

BEE POLLEN ALLEVIATES FIPRONIL AND EMAMECTIN BENZOATE INDUCED-HEPATORENAL TOXICITY IN RATS

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ABSTRACT

Extensive use of pesticide has contributed to environmental pollution and adverse health effects to animals and humans. Fipronil (FIP) and emamectin benzoate (EMB), relatively new and widely used insecticides, are potent environmental toxicants. Therefore, the current study aimed to investigate the protective role of bee pollen suspension (BPS) against FIP- and EMB- induced hepato-renal toxicity in rats. Rats were randomly allocated into six equal groups and treated orally, 3 times weekly for 8 weeks; control group (distilled water), BPS group (120 mg/kg), FIP group (9.7 mg/kg), EMB group, (8.8 mg/kg), BPS+FIP group (BPS one h before FIP) and BPS+EMB group (BPS one h before EMB). The results revealed that FIP and EMB significantly increased serum ALT, AST activities; urea and creatinine levels. Also, there were substantial increases in the liver and kidney malondialdehyde level, reduced glutathione content and catalase activity. Moreover, FIP and EMB caused histopathological alterations in liver and kidney tissues. However, pretreatment with BPS one hour either before FIP or EMB administration ameliorated all the biochemical and histopathological changes indicating its antioxidant properties. In conclusion, BPS can be used as a new preventive supplement against toxic insults of FIP and EMB.

Key words: Fipronil, Emamectin benzoate, Bee pollen, Histopathology, Antioxidant.

INTRODUCTION

Pesticides including insecticides, fungicides, herbicides, rodenticides, and others, are being used extensively throughout the world for controlling agricultural, industrial, domestic and public health pests (Bjorling-Poulsen *et al.*, 2008). The injudicious use of pesticides exerts immense environmental pollution and health hazards to target and non-target organisms (Noaishi *et al.*, 2013). Among them, fipronil and emamectin benzoate, relatively new insecticides, are of a new area of research.

Fipronil (FIP), a phenylpyrazole broad-spectrum insecticide, is widely used in various residential, agricultural and veterinary applications, particularly, against insects that are resistant to pyrethroids, organophosphates, and carbamates (Tingle *et al.*, 2003; Brassard *et al.*, 2011).

Fipronil, a neurotoxic insecticide, is a potent gamma-aminobutyric acid (GABA) gated chloride channel inhibitor and possesses a favorable selective toxicity

towards insects rather than mammals (Stanley *et al.*, 2012). Besides being a neurotoxic agent, FIP exerts hepato-renal (Mossa *et al.*, 2015), development (de Barros *et al.*, 2016), reproductive (de Barros *et al.*, 2017), mutagenic (de Oliveira *et al.*, 2012) and carcinogenic (Jackson *et al.*, 2009) effects.

Emamectin benzoate (EMB), a semi-synthetic derivative of a second-generation avermectin insecticide, is naturally produced by fermentation of the soil bacterium *Streptomyces avermitilis* (Ioriatti *et al.*, 2009). It is extensively used in agro-vet sectors (Castanha Zanoli *et al.*, 2012).

EMB acts as GABA-gated chloride channel activator leading to an increase in membrane chloride-ion permeability, leading to nerve membrane depolarization and eventually blocking the nerve impulse conduction resulting in irreversible paralysis (Jansson and Dybas, 1997). Moreover, EMB possess neuro- (Wise *et al.*, 1997), hepato-renal (Khalidoun-Oularbi *et al.*, 2015, 2016) and developmental (Gabr *et al.*, 2015) toxic effects.

Both fipronil and emamectin induce oxidative stress in different organs, mediated by increasing reactive oxygen species (ROS) production and decreasing the intracellular antioxidants (Badgujar *et al.*, 2015;

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Mossa *et al.*, 2015; El-Sheikh and Galal, 2015), resulting in damage of cellular macromolecules including lipid, protein and DNA.

Because of the implication of oxidative stress in health problems induced by a variety of environmental pollutants, including insecticides, much effort has been expanded in studying the potential ameliorative effect of natural herbal medicine against such toxic effects.

Pollen grains are apicultural honeybee products rich in biologically active substances, including, proteins, amino acids, carbohydrates, lipids and fatty acids, polyphenolic compounds, enzymes, vitamins and bioelements. Pollen and pollen products are valuable traditional herbal medicine widely used in the treatment of various diseases as it has many pharmacological properties including antimicrobial, anti-inflammatory and analgesic effects (Komosinska-Vassev *et al.*, 2015). Furthermore, previous studies proved the immune-stimulant, antioxidant, antimutagenic, hepato-renal protective properties of bee pollen (El-Bialy *et al.*, 2016, Huang *et al.*, 2017, El-Borai *et al.*, 2018).

