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Hepatoprotective, anti-apoptotic and anti-inflammatory efficacy of Quercetin or Rosemary extract against metalaxyl toxicity -induced liver damage in rats: A role of Nrf2/HO-1 signaling pathways

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ABSTRACT

Metalaxyl, a benzenoid fungicide, has hazardous effects on mammalian animals. Exposure to metalaxyl causes oxidative stress as well as clear toxicity. The possible protective impact of quercetin or rosemary extract against liver damage caused by the fungicide metalaxyl in rats were evaluated. Twenty-eight male albino rats split into four equal groups. G1 (control group); Rats were received distilled water. G2 (metalaxyl): Rats were administered oral dosages of metalaxyl (130 mg/kg b.wt) 1/10 LD50 three times a week for six weeks. G3 (metalaxyl + quercetin): Rats were given 50 mg/kg b.wt/day of quercetin in addition to (130 mg/kg b.wt) of metalaxyl. G4 (metalaxyl + Rosemary extract): Rats were given (200 mg/kg b.wt/day) of Rosemary extract in addition to 130 mg/kg b.wt of metalaxyl. The findings showed that rats exposed to metalaxyl had markedly elevated levels of liver marker enzymes and clearly up-regulation of Caspase-3 gene expression. However, rats exposed to metalaxyl showed a considerable downregulation of the expression of the liver HO-1, Nrf2, and Bcl-2 genes. Conversely, Quercetin or Rosmary extract co-treatment with metalaxyl induce significant decreases in serum liver enzymes along with downregulation in caspase 3, while up-regulation of HO-1, Nrf2 and Bcl-2 gene expression in hepatocytes of treated rats. These results suggest that quercetin and rosemary extract may have a potential protective role as strong hepatoprotective, anti-inflammatory and anti-apoptotic properties, mitigate oxidative stress caused by metalaxyl toxicity induced -liver damage by inhibiting caspase 3 mediated initiation of HO-1, Nrf2 and Bcl-2 genes.

Introduction

Metalaxyl, N-(2, 6 Dimethylphenyl)-N-(methoxyacetyl)-DL-alanine methyl ester, is a systemic benzenoid fungicide which represents the most famous member of the amide group. It is used for tropical and subtropical crops in foliar spray mixtures, as a seed treatment and a soil treatment to prevent soil-borne pathogens, fungal infections on ornamentals, fruits, peanuts, grasses, cotton, and soybeans (Ding *et al*., 2012).

Metalaxyl is applied to combat soil-borne fungal infections on grasses, fruits, peanuts, soybeans, and cotton (Sukul and Spiteller, 2000). The fears caused by metalaxyl result from its high residual level in crops, particularly vegetables grown in greenhouses, and other environmental components (Pattanasupong *et al*., 2004). It is highly dissolve in water, capable of penetrating groundwater, and stable in the presence of sunshine possesses a 400-day half-life (United States Environmental Protection Agency, 1994). Yin *et al*. (2017) reported that metalaxyl induces DNA hypomethylation in organism lead to toxic-epigenomics in organism. Who added that, metalaxyl affected on the activity of methyltransferase, which causes modification of DNA methylation levels and pattern.

The liver is the primary organ included in the metabolism of xenobiotics, such as fungicide, and plays a significant role in maintaining homeostasis. Traditional liver function tests are frequently used for evaluation dysfunction of the biliary and hepatocellular systems via assessment of serum levels of alkaline phosphatase (ALP) and aminotransferases (ALT, AST), respectively (Lozano-Paniagua *et al*., 2021).

Many fungicides primarily affect biological targets by electrophilically attacking cellular components related with synchronous generation of reactive oxygen species (ROS) (Al-Attar, 2015). ROS is a primary origin of oxidative stress in tissue (Elzoghby *et al*., 2014). Nuclear factor erythroid 2-related factor 2 (Nrf2) is a known redox controller of cells that regulators the expression of a variety of genes dependent on antioxidant response elements as well as eventually stimulates the body's antioxidant protection abilities (Kim *et al*., 2010). Pesticide exposure-stimulated organism damage is frequently associated with Nrf2-mediated cellular defense mechanisms (Zheng *et al*., 2020). According to Yang *et al*. (2021), Nrf2 with its target gene products, Heme oxygenase-1 (HO-1) and NAD(P)H Quinone Dehydrogenase 1 (NQO1), can be activated in response to a variety of toxins, and they represent the potential of organisms attacking oxidative stress to enhance an anti-oxidative response. In addition, cell apoptosis-linked protein expression Bcl-2 as well as enzyme activities (caspase-3) were associated with the molecular mechanisms of cell apoptosis (Wang *et al*., 2020).

Most plants contain a class of naturally occurring polyphenolic chemicals called flavonoids, which have been shown to have a major effect on the inflammatory process. Common flavonoids like quercetin are found in a variety of fruits and vegetables, including broccoli, soybeans, berries, apples, and grapes. Many fruits and vegetables contain quercetin, (3,3′,4′,5,7-pentahydroxyflavone), one of the plant flavonoids that belongs to the polyphenol group (Bao *et al*., 2017). Several pharmacological investigations have revealed that quercetin possesses strong antioxidant, neuroprotective, anti-inflammatory, anti-apoptotic and anti-angiogenic, properties (Wang *et al*., 2018). Additionally, it has been proposed that due to its potent anti-inflammatory with antioxidant features, it can inhibit diseases including obesity, diabetes, and cancer. Quercetin has been reported to be more effective than vitamins C, E and other natural antioxidants that stop oxidation of lipids, besides being a strong antioxidant as well as free radical scavenger (Smart *et al*., 2018). Quercetin is one of the most prevalent flavonoids, which has demonstrated to exhibit strong antioxidant, anti-inflammatory, anti-diabetic, anti-fibrotic, and anti-carcinogenic properties (Hassan *et al*., 2019). Moreover, quercetin treatment may have hepato-protective benefits via anti-inflammatory activity, capturing of free radical, and the regeneration of endogenous antioxidant defense system mechanisms against oxidative stress and liver damage induced by metalaxyl in rats (Hussein *et al*., 2017).

Rosemary (*Rosmarinus officinalis*) is one common household plant, has several phytochemicals, likewise rosmarinic acid, betulinic acid, camphor, caffeic acid, ursolic acid, and the antioxidants carnosic acid (Akela *et al*., 2018). Rosemary leaves extracts have several bioactivities in vitro, such as antioxidant, antibacterial, anti-tumor, antiulcerogenic, antidiuretic, antidiabetic, anti-inflammatory, and antithrombotic agents (Tousson *et al*., 2019). Accordingly, the current study was designed to explore the deleterious effects of metalaxyl toxicity caused-liver injury, oxidative stress, inflammation, and apoptosis in rats. Also, the possibility for the protective influence of quercetin and rosemary extracts on metalaxyl induced alterations of molecular markers and histopathological examination of liver tissues were evaluated.

Materials and methods

Experimental Animals

Twenty-eight male albino rats, weighing between 100 and 150 g at 4-5 weeks of age, were obtained from Laboratory Animals Research Center, Faculty of Veterinary, Benha University, Egypt. Animals were housed in separated stainless steel cages and kept up 12 hours, light/dark cycle, (24.0±1.0°C and 52.0±12.0 % relative humidity. Water and food were given ad- libitum. Rats were adapted for two weeks before the experiment's beginning. The Experimental protocol was carried out in compliance with the guidelines for Institutional Animals Care as well as Use Committee and adopted by Research Ethics Committee, Faculty of Veterinary Medicine, Benha University (BUFVTM 07-02-23).

