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Paper

Ameliorative impacts of chrysin against gibberellic acid-induced liver and kidney damage through the regulation of antioxidants, oxidative stress, inflammatory cytokines, and apoptosis biomarkers

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Gibberellic acid (GA3), a widely known plant growth regulator, has been mostly used in agriculture. Little is known regarding its toxicity or the impact of its metabolic mechanism on human health. The current study examined the protective impact of chrysin against GA3-induced liver and kidney dysfunctions at biochemical, molecular, and histopathological levels. Forty male albino rats were allocated into 4 groups. The control group received saline; the chrysin group received 50 mg/kg/BW orally daily for 4 weeks; the GA3 group received 55 mg/kg/BW GA3 via daily oral gavage for 4 weeks, and the protective group (chrysin + GA3) was administered both chrysin and GA3 at the same dosage given in chrysin and GA3 groups. Chrysin was administered 1 h earlier than GA3. The GA3 induced liver and kidney injuries as proven by the elevation of hepatic and renal markers with a significant increase in malondialdehyde levels. Furthermore, a decrease of catalase and glutathione was reported in the GA3-administered rats. Pre-administration of chrysin significantly protected the hepatorenal tissue against the deleterious effects of GA3. Chrysin restored the hepatorenal functions and their antioxidant ability to normal levels. Moreover, chrysin modulated the hepatorenal toxic effects of GA3 at the molecular level via the upregulation of the antiapoptotic genes, interleukin-10 (IL-10), hemoxygenase-1, and nuclear factor erythroid 2-related factor 2 expressions; the downregulation of the kidney injury molecule-1 and caspase-3 mRNA expressions; and a decrease in IL-1β and tumor necrosis factor-α secretions. Additionally, the pre-administration of chrysin effectively attenuated the GA3-induced hepatorenal histopathological changes by regulating the immunoexpression of cytochrome P450 2E1 (CYP2E1) and pregnane X receptor, resulting in normal values at the cellular level. In conclusion, chrysin attenuated GA3-induced oxidative hepatorenal injury by inhibiting free-radical production and cytokine expression as well as by modulating the antioxidant, apoptotic, and antiapoptotic activities. Chrysin is a potent hepatorenal protective agent to antagonize oxidative stress induced by GA3.

Key words: chrysin; gibberellic acid; hepatorenal dysfunction; gene expression; oxidative stress; apoptosis.

Introduction

According to the American Society of Agricultural Science, gibberellins are one of the 6 primary types of plant growth regulators (PGRs),[1] and Gibberellic acid (GA3), a naturally occurring PGR of the gibberellin class, has been extensively used in agriculture to support cell division and elongation in plants.[2,3] However, the use of GA3 as an insecticidal phytohormone has been of growing concern as humans are exposed to GA3 through the ingestion of food and byproducts treated with this chemical.[4,5]

In rats, chronic exposure to GA3 has increased tumor growth and oxidative stress, resulting in hepatocellular carcinomas.[6] In mice, GA3 produced breast and lung adenocarcinomas. In human and murine cells, GA3 induced chromosomal aberrations.[7,8] Toxicants frequently impact the kidney as it can accumulate hazardous compounds in high concentrations that can lead to negative alterations in kidney functions and structure.[9] Several chemicals are also dangerous to the liver. Most liver blood is derived from the gastrointestinal

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tract via portal veins that transport medications and toxins in concentrated forms to the liver.[10] The ability to scavenge reactive oxygen species has been diminished in GA3-treated cells, resulting in oxidative stress, cell death, and affecting the antioxidant ability of the cells.[11–13] The lipoxygenase end product, malondialdehyde (MDA), was also shown to be greater in rats treated with GA3. An increase in MDA, a key byproduct of the lipid peroxidation process, has been a consistent predictor for the incidence of diseases.[14] Exposure to GA3 resulted in a significant reduction in the total protein amount of hepatic tissue and induced hepatic biochemical and histochemical changes that manifested by increased liver enzyme markers.[15,16] The liver, the kidney, the testes, and the ovaries are the main organs that may be affected by GA3 intoxication. GA3 has been shown to affect hormonal secretions in both sexes.[17,18]

Flavonoids are polyphenolic chemicals found in plants and fall into numerous categories. Chrysin (5,7-dihydroxy flavones) is found naturally in flowers (e.g. the blue passion and the Indian trumpet), mushrooms, honey, and propolis. It has antioxidant, anti-inflammatory, antiapoptotic, antidiabetic, anticancer, antiestrogenic, and anxiolytic activities.[19–23] In rats, chrysin significantly reduced the nephrotoxicity due to cisplatin and doxorubicin as well as the hepatotoxicity due to methotrexate and carbon tetrachloride.[24–26] Therefore, the present study investigated the putative protective effect of chrysin against hepatorenal toxicity induced by GA3 in rats as well as the protective intracellular pathways of chrysin that may be involved in such protection.

