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Evaluation of the possible ameliorative effect of spirulina on nephrotoxicity induced by methomyl in male albino rats

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Abstract

Methomyl (MET) is a widely used pesticide that has a number of health harmful impacts. Many bioactive and antioxidants components found in spirulina (SP). The target of this study was to estimate the ameliorative role of SP on MET-induced biochemical and histological alterations in rat's kidneys. Rats were divided into four groups; group I employed as a control group, group II was SP-treated rats that received SP orally (500 mg/kg b.wt) for three weeks, group III was MET-treated rats that received MET orally (1/20 of LD_{50}) for three weeks and group IV was rats that received both MET and SP for three weeks with the same previous doses. After three weeks, rats of all groups were weighted and sacrificed. Sera samples were used for biochemical analysis of urea, creatinine and uric acid concentrations and kidney tissues were used for malondialdehyde (MDA), interlekine-6 (IL-6), glutathione (GSH) concentrations and catalase (CAT) activity estimations, besides histological examination and immunohistochemical investigation of tumor necrosis factor-α (TNF-α). The results summarized that MET induced renal impairment and co-treatment of SP and MET caused significant reduction in urea, creatinine, uric acid, MDA, IL-6 concentrations, significant elevation in GSH concentration and CAT activity and lessened histological and immunohistochemical alterations owing to SP high content of antioxidant and free radicals scavenging capacity.

Keywords: Methomyl; Spirulina; Nephrotoxicity; oxidative stress; antioxidants; Rats.

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1. Introduction

Methomyl (MET) {bis[1-methylthioacetaldehyde-O-(N-methylcarbamoyl)oximino]sulfide} is a highly toxic oxime carbamate insecticide that is vastly used in vegetables, fruits, crops, cotton and commercial ornamentals foliar treatment (1). It is also a potential toxic pesticide on aquatic organisms and humans (2). MET causes adverse effects on human health through increment of reactive oxygen species (ROS) production that produces lipid peroxidation leading to degradation of nucleoprotein, nucleic acid and DNA fragmentation (3). Several studies documented MET-induced toxicity including hormonal disruption (4), renal and reproductive toxicity (5), cardiac toxicity (6), genotoxicity and teratogenicity (7).

Spirulina (SP) is a famous genus of cyanobacteria mainly *Arthrospira platensis* and *Arthrospira maxima*. More than 30% of the world microalgal biomass production is obtained from SP and can be used to decrease greenhouse gases and for effluent treatment (8).

SP has an attractive nutritional components of macromolecules such as carbohydrates, lipids, proteins, vitamins and minerals, which are vital for the human nutrition. Besides, it has other compounds that have numerous biological effects such as unsaturated fatty acids, amino acids, carotenoids and phenolic compounds (9). Numerous investigations have been reported on the biological properties of SP, including its immunomodulatory and antioxidant properties (10), antitumorgenic properties (11) and neuroprotective properties (12, 13). The target of this study was to measure the ameliorative role of SP on MET-induced nephrotoxicity.

2. Material and Methods

Spirulina (SP) was purchased as tablets from Puritans Pride, USA, while MET was used as Lannate 90%, USA.

Experimental design:

Forty male albino rats (*Rattus norvegicus*), weight about 150g ± 1.39, were kept in plastic cages and had the standard diet and tape water. Rats randomly divided into four groups, 10 rats in each group, as follows:

Group I (Control group): Rats were orally given distilled water.

Group II (SP-treated group): Rats were orally given SP (500 mg/kg bwt) daily for three weeks (14).

Group III (MET-treated group): Rats were orally given MET (1/20 of LD_{50}) (15) daily for three weeks.

Group IV (MET&SP-treated group): Rats were orally given both MET and SP similar to group II and III.

After three weeks (experimental period), rats were fasted for 12 hours, weighted then anesthetized and dissected. Blood was taken from the heart, placed into plain tubes and centrifuged to obtain sera. Biochemical analysis of urea, creatinine and uric acid were carried out in sera. Kidney tissues from rats of all groups were collected and split into two portions. The first portion was homogenized to measure the concentrations of malondialdehyde (MDA), interleukin-6 (IL-6), glutathione (GSH), as well as the activity of catalase (CAT). The second portion was fixed in 10% neutral formalin for histological examination and immunohistochemical staining of tumor necrosis factor-α (TNF-α).

Biochemical studies:

Urea, creatinine and uric acid concentrations (mg/dl) were measured according to Fawcett and Scott (16), Bartels and Bohmer (17) and Fossati *et al.* (18), respectively by using kits of Biodiagnostic Company, Egypt. Concentrations of MDA (Nmol/g. tissue), GSH (mg/g. tissue) and activity of CAT (U/g. tissue) were determined according to Ohkawa *et al*. (19), Beulter *et al*. (20) and Aebi (21), respectively by using the kits of Biodiagnostic Company, Egypt, while concentration of IL-6 (pg/g. tissue) was determined using an Enzyme-linked immunosorbent assay (ELISA) kit (Elabscience, E-EL-H0102, USA).