Set against the background of the risk of indiscriminate exposure to insecticides and the health hazards effect to animals and humans. This study was designed to evaluate the potential protective role of bee pollen suspension against fipronil- and emamectin benzoate- induced hepato-renal toxicity in rats.

MATERIALS AND METHODS

Experimental Animals:

Sixty mature healthy male albino rats (150-170 g) were obtained from the Animal House of Food Technology Research Institute, Agricultural research Center, Giza, Egypt. Rats were housed in polypropylene cages with mesh wire tops and kept in a natural ventilated room (25±3 °C, 45-60%, 14 h light/ 10 h dark cycle), provided with standard commercial diet and clean tap water *ad libitum*. Rats were acclimatized to the laboratory environmental conditions for 2 weeks prior to the start of experiment. The study was ethically approved by the International Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, University of Sadat City.

Chemicals:

Fipronil (Fipromex® 20% EC, MAC-GmbH, Company, Germany) and Emamectin benzoate (Emamex® 5% EC, MAC-GmbH, Company, Germany) were commercially available. Bee pollen was purchased from apiary at Sadat City, Egypt. Diagnostic kits for assaying serum and tissue biomarkers were purchased from Biodiagnostic

Company, Dokki, Giza, Egypt. Other utilized chemicals were of analytical grade and commercially available.

Preparation of bee pollen suspension (BPS):

Bee pollen grains were grinded carefully to obtain fine powder. The aqueous suspension was freshly prepared daily by adding distilled water to the powdered bee pollen with stirring for 10 minutes on a magnetic stirrer till complete dispersion (Mehraban *et al.*, 2014).

Experimental Design and Animal Grouping:

Rats were weighed, randomly allocated into six equal groups and treated orally, 3 times weekly for 8 weeks. Control group, rats were orally administered distilled water. BPS group, rats were orally administered BPS at 120 mg/kg b.w. (Mehraban *et al.*, 2014). FIP group, rats were orally administered FIP at 9.7 mg/kg b.w. EMB group, rats were orally administered EMB at 8.8 mg/kg b.w. BPS+FIP group, rats were administered BPS one hour before FIP administration. BPS+EMB group, rats were administered BPS one hour before EMB administration. The selected doses of FIP and EMB were 1/10 of oral LD₅₀ of FIP (97 mg/kg b.w) and EMB (88 mg/kg b.w.) in rats (Tingle *et al.*, 2003 and Gabr *et al.*, 2015).

Samples Collection:

At the end of the experiment, rats were fasted overnight, anaesthetized and sacrificed for samples collection. Blood samples were collected in clean and dry glass centrifuge tubes for serum collection and then stored at -20°C for further serum biochemical analysis. Liver and kidney of each rat were collected and divided into two parts, one part was packed in ice bags at -20°C for tissue biochemical analysis, while other parts was fixed in 10% neutral buffered formalin solution for the histopathological investigation.

Serum Biochemical Analysis:

Serum ALT and AST activities (Reitman and Frankel, 1957); urea (Fawcett and Soctt, 1960) and creatinine (Schirmeister, 1964) levels were estimated using commercial kits.

Oxidant/ antioxidant biomarkers:

Malondialdehyde (MDA) and reduced glutathione (GSH) levels and catalase (CAT) activity were measured in liver and kidney tissue homogenates according to Ohkawa *et al.* (1979), Beutler *et al.* (1963) and Aebi (1984), respectively, using commercial kits.

Histopathological examination:

Following necropsy, tissue specimens from liver and kidneys were collected and rapidly fixed in 10% neutral buffered formalin solution. The fixed

specimens were trimmed, washed, dehydrated in ascending grades of ethyl alcohol, cleared in methyl benzoate and processed through the conventional paraffin embedding technique. 3-5 μ m sections were obtained from paraffin blocks using microtome (LEICA RM 2135) then routinely stained by hematoxylin and eosin (H & E) stain according to Bancroft *et al.* (1996) and crossman's trichrome stain according to Crossman (1937). Stained slides were microscopically analyzed using light microscopy. Histopathological Photos were photographed using a digital Leica photomicroscope (LEICA DMLB Germany).

Statistical Analysis:

Values are presented as mean \pm standard error (SE). Statistical significance of data was determined by one-way ANOVA (Analysis of Variance) followed by Duncan's Multiple range test for post hoc analysis.