Chemicals and natural agents

The chemicals and antioxidant employed in the existing study were:
Metalaxyl: Metalaxyl, N-(2,6-Dimethylphenyl)-N-(methoxyacetyl)-DL-alanine methyl ester, technical grade of 98% was purchased from Zhejiang Heben Pesticide and Chemicals Co., Ltd. China. Metalaxyl was dissolved in 860 µL of dimethyl sulfoxide (DMSO) after that, 11.2 mL of propylene glycol was added. Freshly prepared metalaxyl compound was given at a dose of 130 mg/kg b.wt (1/10 of LD50) orally three times a week for 6 weeks (Sakr and Lamfon, 2005).
Quercetin: {2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one dihydrate, 3,3′,4′,5,7-Pentahydroxyflavone dehydrate). Quercetin was purchased from Aktin Chemicals, Inc. company (Nature connecting health); Chengdu; China.

Preparation of Quercetin stock solution

About 200 mg of Quercetin was added to 1 ml of DMSO. About (0.5 ml) of quercetin DMSO stock solution was added to (1ml) Tween 80 and diluted to create 7 ml using normal saline. Each rat received 1 ml of this solution orally (Indap *et al*., 2006). For six weeks, rats were given a daily dosage of (50 mg/kg b.wt) from quercetin, which was prepared by dissolving it in DMSO, Tween 80, and normal saline solution.
Rosemary: Rosemary was purchased from Al-Harraz Co for Agriculture Seeds, Herbs and Medicinal plants, Cairo, Egypt.

Rosemary extract

Preparation of Rosemary leaves extract

In order to inhibit decomposing of chemical contents, dried rosemary leaves about 250 g were ground into a fine powder. Then, in a stoppered container the plant powder was added to ethanol [ethanol/water (70:30)], the mixture was left to stand at room temperature for a minimum of three days. Following this, the mixture was filtered to make a liquid extract, which was then concentrated at 50°C and low pressure using a rotary evaporator. This process was frequented at least three times. Lastly, the extract was weighted and kept at -20°C until it was needed (Abdel-Gawad etal., 2021).

Other chemicals applied in current study were of the highest pure grades obtained from El Gomhouria Company; for Trading Chemicals and Medical Appliances; Egypt.

Design of experimental work

The rats were split up into four equal major groups, each with seven rats, as follow:
G1 (Normal control): rats did not receive any treatment.
G2 (Metalaxyl): Rats given oral dosages of metalaxyl 1/10 LD50 (130 mg/kg b.wt) three times a week for six weeks.
G3 (Metalaxyl + quercetin): Rats were given quercetin orally (50 mg/kg b.wt/day) and given metalaxyl (130 mg/kg b.wt) three times a week.
G4 (Metalaxyl + Rosemary extract): Rats were given rosemary extract orally (200 mg/kg b.wt/day), (Al-Attar and Shawush, 2014) , and given metalaxyl (130 mg/Kg b.wt) three times a week for six weeks.
N.B: During the experimental period, the dosage was adjusted every week according to any change in body weight to maintain similar dose per kg body weight of rat over the entire period of study for each group.

Sampling

Blood samples

At the end of the six-week experiment, Blood samples were obtained by ocular vein puncture into tubes with screw caps, which were subjected to centrifugation at 2900 r.p.m. for 10 minutes for serum separation. The separated serum was preserved in a deep freeze at -20°C. until used for the assessment of liver biomarker enzymes [Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP)] activities.

Liver specimens

After obtaining of blood sample, rats were euthanized in accordance with Animal Ethics Committees. After being dissected, the liver tissue specimens were split into two parts. The first portion was cleaned with sterile physiological saline to eliminate any blood cells and clot, it was placed in Eppendorf tubes, instantaneously stored in liquid nitrogen, and preserved at -80°C until the RNA was extracted to determine the gene expression by reverse transcription polymerase chain reaction (RT-PCR). The second part of the liver was preserved in 10% formalin solution for histopathological investigation.

Analyses

Biochemical analysis

Serum ALT and AST activities were assessed using the kinetic method outlined by (Schumann and Klauke, 2003) while ALP activity was determined following the procedure described by Tietz *et al*. (1983) using a commercial kit supplied by SPINREACT, Santa Coloma, Spain.

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Molecular analysis

The mRNA expression levels of B-cell lymphoma 2 (Bcl-2), Caspase-3, and heme oxygenase-1 (HO-1), nuclear factor erythroid 2–related factor 2 (Nrf2) in livers of rats were evaluated using real-time qPCR (Table 1). Using a complete RNA purification kit and the manufacturer's instructions, pure RNA was isolated from liver tissues (Thermo Scientific, Fermentas,

Table 1. Sequences of forward and reverse primers for genes used in RT- qPCR.

Gene	Forward primer (/5 ------ /3)	Reverse primer (/5 ------ /3)
Caspase3	GGTATTGAGACAGACAGTGG	CATGGGATCTGTTTCTTTGC
Bcl-2	ATCGCTCTGTGGATGACTGAGTAC	AGAGACAGCCAGGAGAAATCAAAC
Nrf2	CACATCCAGACAGACACCAGT	CTACAAATGGGAATGTCTCTGC
HO-1	GGAAAGCAGTCATGGTCAGTCA	CCCTTCCTGTGTCTTCCTTTGT
GAPDH	CAACTCCCTCAAGATTGTCAGCAA	GGCATGGACTGTGGTCATGA

#K0731). Every cDNA sample was reverse transcribed via used the Revert Aid TM First Strand CDNA synthesis kit (#EP0451, Thermo Scientific, Fermentas, USA). Next, using gene-specific primers and the manufacturer's protocol (Thermo Scientific, USA, # K0221), RT-PCR with SYBR Green was utilized to evaluate gene expression. The target genes expression normalizes with glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) using 2-ΔΔCt technique (Livak and Schmittgen, 2001).

Histopathological examination

Liver tissue specimens for histopathological examination were placed in 10% neutral buffered formalin solution (Bancroft and Gamble, 2008). Following appropriate fixation, the specimens underwent dehydration in increasing grades of ethyl alcohol, were subsequently cleared with xylol, encased in paraffin, and then blocked. For microscopy, these specimens were split at a thickness of 5 µm as well as subjected to staining with hematoxylin and eosin (H and E).

Statistical analysis

The means ± SE were applied to express all the data. Using SPSS, 18.0 software 2011, a one-way analysis of variance (ANOVA) was employing to evaluate the statistical significance, and the Duncan's multiple range test (DMRT) was applied to generate individual comparisons. The statistical significance was determined as p ≤ 0.05.

Results

Rats intoxicated with metalaxyl showed a marked elevation in serum activities of ALT, AST and ALP in comparison to the control group. While rats treated with quercetin and rosemary extract showed a highly significant reduction of ALT, AST, and ALP activities compared to the metalaxyl-exposed rats (Table 2).

Table 2. Effects of Quercetin or Rosemary extract administration on the activity of serum AST, ALT and ALP in rats exposed to metalaxyl.