Histological study:

Fixed kidney tissues from all experimental groups were processed for histological technique and stained with hematoxylin and eosin (H&E) (22). Ultimately, sections were examined and photographed by a digital light microscope (Olympus, Tokyo, Japan) with objective lenses 40X and 100X.

Immunohistochemical study of TNF-α: Immunohistochemical processing of TNF-α was performed in accordance with Hsu *et al*. (23). Hematoxylin was used as a counterstain after sections were cleaned in distilled water. A light microscope with a digital camera was used to photograph the stained sections (Olympus, Tokyo, Japan).Using Image J software, the intensity of TNF-α expression was quantitatively assessed (24).

Ethical approval: The ethical approval of this study was obtained from Faculty of Science, Damanhour University, Egypt (MU-SCI-CSRe-24- 02 -06).

Statistical analysis:

The current study results are represented as mean ± SE. The data were analyzed using SPSS software (25). The statistical significance keys in the present study between the experimental groups are $^{***}p \leq 0.001$, $^{**}p \leq 0.01$ and $^{*}p \leq 0.05$ comparing with the control group and $^{\#\#\#}p \leq 0.001$, $^{\#\#}p \leq 0.01$ and $^{\#}p \leq 0.05$ comparing with MET group.

3. Results

Body weight changes:

Data of initial, final and body weights change percent of the different experimental groups were showed in table 1. We started the experiment with initial body weights of rats that recorded an insignificant difference between the studied groups. At the end of the experiment, we recorded the rat's final body weights to measure the effect of MET and SP treatments on body weights change. From the obtained data, final body weights of MET-treated rats recorded a significant decrease (p= 0.000) as compared with the control rats, while co-treatment of rats with MET and SP resulted in a significant increase (p= 0.004) in their final body weights comparing with MET-treated rats and an insignificant decrease (p= 0.257) comparing with the control rats. Furthermore, MET-treated rats recorded a significant decrease (p= 0.000) in body weights change percent comparing with the control group, while co-treatment of MET and SP caused a significant increase (p= 0.000) in body weights change percent comparing with MET-treated rats and a significant decrease (p= 0.037) comparing with the control group.

Kidney function (creatinine, urea and uric acid) results:

Creatinine concentrations of all experimental groups were shown in table 2 and figure 1. Creatinine concentrations recorded an insignificant increase (p= 0.995) in SP-treated rats, while it recorded a significant increase (p= 0.000) in MET-treated rats as compared with the control rats. On the other hand, co-treatment of MET and SP resulted in a significant decrease (p= 0.027) in creatinine concentration as compared with MET-treated group and an insignificant increase (p= 0.066) comparing with the control group.

Urea concentrations of all experimental groups were shown in table 2 and figure 2. Comparing with the control group, SP-treated rats recorded an insignificant increase (p= 0.928) and MET-treated rats recorded a significant increase (p= 0.01) in urea concentrations. Co-treatment of MET and SP caused a significant decrease (p= 0.036) in urea concentration comparing with MET-treated rats and an insignificant increase (p= 0.948) comparing with the control group.

Uric acid concentrations of all experimental groups were shown in table 2 and figure 3. Uric acid concentrations recorded an insignificant increase (p= 0.993) in SP-treated group, while it recorded a significant increase (p= 0.000) in MET-treated group comparing with the control group. Co-treatment of MET and SP caused a significant decrease (p= 0.022) in uric acid concentration comparing with MET-

treated rats and a significant increase (p= 0.006) comparing with the control group.

Lipid peroxidation end product (MDA) and pro-inflammatory biomarker (IL-6) in the kidney tissues:

MDA and IL-6 concentrations in kidney tissues were shown in table 3 and figure 4A&B. SP-treated rats didn't record a significant difference (p= 0.959 & 0.717) in both MDA and IL-6 concentrations comparing with the control group. In the contrary, MET-treated rats recorded a significant increase at p= 0.000 & 0.001 in both MDA and IL-6 concentrations, respectively comparing with the control group.

As a result of co-treatment of MET and SP, MDA and IL-6 concentrations recorded a significant decrease at p= 0.006 & 0.050, respectively comparing with MET-treated group, while comparing with the control group, they recorded an insignificant increase (p= 0.066) in MDA concentration and a significant increase (p= 0.022) in IL-6 concentration.

Antioxidant biomarkers (GSH and CAT) in the kidney tissues:

GSH concentration and CAT activity were shown in table 3 and figure 4 C&D. Both GSH concentration and CAT activity recorded an insignificant increase (p= 0.990 & 0.888) in SP-treated group and a significant decrease (p= 0.000) in MET-treated group as compared with control group. Co-treatment of MET and SP caused a significant increase (p= 0.000) in GSH concentration and a significant increase (p= 0.019) in CAT activity when compared with MET-treated group, but they recorded a significant decrease at p= 0.006 and 0.050 in GSH concentration and CAT activity, respectively when compared with the control group.