All statistical analyses were performed using SPSS (Statistical Package for Social Sciences) Version 16 released on 2007.

RESULTS

Tissues oxidant/ antioxidant biomarkers:

No significant variations ($P < 0.05$) were observed concerning the hepatic and renal MDA and GSH levels; CAT activity between control and BPS groups. Fipronil and emamectin benzoate were able to significantly increase hepatic and renal MDA level and decrease GSH content and CAT activity, compared to control group. Administration of BPS either with FIP or EMB ameliorated these toxic effects and restored the normal control values of all parameters (Fig. 2).

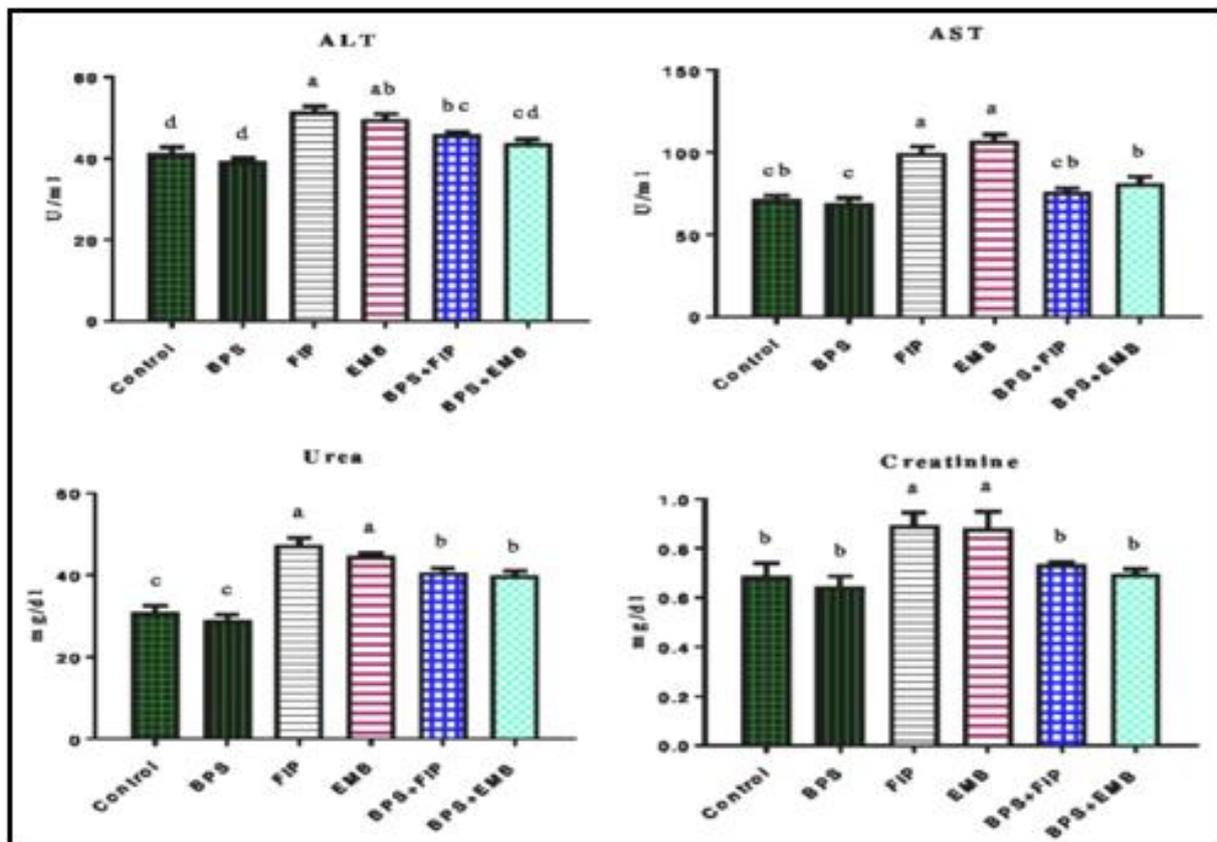


Fig. 1: Serum biochemical parameters of the different groups. Different letters indicate significant differences at $P < 0.05$. BPS: Bee pollen suspension; FIP: Fipronil; EMB: Emamectin benzoate.

