



Official Journal Issued by
Faculty of
Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: <https://bvmj.journals.ekb.eg/>



Since 1990

Original Paper

Cytoprotective, anti-apoptotic and anti-inflammatory effects of lycopene against mercuric chloride-induced hepatorenal injury in rats: Involvement of TNF- α / NF- κ B/ and p53 signaling pathways

Samy A. Hussein¹, Mohamed Azab², Tahia E. Ahmed³, Aziza Amin⁴, Mona M. M. Abu Eldahab⁵

¹ Department of Biochemistry, Faculty of Veterinary Medicine, Benha University, Egypt

² Department of Physiology, Faculty of Veterinary Medicine, Benha University, Egypt

³ Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Benha University, Egypt

⁴ Department of Pathology, Faculty of Veterinary Medicine, Benha University, Egypt

⁵ Veterinarian

ARTICLE INFO

Keywords

Apoptosis
Histopathology.
Inflammatory mediators
Lycopene
Mercuric chloride

ABSTRACT

The purpose of this study was to evaluate the protective and anti-inflammatory effect of lycopene against mercuric chloride (HgCl₂)-induced hepatorenal injury and oxidative stress in rats. Thirty male albino rats were divided into three main equal groups. Group I (control): rats administered distilled water. Group II (mercuric chloride exposed group): rats received 1/20th of LD50 of mercuric chloride orally (2 mg/kg b. wt./day) over a period of 4 weeks. Group III (HgCl₂+lycopene treated group): rats received mercuric chloride (2 mg/kg b. wt.) and treated with lycopene at a dose of (20 mg/kg b. wt./orally) for 4 weeks. Obtained results showed significant increase in serum ALT, AST and ALP activities, urea and creatinine concentrations and liver tissue MDA level in HgCl₂ exposed rats. However, a significant decrease in liver tissue GSH concentration with down-regulation in hemoxygenase 1 (HO-1) gene expression and the anti-apoptotic protein Bcl-2 gene in kidney tissue were observed in HgCl₂ intoxicated group. Moreover, the qPCR results of kidney tissue revealed a significant up-regulation of mRNA gene expressions levels of TNF- α , NF- κ B, Bax and p53 in HgCl₂ exposed rats when compared with control group. Administration of lycopene with HgCl₂ exposed rats caused significant improvement of all previous parameters towards its normal ranges. Various histopathological alterations were detected in kidneys and liver of rats treated with HgCl₂. Interestingly, rats treated with lycopene plus HgCl₂ showed marked reduction in these pathological alterations in comparison to HgCl₂ intoxicated rats. These results suggested that the potential ameliorating role of lycopene as potent cytoprotective, anti-inflammatory and anti-apoptotic against HgCl₂ induced hepatorenal damage

1. INTRODUCTION

Heavy metals such as mercury, lead and arsenic are elements that promote the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) involved in the etiology of oxidative stress. They cause oxidative damage to membrane lipids, proteins and DNA, thereby activating pathways of apoptosis and tissue degeneration (Salazar-Flores *et al.*, 2019). Mercury is a dangerous industrial and environmental pollutant which induces severe damage in diverse organs in animal and humans (Caglayan *et al.*, 2019).

Mercury (Hg) toxicity continues to represent a global health concern. Given that human populations are mostly exposed to low chronic levels of mercurial compounds (methyl mercury through fish, mercury vapor from dental amalgams and ethyl mercury from vaccines).

Mercury can cause biochemical destruction to tissues and genes through various mechanisms, such as intervention intracellular calcium homeostasis, disrupting membrane

potential, altering protein synthesis. The health consequences of human exposure to Hg can be severe (Branco *et al.*, 2017; Sundseth *et al.*, 2017) and include renal injury (Miller *et al.*, 2013), neuronal disorders and is well-known for its hematotoxic, hepatotoxic, neurotoxic, nephrotoxic, and genotoxic effects (Goudarzi *et al.*, 2017). In this respect, Hg is considered a potential contributing factor to Alzheimer's and Parkinson's diseases (Chin-Chan *et al.*, 2015). Exposure to Hg is highly hazardous, especially in regions with high levels of pollution in air, water, and food. In addition, Hg exerts negative impacts on the reproductive system of male rats (Celikoglu *et al.*, 2015). Mercury exists naturally as elemental mercury, inorganic and organic mercury compounds. These 3 types of Hg are known to have different toxicity and health effects (Genchi *et al.*, 2017). Mercury is primarily accumulated and expresses toxicity to the kidney. Environmental and industrial pollutant which induces the severe changes in the tissues of the body in both humans and the animals and cause nephrotoxic effects (Bridges and Zalups, 2017) and has

* Corresponding author: Prof. Samy A. Hussein, Department of Biochemistry, Faculty of Veterinary Medicine, Benha University, Egypt

variety of industrial applications. The liver and kidneys are the primary organs involved in the elimination of Hg and are sensitive to its harmful effects (Mesquita et al., 2016). Natural products, in general, are known to exhibit better safety profile in toxicity studies and are non-mutagenic and non-genotoxic in nature. Lycopene is a carotenoid with known antioxidant and anti-inflammatory properties (Campos et al., 2017). Lycopene is a major pigment found in red fruits, such as tomatoes, which are the largest contributor to the human dietary intake of lycopene (Sokoloski et al., 2015). It is well known that lycopene is a potent antioxidant. It is almost 100 times more efficient in quenching singlet oxygen than vitamin E. The asymmetric carbon skeleton and unsaturated bonds grants lycopene with antioxidant capacity (Sadek et al., 2016). Also, Krishnamoorthy et al. (2013) reported that Lycopene is presumed to protect cells from oxidative damage by stabilization of the membrane and/or scavenging free radicals generated within the tissue. This study was designed to investigate the possible beneficial action of natural antioxidant (lycopene) against deleterious effect of mercuric chloride intoxication in adult male rats through investigation of hepatorenal functions, oxidative stress and antioxidant biomarkers, Pro-inflammatory status and apoptotic markers gene expression in kidney tissues.

2. MATERIAL AND METHODS

2.1. Experimental animals:

Thirty white male albino rats of 6-8 weeks old and weighing 160-200 g were used in this study. Rats were housed in separated metal cages and kept at constant environmental and nutritional conditions throughout the period of experiment. The rats were fed on constant ration and fresh, clean drinking water was supplied *ad-libitum*. All rats were acclimatized for minimum period of two weeks prior to the beginning of study.

2.2. Chemicals and antioxidant agent:

All chemicals were of analytical grade and obtained from standard commercial suppliers. The antioxidant and chemicals used in the present study were:

2.2.1. Inorganic mercuric chloride: mercuric chloride has molecular weight 200.9 g. mol and Atomic number 80. It obtained by Sigma Aldrich as solid material with high purity 99%.

2.2.2. Lycopene: Lycopene was purchased from Aktin Chemicals, Inc. company (Nature connecting health), Chengdu, China. Lycopene (100 mg) was mixed in 2 ml Tween-80 at room temperature until a homogeneous paste was obtained. Physiologic saline at room temperature was added, drop wise and with vigorous stirring, to a final concentration of 20 mg lycopene/ml of suspension (Matos et al., 2000).