Parameters / Animal groups	ALT (U/L)	AST (U/L)	ALP (U/L)
G1: Normal control	56.05^{c}±2.27	69.90^{c}±3.65	120.33^{c}±7.27
G2: Metalaxyl	110.62^{a}±4.29	152.43^{a}±7.73	275.44^{a}±12.64
G3: Metalaxyl + Quercetin	76.18^{b}±3.10	100.35^{b}±5.21	210.35^{b}±9.21
G4: Metalaxyl + Rosmary extract	79.25^{b}±3.33	92.25^{b}±4.33	192.25^{b}±9.33

The data is displayed as Mean ± Standard Error of the Mean (SEM). Means within the same column carrying different superscript letters are significantly different (P≤ 0.05).

The livers of rats intoxicated with metalaxyl displayed a notable up-regulation of the liver Caspase-3 gene expression level and a significant downregulation of Bcl-2, Nrf2 and HO-1 when compared to the control normal group. On the other hand, treatment with quercetin or rosemary extract significantly down-regulate liver Caspase-3 gene and up-regulated Bcl-2, Nrf2, and HO-1 genes expressions compared to the metalaxyl exposed group (Table 3).

Histopathological findings

Histopathological investigation of hepatic tissue of the control group displayed normal histological appearance of central veins, portal areas, and homogenous polyhedral hepatocytes (Fig. 1A). Meanwhile, livers of metalaxyl exposed rats showed various histological alterations in the hepatic parenchyma. Hepatic sinusoids and central veins were seen to be noticeably dilated and congested. The hepatic parenchyma showed presence of scattered foci of hepatic degeneration characterized by discrete clear cytoplasmic vacuoles with peripheral flattened nuclei (fatty degeneration) was noticed in the midzonal and peripheral areas of hepatic lobules (Fig. 1B). Additionally, the hepatocytes displayed severe degrees of degenerative alters in the form of hydropic degeneration, which was mostly detected in all zones of the hepatic lobules and was characterized via swollen pale vacuolated cytoplasm with occasional pyknosis, absence of degenerated hepatocyte nuclei or rarely karyolysis.

Multifocally, perivascular mononuclear inflammatory cell aggregates with Von Kuepfer's cells activations were noted (Fig. 1C, D). Occasionally, small parts of hepatocytes exhibit coagulative necrosis that characterized via preservation of cellular contour with pyknotic nuclei and homogenous, hyper eosinophilic cytoplasm with leukocytic cellular infiltrations primarily lymphocytes (Fig. 1E). The perivascular interstitium was expanded through mononuclear inflammatory cells aggregation (Fig. 1F).

Microscopical investigation of the rats' liver treated by metalaxyl and quercetin showed a slight improvement in their hepatic histological appearance in comparable to the group of rats treated with metalaxyl. With the exception of the portal triad, which revealed the presence of a congested portal vein with slight periductal fibrosis and leukocytic cellular infiltration, the majority of the hepatic tissue showed a modest degree of curing (Fig. 2A). The hepatocyte displayed hydropic degeneration, characterized through enlarged, vacuolated cytoplasm, pale with few pyknotic nuclei which was accompanied by the expansion and Von Kuepfer's cells activation (Fig. 2B). In the meantime, the livers of the rats in the rosemary and metalaxyl group showed a significant restoration of the normal hepatocellular architecture, associated with high regular and less changed hepatocytes comparing with the metalaxyl-exposed rat (Fig. 2C). The hepatocytes in the hepatic lobules' centrilobular zone had a normal histological appearance, but the hepatocytes in the periphero-lobular zones displayed mild hydropic degeneration and occasionally

Table 3. The protective impact of Quercetin or Rosemary extract on the relative expression of Caspase 3, Bcl-2 Nrf2, and HO-1 genes in the liver of rats intoxicated with metalaxyl.

Parameters / Animal groups	Caspase-3 Fold change ±SEM	Bcl-2 Fold change ±SEM	Nrf2 Fold change ±SEM	HO-1 Fold change ±SEM
G1: Normal control	1.00^{d}±0.00	1.00^{a}± 0.00	1.00^{a}±0.00	1.00^{a}±0.00
G2: Metalaxyl	6.15^{a}±0.3	0.04^{d}±0.003	0.26^{c}±0.01	0.05^{d}±0.01
G3: Metalaxyl + Quercetin	4.53^{b}±0.21	0.45^{c}±0.02	0.91^{a}±0.05	0.76^{b}±0.04
G4: Metalaxyl + Rosmary extract	3.20^{c}±0.14	0.67^{b}±0.02	0.62^{b}±0.03	0.32^{c}±0.02

The data is displayed as Mean ± Standard Error of the Mean (SEM). Means within the same column carrying different superscript letters are significantly different (P≤ 0.05).

minor portal inflammatory cellular infiltration (Fig. 2D).

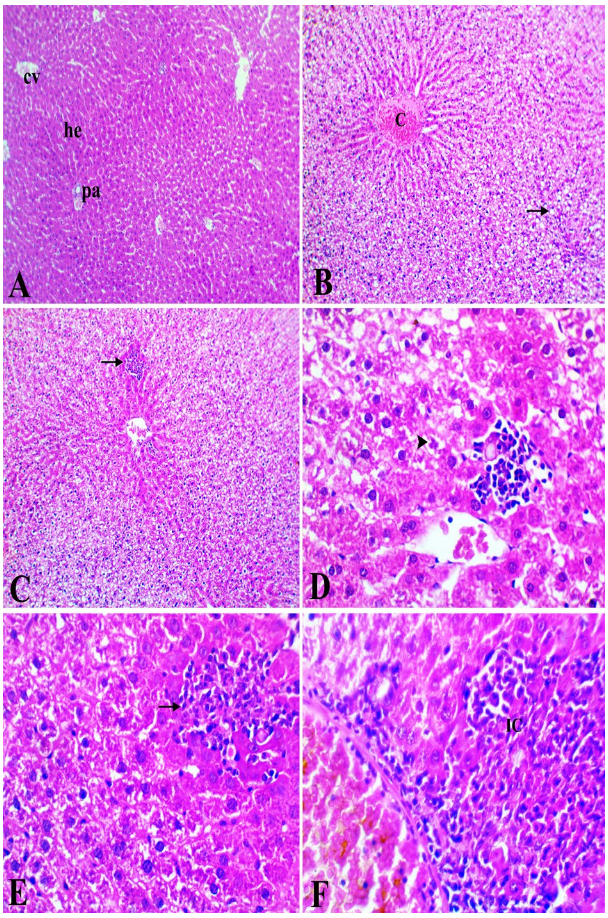

Fig. 1. Liver sections of control (A), and toxic (B- E) groups, H&E. Control group (A), showing normal histological appearance of central veins (cv), portal areas (pa), and hepatocytes (he) (x100). Toxic group (B), showing marked congestion (C) of the central vein and hepatic sinusoids with scattered foci of fatty degeneration (arrow, x100). (C) Diffuse hydropic degeneration of hepatocytes with perivascular mononuclear inflammatory cell aggregates (arrow) between the degenerated hepatocytes (x100). (D) high power of previous figure showing Hepatocyte hydropic degeneration is described by enlarged, pale, vacuolated cytoplasm associated with karyolysis, or by the lack of nuclei (arrowhead, x400). (E) Hepatocyte focal region of coagulative necrosis infiltrated by lymphocytes (arrow, x400). (F) The perivascular interstitium showing aggregates of mononuclear inflammatory cells (IC) (x400).

