

Original Research

Hepatoprotective Effect of Quercetin Nanoparticles Against Fipronil Toxicity in Adult Male Rats.

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ABSTRACT

Fipronil (FPN), a phenyl pyrazole insecticide, has gotten a lot of attention recently because of its toxicity in mammals, mostly mediated by its effects on the antioxidant system. Quercetin is a flavonoid present in many fruits and vegetables that have been demonstrated to have a variety of beneficial biological effects, including anti-inflammatory and antioxidant properties. Nanoparticles of quercetin have been shown to improve its solubility and stability, allowing for improved absorption, cellular absorption, and lower toxicity. The present study was undertaken to explore the prophylactic effect of quercetin nanoparticles (QueNPs) against sub-chronic FPN-induced hepatic injury and oxidative stress in male rats. Experimental animals were orally gavaged for four weeks. It has been shown that FPN elevated the sera of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) whereas serum total protein level was decreased. FIP also caused histological changes in hepatic tissue. The QueNPs counteracted the hepatotoxic effect of FPN exposure.

Keywords: Fipronil; Quercetin; Nanoparticles; Hepatotoxicity; Histopathology; Liver function.

INTRODUCTION

Large-scale anthropogenic activities have primarily resulted in environmental contamination. Pesticides have been connected to the diminishing health of non-target species ranging from frogs to mammals all over the world due to increased chemical persistence in soil and water (Karthek and David, 2018). FPN [5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-fluoromethylsulfinyl pyrazole] is a second-generation phenylpyrazole insecticide that has been causing public health issues since the mid-twentieth century (Elgawish et al., 2019). In insects, fipronil and its main metabolite, fipronil sulfone, block GABA receptors. The chemical interferes with GABA (-aminobutyric acid inhibitor's) function in the central nervous system (CNS) by preventing it from attaching to its receptor, causing neuronal hyperexcitation at low doses and insect paralysis and death at high doses (Koslowski et al., 2020). Despite its positive benefits, excessive use of FPN in high quantities can be detrimental to animals (Elazab et al., 2021). Fipronil's toxicity is based on a disturbance of the antioxidant/oxidant system balance in cells, particularly liver tissue, induced by an excess of reactive oxygen species (ROS) (Wasef et al., 2021). It is

thought to be the most important organ for metabolism and links the digestive tract to the rest of the body (Elgawish et al., 2019).

Quercetin (Que) is a flavonoid found in various fruits, vegetables, herbs, and allied goods, such as apples, onions, *Ginkgo biloba*, and red wine. Que's pharmacological benefits, including anti-tumor, anti-inflammatory, antioxidant, and hepatoprotective properties, have been widely studied (Wu et al., 2008). This molecule has a more potent antioxidant activity than the well-known antioxidant compounds ascorbyl, Trolox, and rutin. The quantity and location of free hydroxyl groups in the Que molecule account for this activity (Kumari et al., 2010). Despite its broad pharmacological activities, Que's usage in the pharmaceutical industry is limited due to its low water solubility.

Furthermore, It's chemically unstable, especially in alkaline aqueous conditions, which might be related to hydroxyl ion attack on quercetin's C-ring (Zhang et al., 2008). As a result, nanoparticles are especially beneficial in the delivery of water-insoluble drugs (Wu et al., 2008). Therefore, our investigation was carried out to examine the ameliorative

effect of Que nanoparticles (QueNPs) against FPN-induced hepatotoxicity in male rats.

MATERIALS AND METHODS

Chemicals Fipronil (FPN) was purchased from Sigma-Aldrich Co., USA. Quercetin was purchased from Sigma-Aldrich Co., USA. Quercetin nanopowder was carried out in Naqaa Foundation, Giza, Egypt. Special diagnostic kits for ALT and AST were purchased from Diamond Company (Heliopolis, Cairo, Egypt). Total protein and albumin assay kits were purchased from BioSystems Company (Cairo, Egypt). The experiments were conducted following the manufacturer's instruction guidelines of supplied kits. **Animals and Ethical Approval** The experimental procedures and all animal treatments were approved by the Institutional Animal Ethics Committee of the Faculty of Veterinary Medicine Animal Ethics Committee, Alexandria University, with Number: 0106672 **Animals and Experimental design:** Thirty-two adult male albino rats (weighting 190 ± 10 g) were obtained from Medical Research Institute, Alexandria University, Alexandria, Egypt. The rats were kept in a regulated dark-light cycle (12/12 hours) at $21 \pm 2^\circ\text{C}$ ambient temperature and $60 \pm 2\%$ relative humidity. To satisfy the rat's nutritional requirements in maintenance, free access to water and a regular rodent meal were provided (Reeves et al., 1993). After a week of adaptation, rats were randomly allocated into four equal groups (8 rats each). The first group served as the control (CTR). The second group (FPN) received FPN at a dose of 5 mg/kg b.wt. orally (1/5 the LD50) (Gupta and Anadón, 2018) by stomach tube 6 days a week for 28 days, the entire experimental period. The third group (QueNPS) was given an aqueous solution of QueNPS at a dose of 30 mg /kg b.wt. orally (Rifaai et al., 2020) by stomach tube 6 days a week. As mentioned above, the fourth group (FPN + QueNPs) received FPN and QueNPs. **Blood and tissue sampling** On the 28th day of the experiment, all rats were euthanized under an anesthesia system containing xylazine and ketamine HCl. The aortic vein was used to collect blood. The serum was isolated and kept at -20°C until liver function assessment. Immediately, the animals were authenticated, and the liver was excised and rinsed using physiological saline (NaCl 0.9%), wiped using filter paper, and kept for histopathological examination after fixing in paraformaldehyde 4% diluted in phosphate buffer saline (PBS) solution. **Serum biochemical studies** ALT and AST, total protein were estimated using diagnostic kits according to the manufacturer's instruction guidelines

