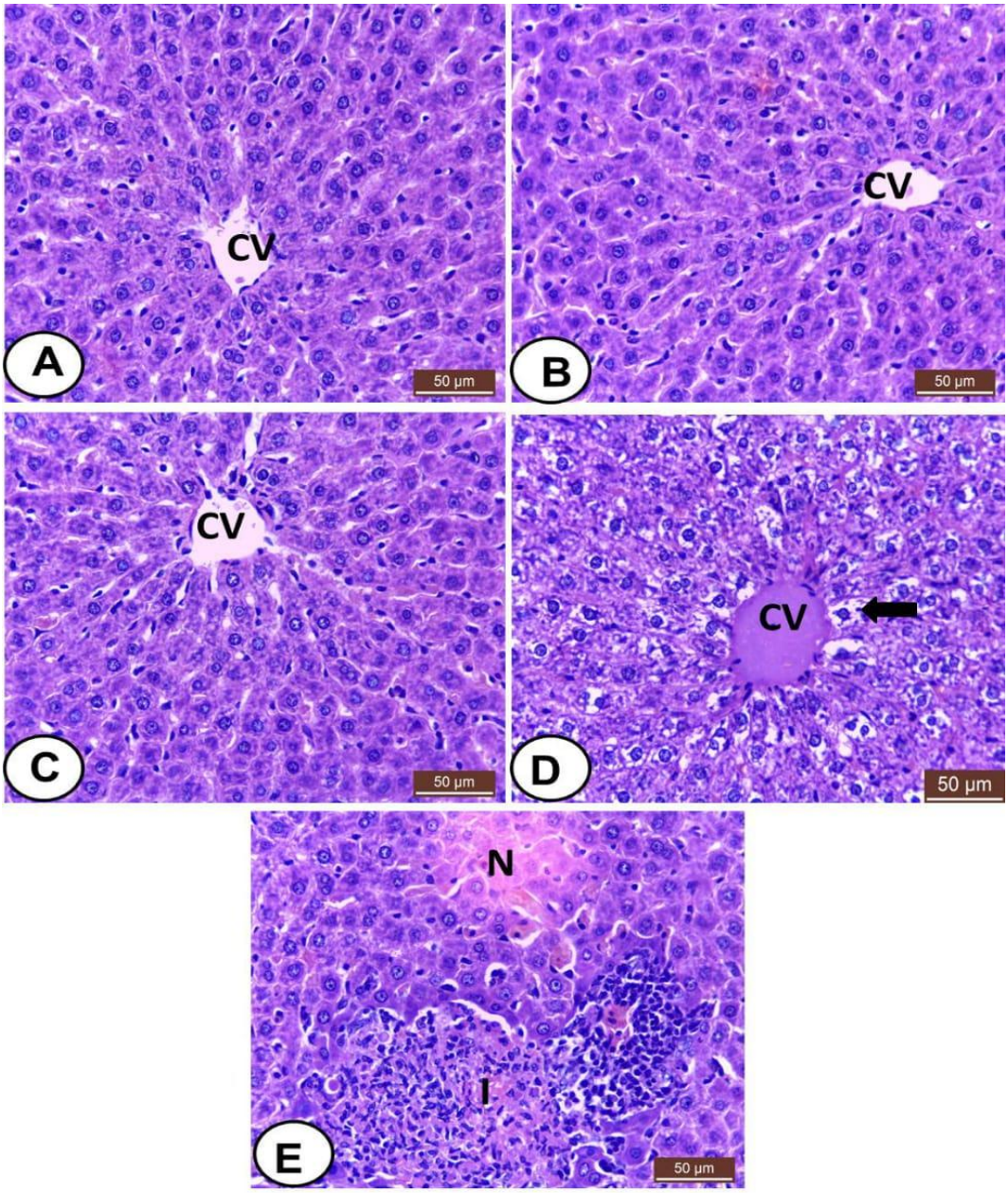

Fig. 2-4. Histopathological sections of livers from control, allicin, omega 3 and paracetamol treated rats A, B and C; Control, allicin and omega 3 groups exhibited normal hepatic histo-architecture. Hepatocytes radiated from central vein (CV) organizing in cords. D-E; Paracetamol intoxicated rats exhibted enormous histological changes. D; severe congestion of the central vein (CV) and hydropic degeneration of the hepatocytes (Thick arrow). E; inflammatory cells aggregation (I). H&E stain, scale bars=50µm.

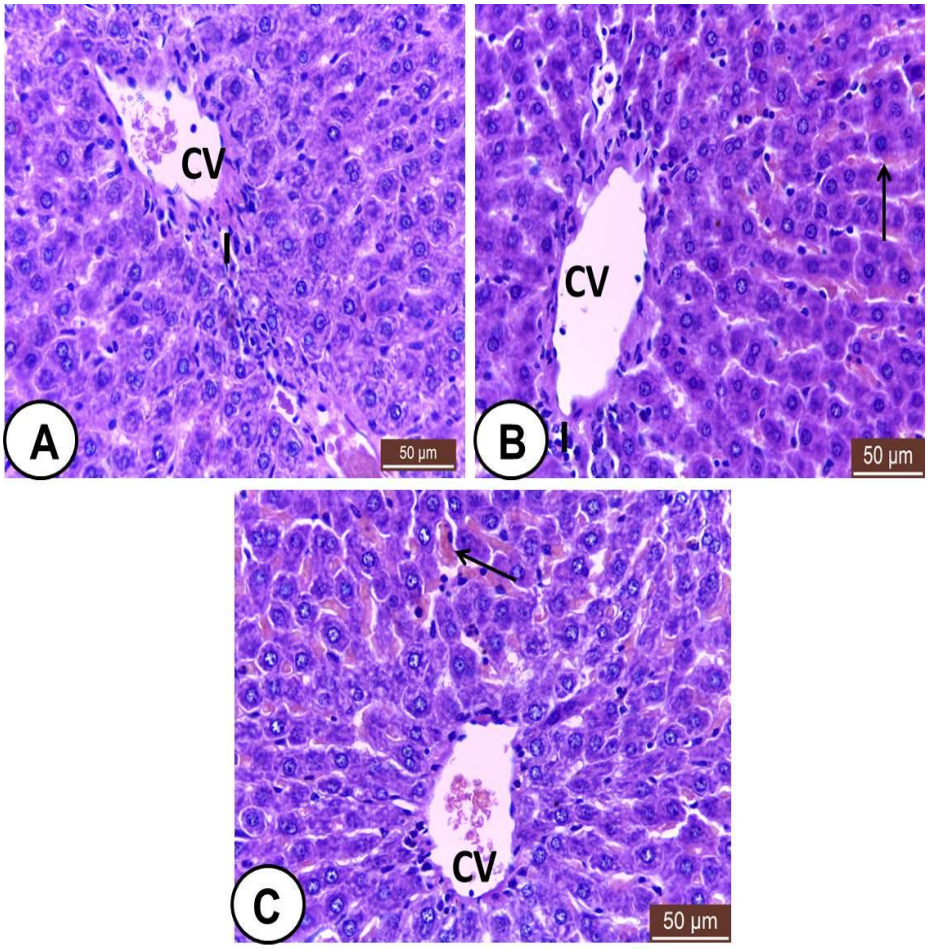

Fig. 2-5. Histopathological sections of livers from the rest of paracetamol+allicin, paracetamol+omega-3, and paracetamol+allicin+omega-3 groups. A, B and C; respectively. A; showed mild congested central vein (CV) with some inflammatory cells infiltration (I). B; showed few inflammatory cells infiltration (I) in addition to congestion of blood sinusoids (thin arrow). C; showed congestion of central vein (CV) without inflammatory cells infiltration. H&E stain, scale bars=50µm.

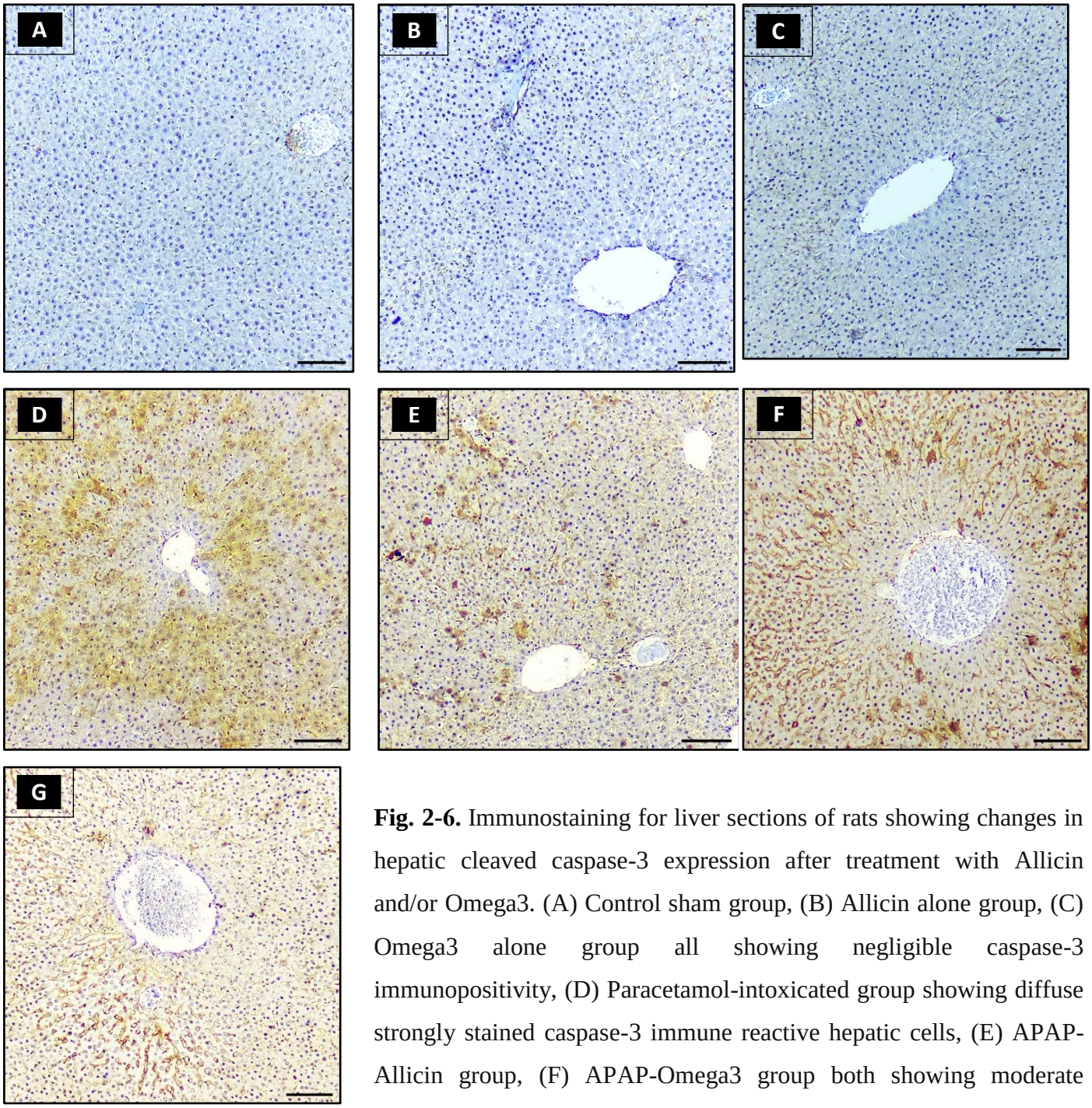

Fig. 2-6. Immunostaining for liver sections of rats showing changes in hepatic cleaved caspase-3 expression after treatment with Allicin and/or Omega3. (A) Control sham group, (B) Allicin alone group, (C) Omega3 alone group all showing negligible caspase-3 immunopositivity, (D) Paracetamol-intoxicated group showing diffuse strongly stained caspase-3 immune reactive hepatic cells, (E) APAP-Allicin group, (F) APAP-Omega3 group both showing moderate immunopositivity, (G) APAP-Allicin+ Omega3 group showing noticeably reduced caspase-3 expression. Brown color indicates immunopositivity.

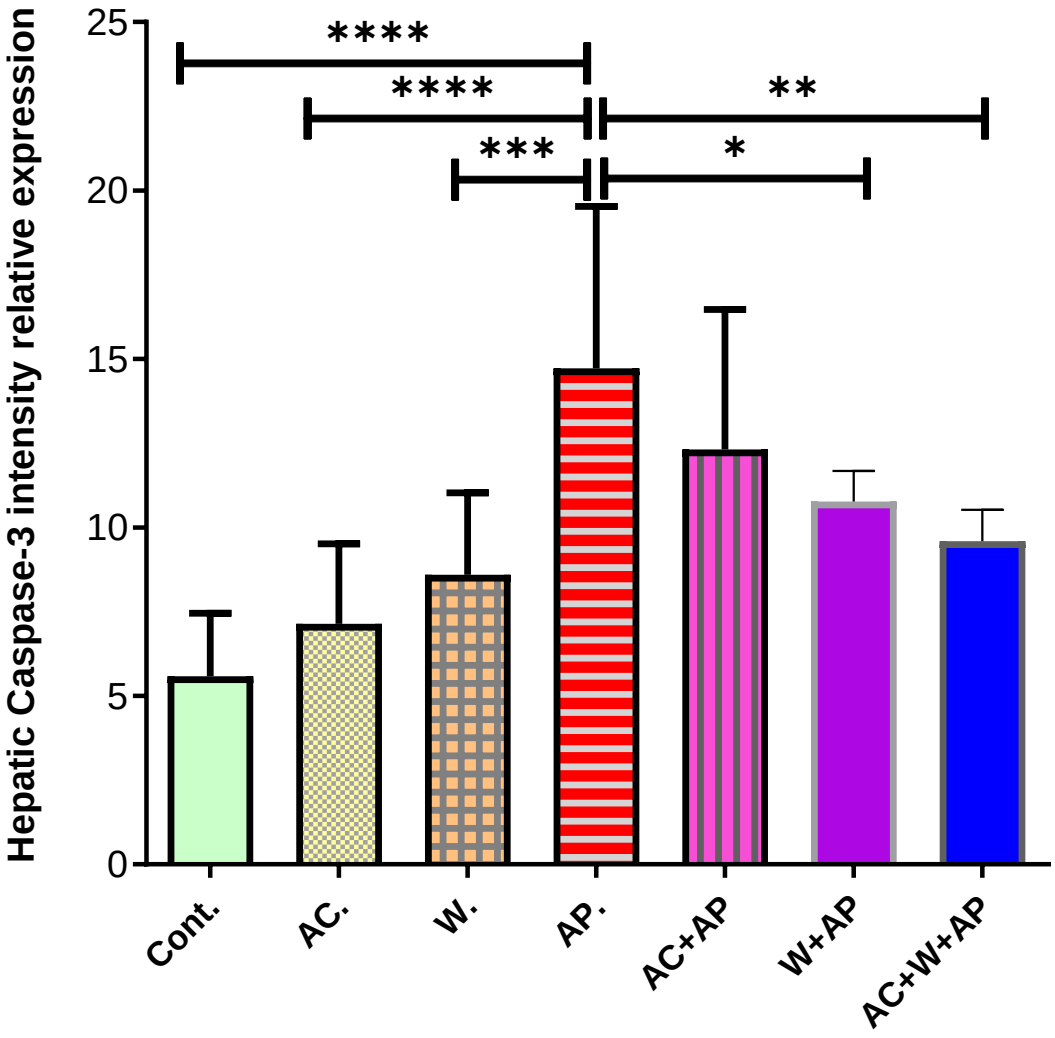

Fig. 2-7. Bar graph of semiquantitative evaluation of Immunostaining intensity for caspase-3 expression in rats from different experimental groups. Data are presented as mean ± standard deviation, *: statistically significant relative to APAP group at **P > .005, ***P > .0005, ****P > .00005 using ANOVA, Bonferroni post hoc

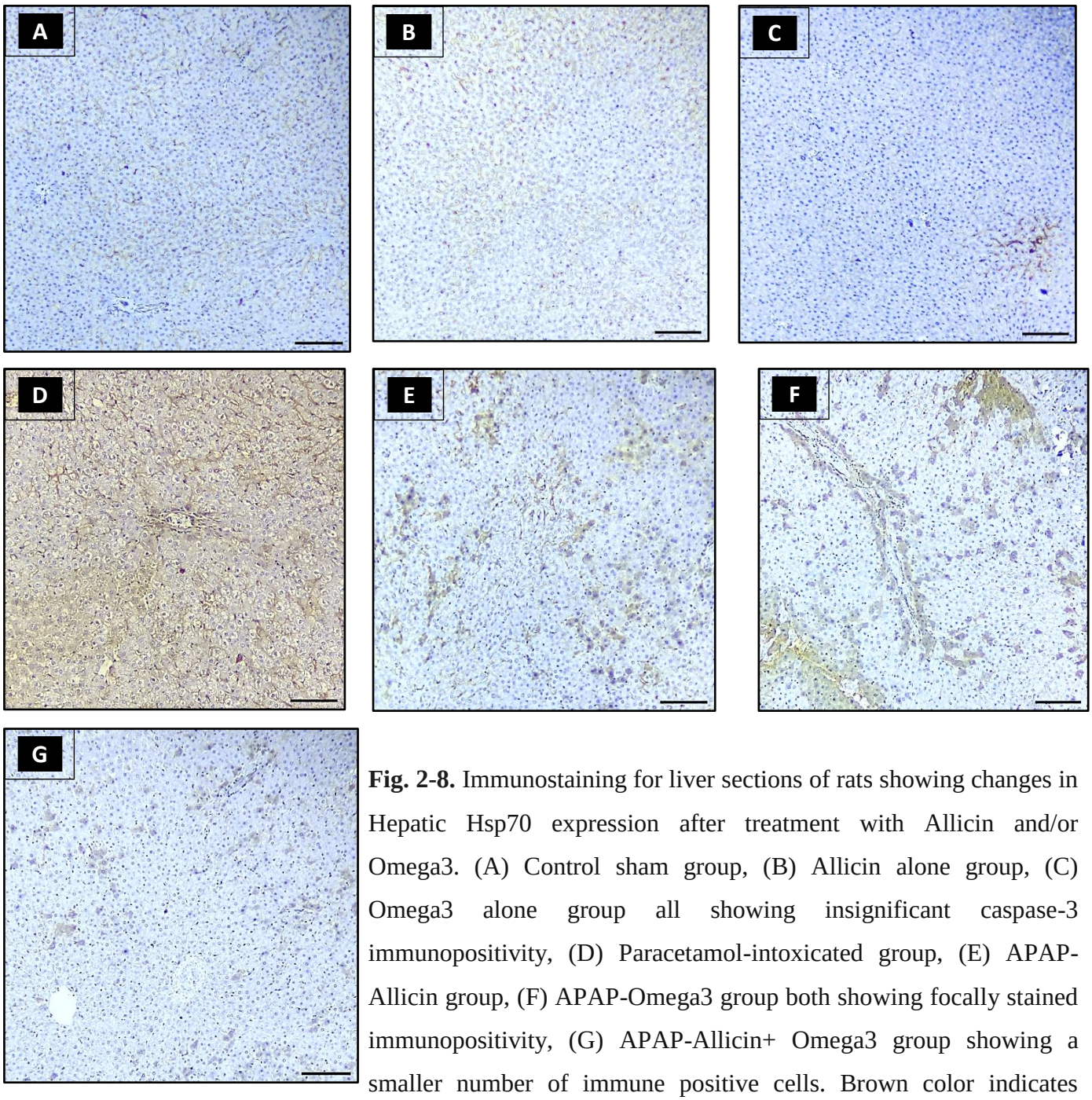

Fig. 2-8. Immunostaining for liver sections of rats showing changes in Hepatic Hsp70 expression after treatment with Allicin and/or Omega3. (A) Control sham group, (B) Allicin alone group, (C) Omega3 alone group all showing insignificant caspase-3 immunopositivity, (D) Paracetamol-intoxicated group, (E) APAP-Allicin group, (F) APAP-Omega3 group both showing focally stained immunopositivity, (G) APAP-Allicin+ Omega3 group showing a smaller number of immune positive cells. Brown color indicates immunopositivity.

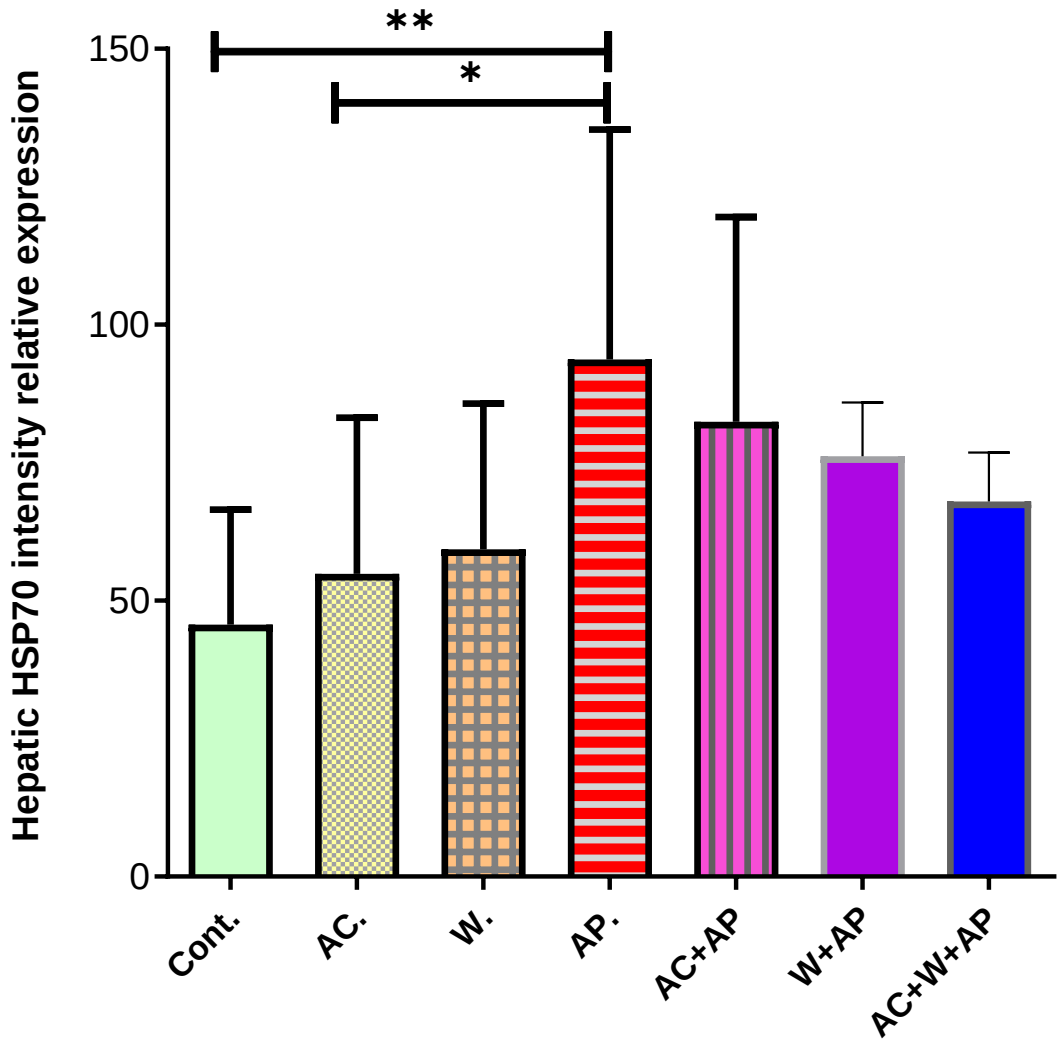

Fig. 2-9. Bar graph of semiquantitative evaluation of Immunostaining intensity for HSP70 expression in rats from different experimental groups. Data are presented as mean ± standard deviation, *: statistically significant relative to APAP group at *P >. 05, **P > .005 using ANOVA, Bonferroni post hoc test.

Protective effect of allicin and omega-3 fatty acids against paracetamol induced renal toxicity.

