

Potential Ameliorative Effect of Crude Honeybee on Monosodium Glutamate Induced Nephrotoxicity to Male Rats

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ABSTRACT

Background: Monosodium glutamate (MSG) is a commercial food improver and is widely marketed as a flavor enhancer. It is now utilized in many processed foods and by most fast-food chains. Honey is a potent antioxidant that acts in the body against many diseases.

Objective: The aim of the current study is to investigate honey's ameliorative effect on kidney damage initiated by monosodium glutamate in adult male rats. Moreover, different polyphenolic compounds in the crude honey were evaluated.

Material and methods: Forty adult male albino rats were equally divided into four groups (N=6): The Control group was administered 1 mL of saline daily orally, the MSG group (30 g/kg on diet), the Honey group (2.5 g/kg body weight/day, orally) and Honey/MSG group as the previous for one month.

Results: Urea, creatinine, and uric acid were significantly increased in the MSG group and decreased in the honey/MSG group compared to the control group. MSG markedly destructed glomeruli of the kidney and increased the immunoexpressing of nuclear erythroid-related factor 2 (Nrf2) and tumor necrosis factor-alpha (TNF- α).

Conclusions: Administration of crude honey attenuates and improves the kidney pathological changes induced by MSG.

Keywords: Kidney, MSG, Honey, Histopathology, Nrf 2, TNF- α .

INTRODUCTION

Monosodium Glutamate (MSG) is added to foods and abundantly found in various types of packed foods such as processed poultry and meat, preserved fish products, and food extras [1].

MSG is a toxic substance that spreads slowly and fleeces behind lots of names, such as natural flavoring and yeast extract [1]. It is not a vitamin, nutrient, or mineral and possesses no beneficial health effects [2]. However, it is added by manufacturers as a food additive to inhibit the microorganisms' growth that causes rot or foodborne ailment and rise consumer acceptability, in addition to its taste and flavor enhancement properties [3].

After MSG metabolism, 78% glutamate, 12% sodium, and about 10% water is released [1]. Glutamate, not sodium, is the component of MSG that negatively affects the body. Due to the kidney's abundance of glutamate receptors, MSG consumption makes the kidney a target for the glutamate [4].

The renal corpuscles filter the glutamate, which then passes via the convoluted tubules of the cortex, making them vulnerable to the harmful effects of MSG [5]. Elbassuoni *et al.* [5] found that there were areas of renal interstitial hemorrhage and congested blood capillaries in rats treated with high-dose MSG. Long-term MSG administration has harmful properties on the internal organs such as renal upsetting redox

homeostasis and encouraging histological lesions as well as lipid peroxidation [4].

Natural products constitute the requisite keystone in the remedy of various diseases for several centuries being superior to traditional medicine in their bioavailability and wide safety. The usage of natural remedies from medicinal plants or food is safe and effective for nephrotoxicity, mainly due to the presence of several antioxidant ingredients [6].

Among them having extremely auspicious beneficial health influences, honey formed from nectar by honeybees (*Apis mellifera*), is one of these natural products. It is an indispensable micronutrient required for normal metabolic function in the body and acted as a powerful antioxidant by scavenging many Reactive Oxygen Species (ROS) [7]. The phytochemical analysis of the honey ingredients showed the highest quantity of polyphenolics, anthocyanins, carotenoids, and flavonoids [6].

Numerous research groups have informed that crude honey participated in hypertension prevention, hyperglycemia, nephrotoxicity, enhancing serum antioxidant status, oxidation of lipoproteins, and lessening the injurious impacts of oxidative stress and inflammation on the excretory system [8].

However, no research has inspected the useful effect of crude honey against kidney damage induced by MSG. According to a previous study by Laaroussi *et*

al.^[8], crude honey was found to be kidney-protective against damage induced by xenobiotics. Therefore, the present study was designed to investigate the protective effect of crude honey, against MSG-induced nephrotoxicity in male Wistar rats.

