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Ginger and Honeybee Modulates MTX-induced Oxidative Stress in Kidney of Rats

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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Original Research Article

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ABSTRACT

Aim: The purpose of this study was to examine the curative effects of aqueous extract of ginger and honey bee solutions on methotrexate (MTX) induced kidney toxicity.
Materials and Methods: Twenty-eight adult female Wistar albino rats (aged 8–12 weeks). It is divided into four groups (n = 7): group I used as control negative; 2 methotrexate groups: G2, G3 and G4 injected with vehicle intraperitoneally of MTX (20 mg/kg body weight) one dose only. In treating groups 3&4 rats were pre-treated and continue seven days with aqueous extract of ginger and the honeybee solution, respectively.
Results and Discussion: It had indicated the treated rats with aqueous extracts of ginger and\or honey bee solution had caused the lowest significantly decreased ($P \leq 0.05$) at urea concentration in blood. As well as significantly increased at ($P \leq 0.05$) in Hb, RBC,S and WBc.s markers compared to the control +ve. In addition, treatment rats with aqueous extracts of ginger and\or honey bee solution had caused significant increase in total antioxidant levels (1.40±0.22 & 1. 70±0.24 mM / L), respectively, compared the rats give MTX only 1.00±0.96 mM/L. Concerning, the MDA concentration in the treatment groups with aqueous extract of ginger or honey bee solution the data indicated that improvement and significantly decreased in the MDA levels ($P< 0.05$) compared to the control +ve. The histopathology results supported this conclusion.

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Recommendation: Ginger and honey bee solution probably protect from MTX induced kidney damage by scavenging of free radicals and inflammation.

Keywords: Methotrexate; ginger; honey bee; kidney toxicity.

1. INTRODUCTION

Methotrexate (MTX) is an anti-folate metabolite which acts by reversibly inhibiting the enzyme dihydrofolate-reductase, affecting purine/ thymidylate and thus DNA synthesis and cell proliferation. Despite the effectiveness of MTX in both cancer chemotherapy and rheumatoid arthritis management, discontinuation is common due to the occurrence of its adverse side effects [1-3]. Methotrexate is primarily excreted by the renal route. It may cause acute neurotoxicity [4,5] which presumed to result from its precipitation or its relatively insoluble metabolites in acidic urine. This nephrotoxicity leads to delayed MTX elimination, ineffective rescue and a marked increase of other non-hematological and hematological toxicities associated with MTX, such as myelo suppression [6].

In traditional Egyptian medicine, ginger, honey and many other spices have been used as medicine. Ginger (*Zingiber officinale*) belongs to *Zingiberaceae* family. The rhizome of the ginger plant is a rich source of antioxidants, including gingerols, shogaols, zingerones and other ketone derivatives [7,8]. The root contains health benefiting essential oils such as gingerol, zingerone, shogaol, farnesene, small amounts of β-phellandrene, 1,8-cineole, and citral. Gingerols have been anti-inflammatory, painkiller (analgesic), nerve soothing, anti-pyretic as well as anti-bacterial properties [9,10]. Ajith et al. [11] reported that ginger administration prevented the acute kidney injury caused by the chemotherapy agents. Another study Shanmugam et al. [12] reported benefit of ginger on kidney function following alcohol-induced injury. Recently, Nasri et al. [13] reported that ginger could prevent degeneration of the renal cells and reduce the severity of tubular damage caused by gentamicin.

Honey is a natural product of honey bees that is, derived from floral nectar and other plant secretion [14]. Honey is rich in carbohydrates, proteins, vitamins, trace elements, enzymes, and phenolic compounds [15] Scientific studies over the past ten years have shown that honey possesses various important biological active compounds including caffeic acid, caffeic acid phenyl ester and flavonoid glycones. These compounds proved to have an inhibitory effect on tumor cell proliferation and transformation of the down-regulation of many cellular enzymatic pathways, including protein tyrosine kinase, cyclooxygenase and ornithine decarboxylase [16]. It contains many properties, such as wound healing [17,18] and antibacterial [19] antioxidant [20,21], antitumor [22], and anti-inflammatory effects [23,24]. In Egypt, there are several types of honey; the common kind is Emtenan, which used in this study. Furthermore, the regenerative activity of ginger and the honey bee solution on MTX kidney toxicity is not yet investigated. The current study was, therefore, designed to evaluate the pretreatment and curative effects of ginger and honey bee against MTX- induced kidney toxicity in Wistar female rats.