Table 1: Initial, final and body weights (BW) change percent (%) of the different experimental groups. (n=10)

	Initial BW (g)	Final BW (g)	BW change %
Control group	150.90±1.68	176.70±1.35	16.90±0.63
SP-treated group	152.60±1.55	178.80±0.89	17.24±0.89
MET-treated group	151.00±1.25	164.50±1.36***	8.94±0.29***
MET&SP-treated group	151.30±1.10	172.60±2.26$^{\#\#}$	14.02±0.89$^{*,\#\#\#}$

Table 2: Serum creatinine, urea and uric acid concentrations of the different experimental group. (n=5)

	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
Control group	0.62±0.01	48.60±1.50	3.20±0.09
SP-treated group	0.63±0.01	50.40±3.23	3.24±0.09
MET-treated group	0.82±0.04***	59.2±1.85**	4.30±0.08***
MET&SP-treated group	0.71±0.01$^{\#}$	50.2±1.20$^{\#}$	3.80±0.15$^{**,\#}$

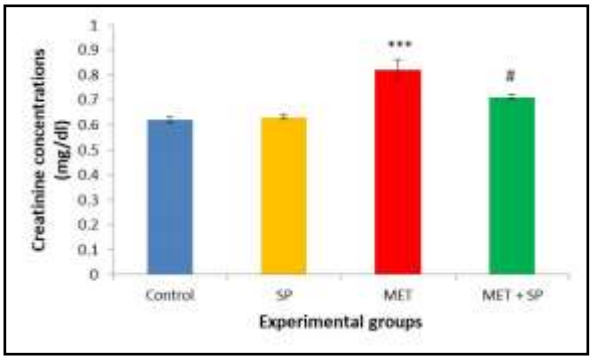

Fig.1: Serum creatinine concentrations of the different experimental groups.

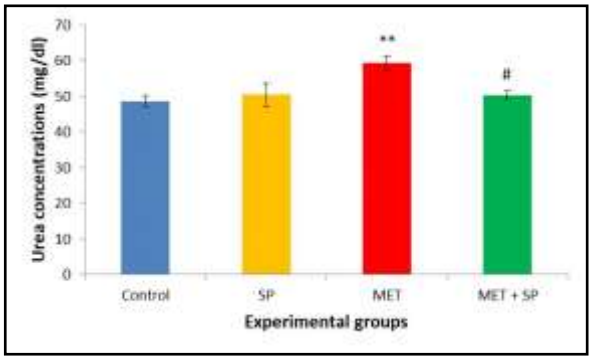

Fig.2: Serum urea concentrations of the different experimental groups.

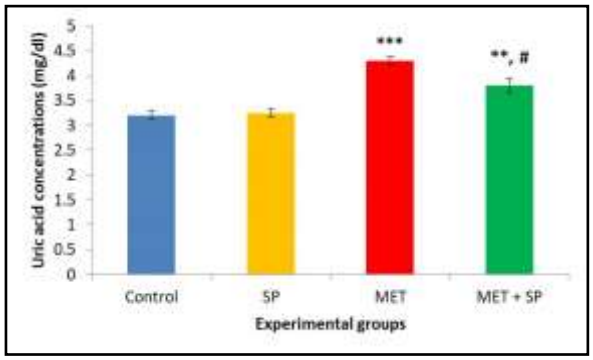

Fig. 3: Serum uric acid concentrations of the different experimental groups.

Table 3: Tissue MDA, IL-6 and GSH concentrations and CAT activity of the different experimental groups. (n=3)

	MDA (Nmol/g. tissue)	**IL-6** (pg/g. tissue)	**GSH** (mg/g. tissue)	**CAT** (U/g. tissue)
Control group	12.53±1.22	29.33±2.03	17.03±0.86	42.00±3.28
SP-treated group	11.87±0.61	33.00±2.65	17.37±0.89	44.90±4.49
MET-treated group	23.13±1.13***	53.00±2.89***	3.47±0.72***	13.13±0.75***
MET&SP-treated group	16.60±0.74##	42.33±2.03*, #	11.67±0.63**, ###	29.00±1.15*, #