Materials and methods

Animal handling and experimental design

Forty male albino rats aged 8 weeks, 150–160 g weight, were maintained at room temperature and gained free access to food and water. The Institutional Animal Care and Use Committee of Turabah University College at Taif University authorized all animal-related procedures for project TURSP-2020-09. After 2 weeks of adaptation, rats were divided into 4 groups of 10 rats each. The control group (CNT) received saline; the chrysin group received an oral administration of 50 mg/kg/BW, dissolved in saline, daily for 4 weeks[27]; the GA3 group received 55 mg/kg/BW of GA3 via daily oral gavage for 4 weeks.[17,28] The protective group (chrysin + GA3) received the same doses of chrysin and GA3 as those administered separately to the chrysin and GA3 groups.[29] When administered together (i.e. in the chrysin + GA3 group), the chrysin was given 1 h earlier than the GA3. On day 28, under isoflurane anesthesia, blood samples were drawn from the tail vein using nonheparinized vacutainer tubes, centrifuged at 3,000×g for 10 min, and kept at −20 °C to observe the changes in serum chemistry and metabolites. After blood sampling, the rats were euthanized by decapitation, and the liver and kidney samples were isolated, rinsed by cold saline, and cleaned before being sliced. Tissue sample slices were kept in either Qiazol for RNA extraction and real-time polymerase chain reaction (RT-PCR) or in a 10% neutral buffer formalin solution for histological and immunohistochemical evaluation.

Biochemical measurements

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were spectrophotometrically assessed (Randox, Antrim, United Kingdom), according to the manufacturer's instructions. ALT and AST activities were assessed in the serum according to Reitman and Frankel,[30] while the alkaline phosphatase was measured following the method of Tietz et al.[31] The total protein and albumin were assessed according to Doumas et al.[32] Urea levels were evaluated following the method of Talke and Schubert.[33] Serum creatinine was assessed by a modified Jaffe's reaction, following the method of Bowers.[34] Uric acid was assessed by the Trinder enzymatic reaction, as previously described.[35] The kits used in the current study were from Biodiagnostic, Dokki, Giza, Egypt.

Measurements of antioxidants and inflammatory cytokines

A colorimetric spectrophotometer was used to measure serum levels of catalase, reduced glutathione (GSH), nitrous oxide (NO), and MDA, based on the supplied instruction manual and using the kits from Biodiagnostic (Dokki, Giza, Egypt). The enzyme-linked immunosorbent assay (ELISA) kits for rattumornecrosisfactor-α (TNF-α, ab46070), interleukin-1β (IL-1β, ab100768), and IL-6 (ab100772) were obtained from Abcam (Tokyo, Japan) and used to determine TNF-α, IL-1β, and IL-6 using an ELISA spectrophotometer, as described in the instruction manual of each kit.

Molecular analysis using quantitative RT-PCR

RNA was isolated from tissues and measured at 260/280 nm. QuantiTect reverse transcription kit produced single-stranded complementary DNA (cDNA) from 2 μg total RNA utilizing a 2-step process with random primer hexamer. The SYBR Green master mix was used for cDNA amplification (Thermo Fisher Scientific, USA). Table 1 shows the primer sequence of genes that were involved in antioxidation, anti-inflammation, and apoptosis. RT-PCR analysis was used to quantify these genes, utilizing the $^{2-\Delta\Delta CT}$ method. The gene β-actin was utilized as a reference and compared to other genes under study. The comparative cycle threshold (CT) values were used to measure the intensity and mRNA expression of the studied genes.

Immunohistochemical investigation for cytochrome P450 2E1 and pregnane X receptor

After euthanasia by decapitation, all animals were necropsied following the guidelines described in,[36,37]

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Table 1. Primers sequence used for quantitative RT-PCR in liver and kidney of rats.

Primer sequence	Direction	Gene	Accession number
ACTCTTCAGGGATGGGGTGA	Sense	Bcl2 (94 bp)	NM_016993
TGACATCTCCCTGTTGACGC	Antisense		
GAGCTTGGAACGCGAAGAAA	Sense	Caspase-3 (146 bp)	NM 012922
TAACCGGGTGCGGTAGAGTA	Antisense		
TGGCACTGTGACATCCTCAGA	Sense	Kim-1 (161 bp)	NM_173149
GCAACGGACATGCCAACATA	Antisense		
TTGTAGATGACCATGAGTCGC	Sense	Nrf2 (141 bp)	NM_031789.2
TGTCCTGCTGTATGCTGCTT	Antisense		
AGAAGCTGAAGACCCTCTGGATAC	Sense	IL-10 (157 bp)	NM_012854
GCTCCACTGCCTTGCTTTTATT	Antisense		
GTAAATGCAGTGTTGGCCCC	Sense	HO-1 (178 bp)	NM_012580.2
ATGTGCCAGGCATCTCCTTC	Antisense		
AGGAGTACGATGAGTCCGGC	Sense	β-actin (71 bp)	NM 031144
CGCAGCTCAGTAACAGTCCG	Antisense		