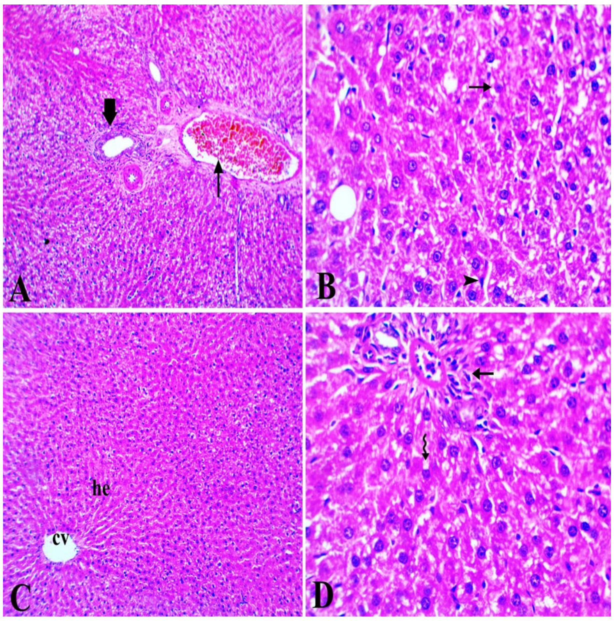

Fig. 2. Liver sections of treated groups with quercetin (A, B) and rosemary (C, D), H&E. (A) Congestion of portal vein (arrow) with mild periductal fibrosis and leukocytic cellular infiltration (thick arrow, x100). (B) Hepatocyte hydropic degeneration is distinguished by enlarged, pale, vacuolated cytoplasm and pyknotic nuclei (arrow) in association with enlargement and initiation of Von Kuepfer's cells (arrowhead, x400). (C) Marked restoration of normal histological appearance of central veins (cv) and hepatocytes (he) (x100). (D) Mild portal inflammatory cell infiltrates (arrow) and mild degree of hepatocyte vacuolation (zigzag arrow, x400).

Discussion

Many pesticides primarily attack cellular components electrophilically while also producing reactive oxygen species (ROS), which leads to the extensive oxidation of biomolecules such as lipids, proteins, and nucleic acids (Zaki, 2012). It has been shown that one of the primary mechanisms underlying pesticide-induced toxicity is the production of oxidative stress. While numerous medications are used to treat people with pesticide intoxication, their application has been restricted due to adverse effects. It makes constant that there is rising interest in determining safeguard naturally antioxidants and determining their ability to reduce pesticide-induced oxidative stress. Flavonoids are important members of the polyphenol family and have potent antioxidant activities (Zeng *et al*., 2021).

The findings of current study's demonstrated that, rats intoxicated with metalaxyl had significantly higher serum ALP, ALT and AST activities in comparison to control group. Similarly, Al-Janabi *et al*. (2023) found that, AST, ALT, and ALP exhibited a greater rise in serum activities due to the toxicity of nano-fipronil pesticides. Additionally, Abolaji *et al*. (2017) suggested that liver damage caused by the co-administration of fungicides or insecticides alone, was indicated by a marked rise in plasma ALT and AST activity. Certain serum biochemical parameter levels are employed as diagnostic evidence of liver injury. The generation of intracellular enzymes, involving ALP, is one of the most dramatic and sensitive signs of hepatocyte injury. The increased activity of these enzymes is an indication of cellular leakage and/or a decline in functional integrity in the liver's cellular membranes both of which are invariably related to hepatic necrosis (Howell *et al*., 2014). Increased AST, ALT and ALP activities were revealed to be associated to liver injury as well as altered hepatic mechanisms (Kanbur *et al*., 2009). This increase may be explained by the enzymes being released from the cytoplasm into the bloodstream, which would indicate inflammation and necrosis (Sidhu *et al*., 2014). Furthermore, the increases of aminotransferases enzymes activities in serum might be related to tissue damage and finally enzymes transfer from the damaged liver tissues into the blood, in addition to enhancing the permeability of cell membranes (Pari and Murugavel, 2004). Whenever a hepatocyte plasma membrane is ruptured, enzymes normally present in the cytosol are generated into the bloodstream. Their evaluation in serum is a useful method for figuring out the nature and extent of hepatocellular injury (Shibabaw *et al*., 2019). Likewise, exposure to other forms of pesticides, as, imidacloprid and chloroquine, was also related to elevate ALT and AST activities (Arfat *et al*., 2014).

The current results exhibited that Quercetin or rosemary extract treatment dramatically lowers ALP, AST, and ALT activities in the serum, suggesting a hepatoprotective effect on the liver of rats exposed to metalaxyl-induced liver damage. In vivo studies have demonstrated the exceptional antioxidant with anti-inflammatory features of the flavonoid quercetin. Moreover, studies have shown that it has cytotoxic, hepatoprotective, antifungal, antioxidant, and anticarcinogenic properties (Batiha *et al*., 2020). Similarly, David *et al*. (2011) demonstrated that quercetin administration to rats intoxicated with thioacetamide for four days resulted in notable decreases in serum activities of AST and ALT as well as effective prevention of morphological changes, El-Shafey *et al*. (2015) also found that giving quercetin daily (15 mg/kg) three weeks before a paracetamol hazardous dose caused significant reduction in AST, ALT and ALP activities. Consequently, it was assumed that quercetin displayed preventive influences against metalaxyl-induced hepatotoxicity in vivo via its antioxidant as well as anti-inflammatory features. Because quercetin diffuses more readily into membranes and can scavenge free radicals at several locations via the lipid bilayer, it may have an antioxidant influence (Moridani *et al*., 2003). Hence, quercetin's protective benefits in adult rats were further supported by its ability to interact with and scavenge several radicals, including peroxyl, hydroxyl, alkoxyl and superoxide. In the same way quercetin acts as antioxidant via its O-dihydroxy structure which provides it the greater stability against free radicals so precipitating them in their delocalized electrons (Padma *et al*., 2012). Also, Hussein *et al*. (2017) stated that, in metalaxyl-exposed rats and received quercetin for 8 weeks, there was a significant reduction in serum activities of ALP, ALT and AST compared to the metalaxyl group. Consequently, it was assumed that quercetin's antioxidant and anti-inflammatory properties prevented metalaxyl-induced hepatotoxicity in vivo. Obvious reduced serum ALT and AST activities were detected in quercetin plus imidacloprid treatment (Hassan *et al*., 2019). The possible protective impact of quercetin on liver function in rats receiving continuous intraperitoneal injection of Clothianidin (CTD) is also observed by Gheshlaghi-Ghadim *et al*. (2022). They found that rats treated with CTD, a pesticide belonging to the neonicotinoid class, had significantly higher serum ALT and AST activities and their activities were decreased significantly in quercetin (10 mg/kg b.wt) + CTD treated animals.

The present study proved that rosemary extract treatment to metalaxyl exposed rats significantly lowered serum ALT, AST and ALP activities. Similarly, Omar (2023) reported significant decline in serum AST and ALT and ALP activities in fish fed 1% rosemary leaf powder suggesting a hepatoprotective effect. Pretreatment with rosemary extract before hepatotoxic azathioprine administration prevent elevated activities of serum ALT and AST in rats. The inhibition of up-regulation in these biomarkers' enzymes suggesting that there was no liver injury, this was confirmed by histopathological findings (Amin and Hamza, 2005). These protective influences were related to the increase levels of antioxidants compounds and free radical fighting properties of rosemary extract (Abdel-Wahhab *et al*., 2011). Also, Shan *et al*. (2015) illustrated the influence of carnosic acid, one of phytochemicals compound of rosemary, on non-alcoholic fatty liver disease progression. They noticed activation of one of the pathways decreasing hepatocyte apoptosis. Rosemary showed positive influences at different stages of liver disease, no matter how advanced development (Al-Attar and Shawush, 2015).