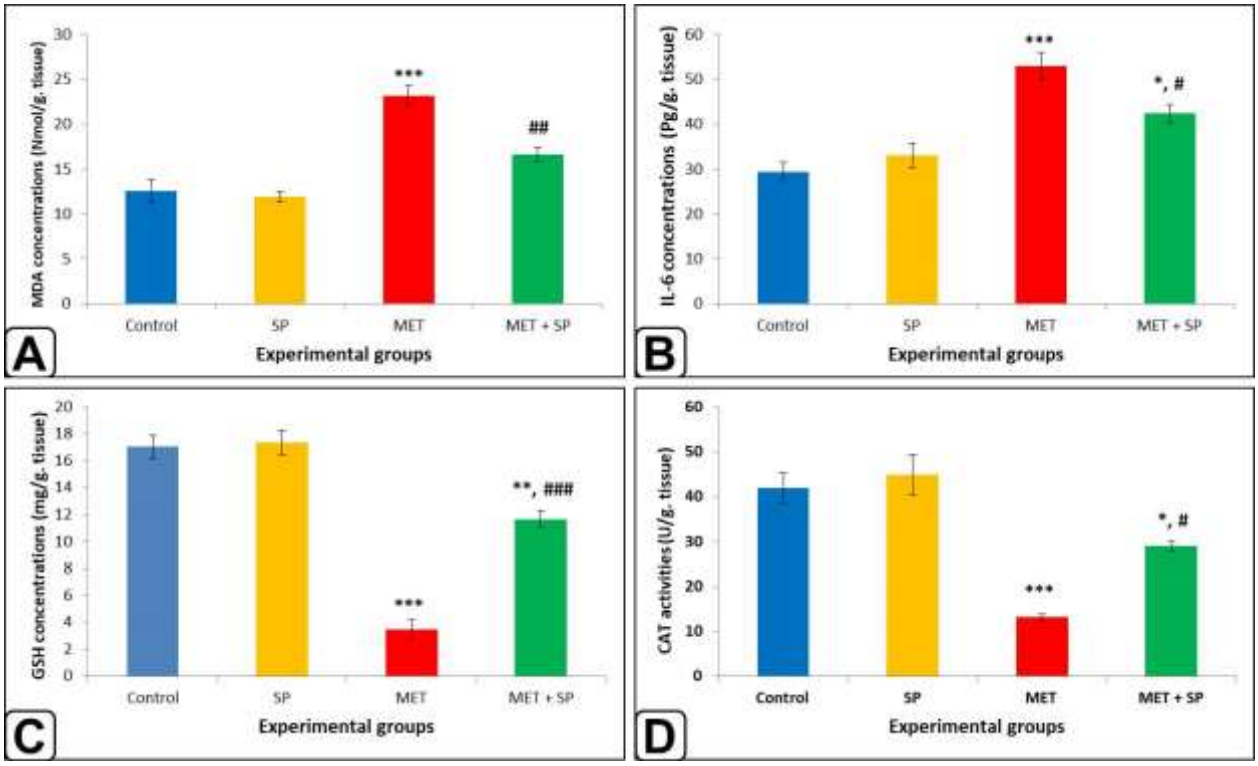

Fig. 4: (A) MDA, (B) IL-6 and (C) GSH concentrations and (D) CAT activity in the kidney tissues of the different experimental groups.

Histological observations:

Histological examinations of the control rat's kidney sections demonstrated a normal structure of renal cortex. Nephron, functional unit in the kidney, cortical parts are Malpighian corpuscle, proximal and distal convoluted tubules. Malpighian corpuscle consists of a glomerulus and parietal epithelium of simple squamous epithelium building up Bowman's capsule, which are separated by a narrow space known as Bowman's space (Fig. 5 A&B). Similarly, SP-treated group kidney sections revealed a similar normal structure to the control rats (Fig. 5 C&D).

Rats treatment with MET caused several histological abnormalities in the kidney cortical components including degenerated Malpighian corpuscles with damaged glomeruli, degenerated Bowman's capsules epithelium and wide Bowman's spaces (Fig. 6 A).

Most of proximal and distal convoluted tubules had degenerated epithelium with pyknotic nuclei and wide lumens (Fig. 6 A&B), besides hemorrhage, congestion of renal arteries, numerous inflammatory leucocytic infiltrations and appearance of fibers between the renal tubules (Fig. 6 C&D). Co-treatment of MET and SP caused an improvement in the histological alterations of renal cortex including nearly normal Malpighian corpuscles and renal tubules (Fig. 6 E&F).

Histological observations grading of H&E-stained kidney sections of all groups were summarized in table 4.

Table 4: Histopathological observations grading of H&E-stained kidney sections of all groups showing the effect of SP and MET on the rat's kidney sections.