Abstract:

Paracetamol (APAP) is a well-known medication with widespread use for its analgesic and antipyretic effects. Renal failure caused by an over dosage of paracetamol is widespread in both experimental animal models and individuals. The study focus was planned to examine the AC and/or OMG-3 ameliorative impact on APAP-caused nephrotoxicity in rats. Randomly, Seventy Westar rats were subdivided into seven groups (10 of each): Control group (given saline), AC (10 mg/kg, PO.), the OMG-3 (100 mg/kg, PO.), APAP (1 g/kg, single dose PO on 27th day), AC+APAP, OMG-3 +APAP, and AC+ OMG-3+APAP group. Following APAP administration, it significantly reduced serum levels of total protein and albumin while noticeably raising serum levels of creatinine and urea. Additionally, reduced glutathione (GSH) concentration, superoxide dismutase (SOD), and catalase (CAT) activity all decreased, and malondialdehyde (MDA) level in the renal tissues dramatically increased. Along with the activation of caspase-3 and HSP70, APAP may also have a substantial impact on the kidney histopathology. As a result, our findings suggest that allicin and/or omega-3 have a potential protective impact through its anti-inflammatory, anti-apoptotic, and antioxidant defense systems.

Keywords: Paracetamol; Nephrotoxicity; Caspase-3; HSP70; Antioxidant; Allicin; Omega-3.

1. Introduction

Nephrotoxicity occurs when kidney-specific detoxification and excretion do not function optimally due to the damage or destruction of kidney function by exogenous, endogenous toxicants (**Kim *et al.*, 2012**) or exposure to drugs **(Lakshmi *et al.*, 2012).** One of these drugs is acetaminophen or paracetamol (N-acetyl-p-aminophenol) (APAP) marketed as Panadol or Tylenol and other preparations belong to a group of drugs called antipyretics (fever reducers) and analgesics (pain killers) (**Coresh *et al.* 2007; Baleni *et al.* 2015).** It occupies a unique position among analgesic drugs. Unlike NSAIDs it is almost unanimously considered to have no anti-inflammatory activity and does not produce gastrointestinal damage (**Bertolini *et al.*, 2006**). APAP is generally considered as safe but overdose of APAP can cause nephrotoxicity (**Chinnappan *et al.*, 2019**).

It has no side effect when used at the therapeutic doses but the chronic use and over dosage may result in hepatic and nephrotoxicity or even death (**Goyal *et al.*, 2011; Fan *et al.*, 2011; Raoof *et al.*, 2012).** In large doses of APAP, significant amounts of NAPQI are produced which overwhelm the cellular antioxidant system through depletion of GSH and GSH dependent enzymes enhancing excessive production of reactive oxygen species (ROS) accompanied by oxidative stress **(Canayakin *et al.*, 2016; Zhang *et al.*, 2016; Wang *et al.*, 2017; El-maddawy and El-sayed, 2018; Abdel-Daim and Abdeen, 2018).** Acute renal failure induced by a toxic dose of acetaminophen (also known as paracetamol, or APAP) is common in both humans and experimental animal models (**Dallak *et al.*, 2022**).

Even if nephrotoxicity is less common than hepatotoxicity in APAP overdose, renal tubular damage and acute renal failure can occur even in the absence of liver injury (**Palani *et al.*, 2009**). Although the exact mechanism of APAP-induced nephrotoxicity remains unclear, oxidative stress and the generation of toxic reactive oxygen species (ROS) induced by NAPQI are known to play major roles in the pathogenesis of APAP-induced renal injury **(Ko *et al.*, 2017).** Therefore, we hypothesized that oxidative damage and lipid peroxidation induced by ROS might be involved in the nephrotoxicity of APAP in rats and that a combination of drug delivery together with potent antioxidant effect and might be able to mitigate toxic effects of APAP on kidney.

Allicin is the first-isolated and main biologically active component of garlic. (**Borlinghaus *et al.*, 2014).** Among the active constituents in garlic, one major component is allicin (thio-2-propene-1-sulfinic acid S-allyl ester), which is formed from the stable precursor S-allyl Cysteine-S-oxide (alliin) by the action of the enzyme alliinase when garlic cloves are crushed or macerated **(Okada *et al.*, 2006).** It possesses antioxidant activity and is shown to cause a variety of actions potentially useful for human health **(El-Kashef *et al.*, 2015).**

The mechanism of the antioxidant activity of allicin may rely on its ability to scavenge oxygen free radicals **(Ghanayem *et al.*, 2005). Chung *et al.*, (2013)** documented that allicin is a natural antioxidant that scavenges the hydroxyl and oxygen free radicals as well as prevents the hydroxyl radical-induced lipid peroxidation of tissue homogenates**. Taubert *et al.*, (2006)** have shown that allicin could inhibit the activity of cytochrome P450 enzyme CYP2E1.In which form

reactive toxic metabolite that in turn produce kidney injury in experimental animals and humans (**Das *et al.*, 2010; Moore *et al.*, 2013).** So, we hypothesized that allicin can reduce oxidative stress and nephrotoxic effect induced by paracetamol.

Omega-3 polyunsaturated fatty acids (OMG-3FAs) are compounds containing more than two double bonds with the first double bond on the third carbon atom from the methyl end of the molecule. Examples of essential dietary OMG-3FAs derived from fish oil are eicosapentaenoic acid, docosahexaenoic acid, and alpha linolenic acid **(Owumi *et al.*, 2020).** These fatty acids are incorporated in many parts of the body, including cell membranes, and play a role in cell signaling and the anti-inflammatory and antioxidant processes **(de Batlle *et al.*, 2012; Avramovic *et al.*, 2012).**

Several studies have demonstrated the use of OMG-3FA in ameliorating oxidative stress and apoptosis as well as pharmaceutical drug in the treatment of inflammatory diseases **(Li *et al.*, 2017; Lee and Kang, 2019; Bäck and Hansson, 2019).** Also, the renoprotective effect of OMG-3FA has been also established **(El-Ashmawy *et al.*, 2018**). Clinical studies suggest that long-term treatment with OMG-3 fatty acids improves renal function and lowers the risk of death or end-stage renal disease **(Hassan and Gronert, 2009).** Therefore, this study aims to clarify the preventative role of AC and/or OMG-3FAs in preventing APAP-induced kidney injury.

2. Materials and Methods

2.1. Chemicals:

Paracetamol (APAP, 1 g) was bought as Panadol® from GlaxoSmithKline Pharmaceuticals Company (Brentford, United Kingdom). Allicin, was bought as pure powder (35% Conc.) from Delta Vet Center (Cairo, Egypt). Omega-3 fatty acids, was bought as pure fish oil (Conc.100%) from Sigma Pharmaceutical Industries (Cairo, Egypt). The used kits were bought from Bio-diagnostic Company (Giza, Egypt).

2.2. Experimental animals:

Seventy male albino Wister rats, 2 months age weighing 160-200 gm were obtained from the Center of Laboratory Animals, Faculty of Veterinary Medicine, Benha University, Egypt. Prior to the experiment, the rats were left for acclimatization for 14 days (temperature 25°C) and were fed ideal laboratory commercial diet and water *ad libitum*. Ethical approval from Animals Care and Use

Committee Research Ethics Board was obtained from Faculty of Veterinary Medicine, Benha University (BUFVTM 08–03-22).

2.3. Experimental design:

Rats were divided into 7 equal groups (10 rats in each group). Group 1 (Control); had been given distilled water. Group (2); AC (10 mg/kg b.wt, orally). Group (3); O3FA (100 mg/kg b.wt, orally). These doses of AC according to **Samra *et al*., (2021)** and for OMG-3FA **(El-Gendy *et al*. 2021)**. Group (4); APAP toxic control group that received saline, orally once daily and a single dose of APAP 1 g/kg b.wt orally on the 27th day of the experiment. Group (5); (AC+APAP). Group (6); (OMG-3FA+APAP). Group (7); (AC+ OMG-3FA+APAP). rats in these groups have been received allicin, omega-3 and APAP as described before. Saline, allicin, and omega-3 were administered for 30 days.

2.4. Sampling:

Rats were anaesthetized at 31st day of the experiment, blood samples were collected from Retro-bulbar venous plexus. Then all rats were euthanized and liver tissues were taken out and cut into two parts, one for histopathological investigation; the other part was preserved at -80°C for biochemical analysis.

2.5. Serum biochemical studies:

The biochemical parameters were urea **(Coulombe and Favreau, 1963)**, Creatinine **(Larsen, 1972),** total protein and albumin. The previous biochemical tests were evaluated in accordance with data protocol provided by using commercial kits (Bio-Diagnostic Company, Giza, Egypt).

2.6. Tissue homogenate preparation for oxidative markers evaluation:

The tissue was cut and rinsed by a PBS (phosphate-buffered saline) solution contain 0.16 mg/ml heparin to separate any RBCs and curd. Tissues were homogenized by sonicator homogenizer using 5 ml of 5-10 ml buffer (i.e., 50 mM potassium phosphate, pH 7.5 1 mM EDTA) added each gram of tissue. Tissue homogenates supernatant were centrifuged in a cooling centrifuge (4000 rpm for 15 min) and then stored at − 80^{o}C. Next, oxidative status was done by determination of malondialdehyde (MDA) level (**Uchiyama and Mihara, 1978**), catalase (CAT) activity (**Aebi, 1984**), SOD and reduced-glutathione (GSH) level (**Beutler, 1963**) using specialized diagnostic kits get from the Laboratory Biodiagnostic Company.

2.7. Histopathological examinations:

The renal tissue of right kidneys from each rat was quickly fixed in 10% neutral-buffered formalin for histopathology. The kidneys were progressively dehydrated, embedded in paraffin, cut into 5-µm sections, and stained with the hematoxylin and eosin (H&E) for histological inspection for histological inspection according to the method described by Bancroft and Gamble (2008). Finally, kidney tissue sections were observed by light microscopy (Leica, Germany).

2.8. Immunological assays:

Kidney tissue sections were heated at 60°C for 25 min in an oven (Venticell, MMM, Einrichtungen. Germany) and then deparaffinized in xylene and rehydrated using graded alcohol. The process of antigen retrieval was performed in 10 mM sodium citrate buffer boiled in a microwave. Immunohistochemistry staining steps were performed following the manufacturer's instructions (DakoCytomation, USA). In brief, endogenous peroxidase was blocked using 0.03% hydrogen peroxide sodium azide for 5 min. Tissue sections were washed gently with wash buffer and then incubated with HSP70 were applied at a dilution of 1:200 and of 1:250 respectively and polyclonal anti-caspase 3 antibodies (Invitrogen, Cat# PA5-77,887, dilution 1/100) for overnight at 4 °C followed by incubation with avidin–biotin complex (ABC kit, Vector Laboratories) at 37 °C for 45 min. Sections were gently washed with wash buffer and kept in the buffer bath in a humid chamber. A sufficient amount of streptavidin-HRP was then added and incubated for 15 min followed by washing. Diaminobenzidine-substrate chromagen was added to the sections and incubated for over 7 min followed by washing and counterstaining with hematoxylin for 5 sec. The sections were then dipped in weak ammonia (0.037 M/L) 10 times, washed and cover slipped. Positive antigens stained brown under light microscopy.

2.9. Statistical analysis:

Statistical analysis was carried out using SPSS (Version 20; SPSS Inc., Chicago, USA). The significant divergence through multiple groups comparisons were analyzed by one-way ANOVA and Duncan test as a post hoc test was used. All values are expressed as mean ± SE, with significance considered at $P \leq 0.05$.

3. Results

3.1. Biochemical study:

Figure 1 indicates that in comparison with control group, the serum creatinine and urea levels exhibited a significant increase and serum albumin and total protein

levels showed a significant reduction in APAP treated group. In contrast, AC+APAP, OMG-3+APAP, and AC+OMG-3+APAP group a marked drop in urea and creatinine and a remarkable elevation in albumin and total protein was recorded in comparison with APAP treated group.

3.2. Oxidative stress markers assay:

Figure. 2. displays the effects of APAP toxicity and treatment with allicin and/or omega-3 on oxidative parameters and lipid peroxidation in the kidney. In renal tissues of APAP-intoxicated rats, MDA levels significantly increased whereas CAT, SOD and GSH levels were decreased when compared to control rats. Besides that, the damaging effects of APAP on renal MDA, CAT, SOD and GSH were almost restored to normal by the administration of allicin and/or omega-3.

3.3. Histopathological changes of kidney:

After 4 weeks of the experiment, kidney tissues were harvested from each animal group and stained with H&E and examined under a light microscope. According to the histologic morphology of the sham control group's kidneys (**Figs. 3, (1A,1B))**, proximal and distal convoluted tubules of renal corpuscles or Malpighian renal corpuscles were normal. The sham + Allicin 10 mg/kg (**Figure 3, (2A,2B)**) and the sham + Omega3 100 mg/kg (**Figure 3, (3A,3B)**) groups showed similar microscopic morphology to the sham control group.

As shown in (**Figs. 4**) by widening of the glomerulus space, tubular dilatation, numerous cellular debris in the renal tubules, as well as vacuolization, and extensive tubular epithelial degeneration, paracetamol substantially damaged kidney tissue. A number of tubules lumina exhibited hyaline casts, desquamated cells, and necrotic cell debris. The interstitium of the renal cortex also showed extravasation and congested blood vessels.

As shown in **Figure 5**, both the paracetamol 1 g/kg + Allicin 10 mg/kg group as well as the paracetamol 1 g/kg + Omega3 100 mg/kg had a better morphology with less tubular necrosis in comparison to the control positive untreated group.

The normal structure of renal corpuscles and convoluted tubules was preserved following allicin plus omega3 treatment (**Figs. 5, (3A,3B)**). There were, however, a few mildly dilated glomeruli spaces in some fields. Many tubules had restored the brush border at the apex and narrow lumina. The presence of mild tubular

epithelial vacuolization, luminal cast formation and cell desquamations was hardly detected.

Besides, the mean thickness of the glomerular space in the Allicin plus Omega3 group was significantly reduced (p>0.0000) compared with the control group but was still significant (p = 0.0000) (**Fig.7)**. In 10 non-overlapping high-power fields/rat of H&E-stained sections, the thickness of glomeruli space was measured using the Image J 1.53q image analyzer (National Institutes of Health, USA).

Using a 4-point scale based on ten randomly chosen non-overlapping fields, a pathologist estimated the percent of tubular injury (criterion: epithelial flattening, tubular dilatation, brush border loss). The scoring system used only cortical tubules, grading the degree of injury from 0 to 4: 0 = no tubular injury; 1 = 10% tubular injury; 2 = 10%-25% tubular injury; 3 = 26%-50% tubular injury; 4 = 51%-75% tubular injury; and 5 = > 75% tubular injury as shown in (**Fig.6**).

In the animals treated with Allicin plus Omega3, the kidney histology displayed the lowest average score of all groups, correspond to the most normal renal architecture with the minimum extent of injury (**Figs. 5,6,7**).

Using analysis of variance (ANOVA) and post-hoc analysis (Bonferroni), quantitative data were tabulated as means and standard deviations (SD). When P-value is (p>0.0000), significant differences are taken into account.

3.4. The expression of Caspase-3 and HSP70 was associated with pathological changes of the kidney:

3.4.1. Immunohistochemical analysis (caspase-3 expression)

Immunohistochemical analysis revealed almost no caspase-3 immune-reactive renal epithelium in kidney sections of normal control rats (**Fig. 8**). On contrary, When kidney tissues were Semi-quantitatively examined (**Figure 9**), Paracetamol-intoxicated group showed significant increase in caspase-3 expressions in comparison to the control sham group (P < .00005). Allicin Omega3 coadministration significantly reduced these expressions when compared to Paracetamol-intoxicated group (P < .00005). The change of Caspase 3 protein expression was consistent with the pathological damage in rats' kidney.

3.4.2. Immunohistochemical analysis (HSP70 expression)

Immunohistochemical study showed that the Paracetamol-intoxicated group HSP70 protein was noticeably expressed in the renal tissue of rats (**Fig. 10**) compared

with that in the sham group which showed completely no HSP70 protein expression in rats' kidney. Besides, some HSP70 proteins spread from the kidney cells and disseminated in kidney tubules and renal interstitium. Meanwhile, Kidney section of allicin only or omega3 only treated groups showed partial inhibition of HSP70 expression as evidenced by weak immune staining in the cortical regions. Moreover, least or modest HSP70 protein expression in rats' kidney was recorded in sections from groups co-treated with Allicin and Omega3.

4. Discussion

The kidney is a vital organ, involved in the removal of metabolic waste products, fluid volume control, and preservation of electrolyte balance. APAP is widely, used as analgesic and antipyretic drug in general medicine hence an assessment of its relative toxicity is important. Acetaminophen (APAP)-induced acute kidney injury is known in human (**Mour *et al.,* 2005**) and animal models (**Karaali *et al.,* 2019**).

Although paracetamol, with the alternative name acetaminophen (APAP), has a reasonable safety profile in therapeutic doses, its overdose remains the most important cause of liver injury and even death in many parts of the world among all drug toxicities (**Larson *et al.,* 2005; Karakus *et al.,* 2013; Yayla *et al.,* 2014**). Hepatotoxic and nephrotoxic effects of paracetamol overdose occur by a complex sequence of events (**Hinson *et al.,* 2010**). Acetaminophen induced nephrotoxicity becomes evident after hepatotoxicity in most cases, but the occurrence of renal tubular damage and acute renal failure, even in the absence of liver injury, should not be ignored (**Eguia & Materson 1997**).

The main objective of our study was to elucidate (i) whether acetaminophen (APAP) overdose can induce alterations to the glomerulus ultrastructure; and (ii) whether the combined two antioxidants, allicin and omega-3 FAs can protect against APAP-induced ultrastructural changes and increment of biomarkers of acute kidney injury. It is well documented that APAP in large doses causes renal damage as a result of the accumulation of higher levels of the APAP-toxic metabolite, NAPQI. The NAPQI-induced renal toxicity is mediated by oxidative stress that occurred due to enhanced ROS formation which oxidize the cellular macromolecules leading to induction of lipid peroxidation, protein oxidation, mitochondrial dysfunction, and

DNA damage (**Das *et al.*, 2010; Yousef *et al.*, 2010; Canayakin *et al.*, 2016; Karthivashan *et al.*, 2016; Murad *et al.*, 2016; Elmaddawy and El-sayed, 2018**).

Urea and creatinine are important indicators of renal damage in clinical findings (**Refaie *et al.*, 2014; Uthra *et al.*, 2017**). These enzymes are very sensitive markers employed in the diagnosis of kidney diseases. Thus serum urea and creatinine were evaluated to demonstrate kidney damage. Our results revealed that the levels of urea and creatinine were significantly increased by APAP intoxication to rats when compared with control group. Also, levels of albumin and total protein exhibited remarkable reduction in APAP intoxicated groups compared with control group, proving that renal function has deteriorated. Our findings are also consistent with those of other researchers who found that APAP-induced kidney damage is manifested by increased serum urea and creatinine levels (**Cekmen *et al.*, 2009**; **Karthivashan *et al.*, 2016**).

It is well known that SOD, GSH, CAT and MDA are important biomarkers of the antioxidant capacity of the body, which protects against oxidative stress-induced damage. Oxidative stress is indicated by an increased lipid peroxidation and/or altered non-enzymatic and enzymatic antioxidant systems. Following the oral administration of paracetamol, we investigated alteration in tissue MDA levels as well as reductions in tissue GSH, CAT level, and SOD activity as markers of the oxidative stress process. The increment in lipid peroxidation was accompanied by a remarkable reduction in the GSH level.

GSH is a tripeptide, that is, found in many mammalian tissues and is an important free radical scavenger and scavenger of NAPQI, which is a reactive intermediate of paracetamol (**Yayla *et al.*, 2014**). It plays an important role in the antioxidant defense system and removes free-radical species, such as hydrogen peroxide and superoxide radicals, and it maintains membrane protein thiols. In current investigation renal MDA level was increased and activities of major renal antioxidant enzymes (GSH, SOD and CAT) were significantly inhibited due to APAP intoxication. The lower SOD, CAT and GSH levels, as well as the higher MDA levels, were consistent with previous research on paracetamol-induced nephrotoxicity. (**Canayakin *et al.*, 2016; Yousef *et al.*, 2010; Abdul Hamid *et al.*, 2012).**

Allicin is the active ingredient of garlic and it is an organic disulfide formed from alliin (**Borlinghaus *et al.*, 2014**). In a study by **Maldonado *et al.*, (2003),** they

revealed that a derivative of allicin called allyl cysteine could ameliorate gentamicin induced acute renal failure in rats via preservation of the antioxidant enzymes in the renal cortex. Previous studies have been shown that allicin has hepatorenal protective effects because it has antioxidant, ROS scavenging, immunomodulatory and anti-inflammatory activities (**Naik and Panda, 2007; MehmetÇik *et al.*, 2008; El-Kashef *et al.*, 2015).**

The present results indicate that rats treated with allicin exhibited decreased serum urea, creatinine and increase albumin and total protein levels towards normal levels as compared with the APAP intoxicated group, suggesting the allicin to some extent protected against the kidney toxicity induced by APAP.

Allicin can protect the cells from oxidative stress, via scavenging free radical, as well as decreasing cytotoxic compounds (**Chan *et al.*, 2013**). Allicin reduced and prevented renal induced lipid peroxidation and deterioration of antioxidant biomarker, confirming that it alleviated APAP-induced oxidative stress in renal tissue. The levels of MDA, an end product of lipid peroxidation, were significantly decreased with the addition of allicin in the current study, as compared with the APAP groups.

The results agreed with the study of **Şener *et al.*, (2000)**, According to his findings, an aqueous garlic extract significantly inhibits the increase of MDA, allowing the MDA concentration to return to baseline levels. GSH is an important constituent of intracellular protective mechanisms against various noxious stimuli including oxidative stresses (**Moskovitz *et al.*, 2002**). SOD is a metalloproteinase to detoxify superoxide anions as an efficient dismutative mechanism and it is the first enzyme involved in the antioxidant defense (**Salvemini *et al.*, 2002**). Administration of allicin increased the levels of GSH, SOD and CAT compared with those in the APAP-intoxicated group.

These findings indicated that the protective effects of allicin might reflect its function as an antioxidant and ant apoptotic agent.

The past three decades have been a period of rapid expansion in the scientific knowledge of OMG-3PUFAs. Recent studies have shown that Fish oil enriched in OMG-3fatty acids retard the progression of various forms of cancers, depression, arthritis, asthma, cardiovascular and renal disorders (**De Caterina *et al.*, 1994**). Also, the renoprotective effect of OMG-3 FAs has been also established (**El-Ashmawy *et al.*, 2018**). Clinical studies suggest that long-term treatment with OMG-3 FAs fatty

acids improves renal function and lowers the risk of death or end-stage renal disease **(Hassan and Gronert, 2009).** The current study was designed to investigate the hypothesis that OMG-3consumption could ameliorate APAP-induced nephrotoxic and other side effects, allowing for further therapeutic use of the drug.

A significant increase in serum creatinine, urea and decrease in albumin and total protein concentrations as illustrated in APAP intoxicated group indicating renal damage. OMG-3fatty acids improved renal function and had renoprotective effects by normalizing serum creatinine, urea, and other biomarker levels. APAP toxicity is mainly associated with the over-production of ROS as well as deterioration of antioxidant capacity (**Hasanein and Sharifi, 2017**).

ROS promote membrane lipid peroxidation, fragmentation of polyunsaturated fatty acids, leading to cellular damage (**El-Ashmawy *et al.*, 2018**). This was indicated by the remarkable increase in renal MDA concentration as an indication of lipid peroxidation as well as a significant reduction in the renal GSH, SOD and CAT. Antioxidant activity of OMG-3fatty acids was evidenced by the ability to decrease MDA concentration, suppressing lipid peroxidation as well as increasing renal GSH, SOD and CAT. Previous studies have also shown the antioxidant potential of OMG-3fatty acids (**Ali and Rifaai, 2019**).