The existing results indicated that, in the liver of rats intoxicated with metalaxyl, there was a notable up-regulation of the liver Caspase-3 gene expression level and a considerable downregulation of Bcl-2, Nrf2 and HO-1 compared to the control group. Similar to this, Li *et al*. (2021) observed that treatment with deltamethrin (DLM) resulted in brain injury by lowering antioxidant defense and mRNA expression of Nrf2 and its downstream proteins levels. Treatment with deltamethrin declined the expression of Bcl-2 gene and increased the level of caspase-3 gene, which in turn caused apoptosis in the cerebrum. According to Yang *et al*. (2021) Nrf2, its target gene product, and HO-1 can be activated in response to a variety of toxins. These responses are indicative of an organism's capacity for an anti-oxidative response against oxidative stress (Wang *et al*., 2020). By reducing the anti-oxidative stress-linked protein or genes, deltamethrin-induced oxidative damage may be the cause of down-regulated Nrf2 as well as HO-1. The organism's ability to defend against oxidative stress was diminished throughout this period, and compensating for the excessive generation of ROS is exciting (Li *et al*., 2020).

Feng *et al*. (2023) stated that, exposure to 30 and 60 μM of organophosphorus triphenyl phosphate (TPhP) led in downregulated protein expression of Bcl-2 as well as caspase-3, with upregulation occurring by 32.4% and 59.9%. Furthermore, Alqahtani and Mahmoud (2016) studied the role of proapoptotic factors in cyclophosphamide (CYC)-induced hepatocyte apoptosis and found elevated levels of caspase-3 and Bax gene protein expression. They reported that in hepatic tissue, CYC enhanced the expression of Bax and Cas-3.

Nuclear factor erythroid 2-related factor 2 signaling pathway is crucial for cellular protection from endogenous and exogenous inflammation and oxidative stress (Wang *et al*., 2022). Excessive ROS levels after fungicide intervention may be enhanced the release of the vital transcription factor Nrf2 and that controls the response of anti-oxidative stress in the tissue (Krajka-Kuźniak *et al*., 2017). Nrf2 translocates to the nucleus and induce the expression of antioxidant enzyme genes involving HO-1and phase II detoxification enzyme genes (Zou *et al*., 2018). Present data display that metalaxyl exposure inhibited the Nrf2 signaling pathway, decreasing antioxidant enzymes activities, involving HO-1.

Elevated oxidative and reduce cellular homeostasis enhanced by metalaxyl exposure linked to elevated apoptosis. Apoptosis is a physiological mechanism of regulated death of cell (Elmore, 2007). These pathways included can be categorized to intrinsic and extrinsic pathway (Morgan *et al*., 2017). Caspase 3 and Bcl2 induce extrinsic/ intrinsic apoptosis pathways (Zou *et al*., 1997), and the last executor in extrinsic pathways is caspase 3 (Falschlehner *et al*., 2007). Our data showed that metalaxyl up-regulated caspase 3 and down-regulated Bcl2 gene expression. These results mean that ROS mediate mitochondrial-dependent pathways included in metalaxyl-induced apoptosis. The involvement of Bcl2 with respect to mitochondrial membrane potential is widely known (Drechsel and Patel, 2008). Also, metalaxyl induced caspase 3 as a repercussion of apoptosis in liver cells. Similarly, Hassan *et al*. (2018) reported that Zineb and metalaxyl fungicid increased Bax levels, inhibited Bcl2 levels, and triggered caspase 3/ caspase 8 activities, which later showed apoptosis through the Bax/Bcl2 and caspase 3/caspase 8 pathway.

Conversely, treatment with Quercetin or rosemary extract on metalaxyl exposed rats displayed notable downregulation of liver Caspase-3 gene expression and up-regulation of Bcl-2, Nrf2 as well as HO-1 gene expression comparing to metalaxyl exposed group. Similarly, Yang *et al*. (2022) demonstrated that quercetin inhibits Caspase-3 activation, hence preventing cell death. In an ischemic brain injury model, quercetin was found to be able to block cell death via decline Caspase-3 activity. Also, Jia *et al*. (2011), described that, quercetin reversed the effects of cadmium on granulosa cells, causing increases in Caspase-3 and Bax levels as well as decreases in Bcl-2 expression. The authors highlighted the potent antioxidant properties of quercetin, which have the ability to inhibit granulosa cell cytotoxicity resulting from cadmium exposure. The current study supports these earlier research findings in the literature by demonstrating that cyclophosphamide (CYC) elevated the expression of Bax and Caspase-3 while quercetin administration dramatically decreased apoptosis in hepatic.

Moreover, rosemary contains the beneficial component carnosic acid (CA). In vitro studies using neonatal rat ventricular cardiomyocytes exposed to doxorubicin (DOX)-induced cardiotoxicity have demonstrated its ability to decrease inflammation and reactive oxygen species. In the rats' hearts, CA significantly reduced pyroptosis and apoptosis responses, which ultimately improved cardiac function. By stimulating Nrf2 and heme HO-1 downstream, CA demonstrated its antioxidant impact. Furthermore, in DOX-induced cardiotoxicity, CA therapy dramatically raised Bcl-2 and prevented caspase-3 cleavage (Hu *et al*., 2023). Carnosic acid (CA) demonstrated its antioxidant activity by initiating HO-1 and Nrf2. Also, by promptly inducing anti-oxidative and phase-2 detoxifying enzymes as well as linked stress-response proteins, Nrf2 is a crucial transcription factor that plays a vital role in cellular defense against oxidative and electrophilic insults (Kim *et al*., 2010). Furthermore, Nrf2 up-regulates HO-1, a significant cytoprotective and anti-inflammatory enzyme. HO-1 is one of the enzymes that Nrf2 upregulates and has strong anti-inflammatory and antioxidative characteristics. Quercetin and rosemary in our data significantly inhibited the metalaxyl-induced liver damage via attenuating different apoptotic markers through increase antioxidant activity by HO-1/Nrf2 pathways and apoptosis regulation.

Histopathological investigation of hepatic tissue of metalaxyl exposed rats showed various histological alterations in the hepatic parenchyma with the presence of scattered foci of hepatic degeneration characterized by discrete clear cytoplasmic vacuoles with peripheral flattened nuclei (fatty degeneration) in the midzonal and peripheral areas of hepatic lobules. Also, hepatic sinusoids and central veins were seen to be noticeably dilated and congested. Additionally, the hepatocytes displayed highly degrees of degenerative changes in the form of hydropic degeneration, which was mostly detected in all zones of the hepatic lobules. Similarly, Lamfon (2011) and Hassan *et al*. (2018) reported that, metalaxyl caused various histopathological changes in the hepatocytes of rats such as marked congestion of intrahepatic blood vessels, tissue impairment and cytoplasmic vacuolization of the hepatocytes. Moreover, the existing study shown that metalaxyl up-regulated caspase 3 and down-regulated Bcl-2 gene expression promoting liver apoptosis. Apoptosis induced by metalaxyl has been reported in hepatocytes of rats (El-Ghonaimy, 2015). Likewise, neuron apoptosis may be associated with the activation of caspase-3 and Bax and the downregulation of Bcl-2 in cerebral ischemia/ reperfusion injury (Liu *et al*., 2013).