2.3. Experimental design:

Rats were randomly divided into three groups (10 rats each) placed in individual cages and classified as follow:

Group I (control normal): Rats received normal saline, served as control non- treated for all experimental groups.

Group II (mercuric chloride): Rats received mercuric chloride 1/20 of LD50 Mercuric chloride was dissolved in

distilled water, freshly prepared, and administered orally and daily at a dose level of 2 mg/kg. body weight orally and once per day over a period of 4 weeks (Kumar et al., 2018).

Group III (mercuric Chloride + lycopene): Rats received mercuric chloride (2 mg/kg. body weight) and treated daily with lycopene (20 mg/kg body weight, orally) (Daniel et al., 2015) over a period of 4 weeks.

2.4. Sampling:

Random blood samples, liver and kidney tissue specimens were collected from all animal groups once at 4 weeks from the onset of rats exposed to mercuric chloride and treated with lycopene.

2.4.1. Blood samples

Twenty-four hours after the last dose of the lycopene treatment and mercuric chloride administration, blood samples were collected by ocular vein puncture in dry, clean tubes and allowed to clot for 30 minutes and serum was separated by centrifugation at 3000 rpm for 15 minute. Serum was taken by automatic pipettes and received in dry sterile tubes, then kept in deep freeze at -20 °C until use for subsequent biochemical analysis. All sera were analyzed for determination of the following parameters: ALT, AST, ALP, urea and creatinine.

2.4.2. Tissue samples:

2.4.2.1. Liver tissue for biochemical analysis

Briefly, 0.5 g of liver tissues were cut, weighed, and minced into small pieces, homogenized with a glass homogenizer in 9 volume of ice-cold 0.05 mM potassium phosphate buffer (pH 7.4) to make 10 % homogenates. The homogenates were centrifuged at 6000 rpm for 15 minutes at 4 °C then the resultant supernatant was used for the determination of L-malondialdehyde (L-MDA) and reduced glutathione (GSH).

2.4.2.2. Liver and kidney tissues for molecular analysis:

About 0.5 g of liver and kidney tissues were collected, put in Eppendorf tubes and immediately kept in liquid nitrogen and stored at -80°C till RNA extraction. The molecular analysis of the relative gene expression in liver tissues was hemoxygenase-1(HO-1). However, in kidney tissues were Tumor necrosis factor-alpha (TNF- α), Nuclear factor kappa B (NF- κ B), Bax; tumor suppressor protein (P53) and anti-apoptotic protein (Bcl-2).

2.4.2.3. Liver and kidney specimens for histopathological examination:

Liver and kidney tissue specimens were taken from different groups. The specimens were preserved in 10% neutral buffered formalin for histopathological examination. The fixed tissues were processed routinely, embedded in paraffin, sectioned, de-paraffin zed and rehydrated according to the technique described by Bancroft and Gamble (2008). Tissue sections were stained with hematoxylin and eosin (H and E) using standard technique (Bancroft and Gamble, 2008) to evaluate the extent of the histopathological changes. The severity of the microscopical lesions mainly in the liver were classified according to Bernet et al. (1999) as following: degree 1, mild pathological changes such as circulatory disturbances with mild degenerative changes, (degree 2); moderate pathological alterations including degenerative changes, in association with cellular deposits with some inflammatory cell

infiltrations; (degree 3); severe alterations such as necrotic areas with leukocytic cellular aggregation.

2.5. Analysis:

2.5.1. Biochemical analysis

Serum, ALT, AST, ALP, urea and creatinine were determined according to the method described by the method described by Schumann et al., (2002), EL-Aaser and EL-Merzabani, (1975), Young, (1997) and Dumas *et al.*, (1971) and Engall, (1980), respectively. Also, liver tissue L-MDA and GSH concentration were determined according to the methods described by Mesbah *et al.* (2004) and Patterson and Lazarow (1955), respectively.

2.5.2. Molecular analysis:

Total RNA was isolated from (liver and kidney) tissue of rats using RNeasy Mini Kit (Thermo Qiagen, #74104) according to the manufacturer's protocol. Following determination of RNA concentration and purity by Quawell nanodrop Q5000 (USA), 5 mg of total RNA from each sample was reverse transcribed using Quantiscript reverse transcriptase. The produced cDNA was used as a template to determine the relative expression of (Heme oxygenase-1 (HO-1), Tumor necrosis factor alpha (TNF- α), NF-kB, Bax, p53 and the anti-apoptotic protein Bcl-2 genes (table 1).

Gene	Forward primer (5'-----3')	Reverse primer (5'-----3')
HO-1	GGAAAGCAGTCATGGTCAGTCA	CCCTTCCTGTGCTTCCTTTGT
TNF- α	GCATGATCCCGACGTGGA	AGATCCATGCCGTTGGCCAG
NF-k β	CCTAGCTTTCTGAACTGCAAA	GGTTCAGAGGCCAATAGAGA
Bax	ACACCTGAGCTGACCTTG	AGCCCATGATGGTCTGTGATC
p53	ATGGCTTCCACCTGGGCTTC	TGACCCACAACGTGACAGGGC
Bcl-2	ATCGCTCTGTGGATGACTGAGTAC	AGAGACAGCCAGGAGAAATCAAAC
β -actin	AAGTCCCTCACCTCCAAAAG	AAGCAATGTCTGCACCTTCCC

2.6. Statistical analysis:

All the data were expressed as means \pm SE. The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS, 18.0 software, 2011 and the individual comparisons were obtained by Duncan's multiple range test. Values were considered statistically significant when $p < 0.05$.

3. RESULTS

The obtained results demonstrated in table (2) revealed that serum ALT, AST and ALP activities, urea and creatinine concentrations were significantly elevated in mercuric chloride intoxicated rats when compared with the control normal group.

However, lycopene treatment to mercuric chloride - exposed rats resulting in a remarkable protection regarding the same parameters with the ability to restore the value of serum ALT, AST and ALP activities, urea and creatinine concentrations nearly to the average level of control group when compared with mercuric chloride exposed group.

The obtained data established in table (3) revealed that, a significant increase in L-MDA and marked decrease in GSH level with significant down-regulation of Heme oxygenase-1 (HO-1) gene expression level were observed in liver tissue of mercuric chloride intoxicated rats after four weeks of experiment when compared with control group. Meanwhile, after lycopene treatment to mercuric chloride intoxicated rats a significant decrease in L-MDA with marked increase in GSH level and significant up-regulation of HO-1 gene expression level were observed in liver tissue when compared with mercuric chloride exposed group.

The obtained results existing in table (4) revealed that significant up-regulation in kidney tissue TNF- α , NF-k β and Bax gene expression levels in mercuric chloride intoxicated rats after four weeks of experiment when compared with normal control group. However, this expression was significantly down-regulated following treatment by lycopene when compared with mercuric chloride exposed group.

The current results presented in table (5) exhibited a significant up-regulation in kidney tissue p53 gene and down-regulation in Bcl-2 gene expression levels in mercuric chloride intoxicated rats when compared with normal control group. Meanwhile, lycopene treatment to mercuric chloride intoxicated rats caused significant down-regulation in kidney tissue p53 and up-regulation in Bcl-2 gene expression levels when compared with mercuric chloride exposed group.