2. MATERIALS AND METHODS

Methotrexate (MTX):MTX 25mg/ml solution for injection produced by Hospira UK Ltd this sterile solution for injection contains the methotrexate sodium equivalent to 25 milligrams of active substance methotn is a yellow and sterile solution in clear glass vials. MTX solution was injected intraperitoneal at a single dose of 5mL.equal (20 mg/kg body weight). Commercial Kits used for determining total antioxidant capacity, malondialdehyde (MDA), kidney function. All chemicals obtained from Sigma Chem. Company, Cairo, Egypt. All other chemicals were of analytical grade.

2.1 Animals

Twenty eight adult female Wistar albino rats (aged 8–12 weeks) weighing 222–265 g obtained from the Laboratory Animal Production Unit of Research Institute of Ophthalmology, Giza Egypt used in the experiment. Rats allowed one week acclimating to the surroundings before beginning any experimentation. Animals housed in individual plastic cages with bedding.

Animals were maintained at free access to tap water and were fed a standard pelleted feed according to the National Research Council, [25] and Reeves et al. [26] duration of the experiments periods. The temperature has

maintained at 22±2℃. A 12/12 h light/dark cycle maintained. All experimental protocols were approved by the locale ethic committee.

2.2 Experimental Design

The animals were randomly divided into four groups (n = 7): group I the group used as a control negative , and placebo (physiologic saline) administrated for seven days; 2 methotrexate groups: G2, G3 and G4 injected with vehicle intraperitoneally for one day as a single dose of MTX (20 mg/kg body weight this method according to All et al. [27].In treating group 3 rats treated with aqueous extract of ginger (4ml \rats) (16 ml/ kg body weight). The treated group 4 has given a solution of honey bee (50% honey: 50% distilled water v\v) for the first day before administrated the MTX drug and following days. After the last doses, all rats fasted about 12 hours, but had free access to water. Blood samples collected from the orbital sinus veins in dry clean centrifuge tubes (for serum) and ethylene diamine tetraacetic acid (EDTA) tubes (for whole blood). After clotting, serum then was centrifuged for 20 min. at 2500 r.p.m to separate serum which transferred carefully into a dry clean, well stopped plastic vials and kept frozen till analysis. The animals sacrificed by cervical dislocation at the end of the experiment. Kidney tissues excised. However, kidneys sample washed in saline and weighted then fixed in 10% neutral buffered formalin during 24 hours and embedded in paraffin for histological examination according to Bancroft et al. [28].

2.3 Aqueous Extract Preparation

The aqueous extract of ginger powder was prepared in hot distilled water, briefly 10 gm. of ginger powder had socked in 100 mL hot water for one hour, then filtered through a sieve and stored in dark bottles and put in the refrigerator immediately. The pure honey bee (Emtenan) was diluted in distilled water (50% honey: 50% distilled water v\v)(1:1 v/v).

2.4 Biological Evaluation

The total food consumption of the experimental period (7 days) calculated. Body weight gain (BWG), food efficiency ratio (FER) and relative kidney wt. determined according to Hsu et al. [29] using the following equations.

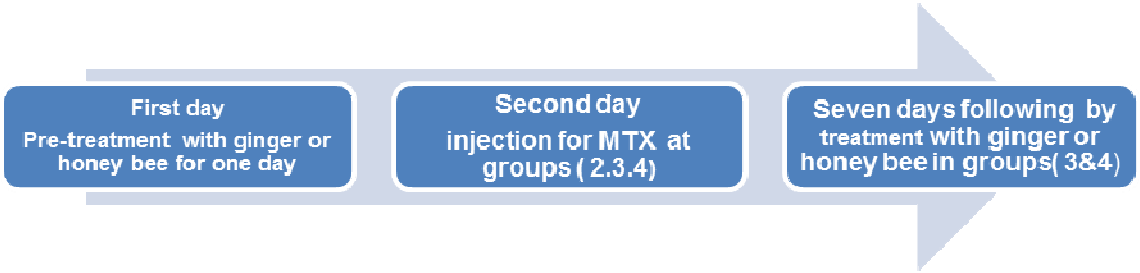
(BWG)= Final wt. – initial wt., (FER) = weight gain (gm.) ÷ Food intake (gm.) and relative kidney wt. = kidney wt.÷ body wt. × 100.

2.5 Biochemical Analysis

Hemoglobin (Hb) had determined according to International Committee [30].Red blood cells (RBCs) count and white blood cells (WBCs) had measured according to Natt and Herrick [31]. Determination of albumin and total protein in serum were carrying out according to Doumas and Biggs [32] and Henry [33], respectively. Determination the creatinine level according to Bartles et al. [34]. Urea concentration in blood determine according to Fawcett, and Soctt [35]. Calcium and phosphorus determined colorimetrically in serum according to Tietz [36] and El- Merzabani et al. [37], respectively. Lipid peroxide (malondialdehyde) in plasma had measured according to the method of Yoshioka, et al. [38]. As regard total antioxidant capacity had determined according to Koracevic et al. [39].