MATERIAL AND METHODS

High-Performance Liquid Chromatography (HPLC) analysis of the crude honey

Honey was purchased in season December 2021 and stored in the Prophetic Medicine Foundation, Ismailia, Egypt with Commercial Registration code No (6332001/111330). The HPLC analysis of the honey polyphenolic compounds was carried out according to Pascual-Maté *et al.*^[9]. Peaks were identified by congruent retention times compared with standards (gallic, protocatechuic, syringic, p-hydroxybenzoic, Genistin, Catechin, Chlorogenic, Ferulic, Sinapic, Caffeic, Syringic, Vanillic, p-Coumaric, Rutin, Rosmarinic, Apigenin-7-glucoside, Cinnamic, Quercetin, Apigenin, Kaempferol, Chrysin, Naringin, Hesperidin) prepared in a concentration of 1 mg/ml. All the chemicals and reagents used in this study were supplied by Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Animal and experimental design

A total number of forty adult male albino rats (120-150 g) were brought from the animal house of veterinary medicine. Rats were reared under the standard conditions of feeding, light/dark ratio (12:12 hrs), temperature (24±2°C), and humidity (50±10%). The animals were allocated randomly into four groups (n= 6): the control group was administrated 1ml of saline daily orally, MSG group was administrated (30 gm/kg in diet), according to Abd-Elkareem *et al.*^[4]. The MSG was obtained from the Morgan Chemical industry, Egypt with a purity of 99%. The honey group was administrated (2.5 gm/kg body weight /day, orally) according to Al-Seeni *et al.*^[10]. Honey/MSG group was administrated honey then after 2 hrs MSG in dose as previously mentioned for one month. The animals were treated and handled according to the guidelines of laboratory animals and the study was approved by the Faculty of Science, Suez Canal University, Ismailia, Egypt (REC93/2022).

Blood Serum and kidney collection

The rats were anesthetized, after overnight fasting, at the end of the experimentation. Blood samples were got by the intra-cardiac route. The serum samples were collected and kept at -20°C until analysis. Animals were euthanized and the kidney was excised quickly, cleaned with (0.9%, w/v) cold physiological saline solution, dried, and weighed. About 1 g of the kidney was used, homogenized in ice-cold buffer, and

centrifuged at 7000 xg for 20 min at 4°C. Supernatants were harvested and stored at -70°C until use. Another part of kidney tissue was fixed in 10% neutral formalin buffer solution for fixation to perform histopathological inspection and immunohistochemical expression at the light microscopic.

Assay for some kidney function, oxidative, antioxidative status in blood serum, and IL-2 level in kidney homogenate:

For the assay for some kidney function, uric acid, creatinine, and urea were measured spectrophotometrically according to Walsh and Dempsey^[11].

For the assay of oxidative, antioxidative status in blood serum and IL2 in kidney homogenate were assayed. Total antioxidant capacity (TAC), total oxidant status (TOS), and superoxide dismutase (SOD) activity were assayed spectrophotometrically as described by Żebrowska *et al.*^[12]. IL-2 level was assayed in kidney homogenate Colorimetric using an Enzyme-linked immunosorbent assay kit (Abcam (Cat. No: ab221834), USA) as described by Alkhedaide^[13].

Histopathological and immunohistochemical analysis:

For the cells/mm². The immunoreactivity for Nrf2 and TNF-α at a magnification of × 400 was calculated from nine non-overlapping fields for each section to determine the immunoreactions and area % of the PAS-positive reaction stain.

Ethical approval:

The study protocol was approved by the Ethical Committee of Suez Canal University. This work has been carried out according to The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Statistical analysis:

The collected data were coded, processed, and analyzed using the SPSS (Statistical Package for Social Sciences) version 20 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi-square test (χ^2) and Fisher's exact test to calculate the difference between two or more groups of qualitative variables. Quantitative data were expressed as mean and standard deviation (SD). Independent samples t-test was used to compare between two independent groups of normally distributed variables (parametric data). One-way ANOVA was used to compare the means of these variables between different groups followed by the Duncan test. P value ≤ 0.05 was considered significant.

RESULTS

HPLC analysis of the crude honey

The analysis of flavonoid compounds and phenolic acids in the honey was made utilizing HPLC and is revealed in **Table 1**. A total of 15 polyphenolic compounds were found. The content of hesperidin (63.791 µg/100 g FW) was higher among the identified compound, followed by Naringin (34.062 µg g/100 g FW).