Histopathological examination:

Liver:

The microscopical examination of the hepatic tissues obtained from rats treated with mercuric chloride Revealed various histopathological alterations in the hepatic parenchyma of the examined rats. There was marked congestion and dilatation of central and portal veins and blood sinusoids with perivascular edema admixed with erythrocytes as well as diffused areas of hemorrhage in the hepatic parenchyma (Fig. 1a). The portal areas were expanded by extensive aggregates of mononuclear inflammatory cells mainly lymphocytes and macrophages in association with mild to moderate hyperplastic proliferation of the lining epithelium of bile duct with formation of newly formed bile ductless (Fig. 1b). There were focal aggregates of lymphocytes in the hepatic parenchyma (Fig. 1c).

Table 2 Protective effect of lycopene (20 mg/kg b. wt.) administration on serum liver marker enzymes activities and kidney function tests in mercuric chloride (2 mg/kg b. wt.) intoxicated rats.

Animal groups	ALT (U/L)	AST (U/L)	ALP (U/L)	Urea (mg/dl)	Creatinine (mg/dl)
Group I: Normal control	40 \pm 0.82 ^b	45 \pm 0.91 ^b	97 \pm 2.04 ^b	29 \pm 0.91 ^c	0.68 \pm 0.08 ^b
Group II : Mercuric chloride	148.25 \pm 5.07 ^a	143.50 \pm 3.27 ^a	180.75 \pm 3.25 ^a	83 \pm 2.38 ^a	1.88 \pm 0.08 ^a
Group III: Mercuric chloride + Lycopene	94.2 \pm 2.78 ^c	113.75 \pm 4.27 ^c	139.50 \pm 2.90 ^c	49.25 \pm 1.25 ^b	1.05 \pm 0.06 ^c

Data are presented as (Mean \pm S.E). Mean values with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

Table 3 Protective effect of lycopene administration (20 mg/kg b. wt.) on liver L-MDA and GSH concentrations and Hemoxygenase-1 (HO-1) gene expression level in mercuric chloride (2mg/kg b. wt.) intoxicated rats

Animal groups	L-MDA (mmol/g. tissue)	GSH (ng/g. tissue)	Heme oxygenase-1(HO-1) (Fold change mean \pm SEM)
Group I: Normal control	5.24 \pm 0.89 ^b	372.46 \pm 11.25 ^a	1.00 \pm 0.06 ^a
Group II : Mercuric chloride	19.13 \pm 1.31 ^a	135.50 \pm 7.80 ^b	0.07 \pm 0.005 ^b
Group III: Mercuric chloride + Lycopene	10.28 \pm 0.54 ^c	259.11 \pm 9.51 ^c	0.42 \pm 0.04 ^c

Data are presented as (Mean \pm SEM). Mean values with different superscript letters in the same column are significantly different at (P \leq 0.05).

Table 4 Protective effect of lycopene administration (20 mg/kg b. wt.) on kidney tissue TNF- α , NF-k β and Bax gene expression levels in mercuric chloride (2 mg/kg b. wt.) intoxicated rats

Animal groups	Tumor necrosis factor alpha (TNF- α)		Nuclear factor kappa B(NF-k β)		Bax	
	Fold change mean	SEM	Fold change mean	SEM	Fold change mean	SEM
Group I: Normal control	1.00 ^b	0.08	1.00 ^b	0.06	1.00 ^b	0.08
Group II : Mercuric chloride	6.68 ^a	0.34	7.78 ^a	0.24	9.00 ^a	0.35
Group III: Mercuric chloride + Lycopene	2.20 ^c	0.11	3.48 ^c	0.14	3.25 ^c	0.21

Data are presented as (Mean \pm SEM) Mean values with different superscript letters in the same column are significantly different at (P \leq 0.05).

Table 5 Protective effect of lycopene administration (20 mg/kg b. wt.) on kidney tissue p53 and Bcl-2 gene expression levels in mercuric chloride intoxicated rats (2mg/kg b. wt.).

Animal groups	P53		Bcl-2	
	Fold change mean	SEM	Fold change mean	SEM
Group I: Normal control	1.00 ^b	0.06	1.00 ^a	0.04
Group II: Mercuric chloride	5.78 ^a	0.27	0.15 ^c	0.01
Group III: Mercuric chloride + Lycopene	2.73 ^c	0.13	0.51 ^b	0.02

Data are presented as (Mean \pm SEM). Mean values with different superscript letters in the same column are significantly different at (P \leq 0.05).

The hepatocytes showed variable degrees of degenerative changes such as marked and diffuse vacuolar and hydropic degeneration that characterized by swollen, pale, vacuolated cytoplasm of hepatocytes (Fig. 1d). Occasionally, fatty change of the hepatocytes, predominantly in the centrilobular zones of hepatic lobules characterized by marked enlargement of the cells by multiple variably sized discrete empty vacuoles that distend the cell cytoplasm were seen. Multifocally, there were focal areas of coagulative necrosis of hepatocytes characterized by preservation of hepatic architecture and loss of cell details and aggregates of mononuclear inflammatory cells mainly lymphocytes and macrophages (Fig. 1e). Additionally, scattered areas of lytic necrosis that characterized by loss of hepatic tissue and its replacement by vacant space admixed with erythrocytes (Fig. 1f). Degree 3 was observed in most treated animals. Accordingly, rats treated with mercuric chloride had severe liver damage with a mean score of 3. In contrast, marked improvement in the hepatocellular architecture with more regular and less altered hepatocytes were noticed in the hepatic tissue obtained from rats of group 3 (lycopene) when compared to mercuric chloride treated rats (Group 2). The hepatic tissue restored its normal histological structure in comparison to the negative control group. Most of the hepatic parenchyma displayed a certain degree of recovery besides the portal area appeared normal and contained normal bile duct with mild congested portal vein only. The livers of these treated rats showed mild congestion and dilatation of central and portal veins, the blood sinusoids with activation of Von Kuepfer cells (Fig. 1g & h). Interestingly, the hepatocytes showed normal histological appearance with rare mild vacuolar degeneration of individual hepatocytes (Fig. 1g & h). Collectively, the liver of rats obtained from this group appeared to be with a mean liver score 1, significantly better scores than animals had mercuric chloride only. On the other side, the microscopic examination of the hepatic tissue taken from rats injected with normal saline revealed normal histological structure of

hepatic parenchyma, portal area and hepatic blood vessels (group 1).