Histopathological alters in the livers of treated rats with metalaxyl and quercetin displayed a slight improvement in their hepatic histological appearance compared with metalaxyl treated group. Similarly, Bahar *et al*. (2017) displayed that, administration of quercetin (25 or 50 mg/kg) to Mn-exposed rats displayed improvement of histopathological alteration compared with Mn-treated rats. Also, Sakr and Lamfon (2010) showed that treating animals with metalaxyl followed by antox revealed an improvement in the histological changes observed in animals treated with metalaxyl alone. Who added that, antox treatment leads to a decrease in the percent of apoptotic cells. In current study, livers of the rats in the rosemary and metalaxyl group showed a significant restoration of the normal hepatocellular architecture. These results coincided with that recorded by Morsi *et al*. (2024) who reported that, liver of the rats treated with Etoposide chemotherapy with rosemary group displayed a significant improvement as a majority of hepatocytes revealed normal appearance. The generation of free radicals is highly responsible for the massive degenerative changes produced by metalaxyl. The protective influences of quercetin and rosemary extract were also verified by histopathological examination, demonstrating that, they had the potential to scavenge free radicals. Similarly, Al-Attar and Shawush, (2015) recorded that, administering of rosemary extract before or during thioacetamide therapy improvement of the histological picture of the liver in rats.

Conclusion

Quercetin and rosemary extract are potent antioxidants that inhibit the generation of reactive oxygen species (ROS), alleviate oxidative stress caused by metalaxyl, and protect the liver via activating the Nrf2/HO-1 pathway. Also, Quercetin and rosemary extract has strong anti-apoptotic and anti-inflammatory properties by suppress caspase 3 mediated activation of the Bcl-2/Nrf2 signaling.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

Abdel-Wahhab, K.G., El-Shammy, K.A., El-Beih, N.A., Morcy, F.A., Mannaa, F.A., 2011. Protective effect of a natural herb (*Rosmarinus officinalis*) against hepatotoxicity in male albino rats. Comunicata Sci. 2, 9-17. DOI: https://doi.org/10.14295/cs.v2i1.82

Abdel-Gawad, H., Soliman, M.S., Hamdy, T., Sayed Aly, M.A., Monira, A.A., Bahira, H., 2021. Pathogenic Effects of Ethion Residues and the Expected Protective Role of the Ethanolic Extract of Rosemary (*Rosmarinus officinalis* L.) Leaves in Male Rats. Egypt. J. Chem. 64, 1817 – 1829. DOI: 10.21608/EJCHEM.2021.59950.3282

Abolaji, A.O., Awogbindin, I.O., Adedara, I.A., Farombi, E.O., 2017. Insecticide chlorpyrifos and fungicide carbendazim, common food contaminants mixture, induce hepatic, renal, and splenic oxidative damage in female rats. Human and Experimental Toxicology 36, 483-493. doi:10.1177/0960327116652459

Akela, M.A., El-Atrash, A., El-Kilany, M.I., Tousson, E., 2018. Qualitative and quantitative characterization of biologically active compounds of Rosemary (*Rosmarinus officinalis*) Leaf Extract. Journal of Advanced Trends in Basic and Applied Science 2, 59-64.

Al-Attar, A.M., Shawush, N.A., 2014. Physiological investigations on the effect of olive and rosemary leaves extracts in male rats exposed to thioacetamide. Saudi J. Biol. Sci. 21, 473-480. doi:10.1016/j.sjbs.2014.08.004

Al-Attar, A.M., Shawush, N.A., 2015. Influence of olive and rosemary leaves extracts on chemically induced liver cirrhosis in male rats. Saudi J. Biol. Sci. 22, 157-163. https://doi.org/10.1016/j.sjbs.2014.08.005

Al-Attar, A.M., 2015. Effect of grapeseed oil on diazinon-induced physiological and histopathological alterations in rats." Saudi Journal of Biological Sciences 22, 284-292. doi: 10.1016/j.sjbs.2014.12.010.

Al-Janabi, A.A.Q., Hamed, L.N., Ibraheem, K.A., 2023. Effects of Nano-Fipronil on Male Rats' Biochemical, Liver, and Renal Functions. IOP Conf. Ser.: Earth Environ. Sci. 1259, 012033. DOI 10.1088/1755-1315/1259/1/012033

Alqahtani, S., Mahmoud, A.M., 2016. Gamma-glutamylcysteine ethyl ester protects against cyclophosphamide-induced liver injury and hematologic alterations via upregulation of ppary and attenuation of oxidative stress, inflammation, and apoptosis. Oxid. Med. Cell Longev. 2016,4016209. doi:10.1155/2016/4016209

Amin, A., Hamza, A.A., 2005. Hepatoprotective effects of Hibiscus, Rosmarinus and Salvia on azathioprine-induced toxicity in rats. Life Sci. 77, 266-278. doi: 10.1016/j.lfs.2004.09.048.

Arfat, Y., Mahmood, N., Tahir, U. M., Rashid, M., Anjum , S., Zhao, F., Li, D., Yu-Long S., Lifang Hu, L., Zhihao, Yin, C., Shang, P., Ai-Rong, Q., 2014. Effect of imidacloprid on hepatotoxicity and nephrotoxicity in male albino mice. Toxicology Reports 1, 554–561. https://doi.org/10.1016/j.toxrep.2014.08.004

Bahar, E., Ji-Ye Kim, J.Y., Yoon, H., 2017. Quercetin Attenuates Manganese-Induced Neuroinflammation by Alleviating Oxidative Stress through Regulation of Apoptosis, iNOS/NF-kB and HO-1/Nrf2 Pathways. International Journal of Molecular Sciences 18, 1-19. doi: 10.3390/ijms18091989

Bancroft, J.D., Gamble, M., 2008. Theory and Practice of Histological Techniques. 6th Edition, Churchill Livingstone, Elsevier, China.

Bao, D., Wang, J., Pang, X., Liu, H., 2017. Protective effect of quercetin against oxidative stress-induced cytotoxicity in rat pheochromocytoma (PC-12) cells. Molecules 22, 1122. doi: 10.3390/molecules22071122.

Batiha, G.E., Beshbishy, A.M., Ikram, M., Mulla, Z.S., El-Hack, M.E.A., Taha, A.E., Algammal, A. M., Elewa, Y. H. A., 2020. The Pharmacological Activity, Biochemical Properties, and Pharmacokinetics of the Major Natural Polyphenolic Flavonoid: Quercetin. Foods 9, 374. doi: 10.3390/foods9030374.

David, C., Rodrigues, G., Bona, S., Meurer, L., González-Gallego, J., Tuñón, M.J., Marroni, N.P., 2011. Role of Quercetin in Preventing Thioacetamide-Induced Liver Injury in Rats. Toxicol. Pathol. 39, 949-957. DOI: 10.1177/0192623311418680

Ding. F., Li, X.N., Diao, J.X., Sun, Y., Zhang, L., Sun, Y., 2012.Chiral recognition of metalaxyl enantiomers by human serum albumin: evidence from molecular modeling and photophysical approach. Chirality 24, 471-480. doi:10.1002/chir.22024.

Drechsel, D.A., Patel, M., 2008. Role of reactive oxygen species in the neurotox¬icity of environmental agents implicated in Parkinson's disease. Free Radic. Biol. Med. 44,1873–1886. doi: 10.1016/j.freeradbiomed.2008.02.008.

Elmore, S., 2007. Apoptosis: A review of programmed cell death. Toxicol. Pathol. 35, 495–516.