Table (1): The analysis of phenolic acids and flavonoid compounds in honey aqueous extract using HPLC.

Compound	Concentration (µg/g)
Gallic	1.188
Protocatechuic	ND
p-hydroxybenzoic	0.329
Gentisic	ND
Catechin	ND
Chlorogenic	0.691
Caffeic	0.061
Syringic	0.277
Vanillic	ND
Ferulic	0.025
Sinapic	0.013
p-coumaric	ND
Rutin	ND
Rosmarinic	0.172
Apigenin-7-glucoside	ND
Cinnamic	0.049
Quercetin	0.084
Apigenin	0.064
Kaempferol	0.085
Chrysin	0.042
Naringin	34.062
Hesperidin	63.791

Relative kidney weights

The relative kidney weight of male rats subjected to different treatments showed a non-significant difference among all the experimental groups as shown in **Table 1**.

Assay for some kidney function, oxidative, antioxidative status in blood serum, and IL-2 in kidney homogenate.

The results gained from estimating the biochemical parameters were summarized in **Table 1**. The mean values of kidney functions (urea, uric acid, and creatinine) were significantly (P <0.05) promoted in the MSG-administered rats as compared to the control group. On the other side, the determined mean value of kidney function of the treated rats with honey and

MSG declined significantly (P <0.05) matched to the MSG group. Moreover, in comparison with the control rats, the TAC and SOD activity of the MSG-administered group showed a significant reduction. The levels of TOS and IL-2 in the MSG-administered group were significantly upregulated compared to the control rats. While, using honey supplements before treatment with MSG revealed a decrease in TAC, SOD, TOS, and IL-2 levels nearest to the control levels.

Table (1): Effect of honey on relative kidney weight, kidney function, oxidative, antioxidative status in blood serum, and IL-2 level in kidney homogenate on treated rats with MSG

Parameters	Treatment			
	Control	Honey	MSG	Honey/MSG
Relative weight of the kidney	0.268 ± 0.007	0.288 ± 0.02	0.281 ± 0.01	0.280 ± 0.08
Urea (mg/dl)	38.33 ± 1.1 ^c	34.7 ± 0.6 ^d	55.43 ± 1.2 ^a	50 ± 1.7 ^b
Uric acid (mg/dl)	4.33 ± 0.1 ^b	3.93 ± 0.1 ^b	6.790 ± 0.3 ^a	4.5 ± 0.2 ^b
Creatinine (mg/dl)	0.979 ± 0.1 ^c	0.754 ± 0.1 ^d	1.85 ± 0.2 ^a	1.27 ± 0.1 ^b
TOS (mmol Trolox Eq./l)	5.52 ± 0.2 ^c	3.62 ± 0.2 ^d	11.14 ± 0.8 ^a	7.51 ± 0.4 ^b
TAC (mmol Trolox Eq./l)	1.373 ± 0.07 ^a	1.83 ± 0.05 ^{ab}	0.676 ± 0.042 ^b	3.69 ± 0.67 ^a
SOD (U/ml)	485.81 ± 10.4 ^b	818 ± 31.2 ^a	358.00 ± 18.4 ^c	451.33 ± 16.0 ^b
IL-2 (pg/mL)	1.463 ± 0.07 ^{bc}	1.333 ± 0.05 ^c	1.986 ± 0.04 ^a	1.639 ± 0.06 ^b

Data are presented as means ± SE (n= 6). Mean values with different superscript letters within the same row are significantly different at P ≤0.05 using ANOVA followed by a Duncan multiple comparison test. MSG: monosodium glutamate; TOS: total oxidative stress; TAC: total antioxidant capacity; SOD: Superoxide Dismutase; IL-2: Interleukin 2.

Histopathological and immunohistochemical analysis:

The control group's kidney sections showed the cortex's normal structure consisting of glomeruli with neighboring renal tubules (proximal and distal convoluted tubules) (**Figure 1a**). The Honey group did not reveal any histoarchitectural alterations (**Figure 1b**). The changing of histopathological changes in the renal tissue of the MSG-administered group were noted as atrophic shrinkage glomerulus and mild to moderate infiltration with lymphoid cells in the interstitial tissue (**Figure 1c**). However, these renal deviations were markedly ameliorated with honey administration, as renal tubules and glomerulus were protected.

