Protective Effect of Royal Jelly against Hepatorenal toxicity induced by Deltamethrin in Male Albino Rats: Biochemical, Histopathological studies

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Protective Effects of Royal Jelly against Hepatorenal Toxicity Induced by Deltamethrin in Male Albino Rats: Biochemical and Histopathological Studies.

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Abstract

One of the most environmental and industrial pollutants that are toxic to humans, animals, fishes, and birds. The most common sources of human and animal exposure to deltamethrin (DM) are polluted water and food**.** This study was done to evaluate the nephrohepatic toxicity of deltamethrin. Twenty-four female rats were used. The first group used as control. The second and third groups givens deltamethrin orally in dose of 1/10 % of the LD50 equal to 0.6mg\kg bw alone and plus Royal Jelly (RJ) at dose of 100 mg/kg/day for two month, respectively. Oral administration of DM induced biochemical and histopathological alterations. DM toxicity exhibited changes in the liver and kidney function tests manifested by increase AST, ALT, urea, uric acid, and creatinine with no changes were noticed in plasma proteins when compared to control group. Giving RJ ameliorated the hepatorenal toxicity by causing recovery in both liver and kidney functions in comparison to DM given group. Pathologically, severe degenerative and necrotic changes in livers and kidneys showed in deltamethrin group, where it improved to moderate to mild lesions by protective royal gel substance. This study concluded that royal gel substance has been shown benefit in lower down the side effects and increasing the rate of improvement of injury induced by of deltamethrin.

Key words:

Deltamethrin, Hepatorenal, Royal Jelly, Biochemical, Histopathological

I- Introduction:

Deltamethrin (DLM) a synthetic pyrethroid widely used for agricultural, veterinary, and public health purposes to control insects. However, DLM is regarded as highly effective insecticide and acaricide with an appreciable safety margin, the widespread and indiscriminate application of DLM led to high concentrations of DLM in the air, agriculture products, and the surface waters. Consequently, DLM has become one of the most environmental and industrial pollutants**.** Exposure to DLM may occur directly or indirectly through skin contact, inhalation, or food/water ingestion **(Chrusteke*t al.,* 2018; Bouzar *et al.,* 2020).** Moreover, DLM has been proven to have different toxic effects on the non-target organisms such as hemolysis, neurotoxicity, hepatotoxicity, genotoxicity, reproductive damages, and pulmonary disorders that threaten the health of livestock, poultry, fish stocks and human beings **(Shi *et al.*, 2019; Li *et al.*, 2021).**Nowadays, seeking to the application of natural supplements, which might be a promising therapy of some diseases, has developed innovative market segments focusing on enhancing health and offering well-being using naturals**(Mahgoub*et al.*, 2019).**The liver and kidney are reported among the most common tissues that are affected due to DM exposure since the liver is the main site of DM metabolism and the kidney is the principal organ of its elimination **(Abdel-Daim et al., 2013)** . Deltamethrin is oxidized by cytochrome P450 enzymes in the liver and elevates the reactive oxygen species (ROS) level **(Rehman et al., 2017)** Critical events in the toxicity of DM are the induction of apoptosis in various cells **(Kumar et al., 2015)** and oxidative stress which can further cause mitochondrial damage and lead to liver injury and nephrotoxicity. Royal jelly (RJ) is a natural thick and milky viscous material. It is partially soluble in water and has a color around whitish to yellow. It secreted by some glands (the hypopharyngeal and mandibular glands) in the heads of nursing worker honeybees (*Apismellifera*) that serves as a primary food for young larvae during their first three days of life and to the queen honeybee **(Chi *et al.*, 2021).** RJ was used in traditional and folk medicine for human health care. Recently, there have been several research studies on RJ highlighting their therapeutic properties. It was found that it exhibits a large spectrum of biological and pharmacological potential including antibacterial, antioxidant, anti-inflammatory, immunomodulatory and antitumor activities among others, which might be of high importance in modern medicine for the development of new drugs **(El-Guendouz *et al.*, 2020).** Officially, German Federal Board of Health classifies RJ as a medicine. Also, RJ was used as a food and medicine in China(**Abdelnour*et al.*, 2020).**It was

found that the regular intake of RJ has beneficial influences on many disorders, e.g., Alzheimer, depression, infertility, digestive problems, ageing, anemia, stress-related ailments and weakened immune function, in addition to its antioxidant, antineoplastic, antibiotic and anti-diarrheal properties **(Zhang *et al.,* 2019).** Even if RJ is known since ancient times; research papers concerning its investigation are not this much developed as compared to the other beehive, products such as honey or propolis **(Guendouz *et al.*, 2020**). So that, the current study aims to investigate the ameliorative effects of RJ on the DLM-induced hepatorenal toxicity in male albino rats.