Hepato-histological examination The fixed samples were treated using the traditional paraffin embedding method, including dehydration in alcohol and clearing from fat in xylol, and melted paraffin finished by inserting it at 65°C in paraffin wax for 24 hrs. Paraffin bees wax tissue blocks were prepared for sectioning at $5 \mu\text{m}$ by slide microtome. The obtained tissue sections were collected on glass slides, deparaffinized (xylene), and stained with hematoxylin and eosin stains for

histopathologic. A Leica microscope was used to do the histological investigation. Examination (Leica DM500, Leica, Germany) with a digital camera (Leica EC3, Leica, Germany).

RESULTS

During the entire experimental period, no evident clinical indications or symptoms were observed in FPN or/and QueNPs-treated rats.

Serum biochemical findings

Liver function data, including serum ALT, AST, and TP of adult male rat in all experimental groups, are illustrated in Table (1). Compared to control, there was a significant increase in serum ALT and AST (≈ 2.2 , 3.9 -folds, respectively) with a significant decline in serum TP (86.5%) of the FPN-treated rats. Meanwhile, there was no substantial change in QueNPs rats' serum ALT and AST activities and TP content (≈ 107.9 , $97,101\%$, respectively). The combined treatment of FPN + QueNPs was markedly decreased serum ALT and AST enzymes activity (≈ 0.37 and 0.63 - fold-decrease) without significant change in total protein content ($\approx 101.5\%$).

Histopathological investigation of hepatic tissue.

Figure (1) exhibited histopathological changes in the liver after treatment with FPN, QueNPs, or their combination. The CTR and QueNPs-treated rats showed typical hepatocytes architecture. The liver section of the FPN-treated rats showed diffuse granular hepatic vacuolation associated with foci of mononuclear cells infiltration consisting primarily of macrophages. However, FPN + QueNPs treated group showed a marked decrease in hepatic vacuolation around the portal area

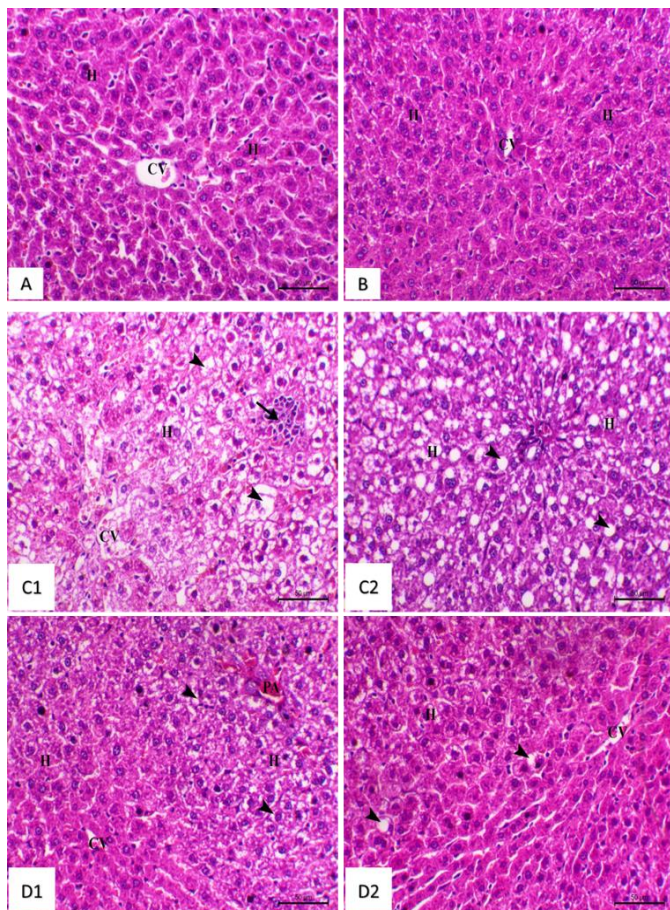


Figure (1) A: control saline showed normal histology of hepatic tissues B: QUENPs treated group presented normal hepatocytes C: FPN treated group showed diffuse granular hepatic vacuolation (arrowheads) associated with foci of with mononuclear cells infiltration mainly consisted of macrophages (arrow) D: FPN+QUENPs treated group exhibited marked decrease of hepatic vacuolation around the portal area (arrowheads) (H letters indicate hepatocytes, PA area indicates portal and CV indicates central vein), (H&E stain), $\times 200$, bar= 50 μm