Ameliorative potential effects of allicin and/or OMG-3 FAs on histopathological changes caused by APAP were significant, in that they reduced damage greatly, but the tubular scores of the allicin and/or OMG-3 FAs group were significantly higher than the control. This suggests that although there was a reduction in renal damage of APAP with the administration of allicin and/or OMG-3 FAs, the result was not a return to normal values of the control group. Also, to emphasize, the effects of allicin and/or OMG-3 FAs were remarkably similar.

Our biochemical investigations and oxidative indications are also supported by histological observations. In the current study, following acute APAP intoxication, histopathological examinations revealed clear evidence of nephrotoxicity. The most important histological change was acute tubular necrosis. Furthermore, it causes severe damage to the kidney, with tubular degeneration, wide lumina, damaged glomeruli, interstitial vascular congestion, epithelial degeneration and extensive loss of brush border in the tubular epithelium cells which may have a role in APAP-induced lipid peroxidation. These findings are consistent with a previous study that

described renal histological changes following APAP administration (**El-maddawy and El-sayed 2018; Reshi *et al.*, 2020**). While the normal structure of renal corpuscles and convoluted tubules was preserved following allicin plus omega3 treatment as indicated in results. There were, however, a few mildly dilated glomeruli spaces in some fields. Many tubules had restored the brush border at the apex and narrow lumina. The presence of mild tubular epithelial vacuolization, luminal cast formation and cell desquamations was hardly detected. In conclusion, the administration of allicin and/or omega-3 significantly reduced the toxic effects of paracetamol induced renal damage.

Moreover, caspases are important mediators of apoptosis. The most important one of them is caspase-3 which is activated death protease, stimulating the specific cleavage of many key cellular proteins. Caspase-3 is also needed as a hallmark of apoptosis. Therefore, caspase-3 is essential for specific processes relating to cell death and the synthesis of apoptotic bodies **(Porter and Jänicke 1999).** APAP-induced apoptosis is a process that depends on caspase which includes activation of the initiator active caspase-9 and effector caspase-3 in renal tubular cells **(Lorz al. 2004)**.

The current study showed that APAP caused an elevation in expression of caspase-3 (nuclear and cytoplasmic) within the renal tubules when compared with the control group which could be explained by oxidative stress induced by APAP which matched with **Hong- Min *et al.*, (2018)** who proved that APAP-induced kidney injury significantly increased cell apoptosis in renal tubules. However, allicin and/or OMG-3 FAs pretreatment lowered the expression of caspase-3 induced by APAP.These findings suggest that allicin and/or OMG-3 FAs have anti-apoptotic effects against APAP-induced nephrotoxicity by inhibiting mitochondria-mediated apoptosis in rats, as indicated in the results.

Additionally, Immunohistochemical analysis af HSP70 expression showed that the Paracetamol-intoxicated group HSP70 protein was noticeably expressed in the renal tissue of rats compared with that in the control group which showed completely no HSP70 protein expression in rats' kidney. Besides, some HSP70 proteins spread from the kidney cells and disseminated in kidney tubules and renal interstitium. Meanwhile, Kidney section of Allicin only or Omega3 only treated groups showed partial inhibition of HSP70 expression as evidenced by weak immune staining in the

cortical regions. Moreover, least or modest HSP70 protein expression in rats' kidney was recorded in sections from groups co-treated with Allicin + Omega3.

Impressively, the combination of omega-3 and allicin had a greater antioxidant and anti-inflammatory effect than either agent alone. This shows that by optimizing cellular antioxidant defenses and reducing the expression of active caspase-3 and HSP70. Based on the findings of this investigation and the literature, both medicines appear to be good candidates for nephroprotection, and their combination may provide greater cytoprotective effect. More investigation into the chemico-biological interactions between allicin and omega-3 is recommended.

5. Conclusion

The results of the current study suggest that concurrent administration of allicin with OMG-3 FAs eevated the development of APAP-induced nephrotoxicity. This shows that by optimizing cellular antioxidant defenses, restoring almostly hepatorenal biomarkers to normal and reducing the expression of active caspase-3 and HSP70.

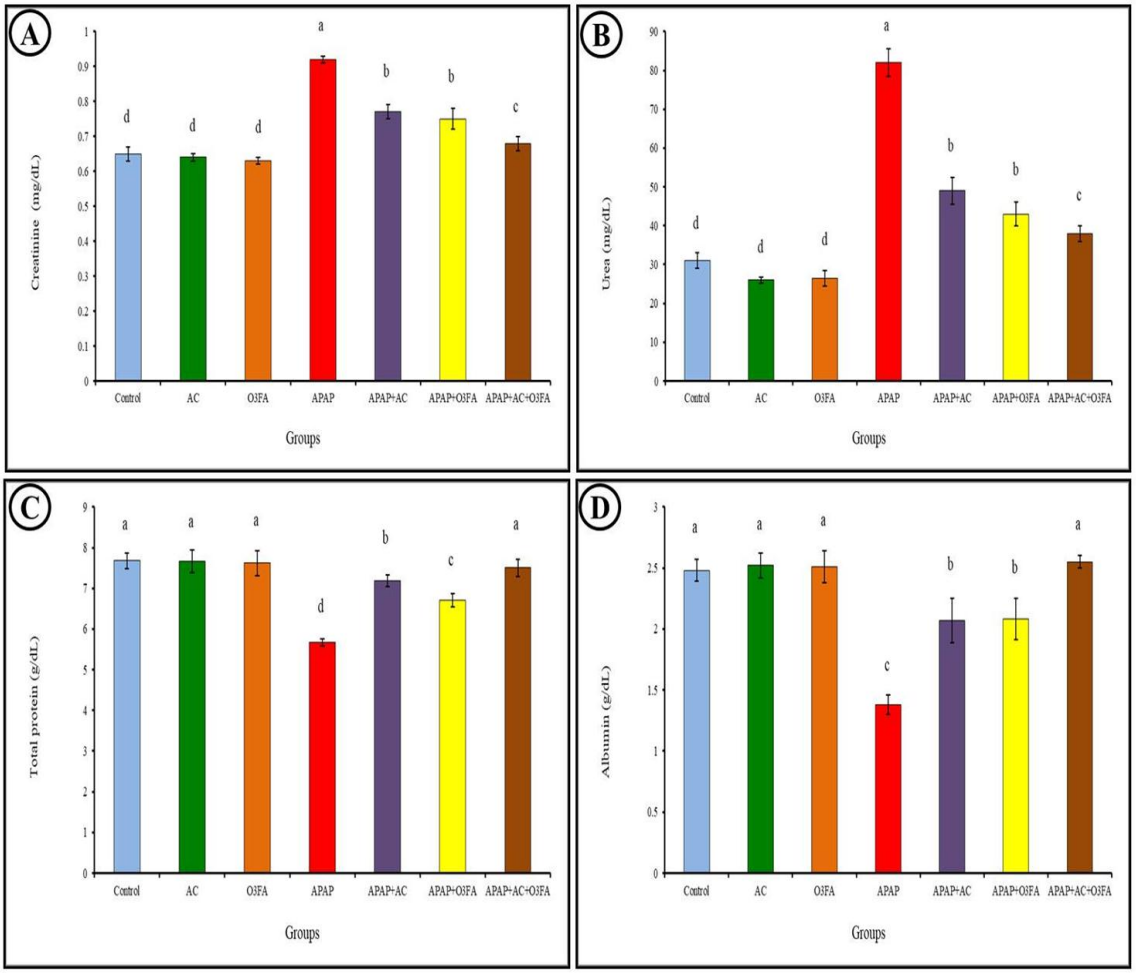

Fig. 3-1. Effect of allicin and/or omega3 on renal damage induced by paracetamol indicated by kidney biomarkers including, creatinine, urea, total protein and albumin.

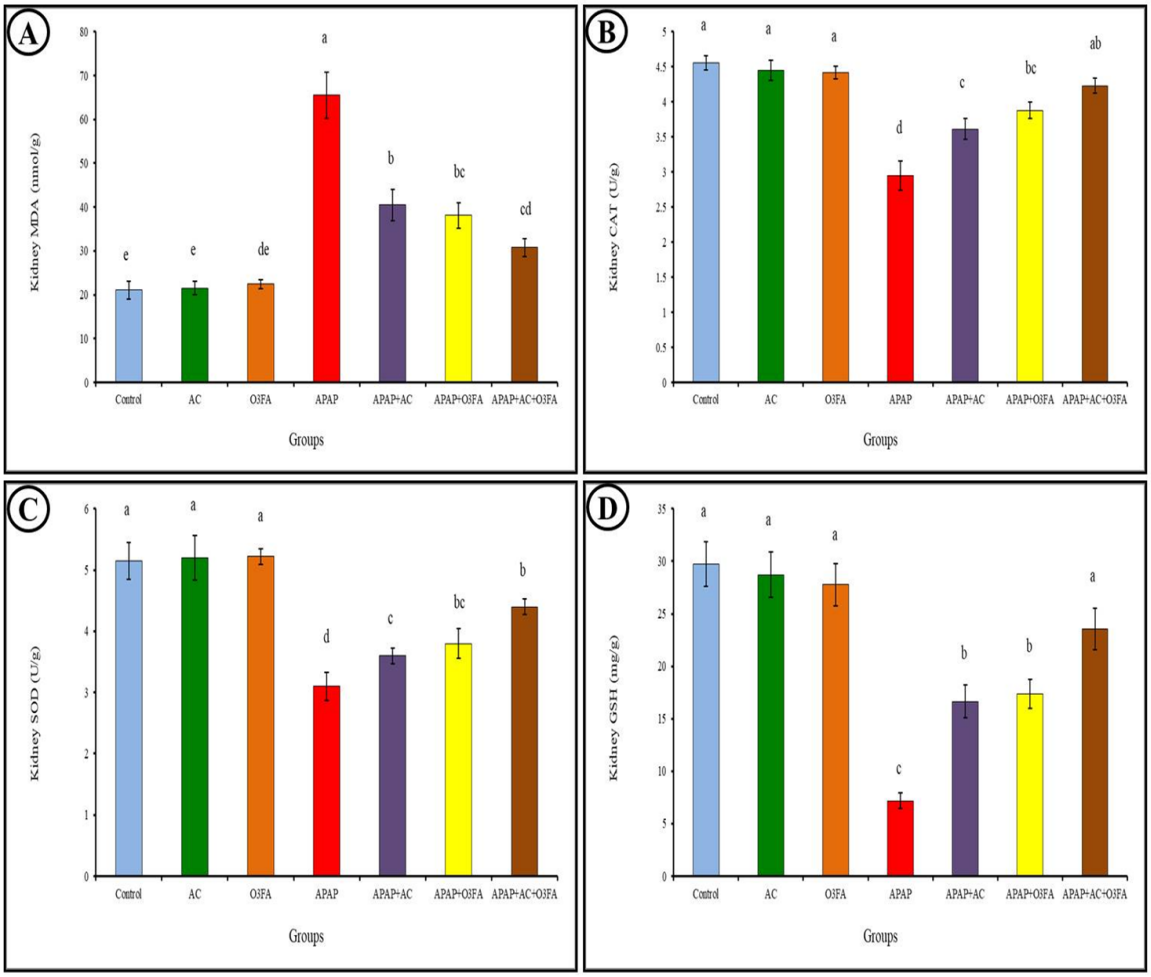

Fig. 3-2. Ameliorative effect of allicin and/or OMG-3 on renal MDA, CAT, SOD and GSH level in rats against damaging effect of acute paracetamol (APAP) exposure.

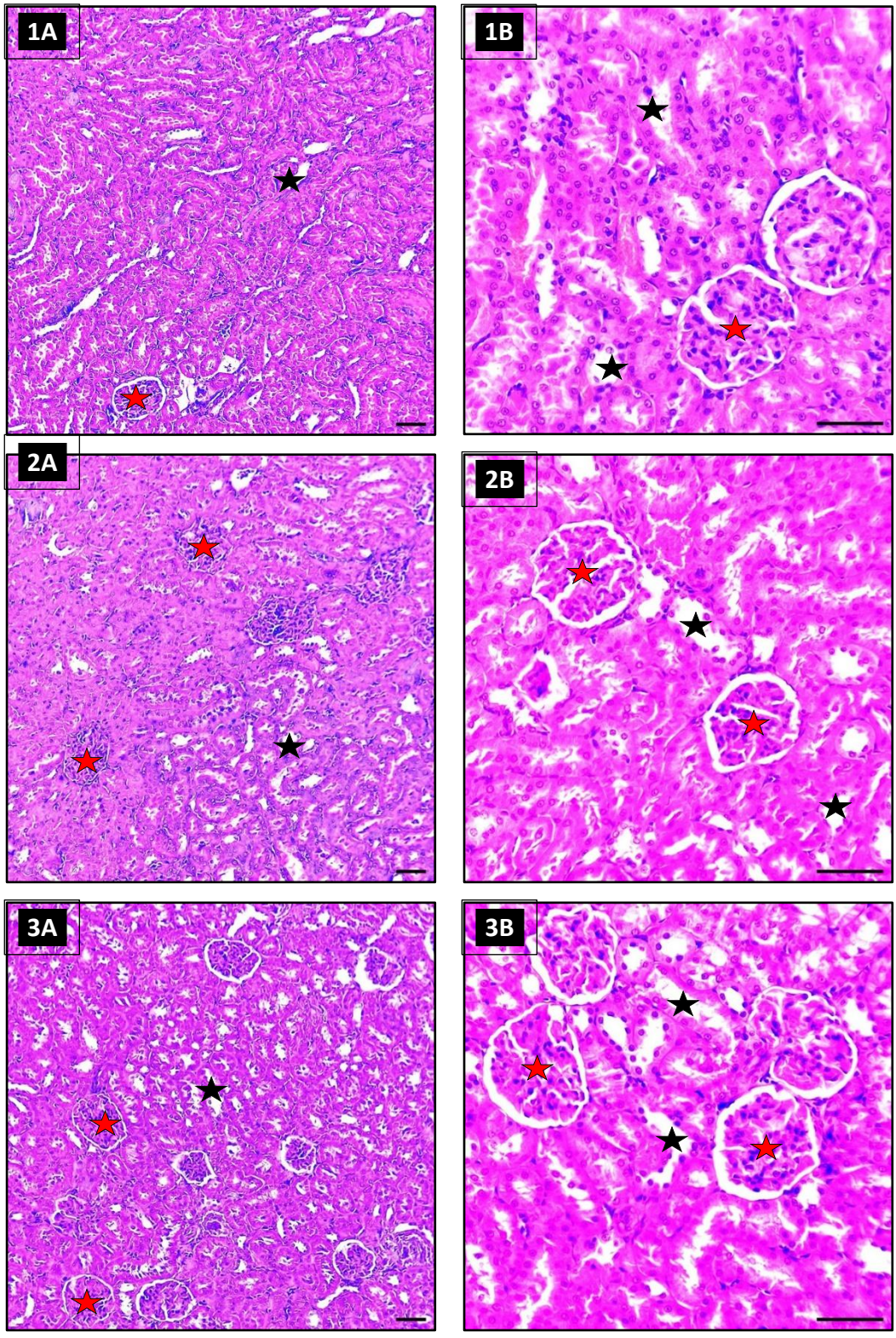

Figure 3-3. Light microscopic micrographs of rat kidney sections stained with H&E obtained at the end of the experiment from control, sham **(1A,1B)**, Allicin **(2A,2B)**, and Omega3 **(3A,3B)** groups. Structure of kidney glomerular (red star), and renal tubules (black star) with normal histological structure and intact well-organized cellular boundary.

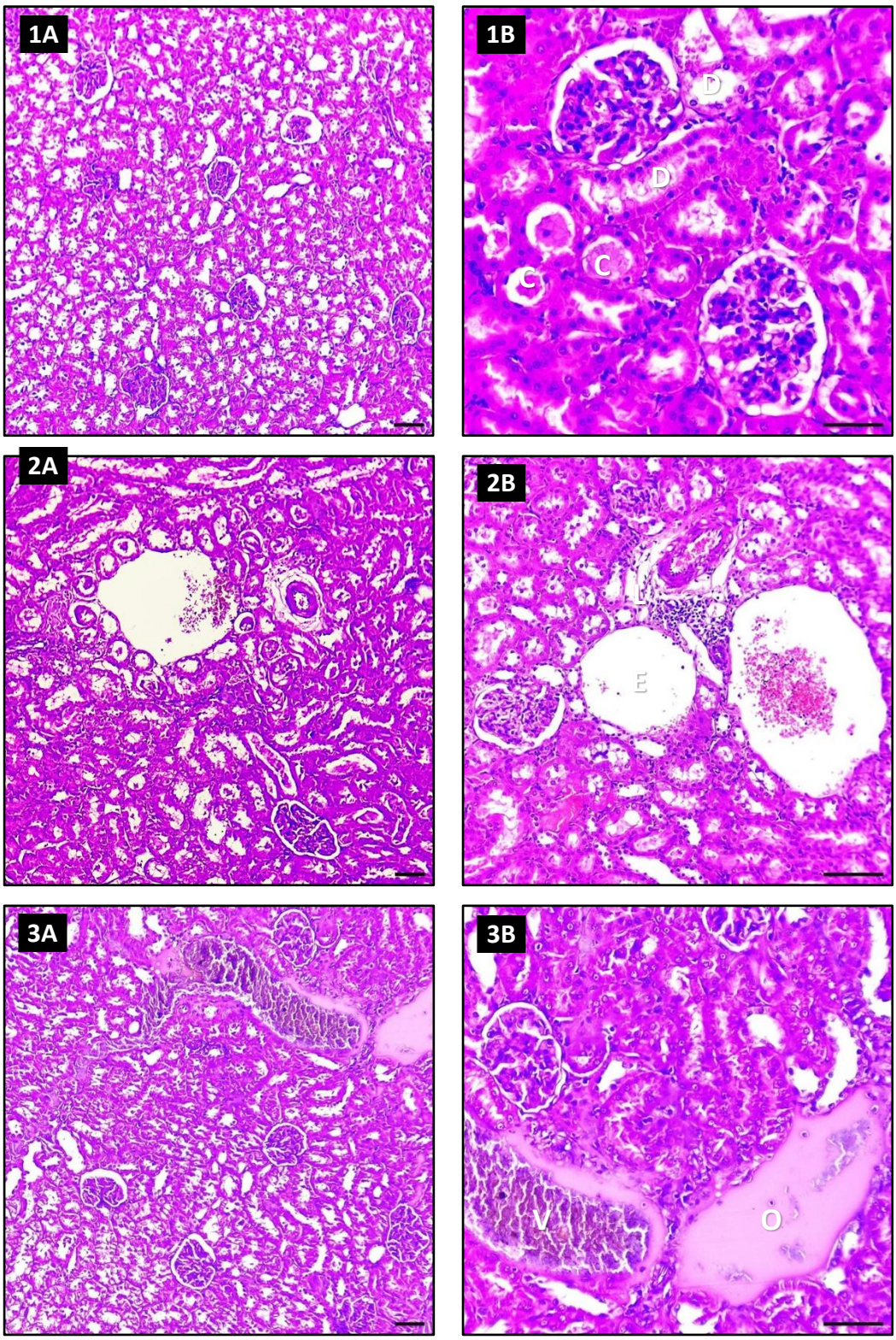

Figure 3-4. Photomicrograph of the kidney of rat from the paracetamol control positive untreated group showing **(1A,1B)** severe loss of brush border, tubular casts (C), tubular degeneration (D), **(2A,2B)** tubular cystic enlargement and lymphocytic infiltration (L). **(3A,3B)** Congested renal blood vessels (V) with proteinaceous fluid deposition (O) were also seen.

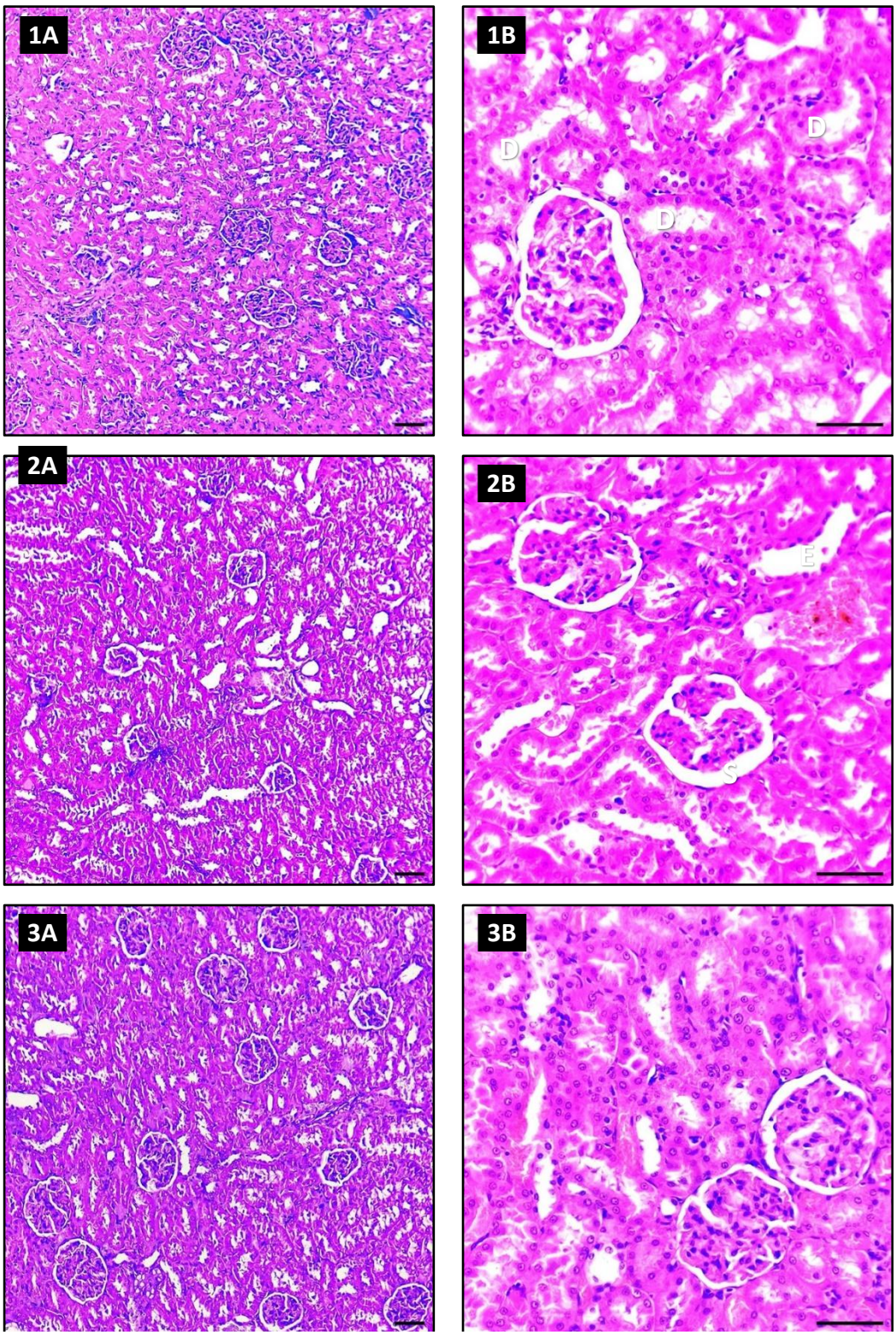

Figure 3-5. Histological evidence regarding the effect of Allicin (AC) and or Omega3 (OMG-3) on paracetamol (APAP)-induced nephrotoxicity. **(1A,1B)** Stained sections of kidney of paracetamol 1 g/kg + AC 10 mg/kg. Slightly to mild degenerations **(D)** were seen. **(2A,2B)** Paracetamol 1 g/kg + OMG-3 100 mg/kg group showing slightly to mild constriction of renal corpuscles **(S)** and tubular enlargement **(E)**. **(3A,3B)** Paracetamol 1 g/kg + AC 10 mg/kg + OMG-3 100 mg/kg group. Structures of kidney were comparable to the control group.