2.6 Statistical Analysis

One–way analysis of variance (ANOVA) test and Duncan's multiple range tests had used to show the differences among means at $P \leq 0.05$ [40].



Experimental Design

3. RESULTS

The effect of MTX and treatment of aqueous extract of ginger and honey bee solution each other at (16 ml/ kg body weight) of intake on body wt. gain, food intake (FI) and feed efficiency ratio (FER), had shown in (Fig. 1). A group of rats give (MTX) alone at 20 mg/ kg.b. wt. (control +ve) have shown significantly decreased ($P \leq 0.05$) in body wt. gain (- 13.60±2.88gm), food intake (4.70±0.50gm) and food effectively ratio (-2.90±0.50) compared with the control –ve, which showed the values of 5.10±2.00gm, 8.60±0.50 gm and 0.60±0.20% for body wt. gain, food intake and food efficiency ratio, respectively. Data indicated also, that both of body wt. gain, food intake and feed efficiency ratio had significantly decreased at ($P \leq 0.05$) by giving rats the aqueous extract of ginger of intake. But also, the same indicators improvement when rats given the honey bee solution compared the control -ve except the results of food intake. Results in (Fig. 1) also showed that the relative kidney weight, there were no significant differences between all groups.

Results in (Table1) showed the effect of aqueous extract of ginger or honey bee solution on the hematological parameters. The data indicated that rats injected by (MTX) alone had significantly decreased at ($P \leq 0.05$) in hemoglobin, RBC, S and WBc.s with the means value of 9.80±0.94gm\dl, 3.20±0.30 and 00.70±00.11, respectively, compared to the control –ve at (13.33±0.20gm\dl & 4.4±00.8 & 5.40±00.40), respectively. The treated rats with aqueous extracts of ginger and\or honey bee solution significantly increased at ($P \leq 0.05$) in Hb, RBC, S and WBc.s markers compared to the control +ve. Their increased magnitude was similar to the control –ve.

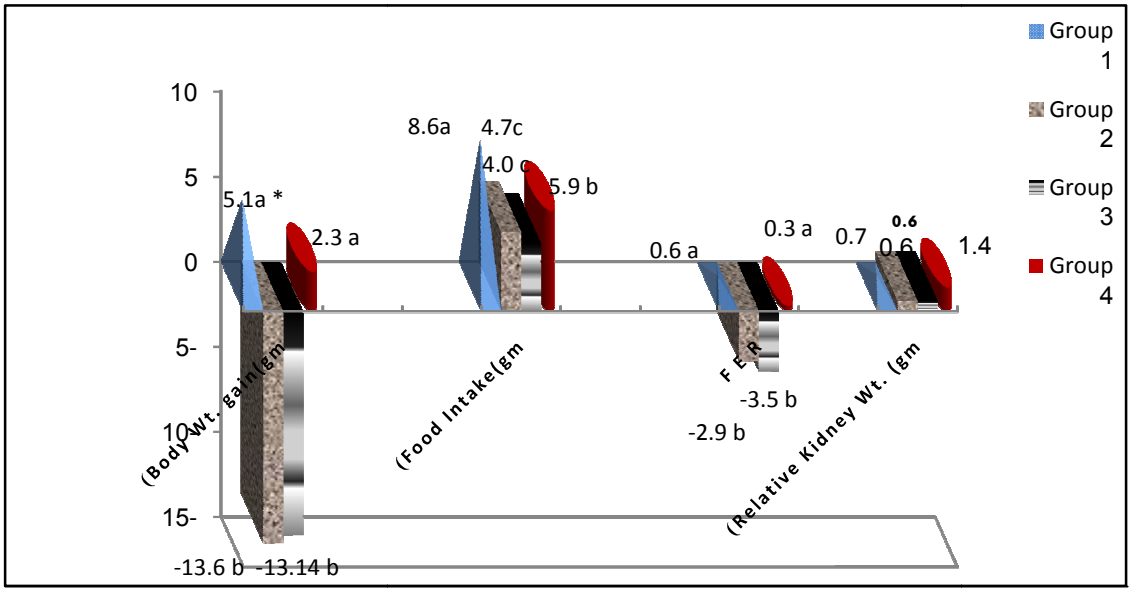


Fig. 1. Effect MTX and treatment ginger and honey bee on body wt. gain, food intake, FER and relative kidney wt.