Kidney:

The histopathological examination of renal tissues obtained from rats treated with mercuric chloride for four weeks revealed congestion of the renal blood vessels and intertubular blood capillaries with vacuolar degeneration of the renal blood vessels with perivascular mononuclear leukocytic cellular infiltration (Fig. 2a) as well as interstitium of renal cortex was expanded by aggregates of lymphocytes and macrophage (Fig. 2b). The glomeruli were variably distorted, with degeneration of glomerular tufts characterized by swelling and vacuolization of mesangial cells with marked congestion and dilatation of glomerular capillaries (Fig. 2c). Occasionally, hyper-segmentation of glomerular tufts with adhesions between glomerular tufts and Bowman's capsule were also observed in association with lytic necrosis of the glomerular tuft that characterized by absence of the glomerular tuft and replaced by eosinophilic debris (Fig. 2d). Additionally, variable degree of degenerative changes of the lining epithelium of proximal and distal convoluted tubules characterized by vacuolar and hydropic degeneration as the epithelial cell lining the renal tubules were swollen and showing clear empty cytoplasm with pyknotic nuclei were seen. Furthermore, entire necrosis of the renal tubular epithelium with presence homogenous eosinophilic casts in the lumen of renal tubules (Fig. 2e). Accidentally, areas of lytic necrosis characterized by loss of renal tissue architecture and replaced by erythrocytes and mononuclear inflammatory cells were also demonstrated (Fig. 2f). Some of the renal tubules showed cystic dilatation lined with attenuated epithelium. In most treated animals, degree 3 was observed. Rats treated with mercury chloride therefore suffered from severe damage of renal tissues with an average score of 3. Interestingly, the microscopical examination of the kidney of treated rats with mercuric chloride and lycopene for four weeks displayed distinct improvement in renal tissue histology in comparable with

that of the control group. Mild congestion of the renal blood vessels with normal histological structure of the glomeruli in some examined cases. Meanwhile, hyper-cellularity of glomerular tuft to the extent of its adhesion with the parietal layer of Bowman's capsule and absence of Bowman's space (Fig. 2g) was also demonstrated in other cases. Hypertrophy of parietal epithelium of Bowman's capsule with necrosis of the glomerular tuft was detected in one examined case (Fig. 2h). However, mild degenerative changes in the epithelial

cell lining of the renal tubules in the form of cloudy swelling in association with few amounts of eosinophilic debris was noticed in the lumen of some renal tubules (Fig. 2h). The kidney of rats collectively obtained from this group appeared to have a mean score 1, significantly better than that of mercury chloride alone. On the other side, the microscopic examination of the renal tissue taken from injected rats with normal saline revealed normal histological structure of renal tissues (group 1).

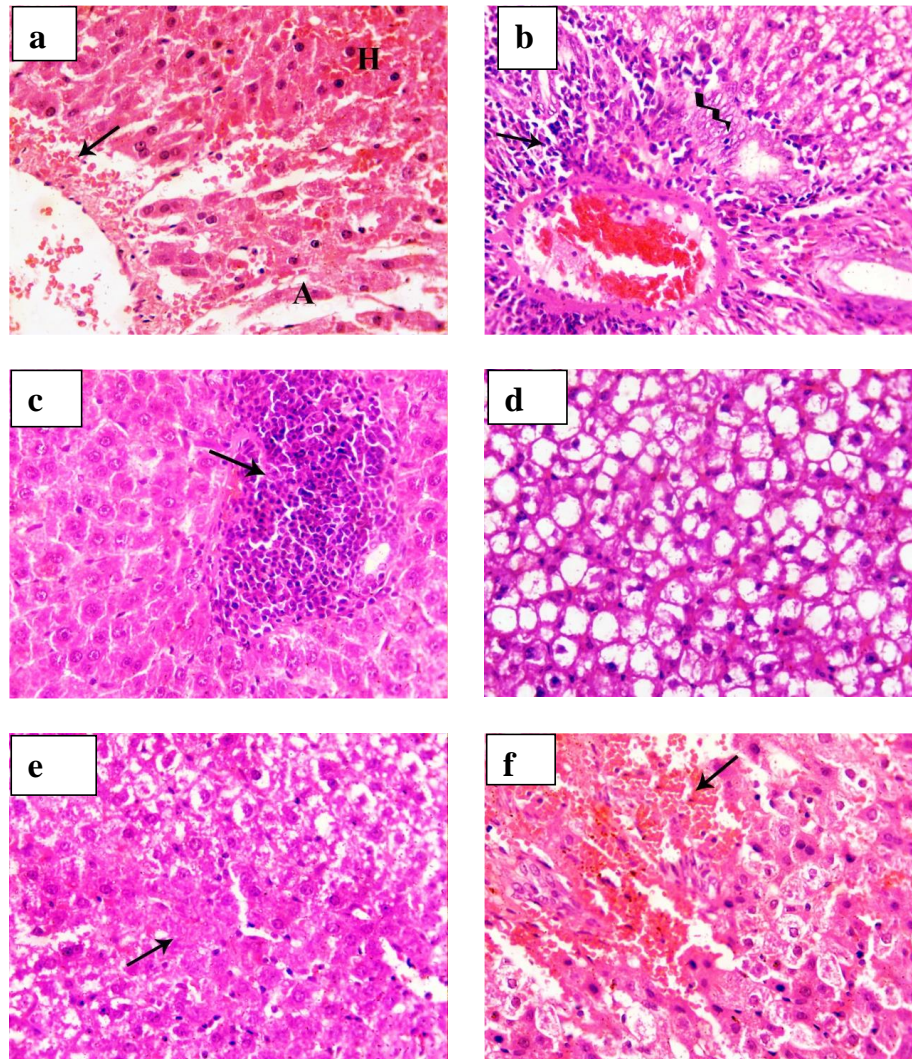


Fig. 1 H&E stained sections of liver tissue taken from mercuric chloride (2mg/kg b. wt.) for four weeks (Group 2) (a-f) and from rats treated with mercuric chloride plus lycopene (20 mg/kg b. wt.) (group 3), showing (a) congestion of central vein, perivascular edema admixed with erythrocytes (arrow) with scattered hemorrhage in hepatic parenchyma (H) and atrophy of the hepatic cords (A, x400), (b) marked mononuclear leukocytic aggregations of inflammatory cells in the portal area (arrow) with proliferation of the biliary epithelium (zigzag arrow, x400), (c) focal aggregation of leukocytes in the hepatic parenchyma (arrow, x400), (d) extensive hydropic degeneration of hepatocytes characterized by swollen, pale, vacuolated cytoplasm (x400), (e) diffuse areas of coagulative necrosis in the hepatic parenchyma (arrow, x400), (f) lytic necrosis, that characterized by loss of cord architecture and replacement of the vacant space by erythrocytes (arrow, x400), (g) congestion of central with mild vacuolar degeneration of hepatocytes (x400), (h) dilatation of hepatic blood sinusoids with activation of Von Kuepfer cells (x400).

4. DISCUSSION

Mercuric chloride intoxicated rats shown a significant increase in serum ALT, AST and ALP activities when compared with normal control group. These results came in accordance with the recorded data of Oliver *et al.* (2017), who recorded that the exposure to HgCl_2 increased hepatic damage and increase serum liver marker enzyme (AST, ALT, and ALP) activities. Also, one of the most sensitive

and dramatic indicators of hepatocyte damage is the release of intracellular enzymes, such as transaminases and serum alkaline phosphatase. The elevated activities of these enzymes are signal of cellular leakage and the loss of the functional integrity of the cell membranes in liver which are always associated with hepatic necrosis (Howell *et al.*, 2014). In the current study, the damaging effects of HgCl_2 may be resulted from its generation of ROS that causes oxidative stress of various organs. The obtained results confirmed that oxidative stress, increased lipid peroxidation,

depletion of antioxidant defenses and increased production of pro-inflammatory mediators are implicated in the pathogenesis of HgCl₂ toxicity induced chronic hepatic damage, inorganic mercury induces severe liver injury as shown by hepatic morphological changes and apoptosis as well as negative effect on hepatic function (Rice et al.,

2014). Moreover, Goudarzi *et al.* (2017) reported that in HgCl₂-treated rats (0.4 mg/kg b. wt./day) for 28 days serum ALT, AST and ALP activities were significantly increased. Treatment with lycopene to HgCl₂ intoxicated rats significantly reduced elevated serum ALT, AST and ALP activities when compared with HgCl₂ exposed group.