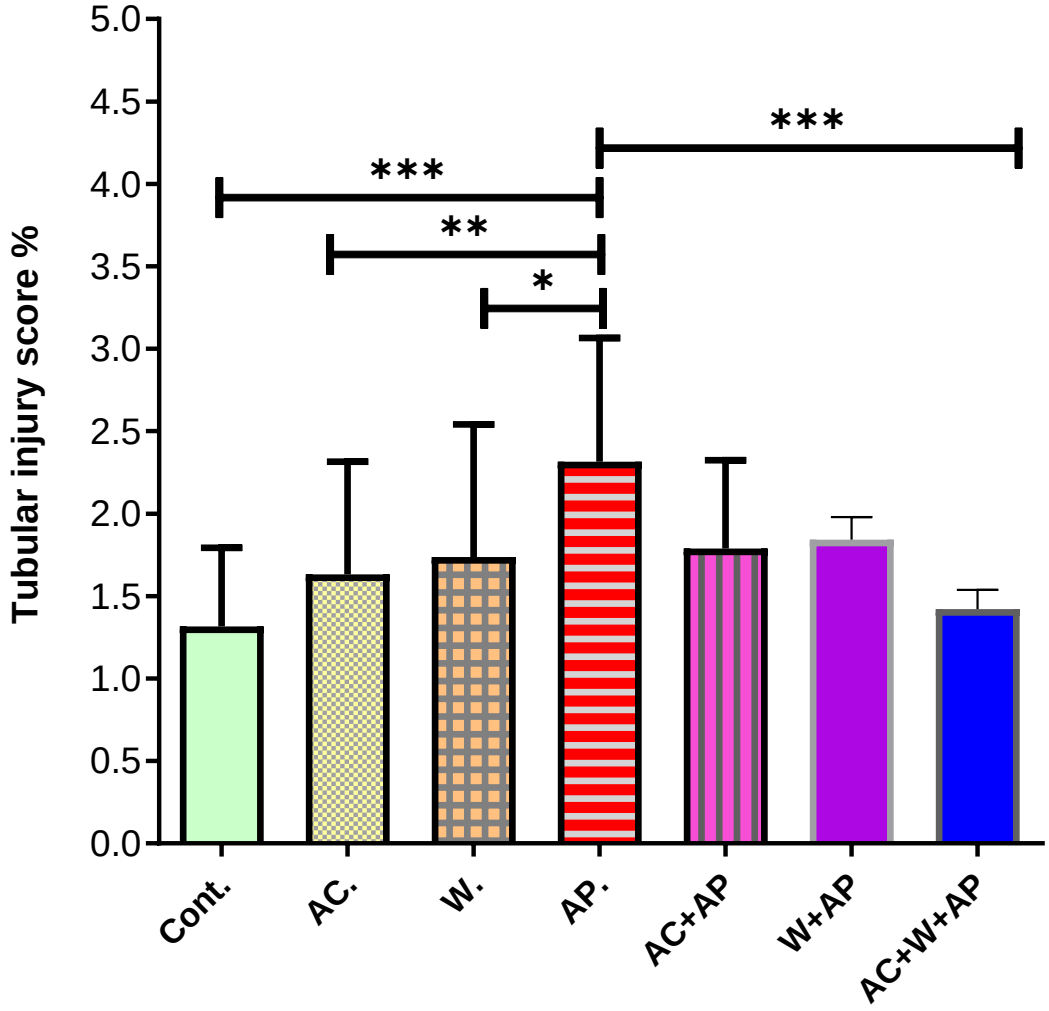

Figure 3-6. Bar graph of tubular injury score. Tubular damage and necrosis was significantly (*P < 0.05) reduced in kidneys of rats treated with Allicin plus Omega3. Data are expressed as mean ± SD for each treatment group.

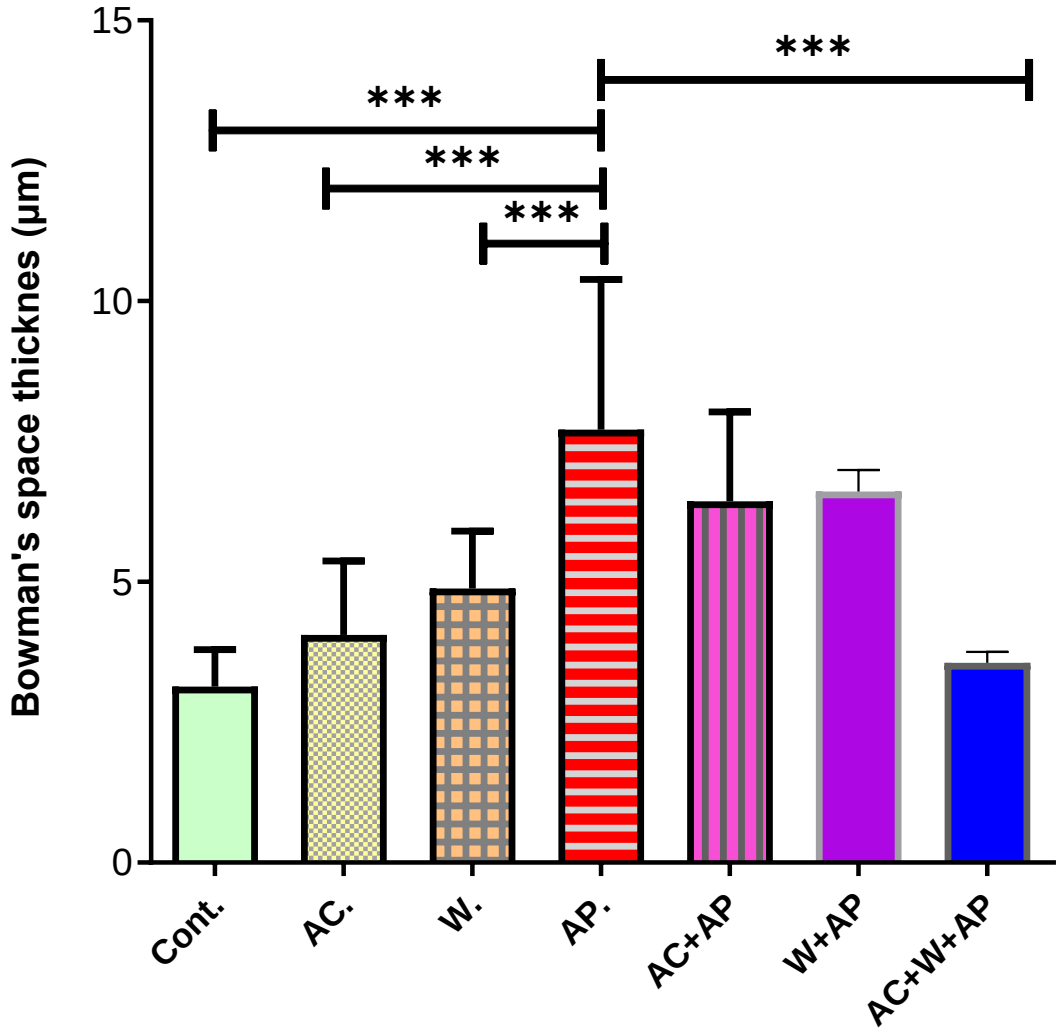

Figure 3-7. Quantitative analysis of the mean thickness of Glomeruli's space is shown.

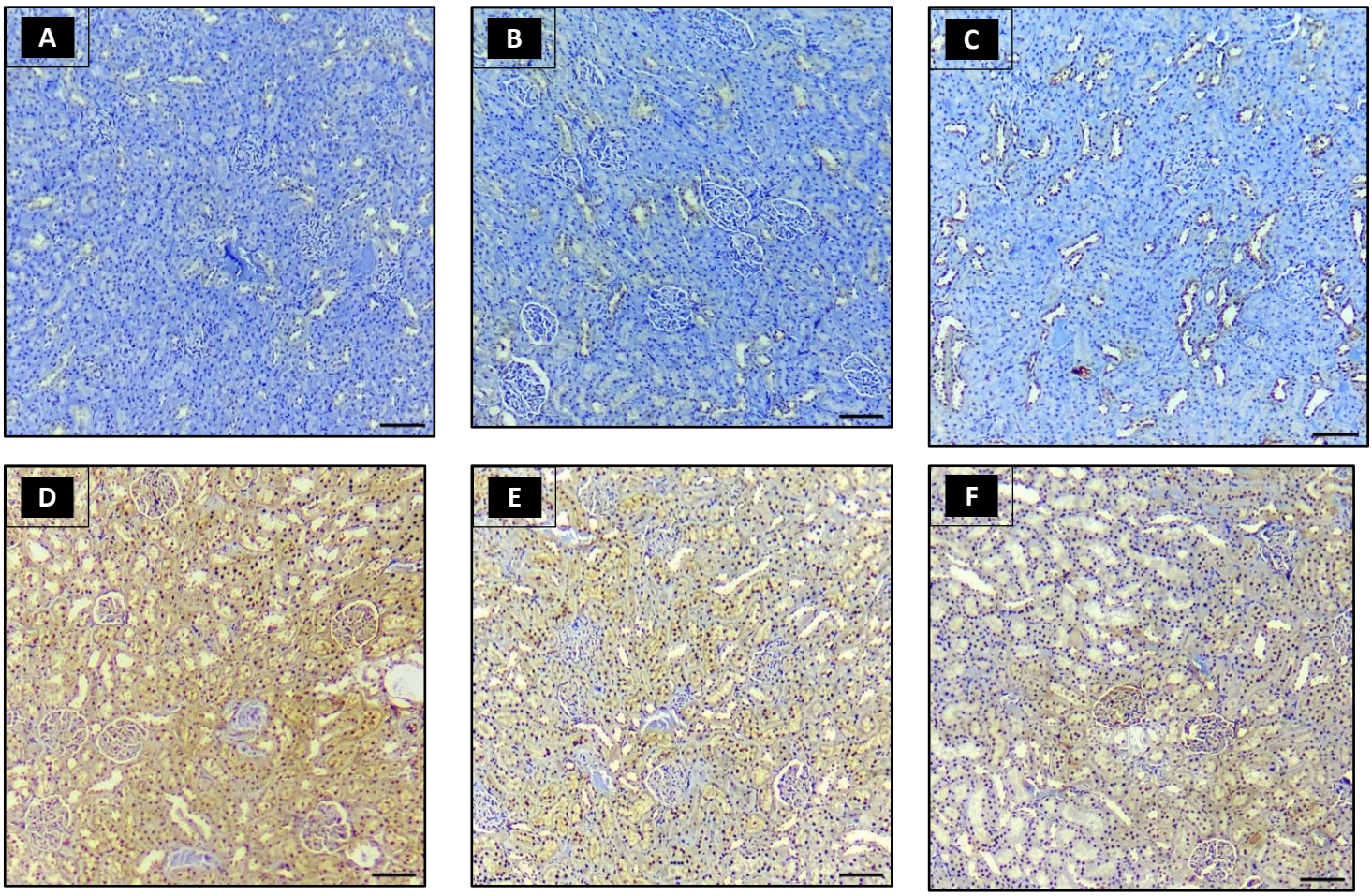

Figure 3-8. Immunohistochemical staining of caspase-3 in rat kidney from: **(A)** Control sham group, **(B)** Allicin alone group, **(C)** Omega3 alone group, **(D)** APAP-intoxicated group, **(E)** APAP-Allicin group, **(F)** APAP-Omega3 group, **(G)** APAP-Allicin+Omega3 group. Immunostaining was performed using anti-Caspase-3 antibody and developed with DAB. Brown color indicates caspase-3 positivity.

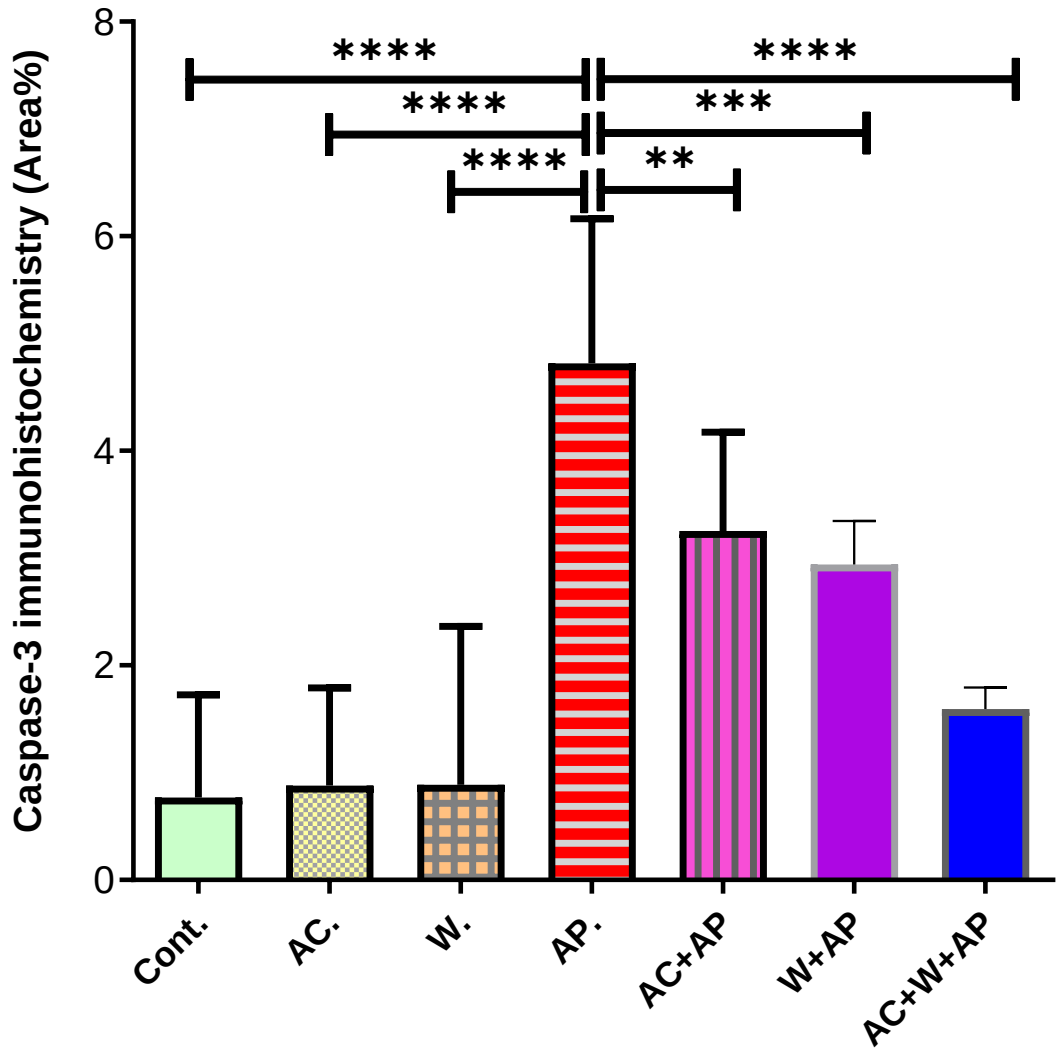

Figure 3-9. Bar graph of caspase-3 immunohistochemical expression in the different study groups. Area percent of immunoreactivity of caspase-3; data are presented as mean ± standard deviation, *: statistically significant relative to APAP group at **P > .005, ***P > .0005, ****P > .00005 using ANOVA, Bonferroni post hoc test.

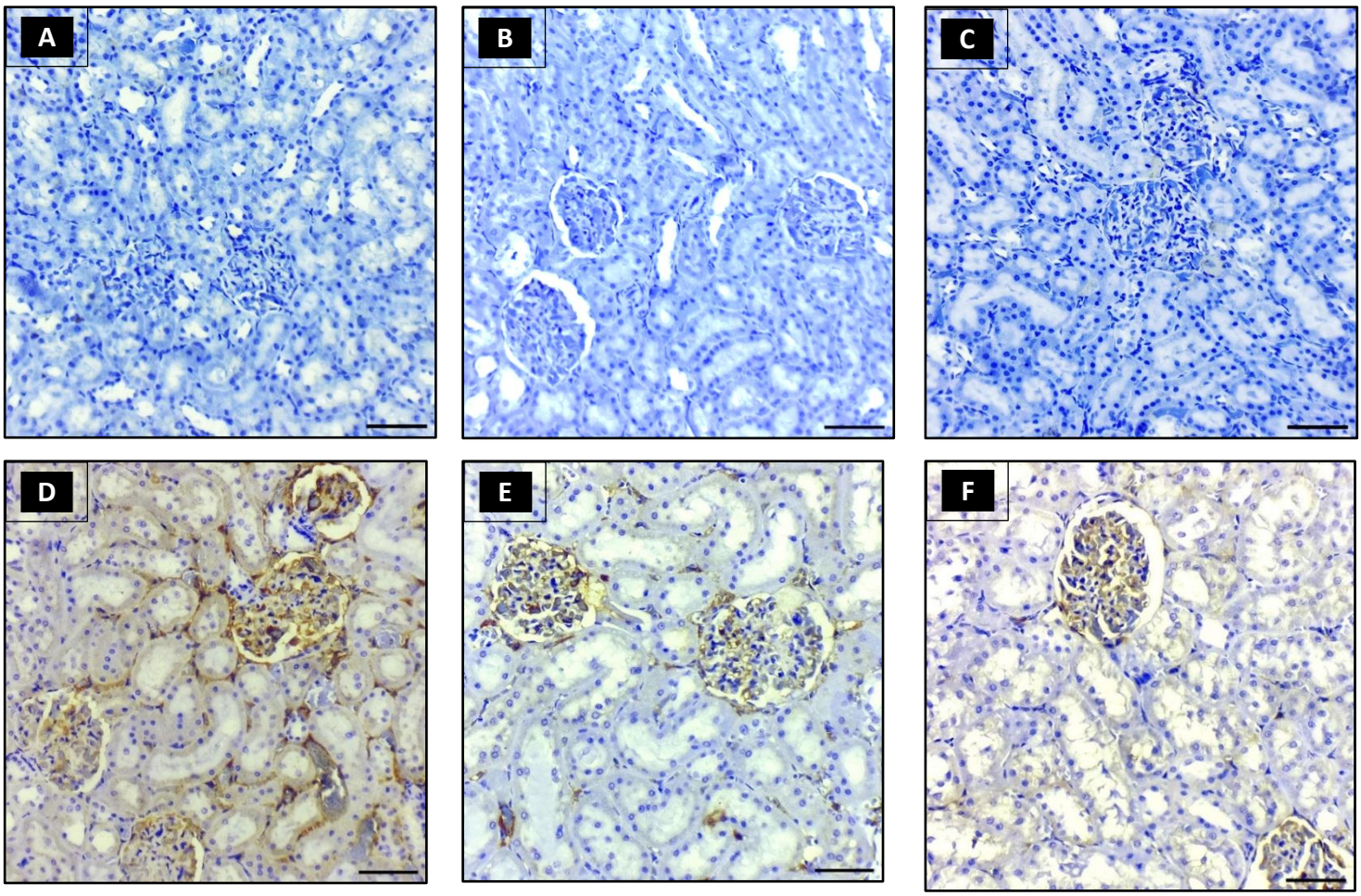

Figure 3-10. Cross section in the kidney of Paracetamol induced nephrotoxicity model showing changes in renal HSP70 Immunohistochemical expression after treatment with Allicin and/or Omega3. **(A)** Control sham group, **(B)** Allicin alone group, **(C)** Omega3 alone group all showing no expression of HSP70 in the cortical regions of kidney, **(D)** Paracetamol-intoxicated group showing diffuse intense expression, **(E)** APAP-Allicin group, **(F)** APAP-Omega3 group both showing moderate immunopositivity, **(G)** APAP-Allicin+ Omega3 group showing partial inhibition of HSP70 expression as evidenced by weak immune staining in the cortical regions. Brown color indicates immunopositivity.

General Discussion and conclusion

Although paracetamol, with the alternative name acetaminophen (APAP), has a reasonable safety profile in therapeutic doses, its overdose remains the most important cause of liver injury and even death in many parts of the world among all drug toxicities (**Larson *et al.*, 2005; Karakus *et al.*, 2013; Yayla *et al.*, 2014**). Hepatotoxic and nephrotoxic effects of paracetamol overdose occur by a complex sequence of events (**Hinson *et al.*, 2010**). The liver is an important organ for the detoxification and deposition of endogenous and exogenous substances. The disease of liver is considered to be a serious health problem (**Zhang *et al.*, 2012**). Drug-induced hepatotoxicity is one of the major reasons for the mortality and morbidity of human beings across the world **(Bhawna & Kumar, 2009)**. Paracetamol (acetaminophen) is used globally for its analgesic and antipyretic properties: however, it causes acute liver damage if administered in overdose **(Akhilraj *et al.*, 2021).** In the body, most of APAP (80–85%) dose is metabolized through glucuronidation or sulfation and excreted out. A small proportion (10–15%) of APAP gets metabolized and produces the active intermediate NAPQI which is detoxified with the help of GSH. APAP overdose increases the expression of CYP enzymes, decrease in GSH level, and repression of glucuronidation formation occur leading to elevated levels of APAP metabolite **(Bromer and Black 2003)**.

The toxic effect of paracetamol on the liver is not only from paracetamol, but also from its metabolite N-acetyl P benzo quinonimine (NAPQI) also known as N-acetyllimidoquimone. NAPQI depletes the liver's natural antioxidant glutathione and directly damages liver cells, leading to liver failure **(Akhilraj *et al.*, 2021).** APAP is also directly involved in the induction of oxidative stress resulting in lipid peroxidation, depletion in antioxidants, and ATP synthesis, and ultimately leading to liver damage **(Rabiul *et al.*, 2011)**.

The current study demonstrated that ingestion of a paracetamol single acute overdose resulted in a significant increase in serum ALT, AST, ALP, triglycerides (TG) and total cholesterol as well as a significant reduction in albumin and Total protein level. Particularly serum concentration of ALT and AST use as a biomarker of hepatic necrosis. Both ALT and AST enzymes involve in the reductive transfer of amino acid from alanine or aspartate, respectively to alpha ketoglutarate to form

pyruvate or oxaloacetate, respectively. Damaged hepatocytes release their contents, including ALT and AST into the extracellular space **(Islam *et al*., 2021).**

ALT is present in heart, brain, skeletal muscle, and liver; however, it is present in higher amounts in liver than any other organs. On the other hand, AST is considered to have lower specificity for liver damage due its presence in other organs. The reactive species (NAPQI) produced by paracetamol overdose harm the hepatic cells by lipid peroxidation and thereby damage the cellular permeability resulting higher serum levels of ALT and AST **(Islam *et al*., 2021).**

Moreover, the elevated serum ALP level observed, in this study, could be attributable to defective hepatic excretion or increased ALP synthesis by hepatic parenchymal or duct cells in the presence of increasing biliary pressure as reported by Iyanda and Adeniyi **(Iyanda and Adeniyi, 2011).** The intoxication of APAP seems to cause impairment of metabolism of lipoprotein **(Kobashigawa and Kasiske, 1997).** leading to alteration of cholesterol metabolism. The availability of free acid, slower hepatic release of lipoprotein, and enhanced esterification of free acids may all contribute to the higher blood level of TG produced by APAP.

The reduction in total protein levels seen in APAP-treated rats suggests the destruction of many hepatic cells, which may result in a decrease in hepatic capacity to synthesis protein as most plasma proteins are synthesized by hepatocytes **(Chaphalkar *et al.*, 2017).** Oxidative stress is another possible indicator of liver damage and as reported before it is an important mechanism that has been proposed to have a role in the development of APAP toxicity **(Wang *et al.*, 2017).**

Oxidative stress induces the production of free oxygen radicals, an undesirable by-product. It is the main factor in APAP induced liver and renal toxicity and can exacerbate free radical chain reactions **(Parikh *et al.*, 2015).** In the current study, we observed that the APAP treated animal group showed significant reduction in GSH, SOD, CAT and increased MDA level compared with the vehicle group as indicator of lipid peroxidation in the liver of rats. These changes in these biomarkers are due to depletion of the enzyme substrates and irreversible inactivation of enzyme proteins from increased ROS production **(Verma *et al.*, 2017).**

CAT is one of the enzymatic antioxidants that protect tissues against H2O2. Found in high amounts in liver and erythrocytes and reduce H2O2 to molecular O2 **(Akgun et al., 2021).** The buildup of free oxygen radicals in the APAP group might

be the source of lower CAT activity in liver tissues. GSH is thiol-containing compounds and one of the most important defense systems present in the mammals' cell **(Akgun et al., 2021).**

SOD is an antioxidant enzyme that eliminates reactive oxygen species (ROS). It catalyzes the conversion of superoxide radical to H2O2. Although H2O2 is not a radical, it is rapidly converted to highly reactive hydroxy radical through the activity of CAT **(Pandey and Rizvi, 2010).** So, the overproduction of superoxide radical anions in paracetamol-exposed animals might explain the decrease in SOD activity.