DISCUSSION

Because the liver is a highly perfused organ with a large blood volume, it is the major target organ for FPN poisoning. It also aids in the metabolism and elimination of this insecticide. (AlBasher et al., 2020) The liver is critical in the biotransformation of insecticides, resulting in chemically-induced hepatic damage and oxidant-antioxidant system disruptions. (Abdel-Daim et al., 2018) Transaminases (AST and ALT) are enzymes that can be used to identify liver disease, with ALT serving as the gold standard (Mitchell, 2016). Higher circulating levels of these enzymes might be related to disrupted enzyme production and/or dysregulated hepatic membrane permeability (Goel et al., 2005). increased blood levels of these enzymes may indicate pathologic liver disorders, which decrease total protein synthesis (Badgujar et al., 2015). Increased AST and ALT levels in the blood indicate a loss of biochemical and structural integrity in the liver and

are the most often used diagnostic tool for determining hepatocellular injury (Kartheek and David, 2018).

The current study explained a remarkable increase in serum ALT, AST, and decrease in TP of FPN-treated animals compared with CTR, QueNPS, and FPN+QueNPS groups. The significant changes in liver enzymes might be due to the oxidative stress induced by FPN, leading to hepatocytes damage, decreased blood protein production, and leakage of its enzymes into the circulation. Elgawish et al. (2019) also demonstrated an increase in enzymes of the liver. (AlBasher et al., 2020) opined similar changes in treated adult rats as the elevated levels of these enzymes might be related to a change in the permeability of the hepatic membrane and/or a disruption in their production. Our results of liver biomarkers were confirmed with histopathological examination. As there was marked injury in the liver tissue of FPN treated group that appeared as diffuse hepatocytic cytoplasmic vacuolation and presence of mononuclear cell infiltration foci, this obvious cellular degeneration may be due to the free radicals induced by FPN either ROS or RNS causing a disturbance in the mitochondrial oxidative phosphorylation and ATP exhaustion.

ROS attacks biological components, forming peroxy radicals that are then cyclized to form endoperoxides (Abd Abd Eldaim et al., 2020). FPN promotes oxidative stress and cellular DNA damage in vitro and in vivo, according to several studies (Sayed et al., 2019).

There was a marked decrease in hepatic vacuolation around the portal area in FPN + QueNPS treated group. These findings might be explained by QueNPS' capacity to interact with and scavenge free radicals created by FPN, such as hydroxyl, superoxide, alkoxy, and peroxy radicals. As a result of the QueNPs therapy, the liver enzymes ALT and AST were reduced. (de David et al., 2011) observed the same result and recorded the protective effect of quercetin against thioacetamide (TAA) induced liver injury and decreased ALT and AST enzymes. (Chen, 2010) also found a significant reduction in ALT and AST enzymes with the use of quercetin in a rat model of the ethanol-induced hepatic lesion.

Que is claimed to contain a number of important cytoprotective qualities, including suppressing pro-inflammatory cytokine production, preventing lipid peroxidation, and reducing reactive oxygen species levels (i.e., significant antioxidant actions) (anti-inflammatory effect) (Kampa et al., 2021)

Table 1: Effect of oral administration of fipronil and/or quercetin nanoparticles on serum ALT, AST, and total protein.

Parameters	Experimental groups			
	CTR	QueNPS	FPN	FPN+QUENPS
ALT (U/L)	44.77 ±2.58 ^{cf}	48.32 ±2.82 ^c	99.07 ±3.56 ^a	62.64 ±3.37 ^b
AST (U/L)	63.00 ±2.25 ^c	61.17 ±3.20 ^c	245.67 ±16.06 ^a	91.00 ±3.36 ^b
TP (g/dL)	6.75 ±0.29 ^a	6.82 ±0.33 ^a	5.84 ±0.22 ^c	5.93 ±0.24 ^c

Data are expressed as the mean ± SE (n = 8). Different superscript letters in the same row indicate statistical significance at P ≤ 0.05. Fipronil (FPN) at a dose of 5 mg/kg, quercetin nanoparticles (QueNPS) at a dose of 30 mg/kg, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP).

CONCLUSION

Our findings demonstrate that FPN subchronic exposure exerts hepatotoxic effects on the rat liver. QueNPs could reverse these events at the optimal dose of 30 mg/kg BW. The promising hepatoprotective activities of QueNPs have been confirmed via the enhancement of liver biomarkers and improvement of histopathologic imaging via their free radical scavenging and antioxidant characteristics.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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