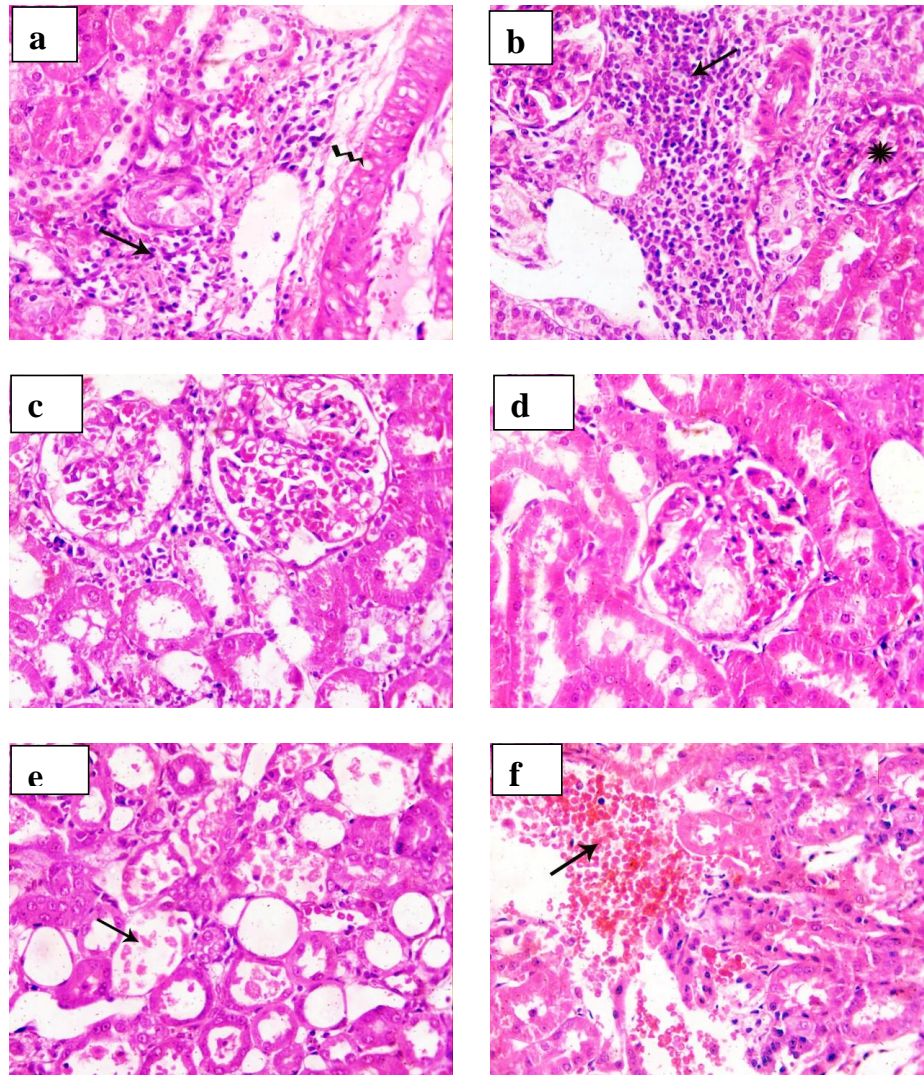


Fig. 2 H&E stained sections of kidney tissue taken from mercuric chloride (2mg/ kg/b. wt.) treated rat (a-f) for four weeks (Group 2) and (g-h) from rats treated with mercuric chloride plus lycopene (20 mg/kg/b. wt. (group 3) showing (a) vacuolar degeneration of the renal blood vessels (zigzag arrow) with intratubular mono-nuclear leukocytic cellular infiltration (arrow, x400), (b) extensive expansion of the interstitium of renal cortex by lymphocytes and macrophage (arrow), necrosis of glomerular tuft (asterisk, x400), (c) swelling and vacuolization of endothelial cells of glomerular tuft with marked congestion of glomerular capillaries and peri-glomerular leukocytic infiltration (x400), (d) lytic necrosis of the glomerular tuft that characterized by absence of the glomerular tuft and replaced by eosinophilic debris (x400), (e) entire necrosis of the lining epithelium of renal tubules with the presence of eosinophilic hyaline casts in the lumen of most renal tubules (arrow, x400), (f) lytic necrosis in the renal cortex that characterized by absence of the renal tissue and replaced by erythrocytes admixed with few leukocytes (arrow, x400), (g) hyper-cellularity of glomerular tuft to the extent of its adhesion with the parietal layer of Bowman's capsule and absence of Bowman's space (x400), (h) mild degenerative changes in the epithelial cell lining of the renal tubules in the form of cloudy swelling, with hypertrophy of parietal epithelium of Bowman's capsule (x400).

Lycopene ameliorate the biomarkers of liver damage and dysfunction by inhibited hepatic oxidative stress caused by HgCl₂. Similarly, Cavusoglu *et al.* (2009) reported that partial improvements in serum ALT and AST activities following treatment with lycopene (20 mg/kg b. wt.) in rats exposed to HgCl₂.

Mercuric chloride intoxicated rats exhibited a significant increase in serum urea and creatinine concentrations. Elevation in serum creatinine and urea resulted from injury in renal tubular cells, which confirmed by obvious alterations in kidney cells of HgCl₂ group. The pathogenesis

of renal damage as a risk factor of renal failure can be due to oxidative stress. The obtained results are in agreement with Abarikwu *et al.* (2018) who, indicated tha urea and creatinine levels were increased after intraperitoneal (i.p.) administration of HgCl₂ (5 mg/kg. b. wt.) daily, 3 times a week, for 21 days. Also, Rojas-Franco *et al.* (2018) recorded that HgCl₂ intoxication causes glomerular dysfunction because it reduces urinary flux, and creatinine clearance with a serum creatinine increase causes electrolytic misbalance because Hg intoxication reduces the urinary excretion of sodium and potassium and its respective clearances respect to electrolytic balance. The kidney is a major target organ of