GSH is thiol-containing compounds and one of the most important defense systems present in the mammals' cell **(Akgun *et al.*, 2021).** The decreased GSH level in APAP group may be due to excessive NAPQI and O2 •- production or peroxides due to APAP administration **(Akakpo *et al.*, 2018).** Therefore, the antioxidant defense mechanism is affected by ROS thereby decrease SOD, CAT, and GSH, leading to hepatic damage **(Ozer *et al.*, 2008).** The uprising level of MDA is considered as an important biomarker of lipid peroxidation in liver tissues **(Attah *et al.*, 2020).** It has been demonstrated that the high dose of APAP causes the oxidation of unsaturated fatty acids in the cell membrane, which is correlated with lipid peroxidation (LPO) **(Zoubair *et al.*, 2013).**

Allicin is the main active ingredient in freshly crashed garlic. It has been reported to have anti-inflammatory and antioxidant properties (**Shang *et al.*, 2019**). Allicin is a natural antioxidant, that not only scavenges oxygen free radicals and hydroxyl radicals, but also prevents the lipid peroxidation of liver homogenates induced by hydroxyl radicals (**Zhang *et al.*, 2012**). Our present study provides that dietary allicin can partially offset the toxicity of APAP. The findings highlight the critical function of dietary antioxidants like garlic in the nutritional protection of oxidative stress-related pathologies.

Cytosolic aminotransferases and ALP are used as an index for hepatocellular membrane damage, as they leak out into the blood stream following exposure to chemicals, including drugs and toxic substances (**Al-Brakati *et al.*, 2019**). Our results showed marked reduction in serum liver functions, ALT, AST, ALP, TG and total cholesterol levels and significant increase in albumin and total protein compared to APAP group that shows significant alterations in these biomarkers. In addition, the elevation of these biomarkers was associated with necrosis, degeneration, and

infiltration of hepatocytes (**Singh *et al.,* 2018**). Interestingly, allicin supplementation reduced the increased serological liver function markers following APAP administration. These results point to allicin having a hepatoprotective effect against APAP exposure by preserving the structure and integrity of the hepatocyte membrane.

Antioxidant enzymes such as CAT, MDA, GSH, SOD can protect cellular compounds against damage induced by free radicals. Therefore, the activities of these enzymes have been used to assess oxidative stress in cells (**Liu *et al.,* 2010**). Allicin can restore the activity of antioxidant enzymes and possibly reduce the generation of free radicals in vitro and in vivo. The antioxidant effect of allicin may be direct through scavenging of ROS or indirect by activating and increasing activity of endogenous cellular antioxidant defenses (**Kelsey *et al.,* 2010**).

In the recent study, we found that allicin can markedly increase the activities of SOD in APAP-treated rats compared to intoxicated group. The levels of MDA, an end product of lipid peroxidation, were significantly decreased with the addition of allicin in the present study, as compared with the APAP groups. The results agreed with the study of **Şener et al., (2000)**, which reported that an aqueous garlic extract significantly inhibits the increase of MDA, thereby allowing the MDA concentration to return back to the baseline levels. Thus, garlic extract can be protective against distant organ damage by preserving cellular integrity.

GSH plays an important role in protecting several tissues and cell lines against injuries by oxidants and reactive electrophiles (**Shan *et al.,* 1990**). Our data show that allicin pretreatment significantly inhibits the APAP-induced depletion of hepatic GSH. The results of the in vivo study also suggested that rats orally administered with allicin can increase GSH levels as compared to APAP group. These findings are consistent with the proposal of **Xie *et al.,*** (**2008**) that the intake of tea polyphenols and diallyl trisulfide can remarkably increase GSH S-transferase activity and GSH content. Furthermore, the results show a significant increase in CAT level in the APAP-intoxicated group, whereas the allicin-pretreated group shows a significant reduction in CAT level.

Omega-3 long-chain polyunsaturated fatty acids (Omega-3 PUFAs) daily doses are nowadays recommended for their antioxidant and anti-inflammatory potentials (**El-Gendy *et al.,* 2021**). They have a variety of proposed mechanisms of

action; the most significant of which would be modulating cell proliferation, regulating fatty acid metabolism, inhibiting lipogenesis as well as suppressing inflammation and oxidative stress (**Huang *et al.*, 2015; Wang *et al.*, 2017**). Omega-3 fatty acids have been shown to have hepatoprotective properties in several investigations (**Adeyemi and Olayaki, 2017**; **Eraky and Abo El-Magd, 2020**).

The mechanisms underlying the hepatoprotective effects of Omega-3 PUFAs includes its ability to increase GSH along with its capability to scavenge free radicals and consequently inhibit lipid peroxidation (**Shaaban *et al.*, 2014; Sohail *et al.*, 2019**). Meanwhile; **Maksymchuk *et al.*, 2016**, previously demonstrated that there was more than two-fold increase in the content of cytochrome P450 2El (CYP2E1) in the liver of rats receiving omega-3 PUFAs for 4 weeks in the standard daily diet. In another study: consumption of OMG-3 PUFAs led to a 3-fold ($p < 0.05$) increase in CYP2E1 content. Such changes in the enzyme expression did not have an impact on the level of lipid peroxidation and on the prooxidant/antioxidant balance in the liver.

The APAP-induced hepatocellular injury was confirmed by a significant increase in serum ALT, AST, ALP, TG and cholesterol activities and decrease in albumin and total protein compared with normal rats, indicating cell damage with cell membrane disruption, leading to cellular leakage and hepatic dysfunction. OMG-3fatty acids ameliorated this increase in these activities and protected against hepatic dysfunction.

APAP toxicity is mainly associated with the over-production of ROS as well as deterioration of antioxidant capacity (**Hasanein and Sharifi, 2017**). ROS promotes membrane lipid peroxidation, fragmentation of polyunsaturated fatty acids, leading to cellular damage (**El-Ashmawy *et al.*, 2018**). This was demonstrated by the significant increase in hepatic MDA concentration as an indication of lipid peroxidation as well as a significant decrease in the hepatic GSH, SOD and CAT as an indication of oxidative stress. overdose of the APAP depletes the level of the GSH (**Eshrati *et al.*, 2021**) which ultimately results in the hepatocellular damage (**Raˇskovi´c *et al.*, 2019**).

Thus, the levels of glutathione (non-enzymatic antioxidant) along with other enzymatic [superoxide dismutase (SOD) and catalase (CAT)] antioxidants are crucial in protecting the liver from the toxicity of acetaminophen. Antioxidant activity of OMG-3fatty acids was evidenced by the ability to decrease MDA concentration, inhibiting lipid peroxidation as well as increasing GSH, SOD and CAT. Previous

studies have also shown the antioxidant potential of OMG-3fatty acids (**El-Ashmawy *et al.*, 2018**; **Ali and Rifaai, 2019**).

Additionally, the results of histopathological examination of the liver confirmed the serum biochemical and oxidative findings which have been reported in other studies and showed that APAP hepatic damages tissue by causing oxidative damage **(Saritas *et al.*, 2014; Uysal *et al.*, 2016)**. The liver tissue was arranged in lobules according to the histological report of the normal control group. Significant and widespread necrosis with degenerative changes, central veins dilatation, were seen in the paracetamol group and these result compatible with previous study **(Akhilraj *et al.*, 2021)**. While the treated rats with allicin or omega 3 represented mild congested central veins with some inflammatory cells infiltration in addition to congestion of blood sinusoids in the combined treated rats, the examined liver showed congestion of central vein and sinusoids without inflammatory cells infiltration. The integrity of the hepatic tissue was significantly distorted by APAP. The administration of allicin and omega-3 showed a more hepatoprotective effect, evident by the less distortion of the histoarchitecture of the liver tissue compared to the APAP intoxicated group.

Besides that, a significant increase in the levels of apoptosis-related proteins such as caspase-3 was observed suggesting a vital role of apoptosis in paracetamol-induced hepatic injury. While our findings showed that allicin and/or OMG-3FAs downregulated the pro-apoptogenic proteins caspase-3 as indicated in the results.

Also, the HSP70 immunohistochemical staining of liver made to elucidate the function of HSP70 more directly during hepatic APAP-injury, we employed HSP70 immunostaining. HSP70 semiquantitative analysis did not differ significantly between the groups in their response to Allicin and or Omega3 treatment. However, APAP-Allicin+ Omega3 group showing a smaller number of immune positive cells. Finally, our results reported that there was a direct link between allicin and/or OMG-3FAs pretreatment and suppression of apoptosis represented by a reduction in active caspase-3 and HSP70.

The kidney is a vital organ, involved in the removal of metabolic waste products, fluid volume control, and preservation of electrolyte balance. APAP is widely, used as analgesic and antipyretic drug in general medicine hence an assessment of its relative toxicity is important. Acetaminophen (APAP)-induced acute

kidney injury is known in human (**Mour *et al.,* 2005**) and animal models (**Karaali *et al.,* 2019**). APAP induced nephrotoxicity becomes evident after hepatotoxicity in most cases, but the occurrence of renal tubular damage and acute renal failure, even in the absence of liver injury, should not be ignored (**Eguia & Materson 1997**).

The main objective here was to elucidate (i) whether acetaminophen (APAP) overdose can induce alterations to the glomerulus ultrastructure; and (ii) whether the combined two antioxidants, allicin and omega-3 can protect against APAP-induced ultrastructural changes and increment of biomarkers of acute kidney injury. It is well documented that APAP in large doses causes renal damage as a result of the accumulation of higher levels of the APAP-toxic metabolite, NAPQI. The NAPQI-induced renal toxicity is mediated by oxidative stress that occurred due to enhanced ROS formation which oxidize the cellular macromolecules leading to induction of lipid peroxidation, protein oxidation, mitochondrial dysfunction, and DNA damage (**Das *et al.* 2010; Yousef *et al.* 2010; Canayakin *et al.* 2016; Karthivashan *et al.* 2016; Murad *et al.* 2016; Elmaddawy and El-sayed 2017**).

Urea and creatinine are important indicators of renal damage in clinical findings (**Refaie *et al.,* 2014; Uthra *et al.,* 2017**). These enzymes are very sensitive markers employed in the diagnosis of kidney diseases. Thus serum urea and creatinine were evaluated to demonstrate kidney damage. Our results revealed that the levels of urea and creatinine were significantly increased by APAP intoxication to rats when compared with control group. Also, levels of albumin and total protein exhibited remarkable reduction in APAP intoxicated groups compared with control group, proving that renal function has deteriorated. Our findings are also consistent with those of other researchers who found that APAP-induced kidney damage is manifested by increased serum urea and creatinine levels (**Cekmen *et al.,* 2009**; **Karthivashan *et al.* 2016**).

It is well known that SOD, GSH, CAT and MDA are important biomarkers of the antioxidant capacity of the body, which protects against oxidative stress-induced damage. Oxidative stress is indicated by an increased lipid peroxidation and/or altered non-enzymatic and enzymatic antioxidant systems. Following the oral administration of paracetamol, we investigated alteration in tissue MDA levels as well as reductions in tissue GSH, CAT level, and SOD activity as markers of the oxidative stress

process. The increment in lipid peroxidation was accompanied by a remarkable reduction in the GSH level.

GSH is a tripeptide, that is, found in many mammalian tissues and is an important free radical scavenger and scavenger of NAPQI, which is a reactive intermediate of paracetamol (**Yayla *et al.*, 2014**). It plays an important role in the antioxidant defense system and removes free-radical species, such as hydrogen peroxide and superoxide radicals, and it maintains membrane protein thiols. In current investigation renal MDA level was increased and activities of major renal antioxidant enzymes (GSH, SOD and CAT) were significantly inhibited due to APAP intoxication. The lower SOD, CAT and GSH levels, as well as the higher MDA levels, were consistent with previous research on paracetamol-induced nephrotoxicity. (**Canayakin *et al.*, 2016; Yousef *et al.*, 2010; Abdul Hamid *et al.*, 2012).**

Allicin is the active ingredient of garlic and it is an organic disulfide formed from alliin (**Borlinghaus *et al.*, 2014**). In a study by **Maldonado *et al.*,** (**2003**), they revealed that a derivative of allicin called allyl cysteine could ameliorate gentamicin induced acute renal failure in rats via preservation of the antioxidant enzymes in the renal cortex. Previous studies have been shown that paracetamol has hepatorenal protective effects because it has antioxidant, ROS scavenging, immunomodulatory and anti-inflammatory activities (**Naik and Panda, 2007; MehmetÇik *et al.*, 2008; El-Kashef *et al.*, 2015).** The present results indicate that rats treated with allicin exhibited decreased serum urea, creatinine and elevated serum albumin and total proteins levels towards normal levels as compared with the APAP intoxicated group, suggesting the allicin potently protected against the kidney toxicity induced by APAP.

Allicin can protect the cells from oxidative stress, via scavenging free radical, as well as decreasing cytotoxic compounds (**Chan *et al.*, 2013**). Allicin reduced and prevented renal induced lipid peroxidation and deterioration of antioxidant biomarker, confirming that it alleviated APAP-induced oxidative stress in renal tissue. The levels of MDA, an end product of lipid peroxidation, were significantly decreased with the addition of allicin in the current study, as compared with the APAP groups.

The results agreed with the study of **Şener *et al.*, (2000)**, According to his findings, an aqueous garlic extract significantly inhibits the increase of MDA, allowing the MDA concentration to return to baseline levels. GSH is an important constituent of intracellular protective mechanisms against various noxious stimuli

including oxidative stresses (**Moskovitz *et al.,* 2002**). SOD is a metalloproteinase to detoxify superoxide anions as an efficient dismutative mechanism and it is the first enzyme involved in the antioxidant defense (**Salvemini *et al.,* 2002**). Administration of allicin significantly increased the levels of GSH, SOD and CAT compared with those in the APAP-intoxicated group. These findings indicated that the protective effects of allicin might reflect its function as an antioxidant and ant apoptotic agent.

The past three decades have been a period of rapid expansion in the scientific knowledge of OMG-3PUFAs. Recent studies have shown that Fish oil enriched in OMG-3fatty acids retard the progression of various forms of cancers, depression, arthritis, asthma, cardiovascular and renal disorders (**De caterina *et al.,* 1994**). Also, the renoprotective effect of OMG has been also established (**El-Ashmawy *et al.,* 2018**).

Clinical studies suggest that long-term treatment with OMG fatty acids improves renal function and lowers the risk of death or end-stage renal disease **(Hassan and Gronert, 2009).** The current study was designed to investigate the hypothesis that OMG-3consumption could ameliorate APAP-induced nephrotoxic and other side effects, allowing for further therapeutic use of the drug.

A significant increase in serum creatinine, urea and decrease in albumin and total proteins concentrations as illustrated in APAP intoxicated group indicating renal damage. OMG-3fatty acids improved renal function and had renoprotective effects by normalizing serum creatinine, urea, and other biomarker levels. APAP toxicity is mainly associated with the over-production of ROS as well as deterioration of antioxidant capacity (**Hasanein and Sharifi, 2017**).

ROS promote membrane lipid peroxidation, fragmentation of polyunsaturated fatty acids, leading to cellular damage (**El-Ashmawy *et al.,* 2018**). This was indicated by the remarkable increase in renal MDA concentration as an indication of lipid peroxidation as well as a significant reduction in the renal GSH, SOD and CAT. Antioxidant activity of OMG-3fatty acids was evidenced by the ability to decrease MDA concentration, suppressing lipid peroxidation as well as increasing renal GSH, SOD and CAT. Previous studies have also shown the antioxidant potential of OMG-3fatty acids (**Ali and Rifaai, 2019**).

Our biochemical investigations and oxidative indications are also supported by histological observations. In the current study, following acute APAP intoxication, histopathological examinations revealed clear evidence of nephrotoxicity. The most important histological change was acute tubular necrosis. Furthermore, it causes severe damage to the kidney, with tubular degeneration, wide lumina, damaged glomeruli, interstitial vascular congestion, epithelial degeneration and extensive loss of brush border in the tubular epithelium cells which may have a role in APAP-induced lipid peroxidation. These findings are consistent with a previous study that described renal histological changes following APAP administration (**El-maddawy and El-sayed, 2018; Reshi *et al.*, 2020**). While the normal structure of renal corpuscles and convoluted tubules was preserved following allicin plus omega3 treatment as indicated in results. There were, however, a few mildly dilated glomeruli spaces in some fields. Many tubules had restored the brush border at the apex and narrow lumina. The presence of mild tubular epithelial vacuolization, luminal cast formation and cell desquamations was hardly detected.

Ameliorative potential effects of allicin and/or OMG-3 on histopathological changes caused by APAP were significant, in that they reduced damage greatly, but the tubular scores of the allicin and/or OMG-3 group were significantly higher than the control. This suggests that although there was a reduction in renal damage of APAP with the administration of allicin and/or OMG-3, the result was not a return to normal values of the control group. Also, to emphasize, the effects of allicin and/or OMG-3 were remarkably similar. corroborating our findings, the protective effect of OMG-3 FAs against APAP-induced nephropathy was demonstrated by reduced morphological changes in kidney tissues.

Moreover, caspases are important mediators of apoptosis. The most important one of them is caspase-3 which is activated death protease, stimulating the specific cleavage of many key cellular proteins. APAP-induced apoptosis is a process that depends on caspase which includes activation of the initiator active caspase-9 and effector caspase-3 in renal tubular cells **(Lorz al. 2004)**.

The current study showed that APAP caused an elevation in expression of caspase-3 (nuclear and cytoplasmic) within the renal tubules when compared with the control group which could be explained by oxidative stress induced by APAP which matched with **Hong- Min *et al.*, (2018)** who proved that APAP-induced kidney injury

significantly increased cell apoptosis in renal tubules. However, allicin and/or OMG-3 FAs pretreatment lowered the expression of caspase-3 induced by APAP.These findings suggest that allicin and/or OMG-3 FAs have anti-apoptotic effects against APAP-induced nephrotoxicity by inhibiting mitochondria-mediated apoptosis in rats, as indicated in the results.

Additionally, Immunohistochemical analysis af HSP70 expression showed that the Paracetamol-intoxicated group HSP70 protein was noticeably expressed in the renal tissue of rats compared with that in the control group which showed completely no HSP70 protein expression in rats' kidney. Besides, some HSP70 proteins spread from the kidney cells and disseminated in kidney tubules and renal interstitium. Meanwhile, Kidney section of Allicin only or Omega3 only treated groups showed partial inhibition of HSP70 expression as evidenced by weak immune staining in the cortical regions. Moreover, least or modest HSP70 protein expression in rats' kidney was recorded in sections from groups co-treated with Allicin + Omega3.

Conclusion

From the present study it was concluded that:

•According to the results of our research, albino rats exposed to Paracetamol (APAP) exhibited toxicological effects. These effects were enhanced by their impact on metabolic parameters, oxidative stress indicators, and immunohistochemistry of the liver and kidney. These results were verified by the liver and kidney's histological structures. We explain the increased formation of reactive oxygen species (ROS) and malondialdehyde, as well as the depletion of naturally occurring antioxidants like CAT, SOD and GSH, for this toxicity. Our results indicate that allicin (AC) and omega-3 fatty acids (OMG-3 Fas) can reduce tissue injury and symptoms of oxidative stress by protecting antioxidant enzymes and reducing lipid peroxidation.

•APAP-induced changes in hepatic function, lipid peroxidation, glutathione activity, and antioxidant enzymes are prevented in APAP-intoxicated rats by AC and OMG-3 FAs, which also has strong protective effects on serum biochemistry and the liver's antioxidant machinery.

• APAP contributed to problems with renal function, which may be linked to the production of free radicals, the imbalance in the redox state, and the significant oxidation of membrane lipids and proteins that resulted in the loss of membrane kidney stability. Due to their strong antioxidant activity, simultaneous AC and OMG-3 FAs supplementation reduced all biochemical and histological abnormalities.

•So the combination of omega-3 fatty acids and allicin impressively had a greater antioxidant and anti-inflammatory effect than either agent alone. This shows that by optimizing cellular antioxidant defenses, restoring almostly hepatorenal biomarkers to normal and reducing the expression of active caspase-3 and HSP70. Based on the findings of this investigation and the literature, both medicines appear to be good candidates for nephroprotection & hepatoprotection, and their combination may provide greater cytoprotective effect. More investigation into the chemico-biological interactions between allicin and omega-3 is recommended.

Summary

The objective of this study was to investigate the protective effects of allicin and omega-3 administration on APAP-induced hepatotoxicity, nephrotoxicity, in rats through investigating some hematological, serum biochemical and tissue oxidative/antioxidant parameters. Both histological alterations and immunohistochemically expressions of caspase3 and HSP70 were also carried out. The present study was carried out on a total number of 70 white Wister albinos male rats weighting 160-200 gm were randomly assigned into 7 equal groups (10 rats each).

- **Group 1**: Rats which served as the control was administered saline (the vehicle) orally once daily for 30 consecutive days.
- **Group 2:** Rats in this group were served as allicin treated group and were orally administered (10 mg/kg b.wt.), once daily for 30 days.
- **Group 3**: Rats in this group were served as omega-3 treated group and were orally administered (100 mg/kg b.wt.), once daily for 30 days.
- **Group 4:** Rats in this group were served as paracetamol treated group and were administered saline orally once daily and a single dose of APAP on the 27^{th} day of the experiment (1 g/kg/day/orally).
- **Group 5:** Rats in this group were administered allicin orally once daily for 30 consecutive days and on the 27^{th} day of the experiment given APAP (1 g/kg/day/orally).
- **Group 6:** Rats in this group were administered omega-3 orally once daily for 30 consecutive days and on the 27^{th} day of the experiment given APAP (1 g/kg/day/orally).
- **Group 7:** Rats in this group were administered both allicin and omega-3 orally once daily for 30 consecutive days and on the 27^{th} day of the experiment given APAP (1 g/kg/day/orally).

Hematological examination revealed that exposure to APAP significantly reduced the values of RBC counts, Hb concentrations, Platelets counts (PL), and packed cell volumes (PCV), while increasing the values of WBC counts when compared to the control group. Allicin and/or omega3 administration reduced the

harmful impacts of APAP by reversing these changes in hematological parameters to the values observed in control rats.

Also in this study, APAP administration significantly increased serum ALT, AST, and ALP activities compared with those in control rats. Similarly, APAP significantly increased the levels of creatinine and urea. In addition, increase the level of triglycerides and total cholesterol levels, in contrast decrease level of albumin and total protein in APAP intoxicated group when compared to those of the other groups. Allicin and/ or omega-3 administration with APAP restored these parameters towards the normal values.

In the current study, there were substantial increases in MDA level along with dramatic decreases in GSH, SOD and CAT in the liver and kidney tissues of APAP-intoxicated rats. Meanwhile, allicin + APAP and/ or omega-3 + APAP revealed a decrease in MDA level along with elevations in GSH, SOD and CAT in hepatic and renal tissues compared with APAP treated group.

Histologically, liver sections from control group, allicin group and omega-3 group administered rats exhibited normal hepatic histo-architecture. Hepatocytes organized in cords radiating from the central vein and separated by regular sinusoids. Otherwise, APAP treated rats revealed several histological changes represented by sinusoidal dilation, congestion of blood vessels, disorganized architecture of hepatic lobule, and fatty infiltration in some hepatocytes. The liver section from allicin+ APAP and omega-3 + APAP showed moderate degeneration and allicin + omega-3 + APAP treated rats represented almost normal hepatocytes and sinusoids.