Examination of renal sections of control rats and honey-treated rats that were stained with PAS showed a strong positive histochemical staining in Bowman's capsules and the basement membranes of the proximal and distal convoluted tubules. Intense histochemical staining in the brush borders of proximal convoluted tubules was observed (**Figures 2a and b**). MSG-treated group stained with PAS revealed minimum histochemical staining in Bowman's capsules and tubules. A weak and partial loss of the brush border of the proximal convoluted tubules was noticed (**Figure 2c**). Kidney sections of rats administered MSG stained with PAS revealed minimum positive reaction. Rats

treated with honey before MSG stained with PAS revealed a positive reaction in Bowman's capsules and tubules, in addition to a strong reaction in the brush borders of proximal convoluted tubules (**Figure 2d**). Percentage-positive cells comparison, within the kidney between control and all treatment groups (**Figures 3 and 4**), demonstrated a significant change according to the immunohistochemical expression of Nrf2 and TNF- α . MSG group exhibited the largest mean value of Nrf2 and TNF- α positive cells, while the Honey/MSG administered group demonstrated the lowest significant mean value of Nrf2 and TNF- α positive cells.

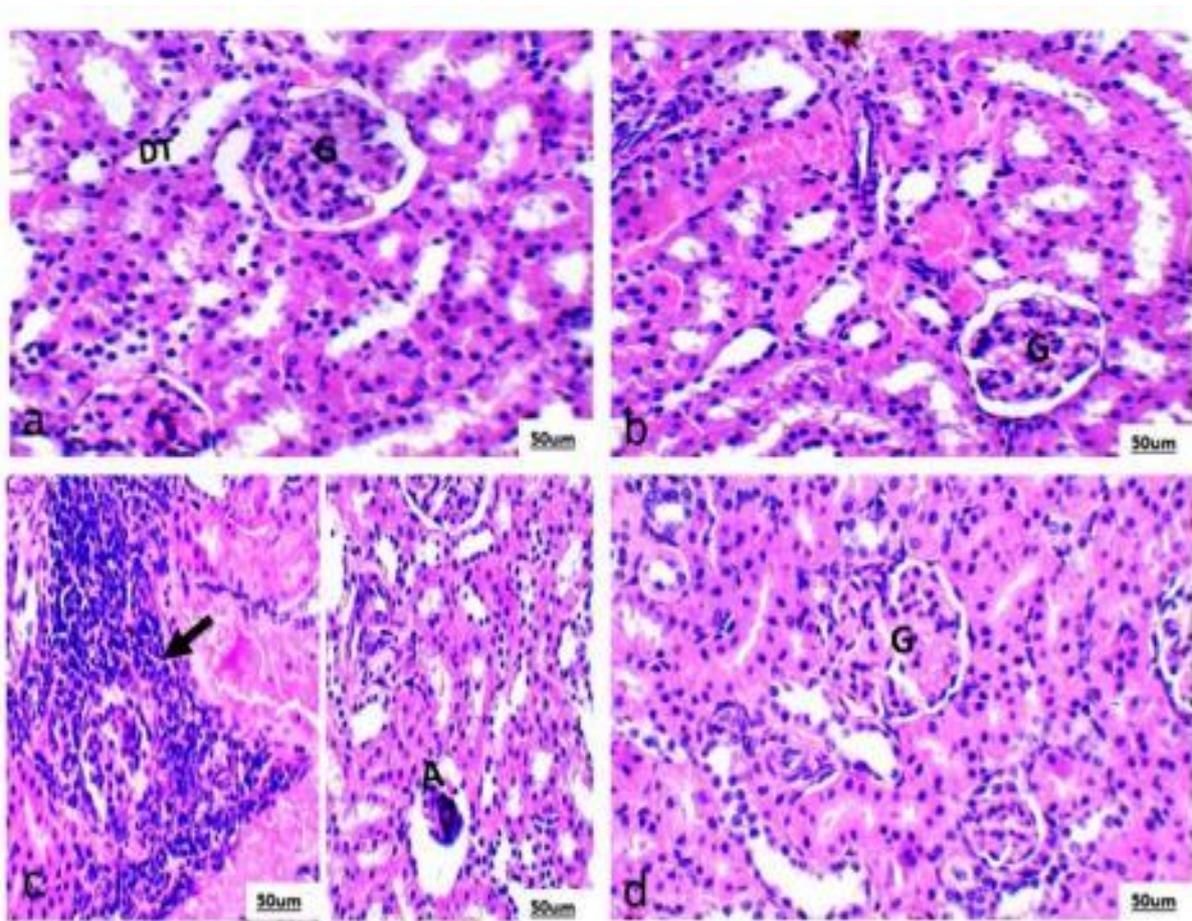


Figure (1): Photomicrograph of the kidney: (a) control and (b) honey group showed ordinary glomerulus with Bowman's space and the neighboring renal distal (D) and convoluted tubules. (c) monosodium glutamate (MSG) group demonstrated interstitial infiltration of inflammatory cells in the cortex (arrow) and atrophic glomerulus (A) with widened Bowman's space and (d) honey and MSG treated group showed ordinary glomerulus with Bowman's space and the neighboring renal tubules. (HE, 200X).

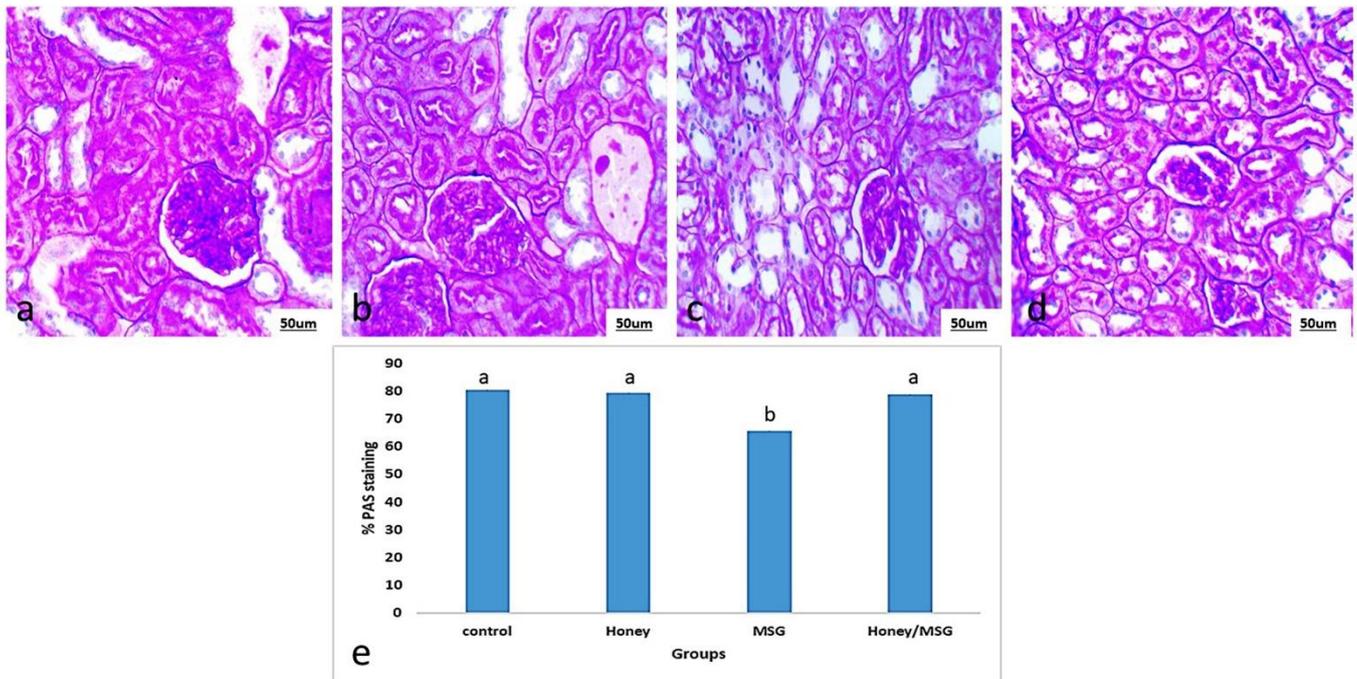


Figure (2): Representative of the kidney using PAS stain to evaluate the glycoprotein of (a and b) control and honey groups showed positive reaction in the Bowman's capsules and the tubules. (c) MSG group showed a weak reaction in the brush borders of proximal convoluted tubules and the Bowman's capsules were noticed. (d) Honey and MSG group showed positive reactions in the Bowman's capsules and the tubules. (PAS X200). (e) Percentage area of glycoprotein stained with PAS in kidney tissue among the different groups. Different superscript letters denote significant differences at $P \leq 0.05$.