inorganic mercury, exhibiting both glomerular and tubular damages. Mercuric chloride reduced the mitochondrial activity affecting the energy production in renal cells which participate in the apoptosis process in resident renal cells (Venkatesan and Sadiq, 2017). Treatment with lycopene to HgCl₂ intoxicated rats significantly reduced elevated serum urea and creatinine concentrations. These results came in accordance with those recorded by Paula et al. (2007), who indicated that animals treated with lycopene after mercuric chloride challenge significantly reduced serum urea and creatinine levels compared to intoxicated group. Also, lycopene protected against renal toxicity caused by mercuric chloride (5mg/kg b. wt.) in rats by inhibiting lipid peroxidation. Likewise, lycopene can remove free radicals, prevent the oxidative damage caused by HgCl₂, enhancing the body antioxidant capacity, reducing the levels of lipid peroxidation, and maintaining cell membrane permeability. A significant increase in L-MDA was observed in liver tissue of mercuric chloride intoxicated rats. The mechanism of liver injury induced by HgCl₂ is believed to involve ROS production and free radical mediated damage. Consequently, oxidative stress may be one of the main contributing causes of HgCl₂-induced disorders in organs. The obtained results agree with Boroushaki et al. (2016), who recorded that administration of HgCl₂ caused significant enhancement in LPO level. Also, Goudarzi et al. (2017) demonstrated that the MDA level in liver tissues significantly increased in HgCl₂ exposure. On the other hand, a significant decrease in GSH concentration was observed in liver tissue of mercuric chloride intoxicated rats. The administration of mercury cause alterations in the mitochondrial inner membrane, resulting in the increased formation of H₂O₂ in the mitochondrial electron transport chain and depletion of mitochondrial GSH levels (Kalender et al., 2013). The level of GSH, which is the primary line of cellular protection against toxic agents and major intracellular conjugation factors, reduced and displayed damaged function in mercuric toxicity binding of HgCl₂ to GSH reduces GSH level in the cells and decreases the antioxidant capacity of the cells (Bashandy et al., 2016). This result is agreed well with Mehrzadi et al. (2018), who recorded that the GSH level and antioxidant enzyme (SOD, CAT and GPx) activities were significantly decreased in the liver tissues of HgCl₂-treated rats. Also, AL-Zubaidi and Rabee (2015) reported that oral administration of mercuric chloride induced free radicals and resulted in significant decrease in the reduced glutathione (GSH) concentration in mercuric exposed rats. Meanwhile, after lycopene treatment to mercuric chloride intoxicated rats a significant decrease in L-MDA with marked increase in GSH concentrations were observed in liver tissue. Similarly, Paula et al. (2007) reported that administration of lycopene (25 mg/kg/day) after exposure to HgCl₂ (5 mg/kg. b. wt.) significantly decreased liver tissue L-MDA and markedly increased GSH concentration.

The obtained results showed a significant down-regulation in liver tissue Home oxygenase-1 (HO-1) gene expression after exposure to mercuric chloride. Home oxygenase-1 (HO-1) is a microsomal cytoprotective enzyme that is induced in response to injury and cellular stress (Nath, 2014; Wegiel et al., 2014). Heme oxygenase-1 is an enzyme presents in the microsome and plays an important role in suppressing inflammation. The inflammatory responses mediated by macrophage is markedly suppressed after

activation of HO-1. Nuclear factor erythroid 2-related factor 2 (Nrf2), a factor sensing the presence of oxidative stress, regulates transcription of genes encoding for cytoprotective enzymes and other proteins crucial for maintaining cellular homeostasis (Wang et al., 2016). In the presence of ROS, Nrf2 is released from Keap1 and translocate into the nucleus, activating the transcription of heme oxygenase-1 and brain ischemia/reperfusion injury leads a dramatic increase in the generation of ROS. Consequently, nuclear Nrf2 was increased and heme oxygenase-1 was up-regulated following ischemia reperfusion injury (Lei et al., (2016). Meanwhile, the existing data revealed a significant up-regulation of liver HO-1 gene expression after lycopene treatment to mercuric chloride intoxicated rats. Dietary lycopene attenuated chronic liver injury induced by HgCl₂, through regulation of the Nrf2/NF-κB/P53 signaling pathway. Therefore, lycopene administration may be a novel therapeutic approach for treating inorganic mercury poisoning. These results came in accordance with the recorded data of Dai et al. (2015), who reported that lycopene treatment (20 mg/kg/day, b. wt., orally) in mice, significantly up-regulated Nrf2 and HO-1 mRNA expression and down regulated the expression of NF-κB mRNA which can functions as a free radical and singlet-oxygen scavenger in renal toxicity. Also, Lei et al. (2016) reported that lycopene pretreatment significantly induced an increase in the expression levels of Nrf2 and heme oxygenase-1 and it provided significant neuro protection in mice subjected to global cerebral ischemia by inhibiting neuronal apoptosis and attenuating oxidative stress, which is associated with the activation of Nrf2/HO-1 signaling. Who added that, lycopene promotes Nrf2 expression, and reverses the depletion of Nrf2 caused by chronic HgCl₂ exposure, thus improves the ability to resist oxidative stress.

The current results revealed that, a significant up-regulation in kidney tissue TNF-α, NF-κβ, Bax and p53 gene expression levels and down-regulation in Bcl-2 gene were observed in mercuric chloride intoxicated rats. Meanwhile, lycopene treatment to mercuric chloride intoxicated rats caused significant down-regulation in kidney tissue TNF-α, NF-κβ, Bax and p53 and up-regulation in Bcl-2 gene expression levels when compared with mercuric chloride exposed group. These results came in accordance with the recorded data of Caglayan et al. (2019), who recorded that NF-κB, TNF-α, Bcl-3, IL-33, bax, p53 and IL-1β gene levels were significantly upregulated after exposure to mercuric chloride (1.23 mg/kg b. wt. i.p) for 7 days in rats. Tumor necrosis factor-α when binding to tumor necrosis factor receptors (TNFR), binds to the TNFR type 1-associated death domain protein (TRADD) and then activates p38 mitogen-activated protein kinase (MAPK) and NF-κβ (Sprowl et al., 2012). p38 MAPK represents a class of MAPKs that can also activate NF-κβ. NF-κβ and p53 could be up regulated by p38 MAPK. The relative protein levels of Bcl-2, Bax, and NF-κβ show that lycopene suppresses NF-κβ, thereby inhibits apoptosis. NF-κβ and p53 could be up regulated by p38 MAPK (Ghisays et al., 2015). Activation of the p38 pathway significantly stimulated p53 function. Moreover, Saccani et al. (2001) establish that p38 MAPK also affected NF-κβ levels by promoting phosphorylation of IκB, resulting in the dissociation and degradation of NF-κβ and IκB complexes. Who added that, lycopene inhibited p38-activated NF-κβ and p53 pathways, which then contributes to the protection of lycopene against HgCl₂-