	Glomerular degeneration	**Tubular degeneration**	**Inflammatory leucocytic infiltration**	**Vascular congestion and hemorrhage**	**Fibrosis**
Control group	-	-	-	-	-
SP-treated group	-	-	-	-	-
MET-treated group	++	+++	+++	++	+
MET&SP-treated group	+	+	+	+	-

(−) negative, (+) slight, (++) moderate and (+++) severe

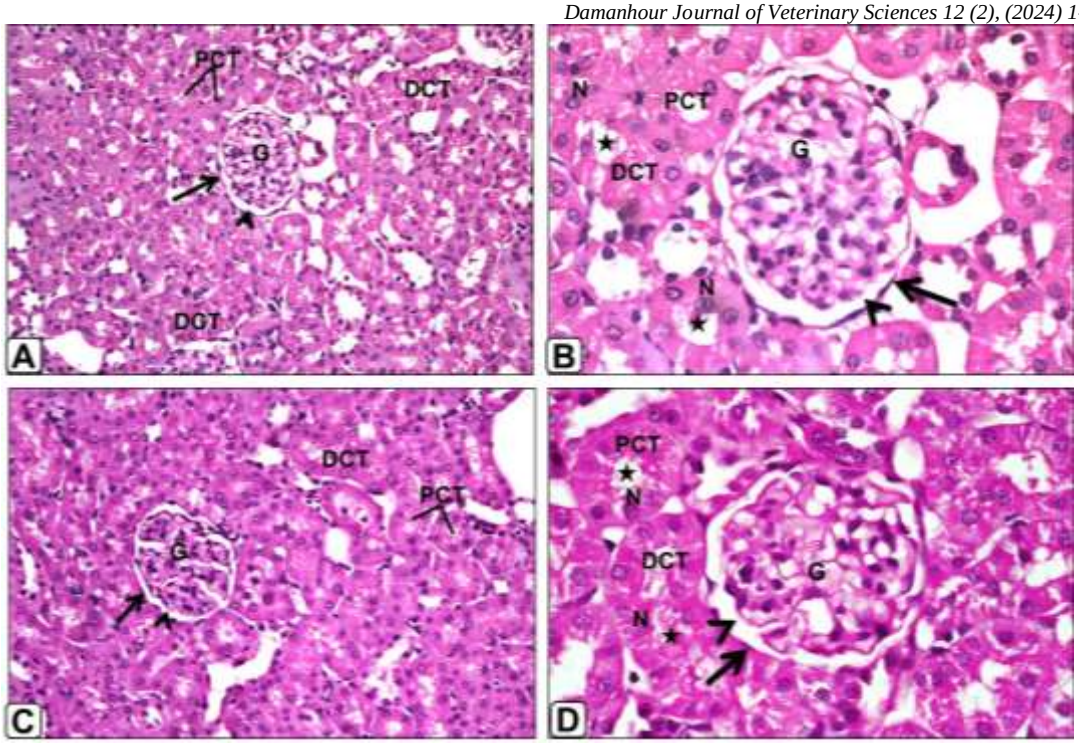

Fig. 5: Photomicrographs of kidney cortex of **(A&B)** control and **(C&D)** SP-treated rats showing normal Malpighian corpuscles consisting of glomeruli (G) and Bowman's capsules (arrows), which are separated by Bowman's spaces (arrow heads) and normal proximal convoluted tubules (PCT) and distal convoluted tubules (DCT) with normal epithelium nuclei (N) and narrow lumens (stars), (H&E-stained sections, magnification; A&C X400, B&D X1000).