**values in a given which have different superscript letters are significantly different (p≤0.05)*

Table 1. Effect of injection MTX and oral treatment of ginger or honey bee on whole blood picture in rats

Groups/parameters	Group 1	Group 2 (MTX)	Group 3 (MTX+ginger)	Group 4 (MTX+honeybee)
Hemoglobin (gm/dl) Mean±SD	13.33±0.24^{b*}	9.80±0.94^{c}	13.30±0.88^{b}	15.20±0.72^{b}
RBc.S (mil/µl)	4.4±0.08^{b}	3.3±0.30^{d}	4.4±0.9^{b}	5.1± 0.26^{c}
WBc,s (10^3/ul)	5.4±0.4^{a}	0.7±0.11^{b}	5.2±1.7^{a}	5.2±0.74^{a}

** Values in a given column which have different superscript letters are significantly different (p≤0.05).*
all data presented as mean±standard deviation

The (Table 2) presents the effect MTX and treatment ginger and\or honey bee solution on kidney function (albumin, total protein, urea, creatinine, calcium and phosphors). The obtained data revealed a no significant difference in albumin concentration in the blood of rats injected with MTX alone compared with normal rats and treatment groups. The total protein concentration significantly decreased in group 2 (control +ve) with the mean value (5.20±0.96 g\dl) compared with control –ve (6.70±0.29 g\dl). The treatment with ginger and honey solution increased the total protein concentration, but that increased not significantly.

The data illustrate the injection, rats of group2 with MTX alone significantly increased a urea concentration in blood (28.60±1.72 mg/dl) compared with the control -ve and treatment groups. Using ginger extract causes the lowest significant decreases (P< 0.05) at urea concentration in blood. Treatment with honey solution in group 4 significantly decreased (P < 0.05) in urea concentration in the blood compared with group (2). However; the results indicated the creatinine concentration in blood didn't significantly differ between all groups. As regards to calcium level in serum the data indicated that rats have taken MTX alone and\ or aqueous extract of ginger were significantly lower level of calcium (8.90±0.69 & 9. 00±0.50 mg\dl), respectively, compared with healthy or treatment rat with a honey solution with the mean value (10.10±0.31 & 10.10±0.83 mg\dl), respectively. Regarding to phosphorus and Ca/P concentration, in serum there were no significant differences between all groups.

The total antioxidant and oxidative stress markers had presented in (Table 2): Data revealed that rats give MTX alone (G2) had the lowest significantly concentration at (P ≤ 0.05) of total antioxidant capacity in the blood (1.00±0.96mM/L) compared to the normal rats (1.60±0.28mM/L). The groups treated with aqueous extract of ginger or honey bee solution (G3&G4) significantly increased the total antioxidant levels (1.40±0.22 & 1.70±0.24 mM/L), respectively compared the rats give MTX only (1.00±0.96 mM/L).As the regard to oxidative processes that represent by MDA. Data indicated the MDA significantly increased at (9.90±1.06 nmol/ml) in rats injected by (MTX) only compared to the healthy rats and treatment groups. Concerning the MDA concentration, results showed that the treatment groups with aqueous extract of ginger or honey bee solution improvement and significantly decreased in the MDA levels (P< 0.05) compared to the control +ve.

3.1 Histopathology Results

That described in the (Table 3), so microscopically slides examination of kidney of rats from group (1) showed the normal histology structure of the renal parenchyma (Fig. 2). Meanwhile, kidney of rats injected by (MTX) 20 ml.\kg of body weight alone showed revealed granularity of cytoplasm of renal tubular epithelium, pyknosis of their nuclei, vacuolation

Table 2. Affect ginger and honey bee on kidney enzyme, antioxidant and oxidative process

Groups/parameters	Group 1	Group 2 (MTX)	Group 3 (MTX)+ginger	Group 4 (MTX)+honey bee
Albumin (g/dl) Mean ±SD	2.90±0.18^{a}*	2.70±0.23^{ab}	2.50±0.21^{cb}	2.90±0.25^{a}
Total protein (g/dl) Mean ±SD	6.70±0.29^{a}	5.20±0.96^{cb}	5.40 ± 0.57^{cb}	6.01±0.80^{ab}
Urea (mg/dl)	20.40±2.60^{c}	28.60±1.72^{a}	11.11±1.35^{e}	23.70±0.10^{b}
Creatinine (mg/dl)	0.23±0.04^{a}	0.22±0.04^{a}	0.23±0.02^{a}	0.27±0.03^{a}
Calcium (mg/dl)	10.10±0.31^{a}	8.90±0.69^{b}	9.0±0.50 ^{b}	10.10±0.83^{a}
Phosphors (mg/dl)	5.10±0.35^{a}	4.60±0.87^{a}	4.10±0.98^{a}	5.1±1.30^{a}
Calcium \ Phosphors ratio	2.00±0.13^{a}	2.00±0.35^{a}	2.30±0.40^{a}	2.10±0.46^{a}
Total antioxidant capacity (mM / L) Mean ±SD	1.60±0.28^{ab}	1.00±0.96^{c}	1.4±0.22^{b}	1.70±0.24^{a}
M D A (nmol / ml) Mean ±SD	5.90±0.50^{b}	9.90±1.06^{a}	6.40 ± 0.10^{b}	4.50±0.24^{c}