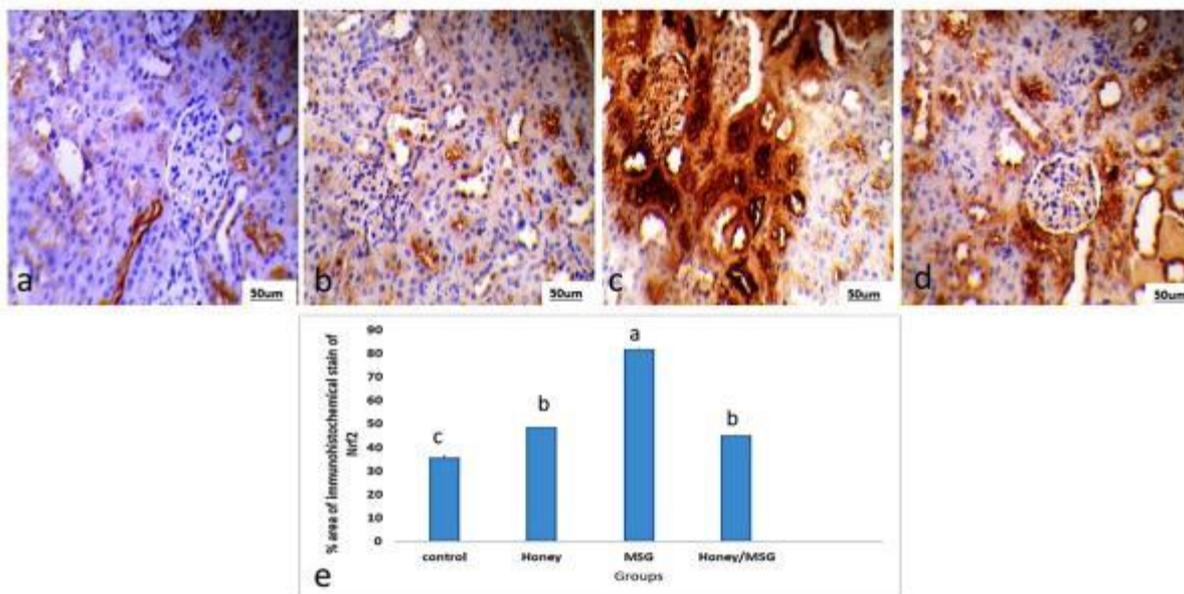


Figure (3): Representative of immunohistochemical staining for nuclear erythroid-related factor 2 (Nrf2) in the kidney of (a) control (b) honey group (c) MSG group (d) Honey and MSG group (X200). (e) Histogram of the mean percentage areas of Nrf2 in the kidney of different groups (n = 6). Different superscript letters denote significant differences at $P \leq 0.05$.