Histologically, Kidney sections from control group, allicin group and omega-3 fatty acids group showed regular renal histo-architecture with normal renal corpuscles and renal tubules; proximal (PCT) and distal convoluted tubules (DCT) and collecting (CT) tubules. In APAP group, many distinguishing histological changes were noted including severe degenerative changes in the renal tubules that notably by hydropic degeneration, pycnotic nuclei, increased cytoplasmic vesicles, cytoplasmic vacuolization, necrosis and apoptosis of tubular cells, and desquamation of necrotic epithelial cells filling the tubular lumens and forming hyaline casts. However, kidney from allicin + APAP and omega-3 + APAP, revealed a moderate tubular degeneration and allicin+OMG-3+APAP treated rats represented almost normal histological structure.

Immunohistochemically, a summary of caspase3 and HSP70 immunohistochemical expressions in the livers and kidneys of all examined groups was recorded.

In liver, control group, allicin group and omega-3 group showed weak expression of caspase3 and HSP70. Meanwhile, APAP -injected rats showed over expression of caspase3 and HSP70. However, Liver from allicin + APAP and omega-3 + APAP treated group showed moderate expression of caspase3 and HSP70 semi quantitative analysis did not differ significantly between the groups in their response to allicin and or omega3. But, allicin + omega-3 +APAP treated group showed mild expression of caspase3 and HSP70.

In kidneys, control group, allicin group and omega-3 group showed weak expression of caspase3 and HSP70. Meanwhile, APAP -injected rats showed over expression of caspase3 and HSP70. However, kidneys from allicin + APAP and omega-3 + APAP treated group showed moderate expression of caspase3 and HSP70. allicin + omega-3 + APAP treated group showed mild expression of caspase3 and HSP70.

From this study, it could be concluded that allicin and /or omega-3 supplementation for patients who regularly take high doses of paracetamol counteracts livers and kidneys toxicity induced by APAP due to its both antioxidant and anti-inflammatory properties.

References

Abdeen, A., Abdelkader, A., Abdo, M., Wareth, G., Aboubakr, M., Aleya, L. & Abdel-Daim, M., 2019, 'Protective effect of cinnamon against acetaminophen-mediated cellular damage and apoptosis in renal tissue'. Environmental Science and Pollution Research international, 26(1), 240-249.

Abdel-Daim, M.M. & Abdeen, A., 2018, 'Protective effects of rosuvastatin and vitamin E against fipronil-mediated oxidative damage and apoptosis in rat liver and kidney'. Food and chemical toxicology, 114, 69-77.

Abdel-Daim, M.M., Abushouk, A.I., Donia, T., Alarifi, S., Alkahtani, S., Aleya, L. & Bungau, S.G., 2019, 'The nephroprotective effects of allicin and ascorbic acid against cisplatin-induced toxicity in rats'. Environmental Science and Pollution Research international, 26(13), 13502-13509.

Abdul Hamid, Z., Budin, S.B., Wen Jie, N., Hamid, A., Husain, K. & Mohamed, J., 2012, 'Nephroprotective effects of Zingiber zerumbet Smith ethyl acetate extract against paracetamol-induced nephrotoxicity and oxidative stress in rats'. Journal of Zhejiang University Science B, 13(3), 176-185.

Abei, H., 1984, 'Catalase in vitro'. Methods Enzymol, 105, 121-126.

Adeyemi, W.J. & Olayaki, L.A., 2017, 'Diclofenac–induced hepatotoxicity: Low dose of omega-3 fatty acids have more protective effects'. Toxicology reports, 5, 90-95.

Akakpo, J.Y., Ramachandran, A., Kandel, S.E., Ni, H.M., Kumer, S.C., Rumack, B.H. & Jaeschke, H., 2018, '4-Methylpyrazole protects against acetaminophen hepatotoxicity in mice and in primary human hepatocytes'. Human & Experimental Toxicology, 37(12), 1310-1322.

Akgun, E., Boyacioglu, M. & Kum, S., 2021, 'The potential protective role of folic acid against acetaminophen-induced hepatotoxicity and nephrotoxicity in rats'. Experimental Animals, 70(1), 54-62.

Akhilraj, A.R., Bhat, S., Priyalatha, B. & Vimala, K.S., 2021, 'Comparative hepatoprotective activity of detoxified roots of Plumbago zeylanica L. and Plumbago rosea L. in Wistar rats'. Journal of Ayurveda and Integrative Medicine, 12(3), 452-457.

Alam, R., Fawzi, E.M., Alkhalf, M.I., Alansari, W.S., Aleya, L. & Abdel-Daim, M.M., 2018, 'Anti-inflammatory, immunomodulatory, and antioxidant activities of allicin, norfloxacin, or their combination against Pasteurella multocida infection in male New Zealand rabbits'. Oxidative Medicine and Cellular Longevity, 2018. doi: 10.1155/2018/1780956.

Al-Brakati, A.Y., Fouda, M.S., Tharwat, A.M., Elmahallawy, E.K., Kassab, R.B. & Abdel Moneim, A.E., 2019, 'The protective efficacy of soursop fruit extract against hepatic injury associated with acetaminophen exposure is mediated through antioxidant, anti-inflammatory, and anti-apoptotic activities'. Environmental Science and Pollution Research international, 26(13), 13539-13550.

Ali, F.F. & Rifaai, R.A., 2019, 'Preventive effect of omega-3 fatty acids in a rat model of stress-induced liver injury'. Journal of Cellular Physiology, 234(7), 11960-11968.

Antagonists, T.N.F., 2012, 'LiverTox: clinical and research information on drug-induced liver injury'. National Institute of Diabetes and Digestive and Kidney Diseases: Bethesda, MD, USA.

Arellano Buendía, A.S., Tostado González, M., Sánchez Reyes, O., García Arroyo, F.E., Argüello García, R., Tapia, E., Sánchez Lozada, L.G. & Osorio Alonso, H., 2018, 'Immunomodulatory effects of the nutraceutical garlic derivative allicin in the progression of diabetic nephropathy'. International journal of molecular sciences, 19(10), 3107.

Attah, A.I., Eneji, E.G. & Hogan, I.E., 2020, 'Effects of Carica Papaya Seeds on Acetaminophen-Induced Hepatotoxicity in Male Wistar Rats'. Tropical Journal of Natural Product Research (TJNPR), 4(8), 463-470.

Avramovic, N., Dragutinovic, V., Krstic, D., Colovic, M.B., Trbovic, A., de Luka, S., Milovanovic, I. & Popovic, T., 2012, 'The effects of omega 3 fatty acid supplementation on brain tissue oxidative status in aged wistar rats'. Hippokratia, 16(3), 241-246.

Bäck, M. & Hansson, G.K., 2019, 'Omega-3 fatty acids, cardiovascular risk, and the resolution of inflammation'. The Federation of American Societies for Experimental Biology (FASEB) journal, 33(2), 1536-1539.

Baleni, R., Bekker, Z., Walubo, A. & Du Plessis, J.B., 2015, 'Co-administration of fresh grape fruit juice (GFJ) and bergamottin prevented paracetamol induced hepatotoxicity after paracetamol overdose in rats'. Toxicology Reports, 2, 677-684.

Bancroft, J.D. & Gamble, M. eds., 2008. 'Theory and practice of histological techniques'. Elsevier health sciences.

Bayan, L., Koulivand, P.H. & Gorji, A., 2014, 'Garlic: a review of potential therapeutic effects'. Avicenna journal of phytomedicine, 4(1), 1-14.

Bellenger, J., Bellenger, S., Bataille, A., Massey, K.A., Nicolaou, A., Rialland, M., Tessier, C., Kang, J.X. & Narce, M., 2011, 'High pancreatic n-3 fatty acids prevent STZ-induced diabetes in fat-1 mice: inflammatory pathway inhibition'. Diabetes, 60(4), 1090-1099.

Bertolini, A., Ferrari, A., Ottani, A., Guerzoni, S., Tacchi, R. & Leone, S., 2006, 'Paracetamol: new vistas of an old drug'. CNS drug reviews, 12(3-4), 250-275.

Beutler, E., Duron, O. & Kelly, M.B., 1963, 'Improved method for the determination of blood GSH'. The Journal of laboratory and clinical medicine, 61, 882–888.

Bhawna, S. & Kumar, S.U., 2009, 'Hepatoprotective activity of some indigenous plants'. International journal of pharmtech research, 4, 1330-1334.

Borlinghaus, J., Albrecht, F., Gruhlke, M.C., Nwachukwu, I.D. & Slusarenko, A.J., 2014, 'Allicin: chemistry and biological properties'. Molecules, 19(8), 12591-12618.

Bouhlali, E.D.T., Derouich, M., Hmidani, A., Bourkhis, B., Khouya, T., Filali-Zegzouti, Y. & Alem, C., 2021, 'Protective effect of Phoenix dactylifera L. seeds against paracetamol-induced hepatotoxicity in rats: a comparison with vitamin C'. The Scientific World Journal, doi: 10.1155/2021/6618273.

Bromer, M.Q. & Black, M., 2003, 'Acetaminophen hepatotoxicity'. Clinics in Liver Disease, 7(2), 351-367.

Calder, P.C., 2012, 'Mechanisms of action of (n-3) fatty acids'. The Journal of nutrition, 142(3), 592S-599S.

Calder, P.C., 2018, 'Very long-chain n-3 fatty acids and human health: fact, fiction and the future'. Proceedings of the Nutrition Society, 77(1), 52-72.

Canayakin, D., Bayir, Y., Kilic Baygutalp, N., Sezen Karaoglan, E., Atmaca, H.T., Kocak Ozgeris, F.B., Keles, M.S. & Halici, Z., 2016, 'Paracetamol-induced nephrotoxicity and oxidative stress in rats: the protective role of Nigella sativa'. Pharmaceutical biology, 54(10), 2082-2091.

Cekmen, M., Ilbey, Y.O., Ozbek, E., Simsek, A., Somay, A. & Ersoz, C., 2009, 'Curcumin prevents oxidative renal damage induced by acetaminophen in rats'. Food and Chemical Toxicology, 47(7), 1480-1484.

Chan, J.Y., Yuen, A.C., Chan, R.Y. & Chan, S.W., 2013, 'A review of the cardiovascular benefits and antioxidant properties of allicin'. Phytotherapy Research, 27(5), 637-646.

Chaphalkar, R., Apte, K.G., Talekar, Y., Ojha, S.K. & Nandave, M., 2017, 'Antioxidants of Phyllanthus emblica L. Bark extract provide hepatoprotection against ethanol-induced hepatic damage: a comparison with silymarin'. Oxidative medicine and cellular longevity, doi: 10.1155/2017/3876040.

Chiew, A.L., Gluud, C., Brok, J. & Buckley, N.A., 2018, 'Interventions for paracetamol (acetaminophen) overdose'. Cochrane Database of Systematic Reviews, (2), doi: 10.1002/14651858.CD003328.

Chinnappan, S.M., George, A., Thaggikuppe, P., Choudhary, Y., Choudhary, V.K., Ramani, Y. & Dewangan, R., 2019, 'Nephroprotective effect of herbal extract Eurycoma longifolia on paracetamol-induced nephrotoxicity in rats'. Evidence-Based Complementary and Alternative Medicine, doi: 10.1155/2019/4916519.

Chiu, H.F., Shen, Y.C., Venkatakrishnan, K. & Wang, C.K., 2018, 'Food for Eye Health: Carotenoids and Omega-3 Fatty Acids'. DOI: 10.1016/B978-0-08-100596-5.21740-X.

Cholewski, M., Tomczykowa, M. & Tomczyk, M., 2018, 'A comprehensive review of chemistry, sources and bioavailability of omega-3 fatty acids'. Nutrients, 10(11), 1662.

Choo, S., Chin, V.K., Wong, E.H., Madhavan, P., Tay, S.T., Yong, P.V.C. & Chong, P.P., 2020, 'Antimicrobial properties of allicin used alone or in combination with other medications'. Folia microbiologica, 65(3), 451-465.

Christen, W.G., Schaumberg, D.A., Glynn, R.J. & Buring, J.E., 2011, 'Dietary OMG-3fatty acid and fish intake and incident age-related macular degeneration in women'. Archives of ophthalmology, 129(7):921-9.

Chung, M.L., Lee, K.Y. & Lee, C.Y.J., 2013, 'Profiling of oxidized lipid products of marine fish under acute oxidative stress'. Food and chemical toxicology, 53, 205-213.

Coresh, J., Selvin, E., Stevens, L.A., Manzi, J., Kusek, J.W., Eggers, P., Van Lente, F. & Levey, A.S., 2007, 'Prevalence of chronic kidney disease in the United States'. Jama, 298(17), 2038-2047.

Coulombe, J.J. & Favreau, L., 1963, 'A new simple semimicro method for colorimetric determination of urea'. Clinical chemistry, 9(1), 102-108.

Dallak, M., Dawood, A.F., Haidara, M.A., Abdel Kader, D.H., Eid, R.A., Kamar, S.S., Shams Eldeen, A.M. & Al-Ani, B., 2022, 'Suppression of glomerular damage and apoptosis and biomarkers of acute kidney injury induced by acetaminophen toxicity using a combination of resveratrol and quercetin'. Drug and chemical toxicology, 45(1), 1-7.

Das, J., Ghosh, J., Manna, P. & Sil, P.C., 2010, 'Taurine protects acetaminophen-induced oxidative damage in mice kidney through APAP urinary excretion and CYP2E1 inactivation'. Toxicology, 269(1), 24-34.

de Batlle, J., Sauleda, J., Balcells, E., Gómez, F.P., Méndez, M., Rodriguez, E., Barreiro, E., Ferrer, J.J., Romieu, I., Gea, J. & Antó, J.M., 2012, 'Association between Ω3 and Ω6 fatty acid intakes and serum inflammatory markers in COPD'. The Journal of nutritional biochemistry, 23(7), 817-821.

De Caterina, R., Endres, S., Kristensen, S.D. & Schmidt, E.B., 1994, 'n-3 fatty acids and renal diseases'. American journal of kidney diseases, 24(3), 397-415.

Doumas, B.T., 1975, 'Standards for total serum protein assays—a collaborative study'. Clinical chemistry, 21(8), 1159-1166.

Doumas, B.T., Watson, W.A. & Biggs, H.G., 1971, 'Albumin standards and the measurement of serum albumin with bromcresol green'. Clinica chimica acta, 31(1), 87-96.

Dwivedi, V.P., Bhattacharya, D., Singh, M., Bhaskar, A., Kumar, S., Fatima, S., Sobia, P., Van Kaer, L. & Das, G., 2019, 'Allicin enhances antimicrobial activity of macrophages during Mycobacterium tuberculosis infection'. Journal of ethnopharmacology, 243, 111634.

Ebada, M.E., 2018, 'Essential oils of green cumin and chamomile partially protect against acute acetaminophen hepatotoxicity in rats'. Anais da Academia Brasileira de Ciências, 90, 2347-2358.

Eguia, L. & Materson, B.J., 1997, 'Acetaminophen-related acute renal failure without fulminant liver failure'. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy, 17(2), 363-370.

El-Ashmawy, N.E., Khedr, N.F., El-Bahrawy, H.A. & Helal, S.A., 2018, 'Upregulation of PPAR-γ mediates the renoprotective effect of omega-3 PUFA and ferulic acid in gentamicin-intoxicated rats'. Biomedicine & Pharmacotherapy, 99, 504-510.

El-Boshy, M., BaSalamah, M.A., Ahmad, J., Idris, S., Mahbub, A., Abdelghany, A.H., Almaimani, R.A., Almasmoum, H., Ghaith, M.M., Elzubier, M. & Refaat, B., 2019, 'Vitamin D protects against oxidative stress, inflammation and hepatorenal damage induced by acute paracetamol toxicity in rat'. Free Radical Biology and Medicine, 141, 310-321.

El-Gendy, Z.A., El-Batran, S.A., Youssef, S., Ramadan, A., Hotaby, W.E., Bakeer, R.M. & Ahmed, R.F., 2021, 'Hepatoprotective effect of Omega-3 PUFAs against acute paracetamol-induced hepatic injury confirmed by FTIR'. Human & Experimental Toxicology, 40(3), 526-537.

El-Kashef, D.H., El-Kenawi, A.E., Suddek, G.M. & Salem, H.A., 2015, 'Protective effect of allicin against gentamicin-induced nephrotoxicity in rats'. International immunopharmacology, 29(2), 679-686.

Ellahi, B., Salman, A.M., Sheikh, S.A. & Summra, E., 2014, 'Hepatoprotective and hepatocurative properties of alcoholic extract of Carthamus oxyacantha seeds'. African journal of plant science, 8(1), 34-41.

El-Maddawy, Z.K. & El-Sayed, Y.S., 2018, 'Comparative analysis of the protective effects of curcumin and N-acetyl cysteine against paracetamol-induced hepatic, renal, and testicular toxicity in Wistar rats'. Environmental science and pollution research international, 25(4), 3468-3479.

Elshal, M. & Abdelmageed, M.E., 2022, 'Diacerein counteracts acetaminophen-induced hepatotoxicity in mice via targeting NLRP3/caspase-1/IL-1β and IL-4/MCP-1 signaling pathways'. Archives of Pharmacal Research, 45(3), 142-158.

Eraky, S.M. & El-Magd, N.F., 2020, 'Omega-3 fatty acids protect against acetaminophen-induced hepatic and renal toxicity in rats through HO-1-Nrf2-BACH1 pathway'. Archives of biochemistry and biophysics, 687, 108387.

Eshrati, R., Jafari, M., Gudarzi, S., Nazari, A., Samizadeh, E. & Ghafourian Hesami, M., 2021, 'Comparison of ameliorative effects of Taraxacum syriacum and N-acetylcysteine against acetaminophen-induced oxidative stress in rat liver and kidney'. The Journal of Biochemistry, 169(3), 337-350.

Fan, Y., Liu, J.H., Lu, H.T. & Zhang, Q., 2011, 'Electrochemical behavior and voltammetric determination of paracetamol on Nafion/TiO2–graphene modified glassy carbon electrode'. Colloids and Surfaces B: Biointerfaces, 85(2), 289-292.

Finley, J.W. & Shahidi, F., 2001, 'The chemistry, processing, and health benefits of highly unsaturated fatty acids: an overview in Omega-3 Fatty Acids'. ACS Publications, 2–11.

Foroutan-Rad, M., Tappeh, K.H. & Khademvatan, S., 2017, 'Antileishmanial and Immunomodulatory Activity of Allium sativum (Garlic) A Review'. Journal of evidence-based complementary & alternative medicine, 22(1), 141-155.

García Trejo, E.M.Á., Arellano Buendía, A.S., Sánchez Reyes, O., García Arroyo, F.E., Arguello García, R., Loredo Mendoza, M.L., Tapia, E., Sánchez Lozada, L.G. & Osorio Alonso, H., 2017, 'The beneficial effects of allicin in chronic kidney disease are comparable to losartan'. International journal of molecular sciences, 18(9), 1980.

Ghanayem, B.I., McDaniel, L.P., Churchwell, M.I., Twaddle, N.C., Snyder, R., Fennell, T.R. & Doerge, D.R., 2005, 'Role of CYP2E1 in the epoxidation of acrylamide to glycidamide and formation of DNA and hemoglobin adducts'. Toxicological sciences, 88(2), 311-318.

Goksu Erol, A.Y., Avcı, G., Sevimli, A., Ulutas, E. & Ozdemir, M., 2013, 'The protective effects of omega 3 fatty acids and sesame oil against cyclosporine A-induced nephrotoxicity'. Drug and chemical toxicology, 36(2), 241-248.

Gong, S., Lan, T., Zeng, L., Luo, H., Yang, X., Li, N.A., Chen, X., Liu, Z., Li, R., Win, S. & Liu, S., 2018, 'Gut microbiota mediates diurnal variation of acetaminophen induced acute liver injury in mice'. Journal of hepatology, 69(1), 51-59.

Goyal, R.N., Rana, A.R.S., Aziz, M.A. & Oyama, M., 2011, 'Effect of gold nanoparticle attached multi-walled carbon nanotube-layered indium tin oxide in monitoring the effect of paracetamol on the release of epinephrine'. Analytica chimica acta, 693(1-2), 35-40.

Gummin, D.D., Mowry, J.B., Spyker, D.A., Brooks, D.E., Beuhler, M.C., Rivers, L.J., Hashem, H.A. & Ryan, M.L., 2019, '2018 Annual report of the American Association of Poison control centers' National Poison Data System (NPDS): 36th annual report'. Clinical toxicology, 57(12), 1220-1413.

Hamam, F. & Shahidi, F., 2006, 'Synthesis of structured lipids containing medium-chain and omega-3 fatty acids'. Journal of agricultural and food chemistry, 54(12), 4390-4396.

Hasanein, P. & Sharifi, M., 2017, 'Effects of rosmarinic acid on acetaminophen-induced hepatotoxicity in male Wistar rats'. Pharmaceutical biology, 55(1), 1809-1816.

Hassan, I.R. & Gronert, K., 2009, 'Acute changes in dietary ω-3 and ω-6 polyunsaturated fatty acids have a pronounced impact on survival following ischemic renal injury and formation of renoprotective docosahexaenoic acid-derived protectin D1'. The Journal of Immunology, 182(5), 3223-3232.

Hawton, K., Simkin, S., Deeks, J., Cooper, J., Johnston, A., Waters, K., Arundel, M., Bernal, W., Gunson, B., Hudson, M. & Suri, D., 2004, 'UK legislation on analgesic packs: before and after study of long term effect on poisonings'. British medical journal, 329(7474), 1076.

Hinson, J.A., Roberts, D.W. & James, L.P., 2010, 'Mechanisms of acetaminophen-induced liver necrosis'. Handbook of experimental pharmacology, (196), 369-405.

Hodgman, M.J. & Garrard, A.R., 2012, 'A review of acetaminophen poisoning'. Critical care clinics, 28(4), 499-516.

Hong-Min, Y., Min, W., Zong-Chao, Y., Yi-Fang, L., Chun-Xin, H., Fang-Xuan, H., Fan-Na, L. & Rong-Rong, H., 2018, 'Antioxidative and antiapoptotic effects

of (+)-clausenamide on acetaminophen-induced nephrotoxicity in mice'. TMR Modern Herbal Medicine, 1(3), 127-135.

Hsu, Y.L., Kuo, P.L., Liu, C.F. & Lin, C.C., 2004, 'Acacetin-induced cell cycle arrest and apoptosis in human non-small cell lung cancer A549 cells'. Cancer letters, 212(1), 53-60.

Huang, W., Wang, B., Li, X. & Kang, J.X., 2015, 'Endogenously elevated n-3 polyunsaturated fatty acids alleviate acute ethanol-induced liver steatosis'. Biofactors, 41(6), 453-462.

Hussain, S., Ashafaq, M., Alshahrani, S., Siddiqui, R., Ahmed, R.A., Khuwaja, G. & Islam, F., 2020, 'Cinnamon oil against acetaminophen-induced acute liver toxicity by attenuating inflammation, oxidative stress and apoptosis'. Toxicology Reports, 7, 1296-1304.

Ishitsuka, Y., Kondo, Y. & Kadowaki, D., 2020, 'Toxicological property of acetaminophen: the dark side of a safe antipyretic/analgesic drug'. Biological and pharmaceutical bulletin, 43(2), 195-206.

Islam, M.T., Quispe, C., Islam, M.A., Ali, E.S., Saha, S., Asha, U.H., Mondal, M., Razis, A.F.A., Sunusi, U., Kamal, R.M. & Kumar, M., 2021, 'Effects of nerol on paracetamol-induced liver damage in Wistar albino rats'. Biomedicine & Pharmacotherapy, 140, 111732.

Iyanda, A.A. & Adeniyi, F.A., 2011, 'Biochemical and histologic presentations of female Wistar rats administered with different doses of paracetamol/methionine'. Nigerian journal of physiological sciences, 26(2), 151-60.