II- Materials and Methods

Materials:

a- Deltamethrin

Commercially grade deltamethrin–based pesticides (butox 5% Ec) were purchased from (Intervet Co., France).

b- Royal jelly (RJ):

Fresh RJ was obtained from local market and stored at 4℃ in the refrigerator until use. About 100 mg\kg of RJ was dissolved in 1 ml distilled water (d.w.) and administrated to rats orally for 30 days according to **Ghanbari*et al.* (2016).**

c- Biochemical kits

The biochemical kits were used for determination of AST, ALT, Bilirubin, Total Protein, Albumin, Globulin, Creatinine, Urea and Uric acid. The kits produced by Ethiopian Institute for Pharmacy, Ethiopia.

A total of twenty-four sexually mature apparently healthy male rats at age (12-14) weeks with average initial body weight (200-250) g was purchased from international animal house, Ethiopia. Throughout the experimental period, each 3 rats were housed in plastic cages with stainless steel cover and provided with standard diet and tap water *ad libitum.* Cages was kept in an air-conditioned room (23±3℃) with 12/12 hrs. light/dark cycle. All rats were reared under the same managerial and hygienic conditions. Cages were cleaned regularly and disinfected. All

experimental protocols that held on animals were done according to regulations set by the Institutional Animal Care and approved by international Ethiopian Institute for Pharmacy.

Methods

1. Experimental design:

Twenty-four rats were randomly allocated into three equal groups (8 rats/each).

Group1, the rats were fed the basal diet and received physiological saline without any supplementation and used as control group.

Group 2, the rats received DLM at dose 1\10 LD_{50} (0.6 mg/kg B.W.) orally using stomach tube.

Group 3, the rats received DLM at dose 1\10 LD50 (0.6 mg/kg B.W.) plus royal jelly (100 mg\kg B.W.).

The animals were examined daily for two months (the end of experiment). The animals were euthanized by ethyl ether and then killed by cervical dislocation. The blood and tissues samples were collected from all live and dead animals. Whole blood samples without anti-coagulant. About 5 ml of whole blood was taken directly from the animal in a sterilized test tube without anti-coagulant, blood samples allowed to stand for clotting from 1-2 hrs. at room temperature. The clot was detached from the wall of the centrifuge tube and then samples were centrifuged at 3000 rpm for 10 mins to separate the serum. Serum samples were then divided into aliquots in Eppendorf tubes and stored at -20˚C until further analysis.

2. Tissue samples:

The livers and kidneys, from each rat were quickly removed, washed in a saline solution (0.9% NaCl) for biochemical examination., and other pieces of each tissue were fixed immediately in 10% neutral buffered formalin, dehydrated, cleared embedded in paraffin wax blocks for histopathological investigation.

3. **Biochemical analysis:**

The biochemical measurements were performed manually using Ultraviolet-visible spectrophotometer using commercial specific kits produced by International Ethiopian Institute for Pharmacy.

4. Liver functions:

Serum ALT and AST were measured according to the method described by **Breuer (1996)** using commercial specific kits produced by International Ethiopian Institute for Pharmacy. Serum total protein and albumin were measured according to **Tietz (1994)** and **Tietze (1990),** respectively, using commercial specific kits produced by International Ethiopian Institute for Pharmacy.

5. Kidney functions:

The BUN, uric acid and serum creatinine were measured according to the method described by according to **Tietz (1990)** using commercial specific kits by International Ethiopian Institute for Pharmacy.

6. Histopathological Evaluation.

Specimens from the livers and kidneys from all groups were performed according to the method of **Bancroft and Gamble (2008).** After fixation with 10 % neutral buffered formalin, specimens were washed in water, dehydrated in ascending grades of alcohol, then in xylene. Then, samples were embedded in paraffin for preparation of 5 μm paraffin sections and then stained with hematoxylin and eosin for histopathological examination.

7. **Statistical Analysis:**

A one-way ANOVA was conducted to examine the effect of our independent variables (the group) on the studied traits under this study. If one-way ANOVA reveled a statistically significant difference, it was followed by a post-hoc test (Duncan's test) for multiple comparisons among experimental conditions. The results were expressed as the mean standard error of mean (SEM). Statistical analysis was performed with IBM® SPSS® (SPSS Inc., IBM Corporation, NY, and USA) Statistics Version 21 for Windows.