and representative tissue specimens from the liver and kidneys were collected and instantly fixed in 10% neutral buffered formalin solution for 24 h. The fixed specimens were washed in distilled water, dehydrated in ascending grades of ethyl alcohol, cleared in UltraClear clearing agent, impregnated, blocked in paraffin wax, and sectioned at 4 μm thickness.[38] These formalin-fixed paraffin-embedded hepatic and renal tissue sections were stained for cytochrome P450 2E1 (CYP2E1) and pregnane X receptor (PXR) using the rabbit polyclonal anti-cytochrome CYP2E1 primary antibody (Cat. No. PIPA579132, Thermo Fisher Scientific, USA) and the rabbit polyclonal anti-PXR primary antibody (Cat. No. ab217375, Abcam USA), 3,30-diaminobenzidine chromogen (DAB), and hematoxylin as a counterstain following the avidin–biotin–peroxidase complex technique.[39] Both PXR and CYP2E1 are transcriptional regulators of xenobiotic metabolizing enzymes in liver. The stained slides were examined microscopically, and 5 nonoverlapped high-power (40 objectives) randomly chosen microscopic fields per marker per organ per animal (5 images per marker per organ per group) were captured using AmScope digital camera attached to Olympus light microscope (BX-52; Olympus, Tokyo, Japan). Next, the hepatic and renal CYP2E1 and PXR immunoexpression were scored by calculating the percentages of the positively stained DAB brown area fractions to the total areas of the images using the image processing program, ImageJ densitometry analysis software, version 1.33 (National Institutes of Health), via the color deconvolution plugins and following the method described by Behairy et al.[40]

Statistical analysis

The data were presented as means ± standard error and analyzed using a one-way ANOVA and Dunnett's post hoc descriptive test using SPSS software for Windows (SPSS, IBM, Chicago, IL). Values with $P < 0.05$ were considered statistically significant.

Results

Impacts of chrysin on GA3-induced hepatorenal dysfunction

The GA3-treated rats revealed a significant ($P < 0.05$) increase in serum levels of liver enzyme markers, AST and ALT, as well as a severe decrease in albumin and total protein levels, as compared to the control group (Table 2). Additionally, there was a significant increase in the kidney injury markers, creatinine, urea, and uric acid, as compared to the control group (Table 2). However, the rats in the chrysin + GA3 group showed a significant decrease in liver enzymes, kidney injury markers, albumin, and the total protein, as compared to the GA3-treated group. These results indicated that chrysin restored the GA3-induced changes in the hepatorenal biomarkers.

Impacts of chrysin on GA3-induced oxidative stress in rat livers and kidneys

Table 3 shows considerable increases in serum MDA levels and substantial decreases in GSH and catalase in the GA3-treated group, as compared to the control group. The chrysin + GA3 group revealed a significant increase in catalase and GSH levels and a significant decrease in the MDA levels, as compared to the GA3-treated group. In addition, Table 4 shows significant increases in IL-1β, IL-6, TNF-α, and NO levels in the GA3-treated group, as compared to the control group. The chrysin + GA3 group demonstrated significant decreases in IL-1β, IL-6, TNF-α, and NO levels, as compared to the GA3-treated group. These results indicated that chrysin had an antioxidative effect.

Ameliorative impacts of chrysin on hepatic and renal genes associated with apoptosis and inflammatory cytokines

Figure 1A shows that there was a significant increase ($P < 0.05$) in the mRNA expression of caspase 3 in the GA3-treated group, which was downregulated in the chrysin + GA3 group. A significant increase in the

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Table 2. Impacts of chrysin (50 mg/kg/BW) against GA3 (55 mg/kg/BW)-induced hepatorenal dysfunction.

Parameter	Control	Chrysin	GA3	Chrysin + GA3
ALT (U/L)	18 ± 2.10	2.8 ± 3.10	58.1 ± 1.78[a]	31.1 ± 3.1[b]
AST (U/L)	21 ± 1.0	20.6 ± 1.40	61.1 ± 5[a]	33.1 ± 4.9[b]
Total proteins (g/dL)	7.60 ± 0.50	6.40 ± 0.34	3.60 ± 0.10[a]	5.80 ± 0.30[b]
Albumin(g/dL)	4 ± 0.40	4.50 ± 0.30	2.10 ± 0.15[a]	2.70 ± 0.15[b]
Uric acid (g/dL)	5.1 ± 0.40	6.8 ± 0.40	1.8 ± 0.67[a]	6.10 ± 0.50[b]
Urea (mg/dL)	22.1 ± 2.10	27.1 ± 4.10	45.9 ± 2.2[a]	26.3 ± 5.10[b]
Creatinine (mg/dL)	0.5 ± 0.010	0.80 ± 0.04	1.87 ± 0.070[a]	0.87 ± 0.04[b]

Values are means ± SE for 10 different rats per each experiment. Values are statistically significant at [a]$P < 0.05$ versus control and chrysin groups and [b]$P < 0.05$ versus GA3 group.

Table 3. Impacts of chrysin (50 mg/kg/BW) against GA3 (55 mg/kg/BW)-induced changes on serum MDA, catalase, and reduced GSH levels.

Group	MDA (nmol/mL)	Catalase (U/L)	GSH (nmol/L)
Control	13.6 ± 2.2	218.1 ± 11.4	3.1 ± 0.10
Chrysin	11.1 ± 1.1	225.2 ± 12.2	3.4 ± 0.30
GA3	41 ± 13.4[a]	142.2 ± 10.2[a]	1.05 ± 0.050[a]
Chrysin + GA3	25.2 ± 3.1[b]	197.8 ± 8.12[b]	2.4 ± 0.030[b]

Values are means ± standard error (SE) for 10 different rats per each treatment. Values are statistically significant at [a]$P < 0.05$ versus control and chrysin, and [b]$P < 0.05$ versus GA3 group.

Table 4. Effects of chrysin (50 mg/kg/BW) against GA3 (55 mg/kg/BW)-induced alterations in inflammatory biomarkers.