Jaeschke, H., Akakpo, J.Y., Umbaugh, D.S. & Ramachandran, A., 2020, 'Novel therapeutic approaches against acetaminophen-induced liver injury and acute liver failure'. Toxicological Sciences, 174(2), 159-167.

James, L.P., McCullough, S.S., Lamps, L.W. & Hinson, J.A., 2003, 'Effect of N-acetylcysteine on acetaminophen toxicity in mice: relationship to reactive nitrogen and cytokine formation'. Toxicological sciences, 75(2), 458-467.

Karaali, H.F., Fahmi, R.R. & Borjac, J.M., 2019, 'Effect of Ocimum basilicum leaves extract on acetaminophen-induced nephrotoxicity in BALB/c mice'. Journal of Complementary and Integrative Medicine, 16(2).

Karakus, E., Halici, Z., Albayrak, A., Polat, B., Bayir, Y., Kiki, İ., Cadirci, E., Topcu, A.T.İ.L.L.A. & Aksak, S., 2013, 'Agomelatine: an antidepressant with new potent hepatoprotective effects on paracetamol-induced liver damage in rats'. Human & experimental toxicology, 32(8), 846-857.

Karthivashan, G., Kura, A.U., Arulselvan, P., Isa, N.M. & Fakurazi, S., 2016, 'The modulatory effect of Moringa oleifera leaf extract on endogenous antioxidant systems and inflammatory markers in an acetaminophen-induced nephrotoxic mice model'. PeerJ, 4, e2127.

Kelsey, N.A., Wilkins, H.M. & Linseman, D.A., 2010, 'Nutraceutical antioxidants as novel neuroprotective agents'. Molecules, 15(11), 7792-7814.

Khan, M.W., Priyamvada, S., Khan, S.A., Khan, S., Naqshbandi, A. & Yusufi, A.N., 2012, 'Protective effect of ω-3 polyunsaturated fatty acids on L-arginine-induced nephrotoxicity and oxidative damage in rat kidney'. Human & experimental toxicology, 31(10), 1022-1034.

Kim, S.Y. & Moon, A., 2012, 'Drug-induced nephrotoxicity and its biomarkers'. Biomolecules & therapeutics, 20(3), 268-72.

Ko, J.W., Shin, J.Y., Kim, J.W., Park, S.H., Shin, N.R., Lee, I.C., Shin, I.S., Moon, C., Kim, S.H., Kim, S.H. & Kim, J.C., 2017, 'Protective effects of diallyl disulfide against acetaminophen-induced nephrotoxicity: a possible role of CYP2E1 and NF-κB'. Food and Chemical Toxicology, 102, 156-165.

Kobashigawa, J.A. & Kasiske, B.L., 1997, 'Hyperlipidemia in solid organ transplantation'. Transplantation, 63(3), 331-338.

Kong, X., Gong, S., Su, L., Li, C. & Kong, Y., 2017, 'Neuroprotective effects of allicin on ischemia-reperfusion brain injury'. Oncotarget, 8(61), 104492-104507.

Koyuncuoğlu, T., Yıldırım, A., Dertsiz, E.K., Yüksel, M., Ercan, F. & Yeğen, B.Ç., 2020, 'Estrogen receptor agonists protect against acetaminophen-induced hepatorenal toxicity in rats'. Life Sciences, 263, 118561.

Lakshmi, M.S., Reddy, U.K. & Rani, S.R.K.S., 2012, 'A review on medicinal plants for nephroprotective activity'. Asian journal of pharmaceutical and clinical research, 5(4), 8-14.

Larsen, K., 1972, 'Creatinine assay by a reaction-kinetic principle'. Clinica chimica acta; international journal of clinical chemistry, 41, 209-217.

Larson, A.M., Polson, J., Fontana, R.J., Davern, T.J., Lalani, E., Hynan, L.S., Reisch, J.S., Schiødt, F.V., Ostapowicz, G., Shakil, A.O. & Lee, W.M., 2005, 'Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study'. Hepatology, 42(6), 1364-1372.

Lee, S.I. & Kang, K.S., 2019, 'Omega-3 fatty acids modulate cyclophosphamide induced markers of immunosuppression and oxidative stress'. Scientific reports, 9(1), 1-8.

Li, Q., Yu, Q., Na, R. & Liu, B., 2017, 'Omega-3 polyunsaturated fatty acids prevent murine dilated cardiomyopathy by reducing oxidative stress and cardiomyocyte apoptosis'. Experimental and therapeutic medicine, 14(6), 6152-6158.

Li, S., Hong, M., Tan, H.Y., Wang, N. & Feng, Y., 2016, 'Insights into the role and interdependence of oxidative stress and inflammation in liver diseases'. Oxidative medicine and cellular longevity, doi: 10.1155/2016/4234061.

Liu, C.M., Ma, J.Q. & Sun, Y.Z., 2010, 'Quercetin protects the rat kidney against oxidative stress-mediated DNA damage and apoptosis induced by lead'. Environmental toxicology and pharmacology, 30(3), 264-271.

Lopez, L.B., Kritz-Silverstein, D. & Barrett-Connor, E., 2011, 'High dietary and plasma levels of the omega-3 fatty acid docosahexaenoic acid are associated with decreased dementia risk: The Rancho Bernardo study'. The journal of nutrition, health & aging, 15(1), 25-31.

Lorz, C., Justo, P., Sanz, A., Subirá, D., Egido, J. & Ortiz, A., 2004, 'Paracetamol-induced renal tubular injury: a role for ER stress'. Journal of the American Society of Nephrology, 15(2), 380-389.

Madinah, N., Nozmo, M. & Ezekiel, I., 2015, 'The protective effects of aqueous extract of Carica papaya seeds in paracetamol induced nephrotoxicity in male wistar rats'. African health sciences, 15(2), 598-605.

Maksymchuk, O., Shysh, A., Chashchyn, M. & Moibenko, O., 2016, 'Dietary omega-3 polyunsaturated fatty acids alter fatty acid composition of lipids and CYP2E1

expression in rat liver tissue'. International Journal for Vitamin and Nutrition Research, 85(56), 322-328.

Maldonado, P.D., Barrera, D., Rivero, I., Mata, R., Medina-Campos, O.N., Hernández-Pando, R. & Pedraza-Chaverrí, J., 2003, 'Antioxidant S-allylcysteine prevents gentamicin-induced oxidative stress and renal damage'. Free Radical Biology and Medicine, 35(3), 317-324.

Mathews, V.V., Paul, M.S., Abhilash, M., Manju, A., Abhilash, S. & Nair, R.H., 2014, 'Mitigation of hepatotoxic effects of arsenic trioxide through omega-3 fatty acid in rats'. Toxicology and industrial health, 30(9), 806-813.

Matić, M.M., Paunović, M.G., Milošević, M.D., Ognjanović, B.I. & Saičić, Z.S., 2021, 'Hematoprotective effects and antioxidant properties of β-glucan and vitamin C against acetaminophen-induced toxicity: an experimental study in rats'. Drug and Chemical Toxicology, 44(3), 302-309.

McGill, M.R. & Jaeschke, H., 2013, 'Metabolism and disposition of acetaminophen: recent advances in relation to hepatotoxicity and diagnosis'. Pharmaceutical research, 30(9), 2174-2187.

Mehmetçik, G., Özdemirler, G., Koçak-Toker, N., Çevikbaş, U. & Uysal, M., 2008, 'Effect of pretreatment with artichoke extract on carbon tetrachloride-induced liver injury and oxidative stress'. Experimental and Toxicologic Pathology, 60(6), 475-480.

Metwally, D.M., Al-Olayan, E.M., Alanazi, M., Alzahrany, S.B. & Semlali, A., 2018, 'Antischistosomal and anti-inflammatory activity of garlic and allicin compared with that of praziquantel in vivo'. BMC complementary and alternative medicine, 18(1), 1-11.

Mikhail, A., Tanoli, O., Légaré, G., Dubé, P.A., Habel, Y., Lesage, A., Low, N.C., Lamarre, S., Singh, S. & Rahme, E., 2019, 'Over-the-counter drugs and other substances used in attempted suicide presented to emergency departments in Montreal, Canada: A cross-sectional study'. Crisis: The Journal of Crisis Intervention and Suicide Prevention, 40(3), 166-175.

Moore, J.K., Love, E., Craig, D.G., Hayes, P.C. & Simpson, K.J., 2013, 'Acute kidney injury in acute liver failure: a review'. Expert review of gastroenterology & hepatology, 7(8), 701-712.

Moore, N., Duret, S., Grolleau, A., Lassalle, R., Barbet, V., Duong, M., Thurin, N., Droz-Perroteau, C. & Gulmez, S.E., 2019, 'Previous drug exposure in patients hospitalised for acute liver injury: a case-population study in the French National Healthcare Data System'. Drug Safety, 42(4), 559-572.

Moskovitz, J., Yim, M.B. & Chock, P.B., 2002, 'Free radicals and disease'. Archives of Biochemistry and Biophysics, 397(2), 354-359.

Mour, G., Feinfeld, D.A., Caraccio, T. & McGuigan, M., 2005, 'Acute renal dysfunction in acetaminophen poisoning'. Renal failure, 27(4), 381-383.

Murad, H.A., Habib, H., Kamel, Y., Alsayed, S., Shakweer, M. & Elshal, M., 2016, 'Thearubigins protect against acetaminophen-induced hepatic and renal injury in mice: biochemical, histopathological, immunohistochemical, and flow cytometry study'. Drug and Chemical Toxicology, 39(2), 190-198.

Naik, S.R. & Panda, V.S., 2007, 'Antioxidant and hepatoprotective effects of Ginkgo biloba phytosomes in carbon tetrachloride-induced liver injury in rodents'. Liver international, 27(3), 393-399.

Naqshbandi, A., Khan, M.W., Rizwan, S., ur Rehman, S. & Khan, F., 2012, 'Studies on the protective effect of dietary fish oil on cisplatin induced nephrotoxicity in rats'. Food and Chemical Toxicology, 50(2), 265-273.

Okada, Y., Tanaka, K., Sato, E. & Okajima, H., 2006, 'Kinetic and mechanistic studies of allicin as an antioxidant'. Organic & Biomolecular Chemistry, 4(22), 4113-4117.

Orabi, S.H., Abd Eldaium, D., Hassan, A., El Sabagh, H.S. & Abd Eldaim, M.A., 2020, 'Allicin modulates diclofenac sodium induced hepatonephro toxicity in rats via reducing oxidative stress and caspase 3 protein expression'. Environmental Toxicology and Pharmacology, 74, 103306.

Owumi, S.E., Olayiwola, Y.O., Alao, G.E., Gbadegesin, M.A. & Odunola, O.A., 2020, 'Cadmium and nickel co-exposure exacerbates genotoxicity and not oxido-inflammatory stress in liver and kidney of rats: Protective role of omega-3 fatty acid'. Environmental toxicology, 35(2),231-241.

Oyedeji, K.O., Bolarinwa, A.F. & Ojeniran, S.S., 2013, 'Effect of paracetamol (acetaminophen) on haematological and reproductive parameters in male albino rats'. IOSR Journal of Pharmacy and Biological Sciences, 4(6), 1-6.

ÖZDATLI, Ş., SIPAHI, H., CHAREHSAZ, M., BEHZETOĞLU, Y., DUMAN, G. & AYDIN, A., 2015, 'A Pilot Study on Effects of Concomitant Usage of Acetaminophen and N-Acetylcysteine to Prevent Possible Acetaminophen Toxicity'. Turkish Journal of Pharmaceutical Sciences, 12(1), 45-52.

Ozer, J., Ratner, M., Shaw, M., Bailey, W. & Schomaker, S., 2008, 'The current state of serum biomarkers of hepatotoxicity'. Toxicology, 245(3), 194-205.

Palani, S., Kumar, S.N., Gokulan, R., Rajalingam, D., Kumar, B.S., 2009, 'Evaluation of nephroprotective and antioxidant potential of Tragia involucrata'. Drug invention today, 1(1), 55-60.

Pandey, K.B. & Rizvi, S.I., 2010, 'Markers of oxidative stress in erythrocytes and plasma during aging in humans'. Oxidative medicine and cellular longevity, 3(1), 2-12.

Panyod, S., Wu, W.K., Ho, C.T., Lu, K.H., Liu, C.T., Chu, Y.L., Lai, Y.S., Chen, W.C., Lin, Y.E., Lin, S.H. & Sheen, L.Y., 2016, 'Diet supplementation with allicin protects against alcoholic fatty liver disease in mice by improving anti-inflammation and antioxidative functions'. Journal of agricultural and food chemistry, 64(38), 7104-7113.

Parikh, H., Pandita, N. & Khanna, A., 2015, 'Phytoextract of Indian mustard seeds acts by suppressing the generation of ROS against acetaminophen-induced hepatotoxicity in HepG2 cells'. Pharmaceutical biology, 53(7), 975-984.

Porter, A.G. & Jänicke, R.U., 1999, 'Emerging roles of caspase-3 in apoptosis'. Cell death & differentiation, 6(2), 99-104.

Rabiul, H., Subhasish, M., Sinha, S., Roy, M. G., Sinha, D. & Gupta, S., 2011, 'Hepatoprotective activity of Clerodendron inerme against paracetamol induced hepatic injury in rats for pharmaceutical product'. International Journal of Drug Development and Research, 3(1), 118-126.

Raoof, J.B., Baghayeri, M. & Ojani, R., 2012, 'A high sensitive voltammetric sensor for qualitative and quantitative determination of phenobarbital as an antiepileptic drug in presence of acetaminophen'. Colloids and Surfaces B: Biointerfaces, 95, 121-128.

Rašković, A., Bukumirović, N., Paut Kusturica, M., Milić, N., Čabarkapa, V., Borišev, I., Čapo, I., Miljković, D., Stilinović, N. & Mikov, M., 2019, 'Hepatoprotective and antioxidant potential of Pycnogenol® in

acetaminophen-induced hepatotoxicity in rats'. Phytotherapy Research, 33(3), 631-639.

Refaie, A.A., Ramadan, A. & Mossa, A.T., 2014, 'Oxidative damage and nephrotoxicity induced by prallethrin in rat and the protective effect of Origanum majorana essential oil'. Asian Pacific journal of tropical medicine, 7S1, S506-S513.

Reiter, J., Levina, N., Van der Linden, M., Gruhlke, M., Martin, C. & Slusarenko, A.J., 2017, 'Diallylthiosulfinate (Allicin), a volatile antimicrobial from garlic (Allium sativum), kills human lung pathogenic bacteria, including MDR strains, as a vapor'. Molecules, 22(10), 1711.

Reshi, M.S., Yadav, D., Uthra, C., Shrivastava, S. & Shukla, S., 2020, 'Acetaminophen-induced renal toxicity: preventive effect of silver nanoparticles'. Toxicology Research, 9(4), 406-412.

Reitman, S. & Frankel, S., 1957, 'A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases'. American journal of clinical pathology, 28(1), 56-63.

Saleh, D., Abdelbaset, M., Hassan, A., Sharaf, O., Mahmoud, S. & Hegazy, R., 2020, 'Omega-3 fatty acids ameliorate doxorubicin-induced cardiorenal toxicity: In-vivo regulation of oxidative stress, apoptosis and renal Nox4, and in-vitro preservation of the cytotoxic efficacy'. Plos one, 15(11), e0242175.

Saleh, D.O., Mansour, D.F. & Fayez, A.M., 2021, 'Thioacetamide-induced acute hepatic encephalopathy: central vs peripheral effect of Allicin'. Metabolic Brain Disease, 36(6), 1331-1340.

Salehi, B., Zucca, P., Orhan, I.E., Azzini, E., Adetunji, C.O., Mohammed, S.A., Banerjee, S.K., Sharopov, F., Rigano, D., Sharifi-Rad, J. & Armstrong, L., 2019, 'Allicin and health: A comprehensive review'. Trends in Food Science & Technology, 86, 502-516.

Salvemini, D., Riley, D.P. & Cuzzocrea, S., 2002, 'SOD mimetics are coming of age'. Nature Reviews Drug Discovery, 1(5), 367-374.

Samra, Y.A., Hamed, M.F. & El-Sheakh, A.R., 2020, 'Hepatoprotective effect of allicin against acetaminophen-induced liver injury: Role of inflammasome pathway, apoptosis, and liver regeneration'. Journal of biochemical and molecular toxicology, 34(5), e22470.

SanGiovanni, J.P., Chew, E.Y., Agron, E., Clemons, T.E., Ferris 3rd, F.L., Gensler, G., Lindbald, A.S., Milton, R.C., Seddon, J.M., Klein, R. & Sperduto, R.D., 2008, 'Age-Related Eye Disease Study Research Group The relationship of dietary omega-3 long-chain polyunsaturated fatty acid intake with incident age-related macular degeneration: AREDS report no. 23'. Archives of ophthalmology, 126(9), 1274-1279.

Saritas, A., Kandis, H., Baltaci, D., Yildirim, U., Kaya, H., Karakus, A., Colakoglu, S., Memisogullari, R. & Kara, I.H., 2014, 'N-Acetyl cysteine and erdosteine treatment in acetaminophen-induced liver damage'. Toxicology and Industrial health, 30(7), 670-678.

Sarvizadeh, M., Hasanpour, O., Naderi Ghale-Noie, Z., Mollazadeh, S., Rezaei, M., Pourghadamyari, H., Masoud Khooy, M., Aschner, M., Khan, H., Rezaei, N. & Shojaie, L., 2021, 'Allicin and digestive system cancers: from chemical structure to its therapeutic opportunities'. Frontiers in oncology, 563, doi: 10.3389/fonc.2021.650256.

Scorletti, E. & Byrne, C.D., 2018, 'Omega-3 fatty acids and non-alcoholic fatty liver disease: Evidence of efficacy and mechanism of action'. Molecular aspects of medicine, 64, 135-146.

Şener, G., Şatiroglu, H., Kabasakal, L., Arbak, S., Öner, S., Ercan, F. & Keyer-Uysal, M., 2000, 'The protective effect of melatonin on cisplatin nephrotoxicity'. Fundamental & clinical pharmacology, 14(6), 553-560.

Serhan, C.N. & Petasis, N.A., 2011, 'Resolvins and protectins in inflammation resolution'. Chemical reviews, 111(10), 5922-5943.

Shaaban, A.A., Shaker, M.E., Zalata, K.R., El-Kashef, H.A. & Ibrahim, T.M., 2014, 'Modulation of carbon tetrachloride-induced hepatic oxidative stress, injury and fibrosis by olmesartan and omega-3'. Chemico-biological interactions, 207, 81-91.

Shah, S.S., Shah, G.B., Singh, S.D., Gohil, P.V., Chauhan, K., Shah, K.A. & Chorawala, M., 2011, 'Effect of piperine in the regulation of obesity-induced dyslipidemia in high-fat diet rats'. Indian journal of pharmacology, 43(3), 296.

Shahidi, F. & Ambigaipalan, P., 2018, 'Omega-3 polyunsaturated fatty acids and their health benefits'. Annual review of food science and technology, 9, 45-381.

Shan, X., Aw, T.Y. & Jones, D.P., 1990, 'Glutathione-dependent projection against oxidative injury'. Pharmacology & therapeutics, 47(1), 61-71.

Shang, A., Cao, S.Y., Xu, X.Y., Gan, R.Y., Tang, G.Y., Corke, H., Mavumengwana, V. & Li, H.B., 2019, 'Bioactive compounds and biological functions of garlic (Allium sativum L.) '. Foods, 8(7), 246.

Sinaga, E., Fitrayadi, A., Asrori, A., Rahayu, S.E., Suprihatin, S. & Prasasty, V.D., 2021, 'Hepatoprotective effect of Pandanus odoratissimus seed extracts on paracetamol-induced rats'. Pharmaceutical Biology, 59(1), 31-39.

Singh, C., Prakash, C., Tiwari, K.N., Mishra, S.K. & Kumar, V., 2018, 'Premna integrifolia ameliorates cyclophosphamide-induced hepatotoxicity by modulation of oxidative stress and apoptosis'. Biomedicine & Pharmacotherapy, 107,634-643.

Sohail, N., Hira, K., Tariq, A., Sultana, V. & Ehteshamul-Haque, S., 2019, 'Marine macro-algae attenuate nephrotoxicity and hepatotoxicity induced by cisplatin and acetaminophen in rats'. Environmental Science and Pollution Research international, 26(24), 25301-25311.

Sreevallabhan, S., Mohanan, R., Jose, S.P., Sukumaran, S., Jagmag, T., Tilwani, J. & Kulkarni, A., 2021, 'Hepatoprotective effect of essential phospholipids enriched with virgin coconut oil (Phoscoliv) on paracetamol-induced liver toxicity'. Journal of Food Biochemistry, 45(2), e13606.

Suddek, G.M., 2014, 'Allicin enhances chemotherapeutic response and ameliorates tamoxifen-induced liver injury in experimental animals'. Pharmaceutical biology, 52(8), 1009-1014.

Sun, D., Sun, C., Qiu, G., Yao, L., Yu, J., Al Sberi, H., Fouda, M.S., Othman, M.S., Lokman, M.S., Kassab, R.B. & Abdel Moneim, A.E., 2021, 'Allicin mitigates hepatic injury following cyclophosphamide administration via activation of Nrf2/ARE pathways and through inhibition of inflammatory and apoptotic machinery'. Environmental Science and Pollution Research international, 28(29), 39625-39636.

Swanson, D., Block, R. & Mousa, S.A., 2012, 'Omega-3 fatty acids EPA and DHA: health benefits throughout life'. Advances in nutrition, 3(1), 1-7.

Taubert, D., Glöckner, R., Müller, D. & Schömig, E., 2006, 'The garlic ingredient diallyl sulfide inhibits cytochrome P450 2E1 dependent bioactivation of acrylamide to glycidamide'. Toxicology letters, 164(1), 1-5.

Tietz, N.W., Burtis, C.A., Duncan, P., Ervin, K., Petitclerc, C.J., Rinker, A.D., Shuey, D. & Zygowicz, E.R., 1983, 'A reference method for measurement of alkaline phosphatase activity in human serum'. Clinical chemistry, 29(5), 751-761.

Tittarelli, R., Pellegrini, M., Scarpellini, M.G., Marinelli, E., Bruti, V., Di Luca, N.M., Busardò, F.P. & Zaami, S., 2017, 'Hepatotoxicity of paracetamol and related fatalities'. European review for medical and pharmacological sciences, 21(1), 95-101.

Tripathi, S.S., Singh, S., Garg, G., Kumar, R., Verma, A.K., Singh, A.K., Bissoyi, A. & Rizvi, S.I., 2022, 'Metformin ameliorates acetaminophen-induced sub-acute toxicity via antioxidant property'. Drug and chemical toxicology, 45(1), 52-60.

Uchiyama, M. & Mihara, M., 1978, 'Determination of malonaldehyde precursor in tissues by thiobarbituric acid test'. Analytical biochemistry, 86(1), 271-278.

Uthra, C., Shrivastava, S., Jaswal, A., Sinha, N., Reshi, M.S. & Shukla, S., 2017, 'Therapeutic potential of quercetin against acrylamide induced toxicity in rats'. Biomedicine & Pharmacotherapy, 86, 705-714.

Uysal, H.B., Dağlı, B., Yılmaz, M., Kahyaoğlu, F., Gökçimen, A., Ömürlü, İ.K., Demirci, B., 2016, 'Biochemical and histological effects of thiamine pyrophosphate against acetaminophen-induced hepatotoxicity'. Basic & clinical pharmacology & toxicology, 118(1), 70-76.

Verma, P.K., Raina, R., Singh, M., Wazir, V.S. & Kumar, P., 2017, 'Attenuating potential of Calendula officinalis on biochemical and antioxidant parameters in hepatotoxic rats'. Indian journal of physiology and pharmacology, 61(4), 398-410.

Vimal, V. & Devaki, T., 2004, 'Hepatoprotective effect of allicin on tissue defense system in galactosamine/endotoxin challenged rats'. Journal of ethnopharmacology, 90(1), 151-154.

Wang, E.T., Chen, D.Y., Liu, H.Y., Yan, H.Y. & Yuan, Y., 2015, 'Protective effect of allicin against glycidamide-induced toxicity in male and female mice'. General Physiology and Biophysics, 34(2), 177-187.