El-Ghonaimy, N., 2015. Role of ginger (zingiber officinale) against metalaxyl induced hepatotoxicity in male albino rats: a histological and immunohistochemical study. J. Histol. Histopathol. 2, 1-9. doi:10.7243/2055-091X-2-9.

El-Shafey, M.M., Abd-Allah, G.M., Mohamadin, A.M., Harisa, G.I., Mariee, A.D., 2015. Quercetin protects against acetaminophen-induced hepatorenal toxicity byreducing reactive oxygen and nitrogen species. Pathophysiol. 22, 49–55. DOI: 10.1016/j.pathophys.2014.12.002

Elzoghby, R.R., Hamuoda, A.F., Abdel-Fatah, A., Farouk, M., 2014. Protective role of vitamin c and green tea extract on malathion-induced hepatotoxicity and nephrotoxicity in rats. American Journal of Pharmacology and Toxicology 9, 177-188. DOI: https://doi.org/10.3844/ajptsp.2014.177.188`

Falschlehner, C., Emmerich, C.H., Gerlach, B., Walczak, H., 2007. TRAIL signal¬ing: decisions between life and death. Int J Biochem Cell Biol. 39, 1462–1475. doi: 10.1016/j.biocel.2007.02.007

Feng, Y., Shi, J., Li, M., Duan, H., Shao, B., 2023. Evaluation of the cytotoxic activity of triphenyl phosphate on mouse spermatocytes cells. Toxicol. *In Vitro* 90,105607. doi: 10.1016/j.tiv.2023.105607.

Gheshlaghi-Ghadim, A., Mohammadi, V., Zadeh-Hashem, E., 2022. Protective Effects of Quercetin on Clothianidin-Induced Liver Damage in the Rat Model. Evid Based Complement Alternat Med. 2022, 9399695. doi:10.1155/2022/9399695

Hassan, M.F., Hussein, S., Senosi, Y., El- Mansour, M. K., Amin, A., 2018. Role of Ginger as Anti-inflammatory and Anti-apoptotic in Protection of Liver Damage Induced by Metalaxyl Fungicide in Male Albino Rats. J. Clin. Exp. Pathol. 8, 346. doi:10.4172/2161-0681.1000346.

Hassan, A.M.S., Abo El-Ela, F.I., Abdel-Aziz, A.M., 2019. Investigating the potential protective effects of natural product quercetin against imidacloprid-induced biochemical toxicity and DNA damage in adults rats. Toxicol. Rep. 6, 727-735. doi:10.1016/j.toxrep.2019.07.007

Howell, B.A., Siler, S.Q., Shoda, L.K.M., Yang, Y., Woodhead, J.L., Watkins, P.B., 2014. A mechanistic model of drug-induced liver injury AIDS the interpretation of elevated liver transaminase levels in a phase I clinical trial. CPT Pharmacometrics Syst Pharmacol. 3, 1-8. DOI: 10.1038/psp.2013.74

Hu, S., Liu, B., Yang, M., Mao, S., Ju, H., Liu, Z., Huang, M., Wu, G., 2023. Carnosic acid protects against doxorubicin-induced cardiotoxicity through enhancing the Nrf2/HO-1 pathway. Food Funct. 14, 3849-3862. doi: 10.1039/d2fo03904d.

Hussein, S.A., El Senosi, Y.A., Mansour, M.K., Hassan, M.F., 2017. Potential protective effects of Quercetin on metalaxyl-induced oxidative stress, impaired liver functions and hepatotoxicity in rat. Benha Vet. Med. J. 33, 517-532. . doi: 10.21608/BVMJ.2017.30600

Indap, M.A., Bhosle, S.C., Shinde, A.D., Barkume, M.S., Ingle, A.D., 2006. Tumour response to quercetin, a bioflavonoid with some promises in therapies. Indian J Pharm Sci 68, 570–574.

Jia, Y., Lin, J., Mi, Y., Zhang, C., 2011. Quercetin attenuates cadmium-induced oxidative damage and apoptosis in granulosa cells from chicken ovarian follicles. Reprod Toxicol. 31, 477–485. doi: 10.1016/j.reprotox.2010.12.057.

Kanbur, M., Eraslan, G., Silici, S., Karabacak, M., 2009. Effects of sodium fluoride exposure on some biochemical parameters in mice: Evaluation of the ameliorative effect of royal jelly applications on these parameters. Food Chem. Toxicol. 47, 1184–1189. DOI: 10.1016/j.fct.2009.02.008

Kim, J., Cha, Y.N., Surh, Y.J., 2010. A protective role of nuclear factor-erythroid 2-related factor-2 (Nrf2) in inflammatory disorders. Mutat Res. 690, 12-23. doi:10.1016/j.mrfmmm.2009.09.007

Krajka-Kuźniak, V., Paluszczak, J., Baer-Dubowska, W., 2017. The Nrf2-ARE signaling pathway: an update on its regulation and possible role in cancer prevention and treatment. Pharmacol. Rep. 69, 393–402. doi: 10.1016/j.pharep.2016.12.011.

Lamfon, H., 2011. Protective effect of ginger (*Zingiber officinale*) against metalaxyl induced hepatotoxicity in albino mice. American J. Sci. 7, 1093- 1100.

Li, J., Jiang, H., Wu, P., Li, S., Han, B., Yang, Q, Wang, X., Han, B., Deng, N., Qu, B., Zhang, Z., 2021. Toxicological effects of deltamethrin on quail cerebrum: Weakened antioxidant defense and enhanced apoptosis. Environ Pollut. 286, 117319. doi: 10.1016/j.envpol.2021.117319.

Li, S.Y., Jiang, H.J., Han, B., Kong, T., Lv, Y.Y., Yang, Q.Y., Wu, P.F., Lv, Z.J., Zhang, Z.G., 2020. Dietary luteolin protects against renal anemia in mice. J. Funct. Foods 65, 103740. https://doi.org/10.1016/j.jff.2019.103740.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25, 402-408. doi:10.1006/meth.2001.1262

Liu, G., Wang, T., Wang, T., Song, J., Zhou, Z., 2013. Effects of apoptosis-related proteins caspase-3, Bax and Bcl-2 on cerebral ischemia rats. Biomed Rep. 1, 861-867. doi:10.3892/br.2013.153

Lozano-Paniagua, D., Parrón, T., Alarcón, R., Requena, M., López-Guarnido, O., Lacasaña, M., Hernández, A.F., 2021. Evaluation of conventional and non-conventional biomarkers of liver toxicity in greenhouse workers occupationally exposed to pesticides. Food Chem. Toxicol. 151,112127. DOI: 10.1016/j.fct.2021.112127

Morgan, A.M., Ibrahim, M.A., Hussien, A.M., 2017. The potential protective role of Akropower against Atrazine- induced humoral immunotoxicity in rabbits. Biomed. Pharmacother. 96, 710–715. doi: 10.1016/j.biopha.2017.10.028.

Moridani, M., Pourahmad, J., Bui, H., Siraki, A., O'Brien, P., 2003. 'Dietary flavonoid iron complexes as cytoprotective superoxide radical scavengers', Free Radic. Biol. Med. 34, 245-253. DOI: 10.1016/s0891-5849(02)01241-8

Morsi, R.M., Mansour, D.S., Mousa, A.M., 2024. Ameliorative potential role of *Rosmarinus officinalis* extract on toxicity induced by etoposide in male albino rats. Brazilian Journal of Biology 84, e258234.1-13. doi.org/10.1590/1519-6984.258234.