III- Results:

Liver function test:

To evaluate the liver function's response to DLM exposure, we determined the activities of serum AST and ALT as well as total protein, albumin, and globulin in different groups. The present data showed that in the DM group, significant increases of AST and ALT activities were detected as compared with control and RJ plus DM groups (Table 1). The enzymes' activities were declined in the Royal Jelly plus DM group as compared to the deltamethrin group. On the contrary, co-administration of rats with RJ and DM prevented a DM-induced increase in the enzyme activities when compared to control and RJ groups. From Table (1), we observed also that the DM did not affect the total protein, albumin, and globulin concentrations as compared to the control group and RJ group.

Kidney Function test:

In kidney function tests, there was a significant increase in levels of creatinine in the deltamethrin groups as well as increase in the serum urea and uric acid in comparison to control group (Table 2). From (Table 2) a significant decrease in the concentration of mentioned parameters was reduced when royal jelly was given with deltamethrin in comparison to DM groups.

Histopathological Findings:

Histopathological findings, kidneys, and livers in the rats in gps (2, 3) which givens deltamethrin drugs and treated with royal gel, respectively compared with control group gp (1). The control groups showed normally structures in both livers and kidneys **(Figs 1, 2).** The rats in (gp. 2) suffered from degenerative and necrotic changes of cortical tubule epithelial cells are characterized by cloudy swelling of epithelial cells and pyknosis of the nuclei, besides congestion in all renal blood vessels. In addition to, regenerative changes manifested by angiogenesis in blood vessels in medulla pressured on collecting tubules lead to necrosis with dilation in the lumen of the others. In comparison with (gp 3), which treated with royal gel displayed moderate degenerative and necrotic changes with few casts in renal tubules in most rats **(Figs 3- 6).** Moreover, the livers in (gp. 2) showing hepatitis manifested with severe congestion in portal vein, periportal fibrosis and newborn bile ducts in the portal triads, besides coagulative necrosis and degenerative changes in hepatic cells, comparison with (gp 3) which appeared normal hepatic cells with few inflammatory cells around portal areas (**Figs7-10).**

IV- Discussion

According to previous studies, deltamethrin is rapidly metabolized in the liver and a great concentration of DM metabolites accumulates in the liver causing oxidative stress **(Saoudi et al., 2017**).In the present study, increased AST, ALT and ALP activities in DM treated rats is probably due to the overproduction of ROS that alters the oxidant-antioxidant status and disrupts the integrity of membrane lipid cell resulting in the release of hepatic enzymes from the hepatocytes into the circulation **(Khalaf et al., 2017;Tewari et al., 2018).** Elevation in serum AST and ALT activities after DM exposure have been reported by several studies (**Abdel-Daim et al., 2013; Gunduz et al., 2015; Maalej et al., 2017**). ALT is a liver-specific enzyme and one of the most reliable indexes for hepatic damage. There was no significant difference in serum total protein, albumin and globulins concentrations between the DM group and the control. Our findings are in line with **Tewariet al. (2018)**. However, some previous reports were inconsistent with our result **(Abdel-Daim et al., 2013; Uchenduetal., 2017)** as they found that DM exposure resulted in decreased total protein and albumin levels. This inconsistency may be due to the different exposure time to DM, doses, and conditions among the studies.

For better understanding, the metabolic process of deltamethrin in the body, kidney function tests were analyzed. The increase in serum urea concentration reflects impairment in the renal tubular reabsorption, whiles the elevated serum creatinine, a by-product of muscle metabolism and actively secreted by the proximal tubular cells, concentration indicates impairment of glomerular filtration rate (GFR) **(Adedara et al., 2012).** Therefore, the increased urea and creatinine concentrations in the DM intoxicated rats suggesting renal tissue injury **(Tewari et al., 2018).** Co treatment with Royal jelly in this study was able to decline the elevated activities of AST, ALT, and ALP in the DM plus RJ group. This may be due to the antioxidant role of RJ, which neutralizes the effects of the DM by scavenging of ROS **(Kohno etal., 2004).**

Conclusion: It could be concluded that deltamethrin is a toxic substance induced impairment of hepatorenal functions with toxicity lesions. Meanwhile, royal gel substance declared that beneficial in lower down the side effects and increasing the rate of improvement of injury induced by the deltamethrin

Table (1): Liver functions Differences between the control group (gp.1), deltamethrin group (gp.2), and deltamethrin combined with reoyal gel group (gp.3),.:

Parameter	Groups			P-Value
	Control	DLM	DLM+RJ	
ALT UI/L	29.35±1.55	47.96±2.74[a]	38.78±2.04[b]	<0.001**
AST UI/L	50.31±1.52	76.06±1.82[a]	66.08±2.37[b]	<0.0001***
Total protein	12.66±2.01	12.57±1.38	11.77±1.45	<0.916
Albumin g/dl	4.61±0.76	5.13±0.55	5.11±0.53	<0.803
Globulin g/dl	7.89± 1.6	6.93± 1.4	6.20± 1.6	<0.1865
A/G ratio	0.68± 0.19	0.95± 0.23	1.42 ± 0.73	<0.1523

Table (2): Kidney functions Differences between the control group (gp.1), deltamethrin group (gp.2), and deltamethrin combined with reoyal gel group (gp.3).:

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Parameter	Groups			P-Value
	Control	DLM	DLM+RJ	
Creatinine(mg/dl)	1.16±0.18	1.69±0.20**a**	1.36±0.05**b**	<0.05*
Urea(mg/dl)	42.11±4.44	54.93±2.81a	47.92±1.96	<0.05*
Uric acid(mg/dl)	3.71±0.50	5.62±0.28**a**	4.02±0.28	<0.011**

Data are represented as Mean±SE. The P-values reported are for one-way ANOVA test. The used symbols *, **, and *** to represent significance at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ respectively for ANOVA test. The letters are used to denote the significant differences based on Duncan test at 0.05 significance level as follows: **A** for Control vs. DLM, **B** for Control vs. DLM+RJ, **C** for DLM vs. DLM+RJ.

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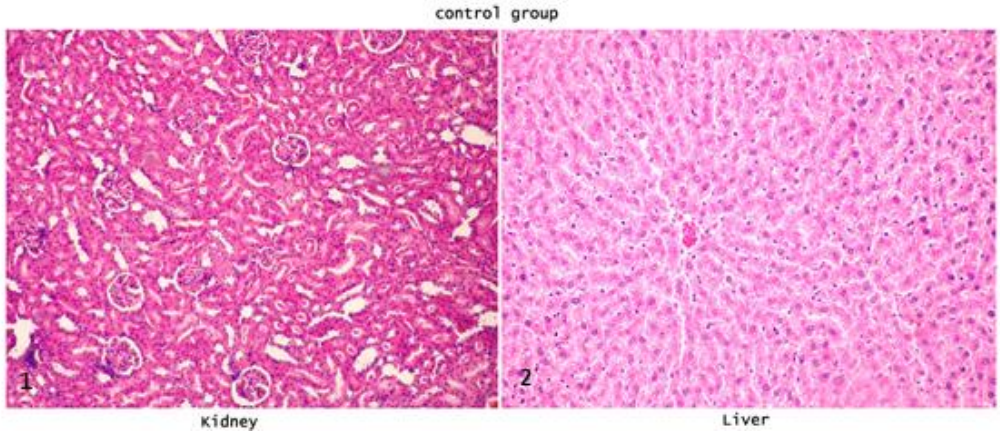
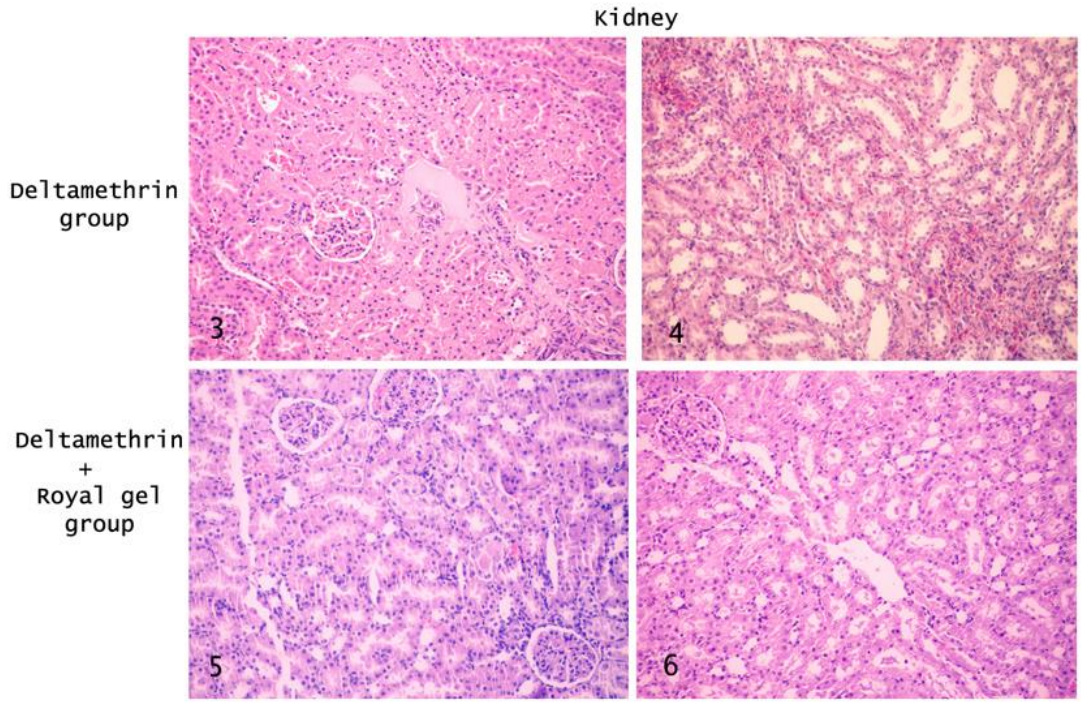