Wang, F.S., Fan, J.G., Zhang, Z., Gao, B. & Wang, H.Y., 2014, 'The global burden of liver disease: the major impact of China'. Hepatology, 60(6), 2099-2108.

Wang, M., Zhang, X., Ma, L.J., Feng, R.B., Yan, C., Su, H., He, C., Kang, J.X., Liu, B. & Wan, J.B., 2017, 'Omega-3 polyunsaturated fatty acids ameliorate ethanol-induced adipose hyperlipolysis: a mechanism for hepatoprotective effect against alcoholic liver disease'. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1863(12), 3190-3201.

Wang, X., Wu, Q., Liu, A., Anadón, A., Rodríguez, J.L., Martínez-Larrañaga, M.R., Yuan, Z. & Martínez, M.A., 2017, 'Paracetamol: overdose-induced oxidative stress toxicity, metabolism, and protective effects of various compounds in vivo and in vitro'. Drug metabolism reviews, 49(4), 395-437.

Wang, Z., Hu, J.N., Yan, M.H., Xing, J.J., Liu, W.C. & Li, W., 2017, 'Caspase-mediated anti-apoptotic effect of ginsenoside Rg5, a main rare ginsenoside, on acetaminophen-induced hepatotoxicity in mice'. Journal of agricultural and food chemistry, 65(42), 9226-9236.

Wans, E.M., Ahmed, M.M., Mousa, A.A., Tahoun, E.A. & Orabi, S.H., 2021, 'Ameliorative effects of corn silk extract on acetaminophen-induced renal toxicity in rats'. Environmental Science and Pollution Research international, 28(2), 1762-1774.

Woolbright, B.L. & Jaeschke, H., 2018, 'Mechanisms of inflammatory liver injury and drug-induced hepatotoxicity'. Current pharmacology reports, 4(5), 346-357.

Wu, H., Zhang, G., Huang, L., Pang, H., Zhang, N., Chen, Y. & Wang, G., 2017, 'Hepatoprotective effect of polyphenol-enriched fraction from Folium Microcos on oxidative stress and apoptosis in acetaminophen-induced liver injury in mice'. Oxidative medicine and cellular longevity, doi: 10.1155/2017/3631565.

Xie, Q., Liu, Y., Sun, H., Liu, Y., Ding, X., Fu, D., Liu, K., Du, X. & Jia, G., 2008, 'Inhibition of acrylamide toxicity in mice by three dietary constituents'. Journal of agricultural and food chemistry, 56(15), 6054-6060.

Xu, J., Feng, Z.P., Peng, H.Y. & Fu, P., 2021, 'Omega-3 polyunsaturated fatty acids alleviate adenine-induced chronic renal failure via regulating ROS production and TGF-β/SMAD pathway'. European Review for Medical and Pharmacological Sciences, 25(22), 6825-6825.

Yang, D., Lv, Z., Zhang, H., Liu, B., Jiang, H., Tan, X., Lu, J., Baiyun, R. & Zhang, Z., 2017, 'Activation of the Nrf2 signaling pathway involving KLF9 plays a critical role in allicin resisting against arsenic trioxide-induced hepatotoxicity in rats'. Biological trace element research, 176(1), 192-200.

Yayla, M., Halici, Z., Unal, B., Bayir, Y., Akpinar, E. & Gocer, F., 2014, 'Protective effect of Et-1 receptor antagonist bosentan on paracetamol induced acute liver toxicity in rats'. European journal of pharmacology, 726, 87-95.

Yousef, M.I., Omar, S.A., El-Guendi, M.I. & Abdelmegid, L.A., 2010, 'Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, impaired liver and kidney functions and haematotoxicity in rat'. Food and Chemical Toxicology, 48(11), 3246-3261.

Yuan, L. & Kaplowitz, N., 2013, 'Mechanisms of drug-induced liver injury'. Clinics in liver disease, 17(4), 507-518.

Zhang, L., Zhang, H., Miao, Y., Wu, S., Ye, H. & Yuan, Y., 2012, 'Protective effect of allicin against acrylamide-induced hepatocyte damage in vitro and in vivo'. Food and chemical toxicology, 50(9), 3306-3312.

Zhang, Y., Zhang, F., Wang, K., Liu, G., Yang, M., Luan, Y. & Zhao, Z., 2016, 'Protective effect of allyl methyl disulfide on acetaminophen-induced hepatotoxicity in mice'. Chemico-Biological Interactions, 249, 71-77.

Zoubair, B.E.N.K.H.A.S.S.I., Azzahra, L.F., Fouzia, H.M.I.M.I.D., Mohammed, L.O.U.T.F.I., Brahim, B.E.N.A.J.I. & Noureddine, B.O.U.R.H.I.M., 2013, 'Evaluation of acetaminophen effect on oxidative stressed mice by peroxide hydrogen'. American Journal of Biomedical Research, 1(4), 75-79.

Appendix I

CURRICULUM VITA

- **Name: Moamen Hussein Elsayed Elsafty**
- The researcher was born on 1 January, 1996 in the village of El-Saadieen, Sharkia governorate, Egypt.
- He got his primary education at El-Saadieen primary school at which he was graduated in 2008.
- He graduated from preparatory education in 2011.
- He joined to Abdel-Hai Mashhour secondary school to be graduated in 2014.
- His B.V.Sc. degree was completed in faculty of Veterinary Medicine, Benha University in 2019.
- The researcher works as a demonstrator in the department of Pharmacology - faculty of Veterinary Medicine - Benha University since March 2020.
- The researcher registered for master degree in (Pharmacology) in September 2020.

Appendix II

Buffers and reagents

- **Chemicals**

Paracetamol (APAP, 1 g) was bought as Panadol® from GlaxoSmithKline Pharmaceuticals Company (Brentford, United Kingdom). Allicin, was bought as pure powder (35% Conc.) from Delta Vet Center (Cairo, Egypt). Omega-3 fatty acids, was bought as pure fish oil (Conc.100%) from Sigma Pharmaceutical Industries (Cairo, Egypt). The used kits were bought from Bio-diagnostic Company (Giza, Egypt).

- **Experimental animals**

Seventy male albino Wister rats, 2 months age weighing 160-200 gm were obtained from the Center of Laboratory Animals, Faculty of Veterinary Medicine, Benha University, Egypt. Prior to the experiment, the rats were left for acclimatization for 14 days (temperature 25°C) and were fed ideal laboratory commercial diet and water ad libitum. Ethical approval from Animals Care and Use Committee Research Ethics Board was obtained from Faculty of Veterinary Medicine, Benha University (BUFVTM 07–03-22). Rats were divided into 7 equal groups (10 rats in each group). Group 1 (Control); had been given distilled water. Group (2); AC (10 mg/kg b. wt, orally). Group (3); OMG-3FA (100 mg/kg b. wt, orally). These doses of AC according to Samra et al. (2021) and for OMG-3FA (El-Gendy et al. 2021). Group (4); APAP toxic control group that received saline, orally once daily and a single dose of APAP 1 g/kg b. wt orally on the 27th day of the experiment. Group (5); (AC+APAP). Group (6); (OMG-3FA+APAP). Group (7); (AC+OMG-3FA+APAP). rats in these groups have been received allicin, omega-3 and APAP as described before. Saline, allicin, and omega-3 were administered for 30 days.

- **Blood samples**

Rats were euthanized at 31st day of the experiment, blood samples were collected from Retro-bulbar venous plexus, in clean, dry tubes and it was left in a slope position to clot at room temperature. After blood centrifugation at 2000 g for 10 min, serum samples were obtained and it was kept frozen at-20°C until further use in biochemical analysis.

- **Hematological analysis**

The whole blood samples were used directly after collection on EDTA for estimation of hematological parameters including the red blood cells (RBCs) count, hemoglobin (Hb) concentration, white blood cells (WBCs) count, hematocrit value (APAPV%) and platelets (Plt) count. These parameters were estimated using automated hematology analyzer (Mindray BC-2800, China).

- **Serum biochemical analysis:**

Sera were used for estimation of the liver markers (ALT, AST, ALP) and renal injury biomarkers creatinine, urea & Cholesterol, triglycerides, albumin and total protein concentrations using commercial kits (Biodiagnostic Co., Giza, Egypt.).

- **Detection of oxidative cascade indices:**

The tissues (liver, kidney) were dissected and washed with phosphate-buffered saline solution (pH 7.4) containing heparin to remove any clots or red blood cells. One gram of each tissue was homogenized in buffer (5 ml), using a homogenizer. Tissue homogenates were centrifuged at 4000 rpm for 20 min, then, stored at -20 ºC. The oxidative status was determined by measuring the levels of glutathione (GSH), malondialdehyde (MDA), catalase (CAT) and superoxide dismutase (SOD) using commercial kits (Biodiagnostic Co., Egypt).

• Reagents used for oxidative stress assessment in tissues:

- Phosphate buffered saline (PBS) solution, pH 7.4 containing 0.16 mg/ml heparin.
- Cold buffer (100 ml potassium phosphate, pH 7.0, containing 2 ml EDTA per gram tissue).

• Chemicals used for histopathological examination:

- Formalin (10 %): from Middle East Company, Cairo, Egypt.
- Hematoxylin and Eosin (H&E) stain: from Middle East Company, Cairo, Egypt.

• Chemicals used for immunohistochemistry Examination:

- Formalin (10 %): from Middle East Company, Cairo, Egypt.
- Hematoxylin and Eosin (H&E) stain: from Middle East Company, Cairo, Egypt.
- Caspase 3 marker.
- HSP70 marker.

• **Other standard laboratory chemicals and solutions were also used as:** 70% hydroethanolic alcohol, distilled water, normal saline solution (sodium chloride 0.9 %) … etc.

Laboratory equipment:

• **Apparatus for serum Biochemical studies:**

- Spectrophotometer, JASCO 7800, un/vis, JAPAN.
- Clean and dry Eppendorf labeled tubes for serum preservation.

• **Apparatus for Oxidative cascade:**

- Sonicator homogenizer (COLOMBIA INTERNATIONAL)
- Clean and dry Eppendorf labeled tubes for liver, kidney and testis tissues preservation.
- Cooling centrifuge heraeus, W. GERMANY.
- Refrigerator for preservation of samples.

• **Apparatus for histological examination:**

- Slide microtome.
- Light microscope: NOVEL, model XSZ-N107-1.

Appendix III

1- Paper 1

Title: Protective effect of allicin and omega-3 fatty acids against paracetamol induced hepatic toxicity.

Authors: Moamem Elsafty, Mohamed Aboubakr, Ahmed Abdeen.

Journal name: Benha Veterinary Medical Journal

Year: 2022

Volume and pages: Volume 43, xx-xx.

2- Paper 2

Title: Protective effect of allicin and omega-3 fatty acids against paracetamol induced renal toxicity.

Authors: Moamem Elsafty, Mohamed Aboubakr, Ahmed Abdeen.

Journal name: under puplicaton.

الملخص العربى

استهدفت هذه الدراسة معرفة التأثيرات الوقائية لإعطاء الأليسين والاوميجا-3 على سمية الكبد والكلى التي يسببها عقار الباراسيتامول في الجرذان. وذلك من خلال الفحص الدموى وفحص بعض العوامل البيوكيميائية في الدم وعوامل الأكسدة / المضادة للأكسدة في الأنسجة. أيضًا، كل من التغيرات الهستولوجيه والتغيرات الهستوكيميائية المناعية (Caspase3 and HSP70).

في هذه الدراسه تم اجراء التجربه على سبعين ذكرا من الجرذان البيضاء من نوع الألبينو تتراوح اوزانها ما بين (200-160 جم). وقد تم تقسيم الجرذان الي سبع مجموعات متساوية (10 جرذان لكل مجموعة).

- **المجموعة الاولي (الضابطه):** تم إعطائها محلول ملحي عن طريق الفم مرة واحدة يوميًا لمدة 30 يومًا متتاليًا.
- **المجموعة الثانيه**: تم إعطائها (10 مجم للأليسين / كجم من وزن الجسم) عن طريق الفم مرة واحدة يوميًا لمدة 30 يومًا متتاليًا.
- **المجموعة الثالثه**: تم إعطائها(100مجم للأوميجا-3 / كجم من وزن الجسم) عن طريق الفم مرة واحدة يوميًا لمدة 30 يومًا متتاليًا.
- **المجموعة الرابعه**: لملاحظه التسمم للباراسيتامول وتم اعطاءها محلول ملحي عن طريق الفم مرة واحدة يوميًا وجرعة واحدة من الباراسيتامول في اليوم السابع والعشرين من التجربة (1جم / كجم، عن طريق الفم).
- **المجموعة الخامسه**: تم اعطائها كل من (10مجم للأليسين / كجم من وزن الجسم)، عن طريق الفم مرة واحدة يوميًا لمدة 30 يومًا متتاليًا وجرعة واحدة من في اليوم السابع والعشرين من التجربة (1 جم / كجم، عن طريق الفم).
- **المجموعة السادسه**: تم اعطائها كل من(100مجم للأوميجا-3 / كجم من وزن الجسم)، عن طريق الفم مرة واحدة يوميًا لمدة 30 يومًا متتاليًا وجرعة واحدة من الباراسيتامول في اليوم السابع والعشرين من التجربة (1 جم / كجم، عن طريق الفم).
- **المجموعة السابعه**: تم اعطائها كل من(الأليسين و الأوميجا-3)، عن طريق الفم مرة واحدة يوميًا لمدة 30 يومًا متتاليًا وجرعة واحدة من الباراسيتامول في اليوم السابع والعشرين من التجربة (1 جم / كجم، عن طريق الفم).

في هذه الدراسة؛ أظهر الفحص الدموي أن التعرض للباراسيتامول قلل بشكل كبير من قيم تعداد كرات الدم الحمراء ، وتركيزات الهيموغلوبين ، وعدد الصفائح الدموية(PL) ، ونسب حجم الدم (PCV) ، مع زيادة قيم تعداد كرات الدم البيضاء عند مقارنتها بمجموعة التحكم. ولكن إعطاء الأليسين و / أو أوميغا 3 قلل من الآثار الضارة للباراسيتامول عن طريق عكس هذه التغييرات في قيم الدم إلى القيم التي لوحظت في الجرذان الضابطة.

أيضا؛ أدى إعطاء الباراسيتامول في هذه الدراسة إلى زيادة كبيرة في أنشطة انزيم الالنين امينو ترانسفيريز واسبرتات امينو ترانسفيريز وانزيم الفوسفاتيز القلوي في الدم مقارنة بتلك الموجودة في الجرذان الضابطة. وبالمثل، زاد الباراسيتامول بشكل ملحوظ كل من مستويات الكرياتينين واليوريا في الدم . أيضًا، أدي إعطاء الباراسيتامول الي زياده مستوي الدهون الثلاثية و نسبه الكوليستيرول الكلي في الدم بالمقارنة مع المجموعات الأخرى. على العكس من ذلك، انخفض البروتين الكلي والألبومين في الدم بسبب إعطاء الباراسيتامول مقارنة بالفئران الضابطة. أدى اعطاء عقار الأليسين مع الباراسيتامول والأوميجا-3 مع الباراسيتامول و الأليسين مع والأوميجا-3 مع الباراسيتامول أدي الي عودة هذه المعدلات نحو قيمها العادية.

و كانت هناك زيادات كبيرة في مستوى المالونالدهيد(MDA) إلى جانب انخفاض كبير في جلوتاثيون والكتاليز والسوبر اوكسيديز (GSH وSOD وCAT) في خلايا الكبد والكلى للفئران التي تعاني من تسمم الباراسيتامول وفي الوقت نفسه ، المجموعة التي تم اعطاؤها عقار الأليسين مع الباراسيتامول والأوميجا-3 مع الباراسيتامول و الأليسين مع الأوميجا-3 مع الباراسيتامول ظهر فيها انخفاض في مستوى المالونالدهيد (MDA) جنبًا إلى جنب مع ارتفاع في جلوتاثيون والكتاليز(GSH وSOD وCAT) في خلايا الكبد والكلى مقارنة بالمجموعة المعالجة بـالباراسيتامول.

اما بالنسبه للتغيرات الهستولوجيه للكبد من الفئران المعالجة ب والأوميجا-3 فقد اظهرت خلايا كبد طبيعية. تنتظم خلايا الكبد في حبال تشع من الوريد المركزي وتفصل بينها جيوب دموية طبيعية، وبخلاف ذلك أظهرت الفئران المعالجة بـالباراسيتامول عدة تغيرات نسيجية تتمثل في تمدد الجيوب الأنفية ، واحتقان الأوعية الدموية ، والبنية غير المنتظمة للفصيص الكبدي ، والتسلل الدهني في بعض خلايا الكبد و اتساع الوريد المركزي.

اما بالنسبه للمجموعه المعالجه بـ الأليسين + الباراسيتامول والمجموعه المعالجه ب الأوميجا-3 + الباراسيتامول أظهرت تنكسًا معتدلا و المجموعه المعالجه بالأليسين+ الأوميجا-3 +الباراسيتامول أظهرت خلايا الكبد والجيوب بصوره طبيعية تقريبًا.

أما بالنسبه للتغيرات الهستولوجية في الكلى في كل من المجموعتين الضابطة والمجموعه المعالجه ب الأليسين والأوميجا-3، اظهرت خلايا كلوية طبيعيه ،مع كبيبة كلوية ، قنوات قريبة ، قنوات بعيدة ، و قنوات جامعة طبيعية.

في المجموعة المعالجه ب بالباراسيتامول ، لوحظت العديد من التغيرات الهستولوجيه المميزة متضمنة تغيرات مفرطة مثل التغيرات التنكسية الشديدة في الأنابيب الكلوية التي تحدث بشكل خاص عن طريق التنكس المائي ، والنواة pycnotic ، وزيادة الحويصلات السيتوبلازمية ، والتفريغ السيتوبلازمي ، والنخر وموت الخلايا المبرمج للخلايا الأنبوبية ، وتقشر الخلايا الظهارية النخرية التي تملأ التجويف الأنبوبي وتشكل القوالب وكذلك احتقان الأوعية الدموية والشعيرات الدموية حول الأنبوب.

اما بالنسبه للمجموعات المعالجه بـالأليسين + الباراسيتامول والمجموعه المعالجه ب الأوميجا-3 + الباراسيتامول و المجموعه المعالجه بـالأليسين+ الأوميجا-3 +الباراسيتامول لوحظ تنكس معتدل في الانابيب مع الحد الأدنى من الاحتقان الخلالي.

من الناحية الهستوكيميائية المناعية، تم تسجيل تفاعل (caspase3 and HSP70) المناعية في الكبد والكلى لجميع المجموعات التي تم فحصها.

في الكبد ، أظهر كل من الفئران الضابطة و المعالجة بـالأليسين والأوميجا-3 فقد كانت سلبيه التعبير مع caspase3. وفي الوقت نفسه، أظهرت الفئران المعطاه باراسيتامول زيادة في التعبير ل caspase3. ولكن، أظهرت المجموعة المعالجة بـالأليسين مع الباراسيتامول والأوميجا-3 مع الباراسيتامول تعبيرًا معتدلًا مع caspase3.ولكن، أظهرت مجموعة المعالجة بـالأليسين مع الأوميجا-3 مع الباراسيتامول تعبيرا ضعيفا مع caspase3.

أيضا في الكبد بالنسبة إلي HSP70 ، أظهر كل من الفئران الضابطة و المعالجة بـالأليسين والأوميجا-3 فقد كانت سلبيه التعبير مع HSP70. وفي الوقت نفسه، أظهرت الفئران المعطاه باراسيتامول زيادة في التعبير بشكل ملحوظ ل HSP70. ولكن، المجموعة المعالجة بـالأليسين مع الباراسيتامول والأوميجا-3 مع الباراسيتامول لم تختلف بشكل كبير بين المجموعتين في الاستجابة معHSP70 .ومع ذلك، أظهرت مجموعة المعالجة بـالأليسين مع الأوميجا-3 مع الباراسيتامول تعبيرا ضعيفا مع HSP70.

في الكلى ، أظهر كل من الفئران الضابطة و المعالجة بـالأليسين والأوميجا-3 فقد كانت سلبيه التعبير مع caspase3. وفي الوقت نفسه، أظهرت الفئران المعطاه باراسيتامول زيادة في التعبير ل caspase3. ولكن، أظهرت المجموعة المعالجة بـالأليسين مع الباراسيتامول والأوميجا-3 مع الباراسيتامول تعبيرًا معتدلًا مع caspase3.ولكن، أظهرت مجموعة المعالجة بـالأليسين مع الأوميجا-3 مع الباراسيتامول تعبيرا ضعيفا مع caspase3.

أيضا في الكلى بالنسبة إلي HSP70 ، أظهر كل من الفئران الضابطة و المعالجة بـالأليسين والأوميجا-3 فقد كانت سلبيه التعبير مع HSP70. وفي الوقت نفسه، أظهرت الفئران المعطاه باراسيتامول زيادة في التعبير بشكل ملحوظ ل HSP70. ولكن، أظهرت المجموعة المعالجة بـالأليسين مع الباراسيتامول والأوميجا-3 مع الباراسيتامول تعبيرًا معتدلًا معHSP70 .ولكن، أظهرت مجموعة المعالجة بـالأليسين مع الأوميجا-3 مع الباراسيتامول تعبيرا ضعيفا مع HSP70.

من هذه الدراسة، يمكن استنتاج أن اعطاء الأليسين مع / أو الاوميجا-3 ل الذين يتناولون جرعات عالية بانتظام من الباراسيتامول يقلل من سمية الكبد والكليتين التي يسببها عقار الباراسيتامول. ويمكن ان يكون هذا بسبب خصائص الاليسين والأوميجا-3 المضادة للأكسدة والمضادة للالتهابات.

جامعة بنها
كلية الطب البيطري
قسم الفارماكولوجيا

قرار لجنة الحكم والمناقشه

قررت لجنة الحكم والمناقشة بجلستها في يوم الثلاثاء الموافق ٢٠٢٢/١٠/٢٥م منح السيد **ط.ب/ مؤمن حسين السيد الصفتي** درجة الماجستير في الطب البيطري "مادة الفارماكولوجيا" بعنوان (الأليسين و اوميجا٣ كعلاج مفضل ضد التسمم الكبدي-الكلوي المُحدَث بالباراسيتامول).

اعضاء اللجنة :

١- أ.د/ أشرف عبد الحكيم أحمد الكومي (رئيساً ومحكماً)
أستاذ الفارماكولوجيا المتفرغ- كلية الطب البيطرى- جامعة بنها.

٢ - أ.د/ وليد فتحي خليل محمود (عضواً ومحكماً)
أستاذ ورئيس قسم الأدويه - كلية الطب البيطري- جامعة قناة السويس.

٣- أ.د/ محمد حافظ محمد أبوبكر (عضواً ومشرفاً)
أستاذ الفارماكولوجيا- كلية الطب البيطري- جامعة بنها.

٤- أ.م.د/ أحمد عبدالحافظ عابدين (عضواً ومشرفاً)
أستاذ مساعد الطب الشرعي والسموم - كلية الطب البيطري- جامعة بنها.

جامعة بنها
كلية الطب البيطري
قسم الفارماكولوجيا

الأليسين واوميجا 3 كعلاج مفضل ضد التسمم الكبدي-الكلوي المُحدَث بالباراسيتامول

رسالة مقدمة إلي

كلية الطب البيطري بمشتمر – جامعة بنها

للحصول علي درجة الماجستير

في الطب البيطري (تخصص الفارماكولوجيا)

من

ط.ب / مؤمن حسين السيد الصفتي

بكالوريوس العلوم الطبية البيطرية – كليه الطب البيطري – جامعه بنها (2019)

تحت إشراف

الأستاذ الدكتور

محمد حافظ محمد أبوبكر

أستاذ الفارماكولوجيا

كلية الطب البيطري

جامعة بنها

الدكتور

أحمد عبدالحافظ عابدين

أستاذ مساعد الطب الشرعي والسموم

كلية الطب البيطري

جامعة بنها

2022