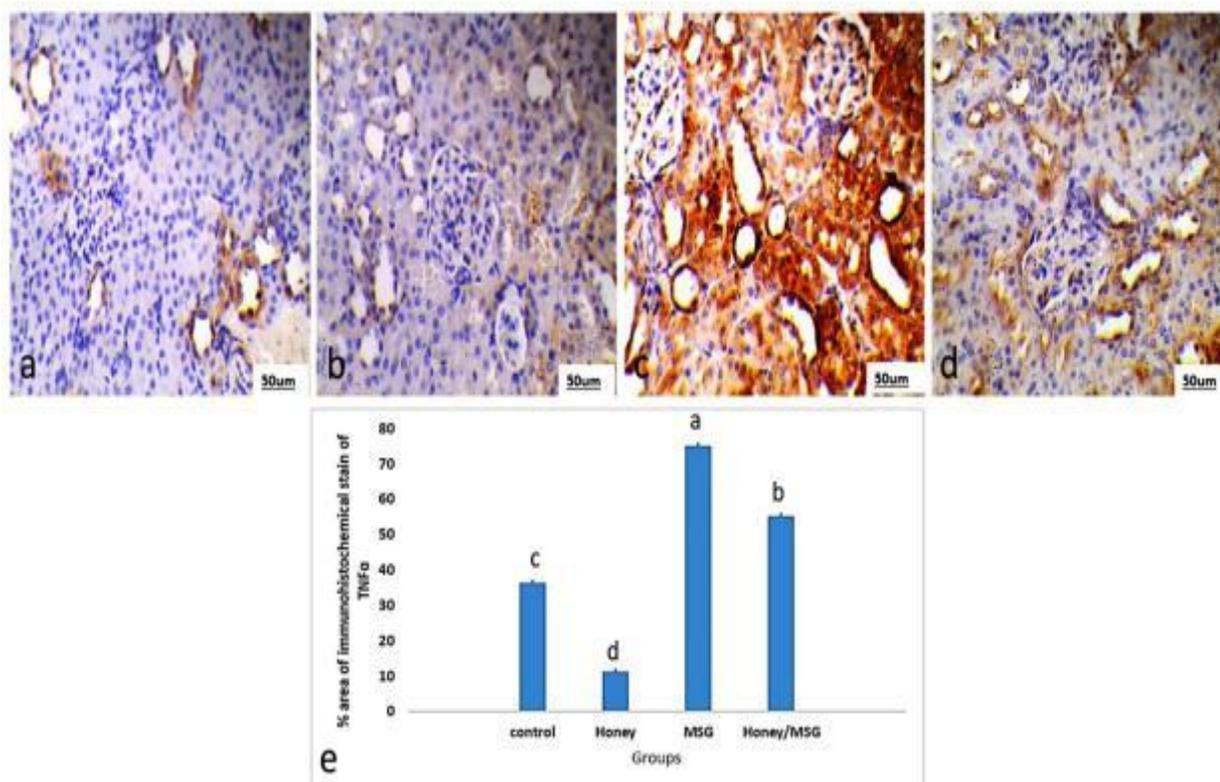


Figure (4): Representative of immunohistochemical staining for tumor necrosis factor-alpha (TNF- α) in the kidney of (a) control (b) honey group (c) MSG group (d) Honey and MSG group (X200). (e) Histogram of the mean percentage areas of TNF- α in the kidney of different groups (n = 6). Different superscript letters denote significant differences at $P \leq 0.05$.

DISCUSSION

The current research article targeted the protective effects of oral administration of honey on MSG-induced kidney dysfunction. Different phenolic and flavonoid compounds were analyzed using HPLC. Gallic, p-hydroxybenzoic, Chlorogenic, Caffeic, Syringic, Ferulic, Sinapic, Rosmarinic, Cinnamic, Quercetin, Apigenin, Kaempferol, Chrysin, Naringin and Hesperidin were detected in the collected honey samples. The phenolic acids and flavonoids that give honey its richness have a variety of biological effects and serve as natural antioxidants. Hesperidin, an abundant compound, has been used as a marker for citrus honey [16]. The detected compounds of Kaempferol and gallic acid are well-identified for their antioxidant criteria, as mentioned by Dar *et al.* [17]. The phenolic and flavonoid compounds that are existed in the honey under investigation own several medicinal criteria, such as antioxidant and anti-inflammatory ones.

Our key findings were that the administration of MSG to experimental rats prompted renal oxidative damage along with inflammation with associated histopathological lesions. Honeybee intervention effectively mitigated these perturbations by ameliorating oxidative stress, the alteration of kidney function, and hindering the renal inflammatory pathway.

Results of the present study showed a marked increase in kidney function in the MSG group. The significant alterations in the kidney function limits of the MSG-administered group agreed with the earlier findings of del Carmen Contini *et al.* [18]. TOS and TAC are proposed to be a highly related biomarkers for evaluating oxidative/reductive potency, taking into consideration the synergistic and cumulative action of all the antioxidants and the oxidative that are existed in the tissue [19].