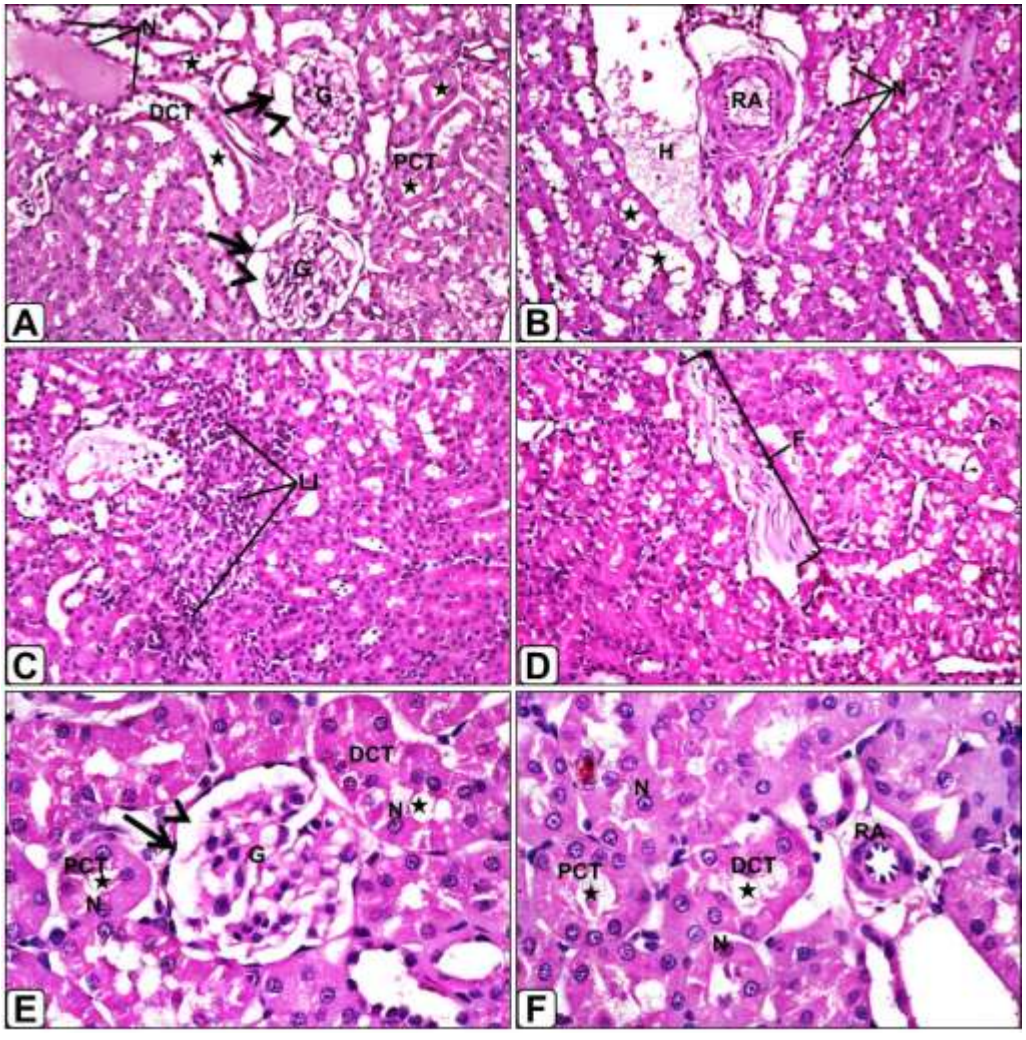

Fig. 6: Photomicrographs of kidney cortex of **(A-D)** MET-treated rats showing degenerated Malpighian corpuscles consisting of damaged glomeruli (G), degenerated Bowman's capsules epithelium (arrows) and wide Bowman's spaces (arrow heads), degenerated proximal convoluted tubules (PCT) and distal convoluted tubules (DCT) with pyknotic epithelium nuclei (N) and wide lumens (stars), hemorrhage (H), congested renal artery (RA), numerous inflammatory leucocytic

infiltrations (LI) and fibers (F) between the renal tubules and **(E&F)** MET & SP-treated group showing relatively normal cortical structure with glomerulus (G), Bowman's capsules epithelium (arrow), Bowman's space (arrow head), normal proximal convoluted tubules (PCT) and distal convoluted tubules (DCT) cuboidal epithelium nuclei (N) and narrow lumens (stars) and normal renal artery (RA), (H&E-stained sections, magnification; A-D&F X400, E X1000).

Immunohistochemical results:

Positive TNF-α immunohistochemical expression was appeared as brown color in the cytoplasm of renal cortex components of glomeruli and tubules epithelium as shown in figure 7. Control group showed a negative expression of TNF-α (Fig. 7 A) and it was quantitatively measured using NIH Image J software (Fig. 7 E), while SP-treated group showed a weak positive expression (Fig. 7 B) with an insignificant increase (p= 0.866) comparing with the control group (Fig. 7 E).

On the contrary, MET-treated group showed a strong positive expression of TNFα (Fig. 7 C). This expression was quantitatively measured by NIH Image J software, which revealed that TNF-α expression recorded a significant increase (p= 0.003) comparing with the control group (Fig. 7 E). Co-treatment with MET and SP caused a week positive expression of TNF-α (Fig. 7 D) with a significant decrease (p= 0.029) as compared with MET-treated group an insignificant increase (p= 0.308) as compared with the control group (Fig. 7 E).