**all data presented as mean ± standard deviation; * values in a given rows which have different superscript letters are significantly different (p≤0.05)*

Table 3. Effect of aqueous extract of ginger and honey bee of intake on histopathological changes in kidney of rats

Groups	Granularity of cytoplasm of renal tubular epithelium	Pyknosis of their nuclei	Vacuolations of endothelial lining glomerular tufts	Hypertrophy of glomerular tufts
Group 1 (control -)	*-	-	-	-
Group 2 (MTX)	++	++	++	++
Group 3 (MTX)+ginger	-	-	-	-
Group 4 (MTX)+ Honey bee	-	-	-	-

**- Normal + Little effect ++ Sever effects*

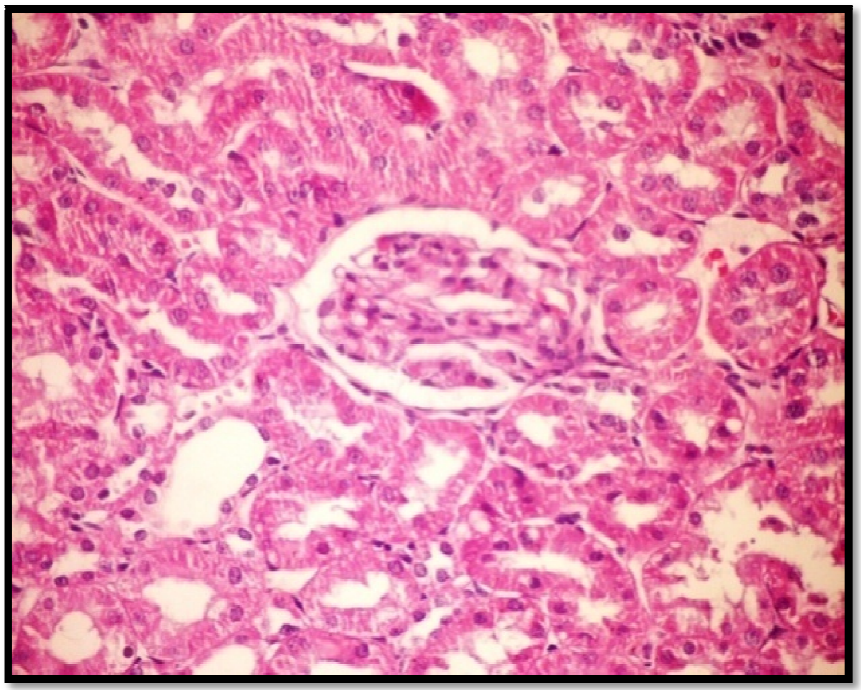


Fig. 2. Kidney of rats from group 1 control negative showing the normal histopathological structure of renal parenchyma (H and E × 400)

of endothelial lining glomerular tufts and hypertrophy of glomerular tufts (Figs. 3 and 4). Some examined sections from group of rats treated with aqueous extract of ginger had noticed no histopathological change (Fig. 5). However, kidney of rats from group 4 that had taken solution of honey bee revealed no histopathological change (Fig. 6).

4. DISCUSSION

Evidenced by earlier findings that injecting drug MTX rat it have led to the significantly decreased in the body wt. gain, food intake and food effective ratio. These results had supported by previous studies. Kolli et al. [41] studied that rats treated with MTX caused diarrhea and weight loss by 6%. As well as Yozai et al. [42] reported that diabetes rat's injection intermittent administrations of MTX were lower in body weights that those of the control group. Also, Anton et al. [43] reported that gastrointestinal toxicity resulted in diarrhea and weight loss in all groups for 5 days after MTX administration. Other literature Scott et al. [44] indicated that methotrexate toxicity has increased in protein calorie malnutrition in 30 adults, female Lewis rats.

The treated rats with aqueous extract of ginger (G3) did not differ in body wt. gain compared with rats had taken MTX alone. The weight gain didn't differ because the aqueous extract of ginger prevented appetite inhuman, was used in diet and reduced feelings of hunger [7,8]. The ginger contains flavor and pungent taste [9] that's can be the affect in rats, caused decreased in food intake, finally a short period of the treatment (one day before and continuous 6 days of the experiment) did not enough to good effect of aqueous extract of ginger. According to food intake, significantly decreased the body wt. gain and FFR also affected.