induced inflammation and apoptosis. Moreover, apoptosis, a phenomenon of programmed cell death, is a cellular process triggered by a variety of chemicals or environmental stimuli (Caglayan et al., 2019). HgCl₂ also prompted the apoptotic pathway by increasing the levels of Bcl-2 associated X protein (Bax) and p53, causes apoptosis by cytochrome c release from mitochondrial leading to caspase-3 and 9 activities. Likewise, apoptosis signaling pathways involves p53 and the Bcl-2 protein family, including pro apoptotic and pro-survival proteins. The tumor suppressor protein p53 influences apoptosis and can modulate levels of the Bcl-2 protein family (Liu and Zhu, 1999). Additionally, tumor suppressor protein P53 is a transcription factor that regulates the DNA repair and apoptosis while Bcl-2 gene is an anti-apoptotic protein, which has important roles in regulating cell survival and is an important tumor suppressor that is widely involved in cellular responses to various stresses. Mercuric chloride reduced the mitochondrial activity affecting the energy production in renal cells which participate in the apoptosis process in resident renal cells. Upon binding to nuclear DNA, activation of proteins involved in apoptosis occurs. Severe DNA damage induced by mercuric chloride leads to activation of p53 which is the master regulator of apoptosis and is known to regulate transcription of several pro-apoptotic proteins, involved in both intrinsic and extrinsic apoptotic pathways (Venkatesan and Sadiq, 2017). In the current study, lycopene suppresses p53, thereby increases Bcl-2 level and decreases Bax level, and finally protects hepato-renal cells against HgCl₂-induced apoptosis. In current study, there was increased in TNF- α , NF- κ B, Bax protein and levels of p53 in HgCl₂-treated group. Similarly, Yang et al. (2016) reported that HgCl₂ induced apoptosis by increasing expression of p53, Bax, and caspase-3 in rat kidney tissues. Conversely, the lycopene showed a strong anti-apoptotic effect by protecting tissues through decreasing TNF- α , NF- κ B, Bax, and p53 protein levels. Also, lycopene has also been found to decrease inflammatory cytokine and chemokine expression by inhibiting TNF- α mediated activation of the NF- κ B signaling pathway both in vitro and in vivo (Luvizotto-Rde et al., 2013). Collectively, these findings demonstrated that, lycopene ameliorated HgCl₂-induced kidney damage, which is associated with the suppression of mitochondria-mediated apoptosis in rats. Furthermore, the rate of apoptosis is decreased to protect against Hg kidney damage and restrain apoptosis by preventing the up regulation of the expression of p53 and caspase-3 mRNA in endothelial cell (Tang et al., 2009).

5. CONCLUSION

It could be concluded that lycopene is a powerful antioxidant agent stops ROS production, attenuates HgCl₂-induced oxidative stress and hepato-renal damage via activating hemoxygenase-1 signaling pathway and enhance antioxidant defense system. Also, lycopene treatment has a strong anti-apoptotic and anti-inflammatory effects through inhibiting TNF- α mediated activation of the NF- κ B, Bax, and p53 signaling pathways.

6. REFERENCES

1. Abarikwu, S.O., Njoku, R.C. and Onuah, C.L. 2018. Aged coconut oil with a High peroxide value induces oxidative stress and tissue damage in Mercury- treated rats. *J Basic Clin Physiol Pharmacol*; 29, 365-376.
2. Al-Zubaidi, E. S., Rabee, A.M. 2015. Effect of mercuric chloride on biochemical and hematologic parameters in male albino mice. *International Journal of Science and Research (IJSR) ISSN (Online): 2319-7064*.
3. Bancroft, J.D. and Gamble, M. 2008. *Theory and Practice of Histological Techniques*. 6th Ed., Churchill Livingstone, Elsevier. ChinaNath, K. A. 2014. Heme oxygenase-1 and acute kidney injury. *Curr Opin Nephrol Hypertens* 23: 17–24.
4. Bashandy, S.A., El Awdan, S.A., Ebaid, H. and Alhazza, I.M. 2016. Antioxidant Potential of Spirulina platensis Mitigates Oxidative Stress and Reprotoxicity Induced by Sodium Arsenite in Male Rats. *Oxid Med Cell Longev*: 7174351.
5. Boroushaki, M.T., Azarhosseini, Dolati, K., Mollazadeh, H. and Arezoorabian 2016. Protective effect of pomegranate seed oil against mercuric chloride-induced hepatotoxicity in rats. *Acta Pol Pharm Jul*;73(4):991-997.
6. Branco, V., Caito, S., Farina, M., Teixeira da Rocha, J Aschner, M. and Carvalho, C. 2017. Biomarkers of mercury toxicity past, present, and future trends. *Journal of Toxicology and Environmental Health., Part B.*, 20(3): 119-154.
7. Bridges, C.C. and Zalups, R.K. 2017. The aging kidney and the nephrotoxic effects of mercuric. *J Toxicol Environ Health B Crit; Rev* 20 (2):55-80.
8. Caglayan, C., Kandemir, F.M., Yildirim, S., Kucuklerb, S. and Eserc, G. 2019. Rutin protects mercuric chloride-induced nephrotoxicity via targeting of aquaporin 1 level, oxidative stress, apoptosis and inflammation in rats, *Journal of Trace Elements in Medicine and Biology*: 54, 69-78.
9. Campos, K.K.D, Araújo, G.R.A., Martins, T.L., Band eirab, A.C.B.B., Costac, G.D., Talvanic, A., Garciad, C.C.M., Oliveirae, L.A.M, Costab, D.C.C., Bezerraa,S.B. 2017. The antioxidant and anti-inflammatory properties of lycopene in mice lungs exposed to Cigarette smoke *Journal of Nutritional Biochemistr*; 48, 9–20.
10. Cavusoglu, K., Oruc, E., Yapar, K. and Yalcin, E. 2009. Protective effect of Lycopene against mercury-induced cytotoxicity in albino mice: Pathological evaluation. *Journal of Environmental Biology*; 30(5) 807-814.
11. Celikoglu, E. and Kalender, Y. 2015. Vitamin E and sodium Selenite against mercuric chloride induced lung toxicity in the Rats. *Braz Arch Biol Technol.*; 58(4): 587-94.
12. Chin-Chan, M., Navarro -Yepes, J. and Quintanilla-Vega, B. 2015. Environmental pollutants as risk factors for Neurodegenerative disorders: Alzheimer and Parkinson Diseases. *Frontiers in Cellular Neuroscience* 9: 124.
13. Dai, C., Tang, S., Deng, S., Zhang, S., Zhou, Y., et al. 2015. Lycopene attenuates colistin-induced nephrotoxicity in mice via activation of the Nrf2/HO-1 pathway. *Antimicrob. Agents Chemother*; 59, 579–585.
14. Engall, E.1980. *Methods in Enzymology*, Volume 70, Van Vunakis, H. and Langone, J. J. (eds.), Academic Press, New York. Pp. 419-492.
15. Genchi, G., Inicropi, M.S. Carocci, A. Lauria, G. and Catalano .2017. Mercury Exposure and Heart Diseases. *Int J Environ Res Public Health*. 14(1):74.
16. Ghisays, F. et al.T. 2015. The N-terminal domain of Sirt1 is a positive regulator of endogenous Sirt1-dependent deacetylation and transcriptional outputs. *Cell Rep* 10, 1665–1673.
17. Goudarzi, M., Kalantar, M. and Kalantar, H.2017. The Hepatoprotective Effect of Gallic Acid on Mercuric Chloride-Induced Liver Damage in Rats, *Jundishapur J Nat Pharm Prod* ;12(4): e12345.
18. Howell, B.A., Siler, S.Q., Shoda, L.K.M., Yang, Y., Woodhead, J.L. and Watkins, P.B. 2014. Amechanistic model of drug-induced liver injury AIDS the interpretation of elevated liver transaminase levels in a phase I clinical trial. *CPT: Pharmacometrics and Systems Pharmacology* 3, 98:1-8.
19. Krishnamoorthy, K Selvakumar, P, Venkataraman, P, Elumalai and Arunakaran, J. 2013. Lycopene supplementation