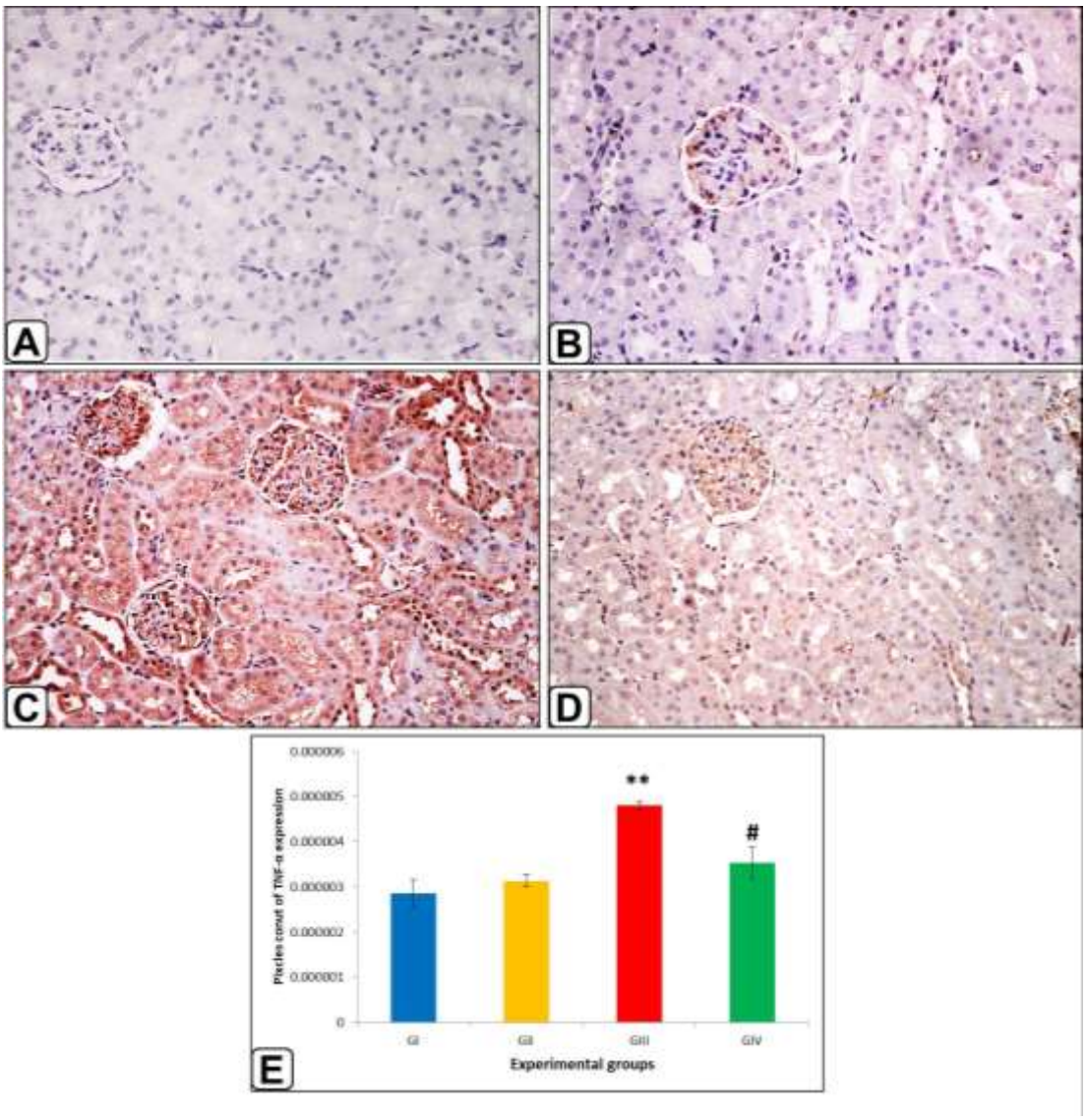

Fig.7: **(A-D)** Photomicrographs of kidney sections showing cytoplasmic TNF-α immunoexpressions of all studied groups; **(A)** a control rat showing negative TNF-α expression, **(B)** SP-treated rat showing a week positive TNF-α expression, **(C)** MET-treated rat showing a strong positive TNF-α expression, **(D)** MET and SP-treated rat showing a week TNF-α expression (TNF-α immunostain, counterstained with H, X400) and **(E)** a Histogram of TNF-α immunoexpression in all studied groups. (n=3)

4. Discussion

The current investigation assessed the impact of SP co-treatment on MET-induced nephrotoxicity. Pesticides consider as one of the major sources of environmental and health problems that induce and raise oxidative stress level causing different diseases (26). MET is a highly toxic substance that can induce oxidative damage through lipid peroxidation and perturbations elevation (27). On the contrary, SP hasn't side effects and is naturally non-toxic (28).

The current study's findings showed that MET administration has obvious effects on body weight gain that may be due to observed decreased food intake as a result of MET-induced loss of appetite and toxicity symptoms. These results were in accordance with Chabane *et al*. (29) who noticed a significant decrease in body weight gain in MET-treated rats in comparison to control rats. In our study, co-administration of SP with MET resulted in an improvement in the body weight gain, which may be caused by an increased in daily food intake and decreased MET-induced toxicity. SP is a rich source of dietary supplements, including minerals, fatty acids, proteins, vitamins and amino acids, that make it to be widely used for animal and human nutrition (30).

Renal markers such as creatinine, urea and uric acid were assessed in the present study to monitor the kidney physiological case. In this experiment, MET-treated rats recorded a significant increase in creatinine, urea and uric acid concentrations comparing with the control rats. Marks *et al*. (31) stated that increased creatinine, urea and uric acid in blood are crucial signs of renal failure. Aziz and Zabut (32) reported that MET-treated rats showed a significant increase in urea, uric acid and creatinine concentrations as clear indicators of renal impairment. Conversely, co-treatment of MET and SP resulted in a significant reduction in creatinine, urea and uric acid concentrations when compared to the MET-treated rats which may be due to SP antioxidant contents that has the ability to restore kidney impairment. SP contains phycocyanin (a super water soluble antioxidant) that can protect the liver and the kidneys during detoxification according to Japanese researches (30). These results are in harmony with Bin-Jumah *et al*. (33) who approved that SP ameliorated the alterations in serum creatinine, urea induced by acrylamide