Parameter	Control	Chrysin	GA3	Chrysin + GA3
IL-1β (pg/mL)	222.43 ± 5.7	249.1 ± 7.3	433.3 ± 15.6[a]	295.4 ± 12.9[b]
IL-6 (pg/mL)	64. 3 ± 3.7	69.7 ± 9.3	153.1 ± 12.1[a]	114.8 ± 6.8[b]
TNF-α (pg/mL)	511.3 ± 33.1	458.2 ± 20.7	744.8 ± 41.1[a]	571.9 ± 19.6[b]
NO (μmol/L)	45.9 ± 1.9	54.3 ± 5.4	86.3 ± 8.3[a]	65.3 ± 3.8[b]

Values are means ± SE for 10 different rats per each experiment. Values are statistically significant at; [a]$P < 0.05$ versus control and chrysin groups and [b]$P < 0.05$ versus GA3 group.

B-lymphoma cell-2 (Bcl-2) genes, which overcame the apoptotic effect found in the GA3-treated group, was determined in the tissues of the chrysin + GA3 group (Fig. 1B). Similarly, the GA3-treated group showed a significant decrease in the mRNA expression of IL-10, whereas a significant upregulation was found in the chrysin + GA3 group (Fig. 1C).

There was a significant upregulation ($P < 0.05$) in the mRNA expression of the Kim-1 genes in the GA3-treated group, which was significantly ($P < 0.05$) downregulated in the chrysin + GA3 group (Fig. 2A). In contrast, the GA3-treated group showed a significant downregulation in HO-1 and Nrf2 mRNA expressions, as compared to the other treated groups (Fig. 2B and C), and the chrysin + GA3 group showed a significant upregulation and normalization, as compared to the GA3-treated group. All these findings indicated the anti-inflammatory potential of chrysin.

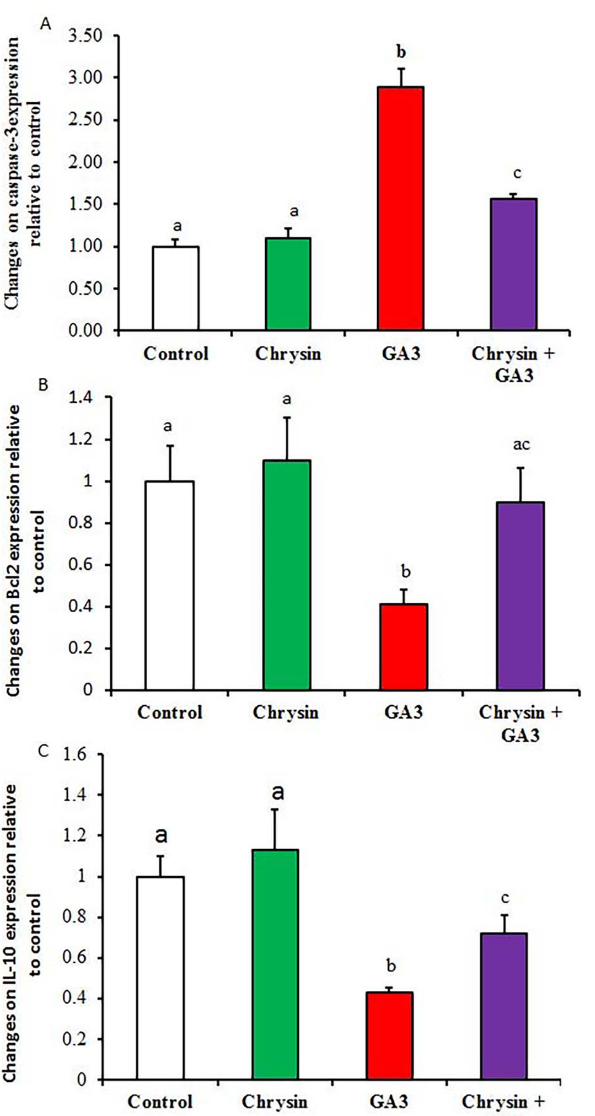

Fig. 1. Protective effect of chrysin (50 mg/kg/BW) against GA3 (55 mg/kg/BW)-induced changes on the expression of caspase-3, Bcl2, and IL-10 in liver of rats. Values are means ± standard error of the mean (SE) for 10 different rats per treatment. Values with different letters are statistically significant at *$P < 0.05$.

Immunohistochemical findings

The immunohistochemical findings and the quantitative scoring for the livers and kidneys (Figs 3 and 4, respectively) for CYP2E1 and PXR immunoexpressions were shown. The image analysis showed that exposure to GA3 significantly upregulated the CYP2E1 and PXR immunoexpressions in the hepatic and renal tissue specimens, as compared to the control and chrysin-treated groups. No