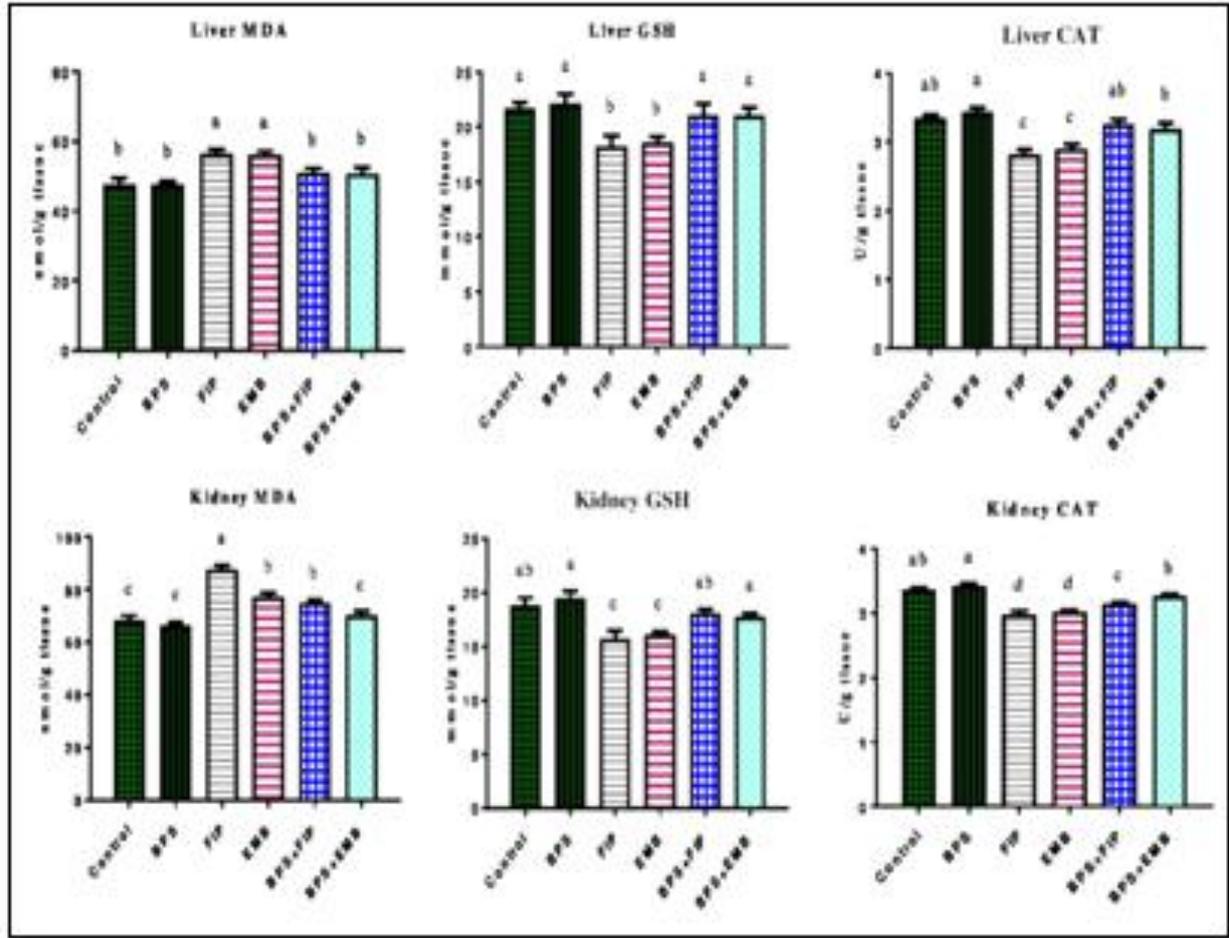


Fig. 2: Oxidant-antioxidant biomarkers in liver and kidney of the different groups. Different letters indicate significant differences at $P < 0.05$. BPS: Bee pollen suspension; FIP: Fipronil; EMB: Emamectin benzoate.

Histopathological findings:

Liver of rats in control (Fig.3A) and BPS (Fig.3B) groups showed normal histological picture of central vein and hepatic parenchyma, while FIP administration induced hydropic degeneration of hepatocytes around central vein (Fig.3C). EMB

caused congestion, edema in the portal area with coagulative necrosis and fatty infiltration in hepatocytes (Fig.3D). Liver sections of BPS+FIP (Fig.3E) and BPS+EMB rats (Fig.3F) showed marked improvement of hepatic architectures and somewhat normal hepatic parenchyma were seen in both groups.