- prevents Reactive oxygen species mediated apoptosis in Sertoli cells of adult albino rats exposed to polychlorinated biphenyls, *Interdisciplinary Toxicology* 6(2): 83–92.
20. Kumar, S., Kumar, R., Mukhopadhyay, S.K. and Singh, K. K. 2018. Protective Effect of *Bryophyllum pinnatum* on mercuric chloride induced oxidative stress in rat; *International Journal of Current Microbiology and Applied Sciences* 2319-7706 Special Issue-7 pp. 4328-4333.
 21. Lei, X., Lei, L., Zhang, Z. and Cheng, Y. 2016. Neuroprotective effects of Lycopene pretreatment on transient global cerebral ischemia reperfusion in rats: The role of the Nrf2/HO Signaling pathway. *Molecular Medicine Reports*. 13: 412-418.
 22. Liu, X. and Zhu, X. Z. 1999. Roles of p53, c-Myc, Bcl-2, Bax and caspases in serum deprivation-induced neuronal apoptosis: a possible neuroprotective mechanism of basic fibroblast growth factor. *Neuroreport*; 10, 3087–3091.
 23. Luvizotto-Rde, A., Nascimento, A.F., Imaizumi, E., Pierine, D.T., Conde, S.J., Correa, C.R., Yeum, K.J. and Ferreira, L. 2013. Lycopene supplementation modulates plasma Concentrations and epididymal adipose tissue mRNA of leptin, resistin and IL-6 in diet-induced obese rats. *British Journal of Nutrition*; 110, 1803-1809.
 24. Mehrzadi, S., Fatemi, I., Esmailizadeh, M., Ghaznavi, H., Kalantar, H. and Goudarzi, M. 2018. Hepatoprotective effect of berberine Against methotrexate induced liver toxicity in rats. *Biomed Pharmacother*; 97:233–9.
 25. Mesquita, M., Pedroso, T.F., Oliveira, C.S., Oliveira, V.A., do Santos, R.F., Bizzi, C.A. and Pereira, M.E. 2016. Effects of zinc against mercury toxicity in female rats 12 and 48 hours after HgCl₂ exposure; *EXCLI J*; 15: 256–267.
 26. Miller, S., Pallan, S., Gangji, A. S., Lukic, D. and Clase, C. M. 2013. Mercury-associated nephrotic syndrome: a case report and systematic review of the literature, *American Journal of Kidney Diseases*. 62 (1): 135–138.
 27. Nath, K. A. 2014. Heme oxygenase-1 and acute kidney injury. *Curr Opin Nephrol Hypertens*; 23: 17–24.
 28. Oliveira, C.S., Piccoli, B.C., Aschner, M. and Rocha, J.B.T. 2017. Chemical speciation of selenium and mercury as Determinant of their neurotoxicity. *Adv Neurobiol* 18: 53-83.
 29. Paula, R., Augusti, Greicy, M., Conteraton, M., Sabrina Somacal, V.C., Lídia Einsfeld, V., Adriano, T.; Ramos, V.; Fernando, Y.; Hosomi, M.; Dominguita, L. and Tatiana Emanuelli. 2007. Effect of Lycopene on Nephrotoxicity Induced by Mercuric Chloride in Rats. *Basic & Clinical Pharmacology & Toxicology*; 100, 398–402.
 30. Rice, K.M., Walker, E.M., Jr., Wu, M., Gillette, C. and Blough, E.R. 2014. Environmental mercury and its toxic effects, *Journal of Preventive Medicine and Public Health*, 47 (2): 74–83.
 31. Rojas-Franco, p.; Franco-li'n, M.; Camargo, M.E.M.; Carmona, M.M.E.; Orti`z- Butro`n, M. R. E.; Blas-Valdivia, V. and Cano-Europa, E. 2018. Phycobiliproteins and phycocyanin of *Arthrospira maxima* (Spirulina) reduce apoptosis promoters and glomerular dysfunction in Mercury-related acute kidney injury.; Volume 2: 1–10.
 32. Sadek, K., Abouzed, T. and Nasr, S. 2016. Lycopene modulates cholinergic Dysfunction, Bcl-2/Bax balance, and antioxidant enzymes gene Transcripts in monosodium glutamate (E621) induced neurotoxicity in a rat model. *Can J Physiol Pharmacol*; 94, 394-401.
 33. Salazar-Flores, J., Torres-Jasso, J.H., Rojas-Bravo, D., Reyna-Villela, Z.M. and Torres- Sánchez, E.D. 2019. Effects of Mercury, Lead, Arsenic and Zinc to Human Renal Oxidative Stress and Functions: A Review. *J Heavy Met Toxicity*; Dis., Vol.4 No.1:2.
 34. Sokoloski, L., Borges, M. and Bagatin, E. 2015. Lycopene not in pill, nor in natura has photo protective systemic effect. *Arch Dermatol Res*; 307:545–549.
 35. Sundseth, K., Pacyna, J., Pacyna, E., Pirrone, N. and R. Thorne. 2017. Global sources and pathways of mercury in the Context of human health, *International Journal of Environmental Research and Public Health* 14(1): 105.
 36. Sprowl, J. A. 2012. Alterations in tumor necrosis factor signaling pathways are associated with cytotoxicity and resistance to taxanes: a study in isogenic resistant tumor cells *Breast Cancer Res*; 14, 1–18.
 37. Sacconi, S., Pantano, S. and Natoli, G. 2001. P38-dependent marking of inflammatory genes for increased NF-kappa B recruitment. *Nat Immunol*; 3, 69–75.
 38. Tang, X., Yang, X. and Peng, Y., et al. 2009. Protective effects of lycopene against H₂O₂-induced oxidative injury and apoptosis in human endothelial cells. *Cardiovasc Drugs Ther*; 23(6), 439-448.
 39. Venkatesan, R. S. and Sadiq, A.M. 2017. Effect of morin-50-sulfonic acid sodium salt on the expression of apoptosis related proteins caspase 3, Bax and Bcl2 due to the mercury induced oxidative stress in albino rats. *Biomed Pharmacother*; 85, 202–208.
 40. Wang, A., Wang, S., Jiang, Y., Chen, M., Wang, Y. and Lin, L. 2016. Bio-assay guided identification of hepatoprotective polyphenols from *Penthorum chinense* Pursh on t-BHP induced oxidative stress injured L02 cells. *Food Funct*; 7, 2074–2083.
 41. Wegiel, B., Nemeth, Z., Correa-Costa, M., Bulmer, A.C. and Otterbein L.E. 2014. Heme oxygenase 1: a metabolic niche. *Antioxid Redox Signal*. 20: 1709–1722.
 42. Yang, D., Tan, X., Lv, Z., Liu, B., Baiyun, R., J. Lu, J. and Zhang, Z. 2016. Regulation of Sirt1/Nrf2/ TNF- α signaling pathway by luteolin is critical to attenuate acute mercuric chloride exposure induced hepatotoxicity. *Sci. Rep.*; Nov 17; 6:37157.
 43. Young D.S. 1997. Effects of Preanalytical Variables on Clinical Laboratory Tests, 2nd ed. Washington, DC: AACC Press; 3-85.
 44. Zalups, R.K. 2017. Molecular interactions with mercury in the kidney, *Pharmacol. Rev* 52, 113–143.