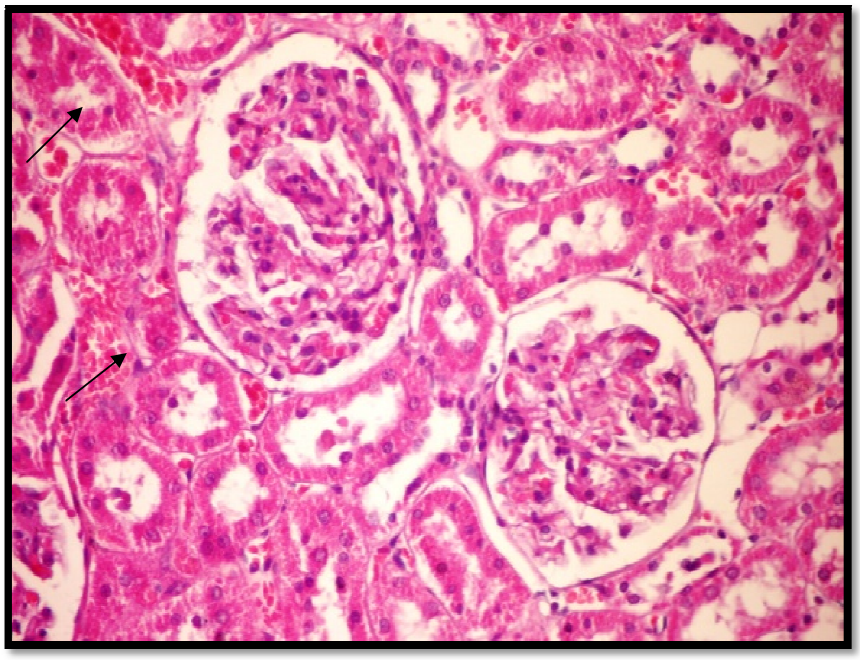


Fig. 3. Kidney of rats from group 2 control positive showing vacuolations of endothelial lining glomerular tufts (H and E × 400)

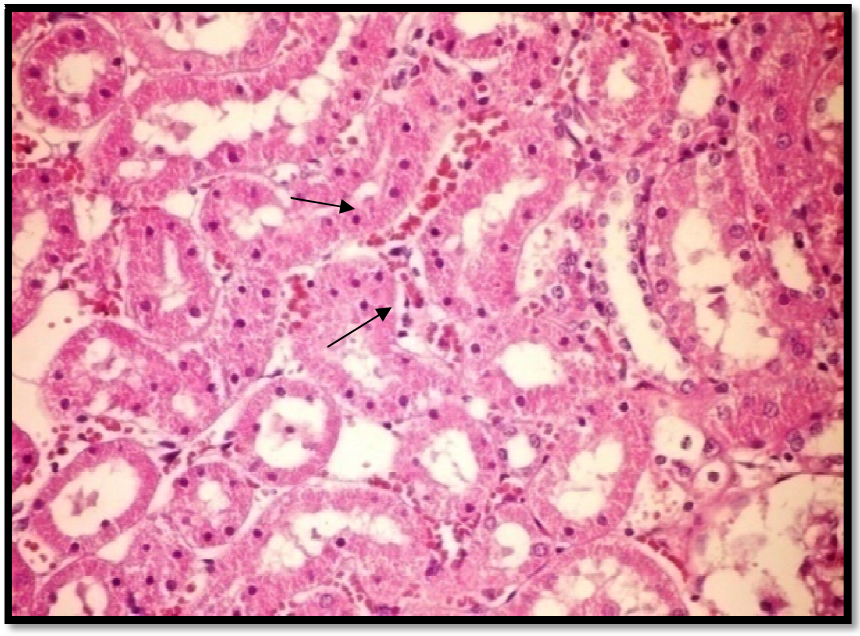


Fig. 4. Kidney of rats from group 2 control positive showing granularity of cytoplasm of renal tubular (epithelium with pyknosis of their nuclei (H and E × 400)