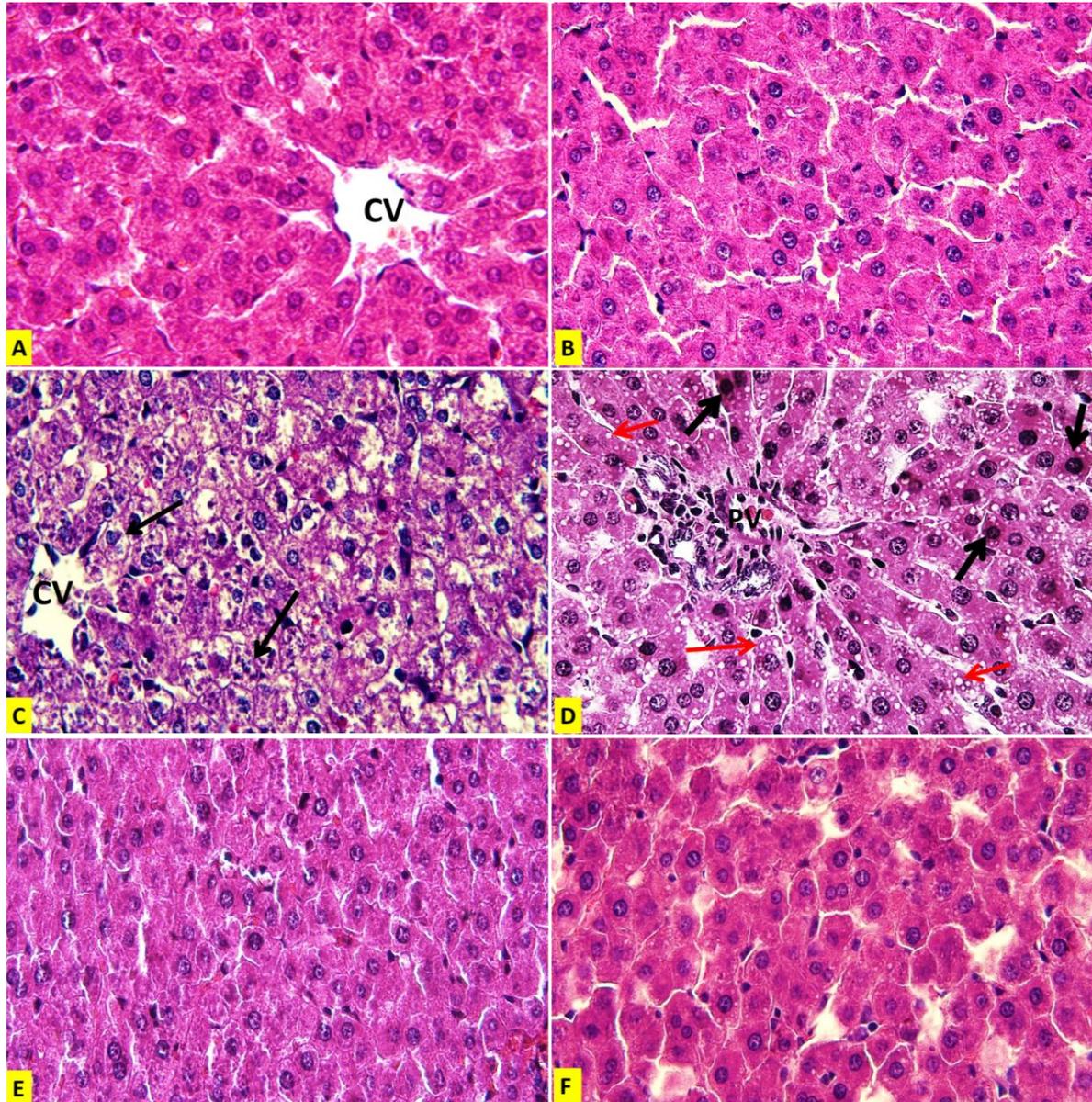


Fig. 3: Representative photomicrographs of liver sections of rats in different groups (H&E stain X₂₀). (A): control and (B): BPS groups showing normal histological structure of hepatic parenchyma. (C): FIP group showing hydropic degeneration of hepatocytes around central vein (arrows). (D): EMB group showing congestion and edema in the portal area with coagulative necrosis of hepatocytes (black arrows) and fatty infiltration in hepatocytes (red arrows). (E): BPS+FIP group showing somewhat normal hepatic parenchyma. (F): BPS+EMB group showing more or less normal hepatocytes.