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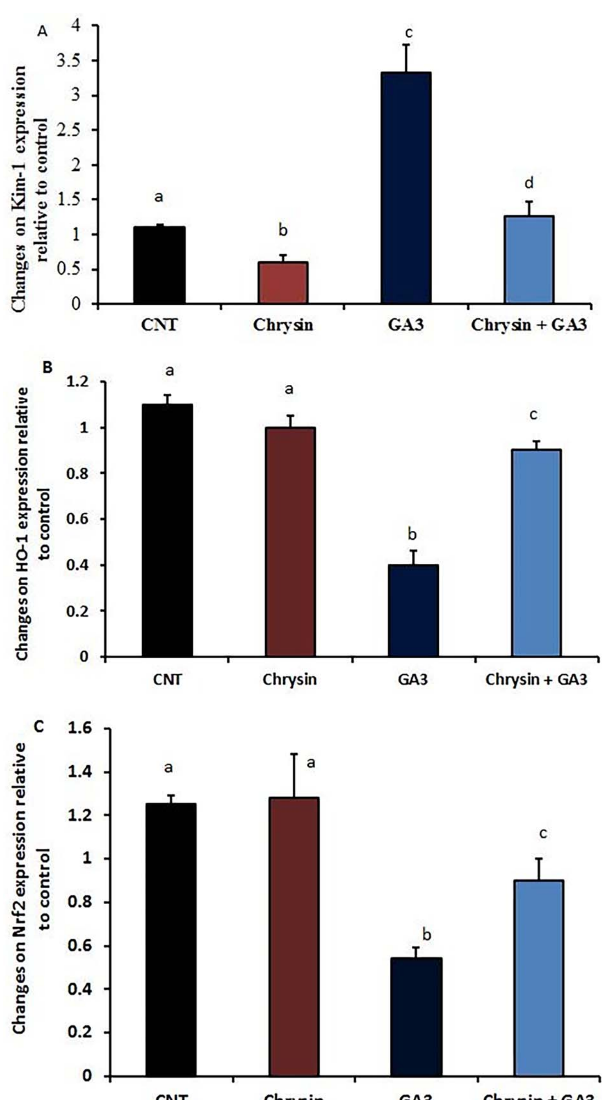

Fig. 2. Protective effect of chrysin (50 mg/kg/BW) against GA3 (55 mg/kg/BW)-induced changes on the expression of Kim-1, HO-1, and Nrf2 in kidney of rats. Values are means ± SE for 10 different rats per treatment. All describe well and we mean that the values with different figures are statistically significant at $P < 0.05$.

differences were observed between the control and the chrysin-treated groups. The upregulation of both markers was more pronounced in the hepatic tissue, as compared to the renal tissue. Chrysin supplementation had significantly downregulated the CYP2E1 and PXR immunoexpressions in the hepatic and renal tissue specimens but did not normalize them. This downregulation was more pronounced in the immunoexpression of CYP2E1, as compared to that of PXR, as well as in the hepatic tissue, as compared to the renal tissue. The degree of positively stained cells in the liver and kidney tissues can be seen in Figs 3I and 4I, respectively. The findings confirmed that chrysin may play a role in metabolizing GA3.

Discussion

The current study showed that chrysin has potential as a protective agent against GA3-induced liver and kidney dysfunction in rats through the regulation of oxidative stress and antioxidants, as well as a positive effect on apoptosis and antiapoptosis markers.

The present study revealed that the liver markers enzymes AST and ALT were significantly increased with a prominent decrease in albumin and total protein levels in the GA3-treated group. Our findings supported previous research findings. GA3 induced liver injury, as evidenced by an increase in the AST and ALT levels in plasma, demonstrating cellular leakage and a loss of cell membrane function integrity.[2,41] As compared to the GA3-treated group, the coadministration of chrysin and GA3 resulted in a substantial decrease in liver enzymes, total protein, and the albumin ratio. These results suggested that chrysin conserved the membranes' structural integrity, and these findings agreed with previous research.[26,42,43]

As compared to other treatment groups, there was a considerable increase in kidney damage indicators in the GA3-treated group; however, there was a significant normalization in the chrysin + GA3 group. Several studies have indicated that the xenobiotic-induced renal function alterations were associated with increased creatinine and urea levels in the blood.[44–46] Female rats fed GA3 had higher levels of creatinine and urea.[2] Furthermore, an increase in blood urea was associated with an increase in protein catabolism.[47,48] Our current findings were in accordance with that of Tahir and Sultana,[49] where chrysin normalized the increase in creatinine and lactate dehydrogenase to control the kidney and liver biomarkers. All indicators revealed a sudden decline in serum blood urea nitrogen and creatinine levels by retaining renal cellular membrane integrity after chrysin administration.

The imbalance of the antioxidant defense system and free-radical production have been shown to be the main factors in tissue damage.[50] In the GA3-treated group, we found significant increases in serum MDA levels. Similarly, Baykalir et al. demonstrated that a GA3-treated rat showed a considerable increase in MDA levels,[51] and chrysin administration reduced MDA levels as well as increased superoxide dismutase (SOD) activity and GSH levels.[51] The GA3-treated group showed considerable decreases in GSH and catalase, which caused an increase in the production of reactive oxygen species (ROS) in tissue. Our data were in agreement with those of Troudi et al., who detected a decrease in the activity of SOD, CAT, and glutathione peroxidase (GPx) in the livers of adult rats administered 75 ppm of GA3.[52] GSH is a sensitive biomarker of oxidative stress and is essential for mitochondrial and cell membrane integrity.[53] The protective effect of chrysin against ethanol-induced liver and kidney injury has been attributed to its antioxidant characteristics, its cell membrane-stabilizing properties, and its inhibition of microsomal CYP2E1 activity.[54] Chrysin increased the biosynthesis of GSH.[55] In addition, Pai et al. showed that the administration of 100 mg/kg body weight of chrysin improved lipid profiles, lowered