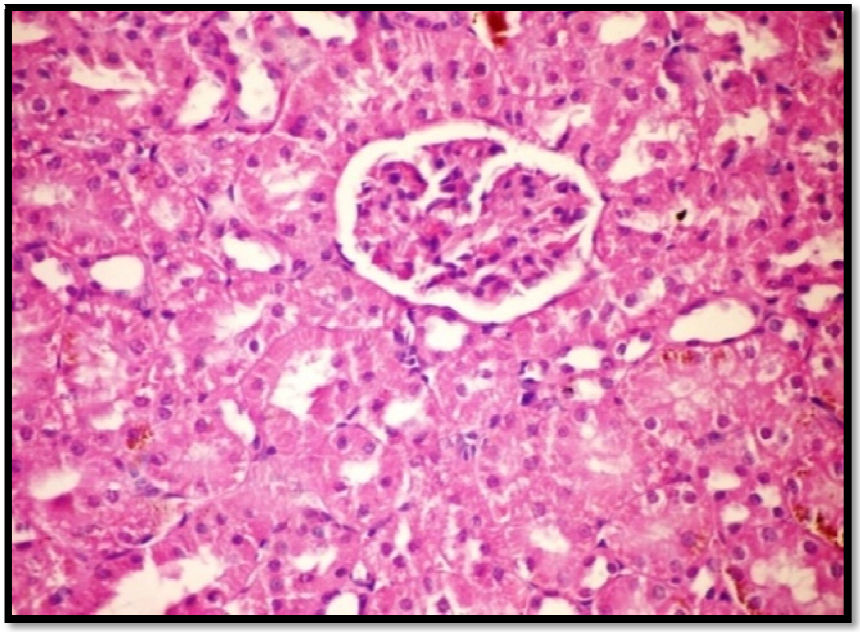


Fig. 5. Kidney of rats from group 3 that is taken aqueous extract of ginger showing no histopathological changes (H and E × 400)

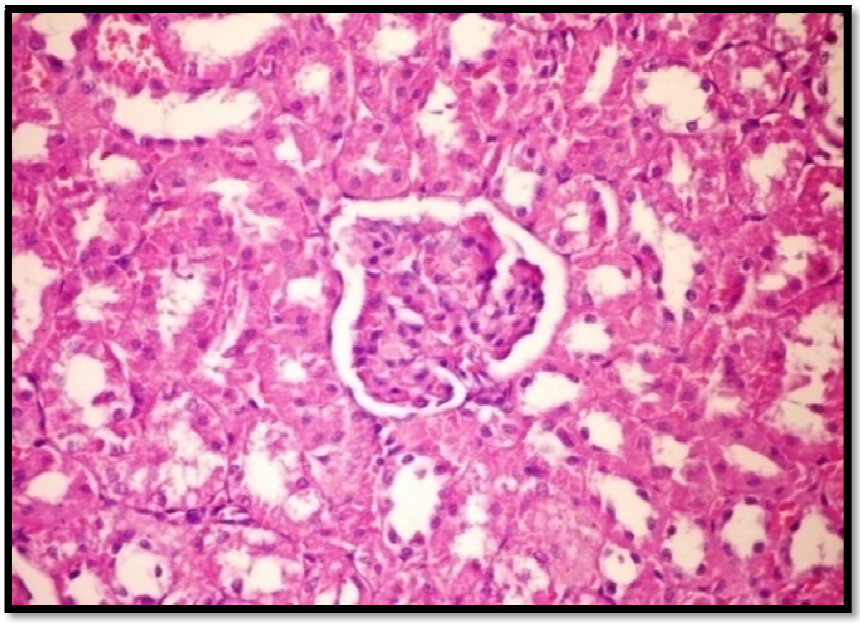


Fig. 6. Kidney of rats from group 4 that taken honey bee solution showing no histopathological changes (H and E ×400)

An addition relative kidney weight, there were no significant differences among all groups because the short period of trail didn't allow weighing the change.

About therapy with aqueous extract of honey the results indicated that animals received the aqueous extract of honey had good body weight gain compared with the group of control -ve. As well as food intake and FER results indicated that theory with honey bee increased the quantity of food intake compared with all experimental groups. These results supported by earlier studies so, honey contain high energy (100 g \

honey contain 304, 8 kcal) Honey is rich in carbohydrates, proteins, vitamins, trace elements, enzymes, and phenolic compounds [15].

The Blood picture results indicated that the group of animals give MTX alone decreased significantly in hemoglobin, RBC,S and WBc.s percentage compared with healthy and treated rats with ginger and honey solution. These findings agreed with Labib et al. [45] and Banji et al. [46]. They founded in injected mice by MTX alone latest significant and substantial changes in the blood picture.

Concerning the rats treated with ginger the results indicated that hematological parameters become normal didn't differ compared the healthy rats. That results because the ginger is rich in nutrition value as iron each 100 g of dry ginger powder contains 19.8 mg of iron equal (152%) of United States recommendations for adults [9,11]. Ginger has been fractionated into at least 14 bioactive compounds, including –4 gingerol, 6-gingerol, 8-gingerol, 10-gingerol,6-paradol,14-shogaol,6-shogaol,1-dehydro-10-gingerdione,10-gingerdione,hexahydrocurcumin, tetrahydrocurcumin, gingerenone A, 1,7-bis-(4′ hydroxyl-3′ methoxyphenyl)-5-methoxyhepthan-3-one, and methoxy-10-gingerol. So that supported the immunity system [8,11,13,15].