Kidneys of control (Fig. 4A) and BPS (Fig. 4B) groups showed normal structure. Sections of FIP-intoxicated rats showed atrophied glomerular tuft, widening of Bowman's space, hyaline casts of most proximal and distal convoluted tubules (Fig. 4C1), severe vacuolar degeneration of renal epithelium of collecting tubules with fibrous tissue formation in between renal tubules (Fig. 4C2) and interstitial connective tissue proliferation with hyperplasia of

fibrocytes in between collecting tubules (Crossman trichrome stain X₂₀) (Fig. 4C3). Kidneys of EMB-intoxicated rats showed congestion of glomeruli and interstitial tissue and necrotic renal tubules (Fig. 4D1) and interstitial hemorrhage between degenerated medullary tubules (Fig. 4D2). However, Pretreatment with BPS improved the observed alterations caused by FIP (Fig. 4E) or EMB (Fig. 4F).

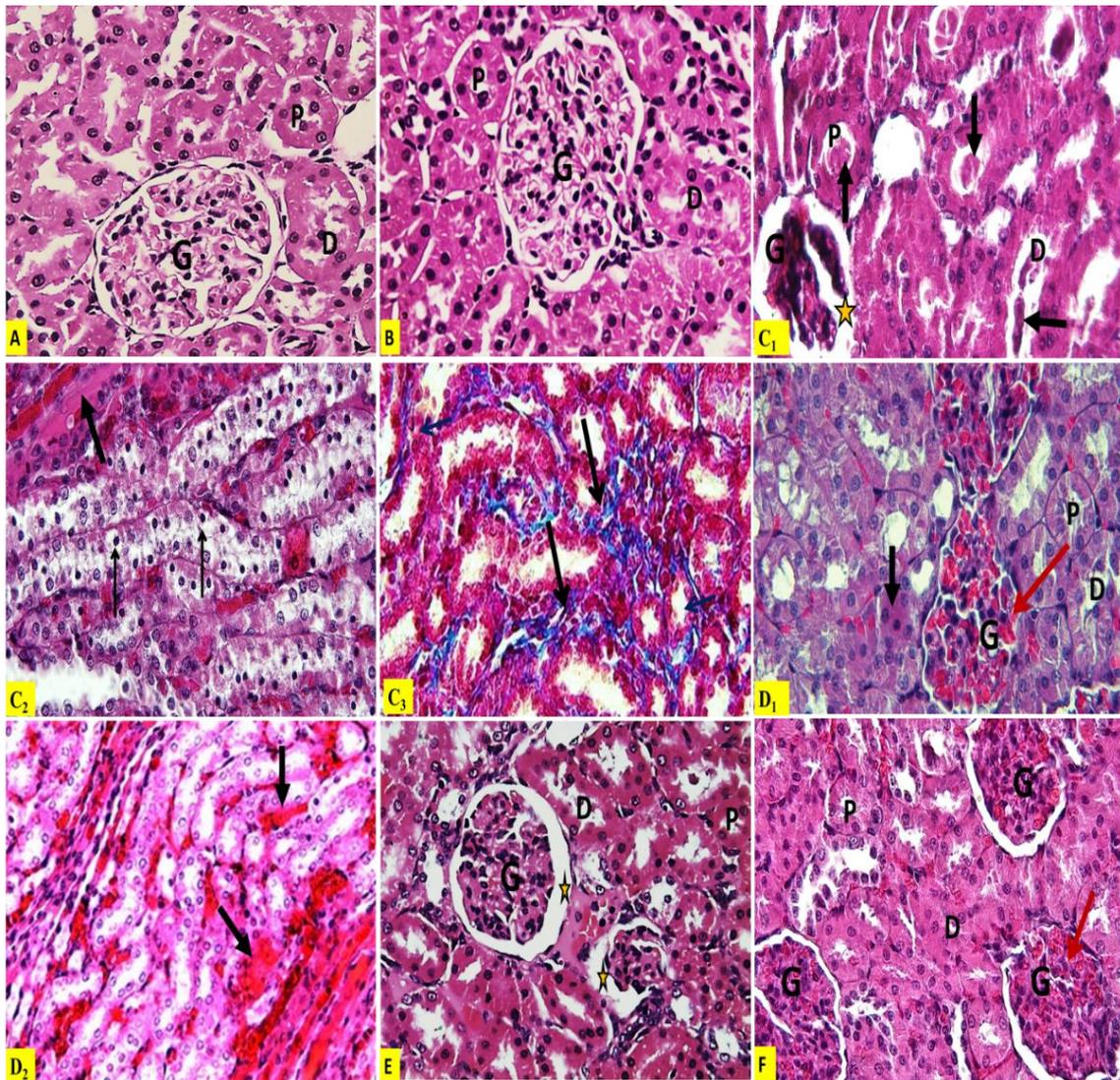


Fig. 4: Representative photomicrographs of Kidney sections of rats in different groups (H&E stain X20 and C3 by Crossman trichrome stain X20). (A): control and (B): BPS groups showing normal structure of glomeruli (G), proximal (P) and distal convoluted tubules (D). (C): FIP groups showing atrophied glomerular tuft and widening of Bowman's space (star) and hyaline casts of most proximal and distal convoluted tubules (arrows) (C1), sever vacuolar degeneration of renal epithelium of collecting tubules (thin arrows) with fibrous tissue formation in between renal tubules (thick arrow) (C2) and interstitial connective tissue proliferation with hyperplasia of fibrocytes in-between collecting tubules (arrows) (C3). (D): EMB group showing congestion of glomeruli and interstitial tissue (red arrow) and necrotic renal tubules (black arrow) (D1) and interstitial hemorrhage between degenerated medullary tubules (arrow) (D2). (E): BPS+FIP group showing normal kidney tissue with mild widening of Bowman's space of glomeruli (stars). (F): BPS+EMB group showing more or less normal kidney tissue with mild congestion of glomeruli (red arrow).

DISCUSSION

Environmental or occupational exposure to insecticides impacts animal and human life and exerts many deleterious effects. Fipronil and emamectin benzoate are relatively new and widely used insecticides causing adverse health effects via by oxidative stress mechanism (El-Sheikh and Galal, 2015; Kartheek and David, 2018). The crucial role of the liver and kidneys in the metabolism of xenobiotics, predisposes them to toxic cellular

damages leading to liver and kidney dysfunction (O'Shaunessy, 2010).