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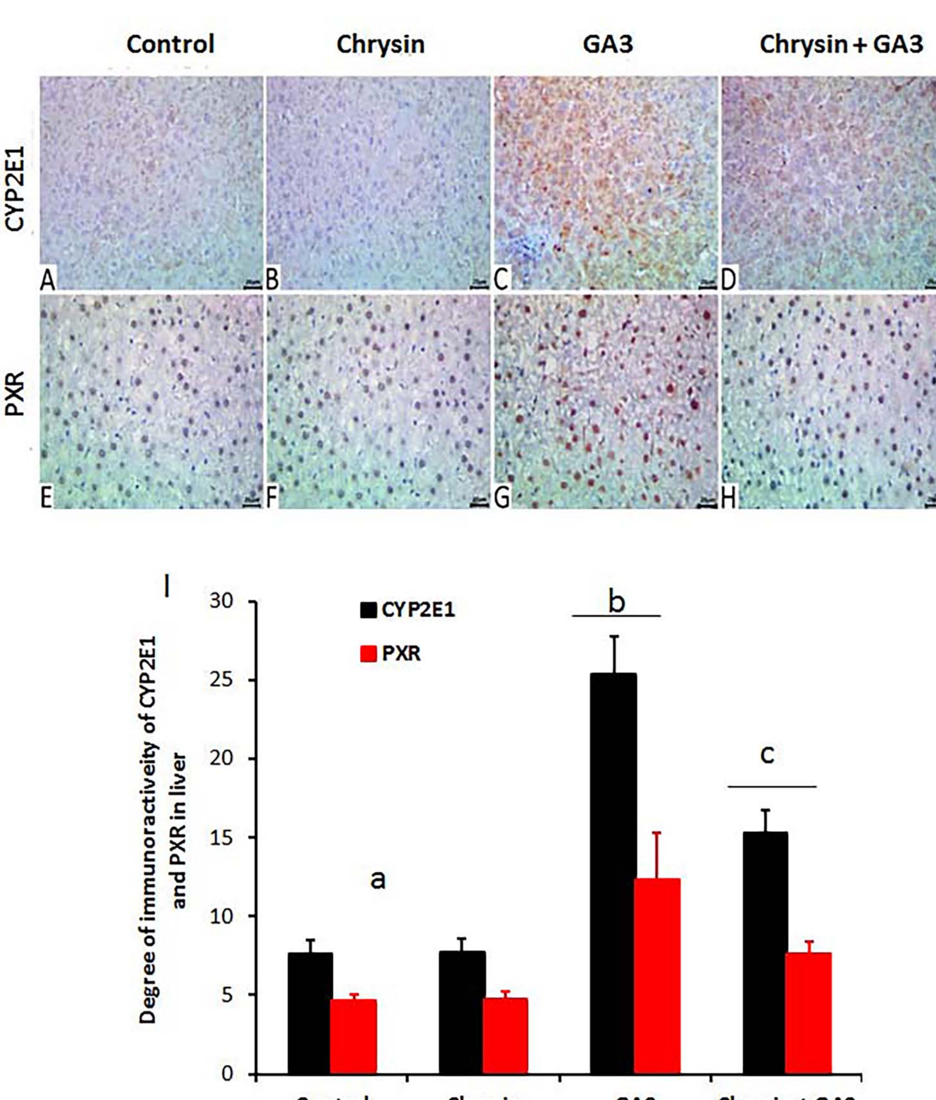

Fig. 3. Representative photomicrograph of A–D) CYP2E1-stained and E–H) PXR-stained hepatic tissue sections showing remarkable upregulation of both biomarkers' immunoexpression in the GA3-treated groups, as compared to the control and chrysin-treated rats, with noticeable downregulation of both markers' immunoexpression in the chrysin + GA3 rats. I) The degree of positive immunoreactivity for CYP2E1 and PXR is represented. Values with different letters a, b and c are statistically significant at $^{*}P < 0.05$.

liver indices (e.g. liver enzymes, liver triglycerides, cholesterol, and collagen), and improved the histology of livers in rats.[56] The inhibition of lipogenesis while increasing fatty acid oxidation was the mechanism through which chrysin reduced nonalcoholic fatty liver disease in rats.

Inflammation plays a significant role in tissue injury and hepatotoxicity through the induction of oxidative stress.[6,57] In addition, inflammation plays a substantial role in GA3-induced injury according to published data that showed increased tissue concentrations of inflammatory mediators (e.g. IL-1β, IL-6, TNF-α). Our results showed a significant increase in IL-1β, IL-6, TNF-α, and NO levels after GA3 administration, as compared to other treated groups. Similarly, Galal et al. reported that GA3-treated rats had higher IL-1β and TNF-α levels than control rats, indicating an inflammatory response.[58] Furthermore, GA3's oxidative ability could be attributed to the increase in the inflammatory response and NO levels.[6,57,59] There was a considerable increase in MDA and NO levels in the hepatic and renal tissues after GA3 administration.[59] Previous research agreed with our results[29]; GA3 treatment increased lipid peroxidation and decreased catalase, SOD, and GPx levels.[29] Pre-administration of chrysin before GA3 showed a significant reduction in IL-1β, IL-6, TNF-α, and NO levels, as compared to the GA3-treated group. Our result was concomitant with Rehman et al. in which they reported that the prophylactic administration of chrysin significantly reduced TNF-α expression and diminished the inflammatory response implicated in the acute liver damage caused by cisplatin.[60] Chrysin was shown to suppress neuroinflammation by lowering TNF-α and IL-6 production as well as oxidative stress.[43] This finding