In the current study, MSG treatment tended to increase TOS levels and decreased TAC and SOD activity statistically significant in comparison to the other groups. One of the main consequences of MSG is oxidative stress. TAC and SOD are the defense mechanism against ROS activities. Unfortunately, antioxidant enzyme levels were reduced in the group treated with MSG. Also, an elevation in the level of TOS in the treated group with MSG was recorded by Yonden *et al.* [20]. In the present study, honeybee supplements given with MSG helped restore kidney function and oxidant/antioxidant equilibrium and normalize the SOD, TOS, and TAC activities. Decreased formation of reactive oxygen species and enhancement of total antioxidant capacity is the main mechanistic methods by which honeybees exhibit antioxidant activity and protect against the adverse effects of MSG [21].

There was a significant elevation in the renal IL-2 levels of the MSG-administered group compared to the control rats. MSG might trigger inflammation because MSG-enhanced renal disease is accompanied by a massive infiltration of macrophages as well as the excretion of inflammatory cytokines into the systemic circulation [22]. IL-2 levels are raised when immune cells are stimulated by numerous pathophysiological triggers such as MSG [23]. The possible mechanisms for an increment of tissue IL-2 level in MSG treated group are renal dysfunction, and oxidative stress [23]. In the current study, the treated group with honey and MSG showed a significant decrease in renal tissue IL-2 levels compared to the control group.

The histopathological variations in the MSG-administered group's renal tissue coincide with that detected in the measured biochemical parameter. The changes in the renal cortex after MSG treatment showed degenerated atrophic glomerular capillaries tufts and widened Bowman's space and interstitial intrusion with inflammatory cells in the renal tissue treated with MSG. Infiltration of inflammatory cells in the renal cortex could demonstrate a defensive slant against MSG [22]. These changes were confirmed in the previous studies by **Nnadozie et al.** [24], which found the same result. The mechanism of renal cortex injury is mainly due to oxidative stress which was detected by the increase of TOS and the decrease of TAC and SOD. Honeybee normalized the kidney histological outlines and protected the renal tubules and glomeruli from inflammatory reactions and degeneration induced by MSG. Following honeybee treatment, MSG-treated rats' renal histoarchitecture improved, in line with **Neamatallah et al.** [25], which showed improvement in kidney histology after using it. Honeybee confers renal protection against MSG by reducing renal oxidative damage and activating antioxidants. Antioxidant and anti-inflammatory are characteristics of the honeybee's active component [26].

The diminution of cellular infiltration in the renal cortex of the honey/MSG-administered group reflects the potent immunomodulatory potential of honey [27] and its prominent role in the promotion of cellular immunity [28]. It reduces the relocation of these cells from the peripheral circulation because it possesses an anti-inflammatory role by suppressing the production of TNF- α [26].

The reduction in histochemical reactivity of PAS in the renal tissue of the MSG group is similar to that observed in former studies of **Bhattacharya et al.** [29] and **Abdel-Aziz and Mohamed** [30] indicating reduced glycoprotein accretion. MSG has worsened drawbacks on carbohydrate metabolism by reducing glucose use via fasting hepatic glucose release to the bloodstream, glycolysis, and exhausting glycogen reserve [31]. On the parallel side, honey returned tissue glycogen reserve storage owing to the promotion of

pancreatic insulin production which stimulates glycogen synthase activity [32].

The positive immunohistochemical expression of Nrf2 and TNF- α in the kidney of the MSG rats significantly upregulated than the control group. The positive immuno-expression of Nrf2 and TNF- α increased inflammatory response oxidative stress in the kidney tissue of the MSG group [33]. Might be concerned with the abridged inflammation and oxidative damage of the kidney. Built on the results of this investigation, it is hypothesized that the machinery of kidney histological deviations is caused in part by Nrf2 and TNF- α activation.

CONCLUSION

MSG altered renal dysfunction markers, elevated inflammation, disrupted histological structure and negatively affected histochemistry. By restoring redox potential and reducing histopathological deteriorations, reduced glycoproteins, and inflammation in the kidney, the nutritional use of crude honey somewhat mitigates the detrimental effects of MSG consumption on renal structure and function. These findings open up new research directions into the efficacy of honeybee supplements in preventing other MSG side effects as well as the potential use of honeybee supplements as a prospective approach against MSG-induced side effects.

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