As regard to treatment by honey bee solution significantly increased inHb, RBC,S and WBc.s markers compared to the control –ve. Their increased magnitude was the best of the control –ve. So honey bee is rich with minerals [15].

It could be seen that (MTX) effect on of the kidney function by raised the urea and significantly decreased the total protein levels and concentrated calcium in the blood. Meanwhile, the drug causes strong binding between total protein and decrease absorption. In addition, increased protein and calorie malnutrition [44]. These finding accomplishments with histopathology results that are supported this conclusion.

Concerning the kidney function the lowest urea concentration in serum has seen significantly in rats treated with aqueous extract of ginger. Because the major pathway of nitrogen excretion is as urea synthesis in the liver that released into the blood and cleaned by the kidney, the ginger extract can be either through a lower rate of urea synthesis in the liver, as well as reduction in food intake and body wt. gain agreement by that results. The reduction of blood urea nitrogen in animals receiving ginger extract is interpreted as suggested a mechanism of reabsorption inhibition of urea in the nephrons [47].

Since the several studies [47,48] indicated that ginger extract markedly decreased the blood urea nitrogen concentrations in experimental mice in a non-linear fashion concerning the administered dose. However, little changes observed in the levels of creatinine in these animals as compared with the control group. It means that the creatinine is filtered only but is not reabsorbed; therefore, ginger might have little influence on its excretion whereas urea is filtered and reabsorbed partly in the nephrons.

Although, some parameter of kidney enzyme didn't improve in the short period at the time of study as (albumin & total protein) because renal injuries may contribute to low- level of serum protein that might have resulted from remarkable leakage into the urine due to injuries in glomeruli and tubules [48].

In this study, it is shown that MTX administration significantly increased at the level of MDA in blood as well as significantly decreased in total antioxidant. In earlier studies Vardi et al. [49] that has observed similarly affected of MTX on intestinal and testicular tissue. Eva et al. [50] reported, the drug pharmacodynamics and toxicity are dependent on its concentrations in plasma, which in turn are directly related to MTX's elimination in the liver and kidney. Moreover, it is known that oxidative stress plays a role in the tissue damage caused by MTX [51]. Methotrexate significantly altered the oxidant/antioxidant balance, increased MDA level accompanied with decreased total antioxidant similar results were previously reported by other investigators [41,51,52].

Besides, treatment with aqueous extract of ginger significantly increased the total antioxidant level, but also significantly decreased the level of MDA in blood that causing because of the ginger contained the highest percentage of antioxidant (3.85 mmol/100 g) of total antioxidants [12]. Also, these results were in harmony with Lebda et al. [53], who reported ginger in its different forms significantly reduced malondialdehyde (MDA) level, glutathione peroxidase (GPX) and glutathione-S-transferase (GST) activities. As well as these results agree with Ajith et al. [11] who reported that the

presence of polyphenols and flavonoids in the ginger extract might be responsible for the antioxidant nephroprotective activities and reduction of serum urea and creatinine levels.

In total, ginger is treating kidney protector of inflammation resulting from MTX drug, where the histopathology results supported these findings. Those results agree with Manal et al. [54] who, reported that treatment with different extracts of ginger ameliorated kidney function parameters. Also, Ali et al. [55] reported that ginger is a strong anti-oxidant substance and may either mitigate or prevent the generation of free radicals. It has considered a safe herbal medicine with only few and insignificant adverse/side effects. Ginger had reported decreasing age-related oxidative stress markers [56].

The honey protects the kidneys rat of the MTX drug by lower significantly the level of kidney enzyme in blood. Many authors reported the benefit the honey. Honey has an element of "natural cancer vaccine" as it can reduce chronic inflammatory processes, improve immune status, reduce infections by hardy organisms and so forth. Some simple and polyphenols found in honey, namely, caffeic acid, caffeic acid phenyl esters, chrysin, galangin, quercetin, kaempferol, acacetin, pinocembrin, pinobanksin, and apigenin , have evolved as promising pharmacological agents in the prevention, treatment of many diseases and cancer [57,20,21]. Our histopathology results participated with our biology and biochemistry results so the honey solution (diluted) protects the kidney tissue from the toxicity of MTX drug.

5. CONCLUSION

As a result, ginger and honey might be an effective prophylaxis in protecting the kidney in rats given MTX drug. However, the future experimental and clinical studies are required to confirm these findings.

CONSENT

Not applicable.

ETHICAL APPROVAL

Author hereby declares that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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