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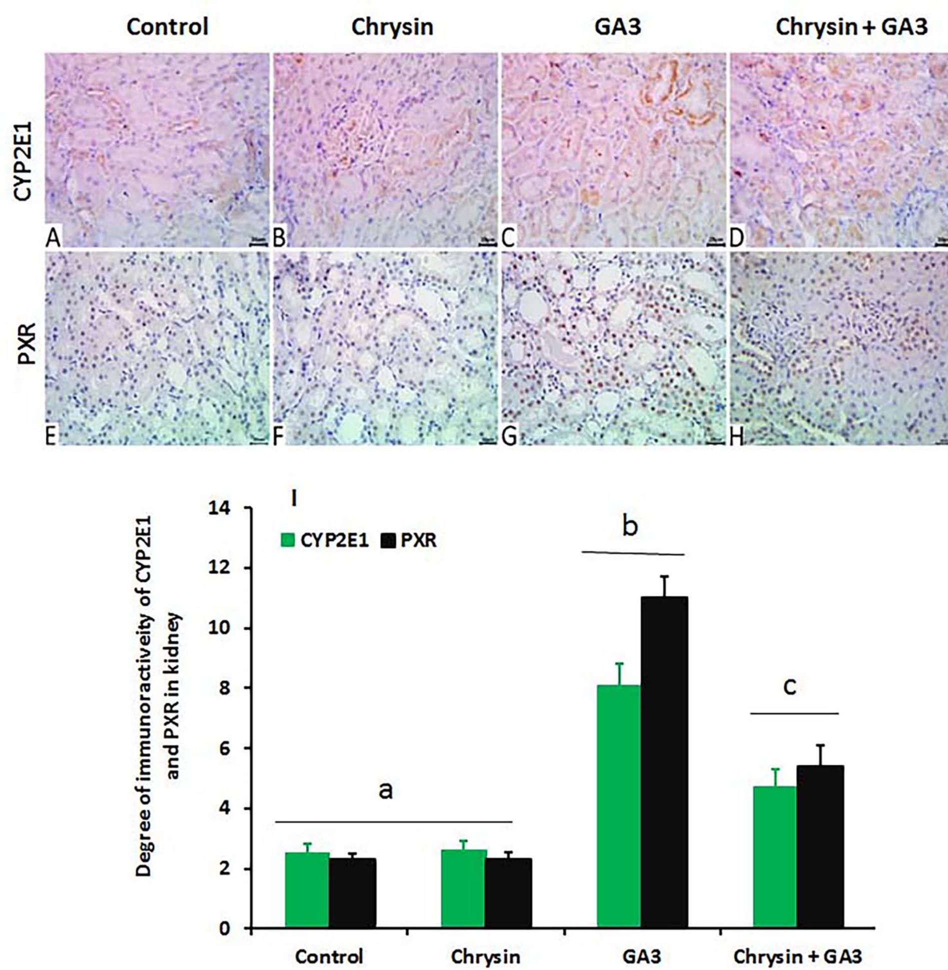

Fig. 4. Representative photomicrograph of A–D) CYP2E1-stained and E–H) PXR-stained renal tissue sections showing remarkable upregulation of both biomarkers' immunoexpression in the GA3-treated groups, as compared to the control and chrysin-treated groups, with noticeable downregulation of both markers' immunoexpression in the chrysin+GA3 group. I) The degree of positive immunoreactivity for CYP2E1 and PXR is presented. Values with different letters a, b and c are statistically significant at $^*P < 0.05$.

was consistent with previous research where chrysin decreased LPS-mediated inflammation by blocking the nuclear factor kappa B pathway.[61]

Kim-1 is a type I transmembrane protein found in proximal tubule epithelial cells after ischemic or toxic injury. Kim-1 regulates cell–cell adhesion and endocytosis. Kim-1 is a sensitive marker of proximal tubule damage produced by several chemicals.[62] Our results revealed an upregulation of the mRNA expression of the Kim-1 gene in the GA3-treated group, which was significantly downregulated with chrysin co-treatment with GA3. Our data were consistent with those of Prozialeck et al., in which they showed that an increase in Kim-1 expression occurred after kidney damage.[63] In addition, Xu et al. suggested that chrysin reduced renal injury by inhibiting tubular apoptosis and inflammation due to its anti-inflammatory and antioxidant activities.[64]

HO-1 has been identified with a cytoprotective function against oxidative stress. The Nrf2 antioxidant system has also been identified as a critical therapeutic target for oxidative stress, as it promotes the development of cytoprotective enzymes and associated proteins.[65,66] Our findings showed that HO-1 and Nrf2 mRNA expressions were significantly upregulated in the chrysin+GA3 group, as compared to the GA3-treated group.[67]

Our data revealed that the GA3-treated group showed a significant upregulation of caspase-3 expression and a subsequent decrease in Bcl-2 mRNA expression. These effects were mediated by chrysin co-treatment that ameliorated the apoptotic effect of the GA3. These findings were in harmony with Alsemeh et al.,[29] as the antiapoptotic marker Bcl-2 expression was downregulated in the GA3-treated group. The apoptotic marker caspase-3 increased, as suggested by Guo et al., by GA3 administration.[68] We concluded that chrysin inhibited apoptosis by increasing Bcl-2 mRNA expression and decreasing proapoptotic mRNA expression.[69] The overexpression of Bcl2 protects the cells from toxic injury, inhibiting cell death as well as downregulating Bax and caspase-3 to apoptosis.[70,71] Our results revealed a significant decrease in IL-10 after GA3 administration, and the chrysin + GA3 group showed an upregulation