Evaluation of serum and tissue biomarkers along with the histopathological changes have a higher predictive value in assessment of the hepatic and renal toxic effects of insecticides and may help in evaluation of the potential ameliorative role of the natural antioxidant against such toxic insults. Therefore, this study was designed to evaluate the potential protective role of BPS against FIP- and EMB- induced hepato-renal toxicity in rats.

In the present study, oral administration of FIP and EMB to rats for 2 months provoked significant elevations in serum ALT and AST activities; urea and creatinine levels as previously reported by (Abdel-Hafez and Osman, 2013; Mossa *et al.*, 2015; Khaldoun-Oularb *et al.*, 2015, 2016; Abdel-Daim and Abdeen, 2018). The aforementioned results reflect the increase in membrane permeability of the damaged hepatocytes (Plaa and Hewitt, 1982) and the reduction in the glomerular filtration in kidney (Walmsley and White, 1994) that may be attributed mainly to the recorded peroxidative effects of FIP and EMB.

Oxidative stress, the pro-oxidant/antioxidant imbalance, leads to oxidation of lipids, proteins and DNA in the cells and hence, tissue damage. It usually occurs due to ROS overproduction and reduction of the intracellular antioxidants resulting in lipid peroxidation, alternations in membrane permeability, DNA damage and different biochemical and histopathological alterations (Sies *et al.*, 1991).

FIP and EMB induce oxidative stress in different organs. FIP causes uncoupling of oxidative phosphorylation leading to inhibition of electron flow through the electron transport chain and resulting in severe ATP depletion with over accumulation of O_2^- at levels that overwhelm the cell (Vidau *et al.*, 2011). H_2O_2 is then generated from O_2^- and further undergoes the Fenton reaction in the presence of transition metals (Fe^{2+}) to produce OH^\cdot , the strongest reactive radical among the ROS (Nordberg and Arnér, 2001; Avery, 2011). Avermectins, including ivermectin and abamectin are substrates for P-glycoprotein-mediated drug efflux (Chinkel *et al.*, 1994) that increases ROS production due to a higher ATP use and the need for higher rates of ATP synthesis (Lespine *et al.*, 2008).

MDA, a lipid peroxidation product along with GSH and CAT, cellular antioxidants, are valuable biomarkers of oxidative tissue damage. Our findings revealed significant elevation of MDA level concomitantly with reductions of GSH content and CAT activity in liver and kidney of FIP- and EMB-intoxicated rats. The accomplished results closely resembled to previous *in vivo* studies, which have confirmed the hepato-renal oxidative damages induced by FIP (Abdel-Daim and Abdeen, 2018) and EMB (El-Sheikh and Galal, 2015), that mainly attributed to their ability to generate free radicals and deplete intracellular antioxidants.