The current study demonstrated that rats received MET recorded a significant increase in MDA concentration and a significant decrease in GSH concentration and CAT activity in the kidney tissues comparing with the control rats, which may be caused by MET-induced ROS production, oxidative stress and antioxidant defense mechanism malfunction inside the kidney tissues. MDA is a biomarker of oxidative stress monitoring as an end product of lipid peroxidation process that causes cellular damage through numerous active compounds formation (34). The antioxidant system protects the body from oxidative stress. The major antioxidant enzymes are superoxide dismutase (SOD), CAT and glutathione peroxidase (33), while GSH is a natural non-enzymatic antioxidant that protects cells against oxygen-derived toxic chemical species. It is believed that GSH plays a major role in scavenging free radicals and acts as an enzyme cofactor in a number of detoxification processes that shield the organism from oxidative stress (35). Meanwhile, increased ROS production and lowered anti-oxidant defense, resulting in defected oxidant/anti-oxidant balance (36). In line with our findings, Aslanturk and Kalender (37) documented that MET caused an increase MDA levels and a decrease in the activities of glutathione peroxidase, glutathione S transferase, SOD and CAT.

On the contrary, co-treatment of SP with MET caused a significant reduction in MDA concentrations and significant increase in GSH concentration and CAT activity in the kidney tissues comparing with MET group, which may be resulted from antioxidant contents capability of SP. Asghari *et al*. (30) revealed that SP contains numerous antioxidants and its health benefits are related to its antioxidant pigments mainly carotenoids (mixture of carotenes and xanthophylls), chlorophyll and phycocyanin (unique blue pigment). Similarly, Bashandy *et al*. (38) reported that co-administration of SP with arsenic increased GSH content and CAT and SOD activity and inhibited MDA production in liver and kidney tissues when compared to rats treated with arsenic alone.

According to the current research, MET-treated group's kidney IL-6 level significantly increased which may be due to MET-induced inflammation due to ROS high production that subsequently stimulates inflammatory cascades. Cestonaro *et al*. (39) documented that exposure to pesticides is related to numerous inflammatory diseases and different effects on immune functions causing adverse effects to the body and these compounds also cause changes in the pro-inflammatory factor IL-6. Inversely, SP co-treatment with MET reduced IL-6 levels in kidney tissues as compared with MET group which supported the anti-inflammatory capability of SP. SP is known to strengthen the immune system, according to Qureshi *et al*. (40) and other studies have demonstrated that algae decrease inflammatory markers expression (41, 42).This emphasizes how algae prevent inflammation and the activity of free radicals, hence safeguarding the structure of the kidneys. These characteristics are probably connected to the phycocyanins and phenolic chemicals that make up its composition. SP has the ability to scavenge radicals, which may stop the release of cellular contents into the bloodstream (43).

In the present study, the noticed histological abnormalities in MET-treated rat's kidney tissues were obvious signs of nephrotoxicity and inflammation which may be induced by MET oxidative stress. Antioxidant enzyme activity inhibition and lipid peroxidation level raise can cause cellular accumulation of ROS leading to tissue damage (44). Pesticides induce oxidative stress that caused by ROS and reactive nitrogen species, which are

companied with different diseases including cancer, inflammation and cardiovascular and neurodegenerative diseases (45). Our histological findings were in agreement with Aslanturk and Kalender (37) who reported MET-treated rat's kidney tissues showed tubular and glomerular degeneration, congestion and debris in tubular lumen and mononuclear cell infiltration. On the other hand, SP co-treatment with MET caused histological improvement in kidney tissues which may be supported by oxidative stress scavenging capacity of SP. Khalil *et al*. (46) was in agreement with our results who demonstrated that SP co-administration has both protective and therapeutic effect against furan-induced hepatic and renal damage.

In the current study, MET treatment caused a significant increase in TNF-α immuoexpression that may be indictor for inflammation induced by MET in rat's kidney. Manar (47) documented that MET activates the inflammatory pathway through NF-κB phosphorylation. On the contrary, SP co-treatment with MET decreased immunoexpression of TNF-α that may be due to its anti-inflammatory effect. It was documented that SP has marked antioxidant activity *in vivo* and *in vitro*, as well as anti-inflammatory activity in certain experimental models (48). Similarly, Zahran and Emam (49) documented that SP supplementation significantly decreased inflammatory markers of IL-6 and TNF-α induced by nicotine.

Conclusion

MET can induce oxidative stress in the kidney tissues through oxidant/antioxidant balance disturbance causing nephrotoxicity that supported by biochemical, histological and immunohistochemical alterations. Inversely, SP has the capacity to lessened MET-induced nephrotoxicity owing to its high content of antioxidant and free radicals scavenging capacity.

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