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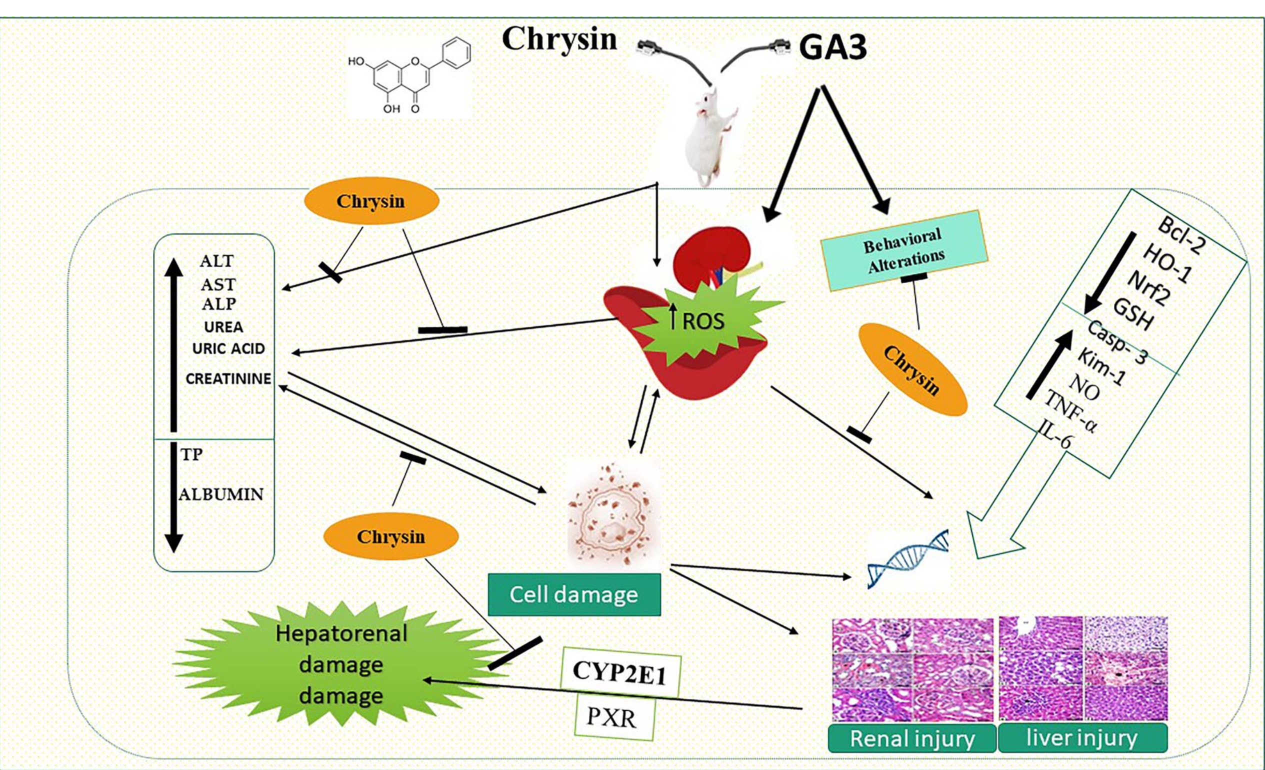

Fig. 5. Schematic graph for the ameliorative impacts of chrysin against GA3-induced oxidative stress in liver and kidney.

in IL-10 mRNA expression. Soliman et al.[59] confirmed same findings after GA3 administration. The anti-inflammatory effect of chrysin was also reported for other mediators.[72] CYP2E1 is a key player in oxidative stress, ROS generation, and hepatotoxic damage.[73,74] PXR is the major regulator of inflammation.[75] In the present study, GA3 administration significantly upregulated the CYP2E1 and PXR immunoexpression in the hepatic and renal tissue specimens, as compared to the control and chrysin-treated rats. However, these changes were normalized in the chrysin + GA3 group. These results were supported by the antioxidant, antiapoptotic, and anti-inflammatory effects of chrysin.[19,64,76]

Conclusion

Chrysin protected the experimental rats against GA3-induced liver and kidney dysfunction through the modulation of the hepatic and renal biomarkers and the normalization of IL-1β, IL-6, TNF-α, and NO values. Moreover, chrysin upregulated HO-1, Nrf2, and Bcl2 mRNA expression and downregulated Kim-1, caspase-3, PXR, and CYP2E1 expression. Based on these findings, chrysin is a viable therapeutic medication against GA3-produced hepatorenal damage. The collective effects of chrysin against GA3 side effects are illustrated in Fig. 5.

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Conflict of interest statement. The authors report no conflicts of interest.

Data availability

Current data are available upon request.

Ethical statement

All experimental procedures of this study were carried out under the National Institutes of Health Guidelines for the care and use of laboratory animals. All steps were followed to minimize the suffering of experimental animals.

Author contributions

All authors contributed equally to finish this work.

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