Omar, S.S., 2023. Evaluation of dietary rosemary leaf powder on growth, carcass composition and hemato-biochemical profiles of common carp (*Cyprinus carpio*) reared in cage system. Cell Mol Biol (Noisy-le-grand). 69,141-148. doi: 10.14715/cmb/2023.69.11.21.

Padma, V.V., Lalitha, G., Shirony, N.P., Baskaran, R., 2012. Effect of quercetin against lindane induced alterations in the serum and hepatic tissue lipids in wistar rats. Asian Pac J. Trop. Biomed. 2, 910-5. doi: 10.1016/S2221-1691(12)60252-4.

Pari, L., Murugavel, P., 2004. Protective effect ofα-lipoic acid against chloroquine-induced hepatotoxicity in rats. Journal of Applied Toxicology 24, 21–26 https://doi.org/10.1002/jat.940

Pattanasupong, A., Nagase, H., Sugimoto, E., Hori, Y., Hirata, K., Tani, K., Nasu, M., Miyamoto, K., 2004. Degradation of carbendazim and 2,4-dichlorophenoxyacetic acid by immobilized consortium on loofa sponge. J. Biosci. Bioeng. 98, 28-33. doi: 10.1016/S1389-1723(04)70238-8.

Sakr, S.A., Lamfon, H.A., 2005. Effect of green tea on metalaxyl fungicide induced liver injury in albino mice. Oxford Research Forum Journal 2, 65-69.

Sakr, S.A., Lamfon, H.A., 2010. Metalexyl fungicide induced oxidative stress and apoptosis in mouse thymus: the effect of antox. International Journal of Immunological Studies 1, 135-149. https://doi.org/10.1504/IJIS.2010.034897

Schumann, G., Klauke, R., 2003. New IFCC reference procedures for the determination of catalytic activity concentrations of five enzymes in serum: preliminary upper reference limits obtained in hospitalized subjects. Clin Chim Acta 327, 69-79. doi:10.1016/s0009-8981(02)00341-8

Shan, W., Gao, L., Zeng, W., Hu, Y., Wang, G., Li, M., Zhou, J., Ma, X., Tian, X., Yao, J., 2015. Activation of the SIRT1/p66shc antiapoptosis pathway via carnosic acid-induced inhibition of miR-34a protects rats against nonalcoholic fatty liver disease. Cell Death Dis. 6, e1833. doi: 10.1038/cddis.2015.196.

Shibabaw, T., Dessie, G., Molla, M.D., Zerihun, M.F., Ayelign, B., 2019. Assessment of liver marker enzymes and its association with type 2 diabetes mellitus in Northwest Ethiopia. BMC Res Notes 12, 707-715. doi:10.1186/s13104-019-4742-x

Sidhu, I.P.S., Bhatti, J.S., Bhatti, G.K., 2014. Modulatory action of melatonin against chlorpyrifos induced hepatotoxicity in Wistar rats. Asian J. Med. Sci. 2, 123-131.

Smart, E., Lopes, F., Rice, S., Nagy, B., Anderson, R.A., Mitchell, R.T., Spears, N., 2018. Chemotherapy drugs cyclophosphamide, cisplatin and doxorubicin induce germ cell loss in an in vitro model of the prepubertal testis. Sci Rep. 8, 1773. doi:10.1038/s41598-018-19761-9

Sukul, P., Spiteller, M., 2000. Metalaxyl: persistence, degredation, metabolism and analytical methods. Reviews of Environmental and Contamination Toxicology 164,1-26.

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, 751–761.

Tousson, E., Masoud, A., Hafez, E., Almakhatreh, M., 2019. Protective role of rosemary extract against Etoposide induced liver toxicity, injury and KI67 alterations in rats.Journal of Bioscience and Applied Research 5, 1-7. doi:10.21608/JBAAR.2019.105884

United States Environmental Protection Agency (UEPA), 1994. R.E.D. FACTS Metalaxyl., Prevention Pesticides And Toxic Substances (7508W). EPA- 738-F-94-013.

Wang, J., Qian, X., Gao, Q., Lv, C., Xu, J., Jin, H., Zhu, H., 2018. Quercetin increases the antioxidant capacity of the ovary in menopausal rats and in ovarian granulosa cell culture in vitro. J. Ovarian Res. 11, 51. doi: 10.1186/s13048-018-0421-0.

Wang, Q., Yang, Z., Zhuang, J., Zhang, J., Shen, F., Yu, P., Zhong, H., Feng, F., 2022 Antiaging function of Chinese pond turtle (Chinemys reevesii) peptide through activation of the Nrf2/Keap1 signaling pathway and its structure-activity relationship. Front Nutr. 9, 961922. doi: 10.3389/fnut.2022.961922.

Wang, X.Q., Han, B., Wu, P.F., Li, S.Y., Lv, Y.Y., Lu, J.J., Yang, Q.Y., Li, J.Y., Zhu, Y., Zhang, Z.G., 2020. Dibutyl phthalate induces allergic airway inflammation in rats via inhibition of the Nrf2/TSLP/JAK1 pathway. Environ. Pollut. 267, 115564. https://doi.org/10.1016/j.envpol.2020.115564.

Yang, R., Shen, Y.J., Chen, M., Zhao, J.Y., Chen, S.H., Zhang, W., 2022. Quercetin attenuates ischemia reperfusion injury by protecting the blood-brain barrier through Sirt1 in MCAO rats. J. Asian Nat. Prod Res. 24, 278–289. doi: 10.1080/10286020.2021.1949302.

Yang, Q.Y., Han, B., Li, S.Y., Wang, X.Q., Wu, P.F., Liu, Y., Li, J.Y., Han, B.Q., Deng, N., Zhang, Z.G., 2021. The link between deacetylation and hepatotoxicity induced by exposure to hexavalent chromium. J. Adv. Res. 35,129-140. doi: 10.1016/j.jare.2021.04.002.

Yin, J., Zhu, F., Hao, W., Xu, Q., Chang, J., Wang, H., Guo, B., 2017. Acylamino acid chiral fungicides on toxiciepigenetics in lambda DNA methylation. Food Chem. Toxicol. 109, 735-745. doi:10.1016/j.fct.2017.04.038

Zaki, N.I., 2012. Evaluation of profenofos intoxication in white rats. Nat. Sci 10, 67-77.

Zeng, X., Du, Z., Ding, X., Jiang, W., 2021. Protective effects of dietary flavonoids against pesticide-induced toxicity: A review. Trends in Food Science andTechnology 109, 271–279. DOI:10.1016/J.TIFS.2021.01.046

Zheng, F., Gonçalves, F.M., Abiko, Y., Li, H., Kumagai, Y., Aschner, M., 2020. Redox toxicology of environmental chemicals causing oxidative stress. Redox Biol. 34, 101475. https://doi.org/10.1016/j.redox.2020.101475.

Zou, H., Henzel, W.J., Liu, X., Lutschg, A., Wang, X., 1997. Apaf1, a human protein homologous to C. elegans CED-4, participates in cytochrome C-dependent activation of caspase-3. Cell 90, 405–413. doi: 10.1016/s0092-8674(00)80501-2

Zou, B., Xiao, G., Xu, Y., Wu, J., Yu, Y., Fu, M., 2018. Persimmon vinegar polyphenols protect against hydrogen peroxide-induced cellular oxidative stress via Nrf2 signalling pathway. Food Chem. 255, 23–30. doi: 10.1016/j.foodchem.2018.02.028.