Figures (1, 2): Kidney and liver of the rats (gp 1) showing nephron consisted of normally glomerulus, primary and distal renal tubules (1). The hepatic lobules noticed in normal architecture around central vein. (2). (H& E.,x 150)



Figures (3- 6): Kidney of the rats in (gp. 2) showing cloudy swelling and necrosis in cortical renal tubules with congestion in glomerular capillaries and renal blood vessels (3), angiogenesis in blood vessels pressured on some collecting tubules leading to compensatory dilation in others (4) but in (gp. 3) moderate degenerative changes with renal casts in cortex (5, 6).
(H& E.,x 150)

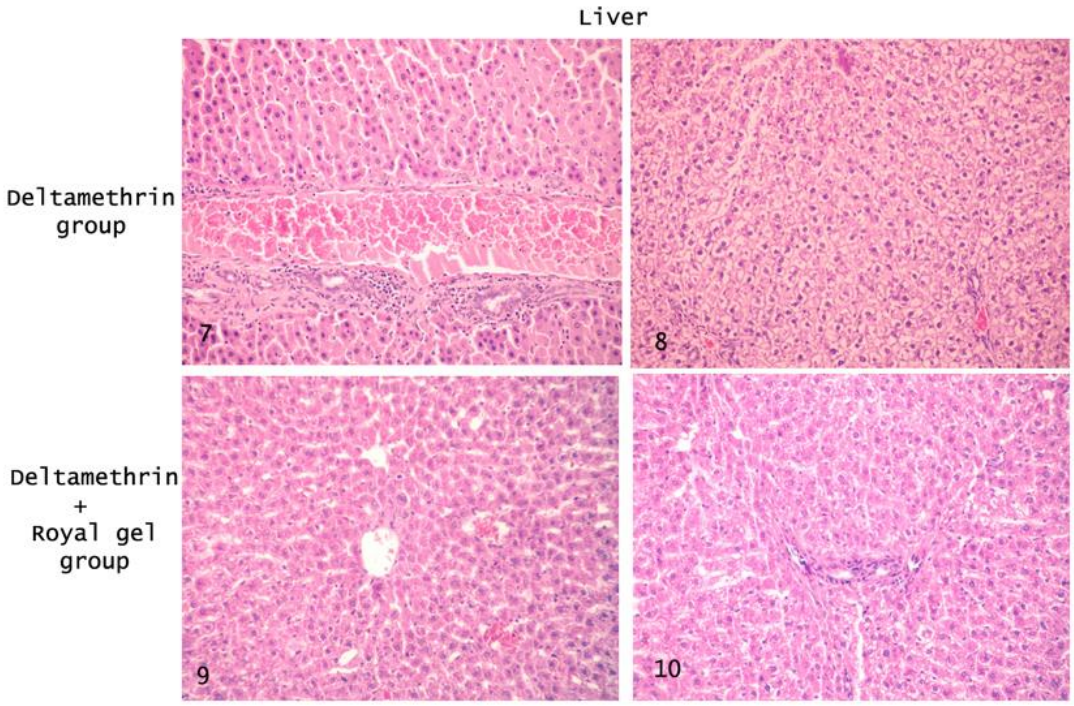


Figure (7-10): Liver of the rats in (gp. 2) showing coagulative necrosis in the hepatic cells with severe congestion in portal vein, periportal fibrosis and newborn bile ducts in the portal triads (7), vascular degeneration in hepatic cells (8), but in (gp. 3) the liver appeared with normal structure and few inflammatory cells showed in portal areas (9, 10).
(H& E.,x 150)