The above findings were confirmed by the observed histopathological changes. In accordance with our histopathological results, previous studies reported pathological changes in liver and kidney tissues of FIP- (Mossa *et al.*, 2015; Abdel-Daim and Abdeen, 2018) and EMB- (Mahmoud and Mahmoud, 2010, Khaldoun-Oularbi *et al.*, 2015, 2016) intoxicated rats.

Bee pollen, an apitherapeutic agent, is rich in proteins, carbohydrates, minerals, lipids, vitamins, phenolic substances, and significant quantities of antioxidant agents (Komosinska-Vassev *et al.*, 2015). It possesses a wide range of biological properties including antimicrobial, antioxidant, anti-inflammatory, anti-apoptotic, anti-carcinogenic properties (Komosinska-Vassev *et al.*, 2015; Huang *et al.*, 2017).

The current results approved the hepato-renal protective effect of BPS that confirmed by the marked reduction of serum ALT, AST activities and urea, creatinine levels as well as significant decrease in MDA level concurrently with elevation of GSH content and CAT activity in liver and kidney. In addition, BPS ameliorated the histopathological changes induced by FIP and EMB. The same findings were reported by Huang *et al.* (2017), who demonstrated the protective effect of bee pollen against cisplatin induced hepato-renal toxicity.

Moreover, bee pollen exhibited protective effect against oxidative tissue damages of CCl_4 (Yıldız *et al.*, 2013), cisplatin (Huang *et al.*, 2017), aflatoxins (El-Borai *et al.*, 2018) and fluoride (Al-Daihan and Bhat, 2019). This protective effect is probably due to its antioxidant property that could be attributed to its phenolic and flavonoids contents which can protect against lipid oxidation in the cell membrane by decreasing ROS production, scavenging free radicals and activating cellular antioxidant enzymes (Procházková *et al.*, 2011).

In the light of the above findings we can conclude that, FIP and EMB induced hepato-renal oxidative damages together with histopathological changes in liver and kidney of intoxicated rats. Bee pollen ameliorated these toxic effects. Therefore, bee pollen can be used as a new therapeutic and preventive supplement against toxic effects a variety of insecticides.

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تخفيف التأثيرات السامة الكبدية والكلىة للفيبرونيل والايماميكتين بنزوات في الفرن باستخدام حبوب اللقاح

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أدى الاستخدام المكثف للمبيدات إلى تلوث البيئة والإضرار بصحة الإنسان والحيوان. الفيبرونيل والايماميكتين بنزوات، مبيدات حشرية جديدة نسبياً وتستخدم على نطاق واسع، تعد من المواد السامة في البيئة. لذلك تهدف الدراسة الحالية إلى تقييم الدور الوقائي لحبوب لقاح النحل ضد التسمم الكبدى والكلىة الناتج عن التعرض للفيبرونيل والايماميكتين بنزوات في الفرن. تم تقسيم الفرن عشوائياً إلى ست مجموعات متساوية وعولجت 3 مرات أسبوعياً لمدة 8 أسابيع عن طريق الفم؛ مجموعة ضابطة (الماء المقطر)، مجموعة حبوب لقاح النحل (120 مجم / كجم)، مجموعة الفيبرونيل (9.7 مجم / كجم)، مجموعة الايماميكتين بنزوات (8.8 مجم / كجم)، مجموعة حبوب لقاح النحل+ الفيبرونيل و مجموعة لقاح النحل+ الايماميكتين بنزوات. أدى التعرض للفيبرونيل او الايماميكتين بنزوات إلى زيادة معنوية في نشاط كل من إنزيم الألانين أمينو ترانسفيريز، أسبرتيت أمينو ترانسفيريز، ومستويات البولينا، والكرياتينين. بالإضافة إلى، زيادة معنوية في مستوى المالدنيالدهيد مع انخفاض معنوي في محتوى الجلوتاثيون ونشاط الكتاليز في الكبد والكلى. علاوة على ذلك، أدى استخدام الفيبرونيل و الايماميكتين بنزوات إلى حدوث تغييرات باثولوجية في أنسجة الكبد والكلى. ومع ذلك فإن استخدام لقاح النحل ساعة واحدة قبل التعرض إلى الفيبرونيل او الايماميكتين بنزوات أدى إلى تحسين جميع التغيرات الكيميائية والنسجية المرضية مما أظهر خصائصه المضادة للأكسدة. وبذلك، يمكن استخدام لقاح النحل كمكمل وقائي جديد ضد التأثيرات السامة لمبيد الفيبرونيل و الايماميكتين بنزوات.