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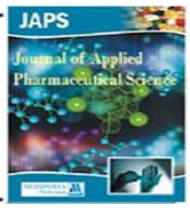

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Effect of leave extract of *Ocimum basilicum* on deltamethrin induced nephrotoxicity and oxidative stress in albino rats

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ABSTRACT

Deltamethrin is a pyrethroid insecticide used extensively in pest control. On the other hand it showed different toxicity in mammalian animals. Basil or sweet basil (*Ocimum basilicum)* is a plant which showed many pharmacological effects. The present work studied the potential protective effect of *Ocimum basilicum* extract on deltamethrin-induced nephrotoxicity in albino rats. Treating animals with deltamethrin induced several histopathological alterations in the kidney. The renal tubules lost their characteristic appearance and their lining epithelial cells were degenerated. The glomeruli were atrophied and the renal blood vessels were dilated and congested. The intertubular spaces were infiltrated by inflammatory leucocytic cells. Marked elevation in serum creatinine and urea was recorded. Moreover, deltamethrin increased significantly the concentration of malondialdehyde (MDA) and decrease the activities of superoxide dismutase (SOD) and catalase (CAT) in renal tissue. Treating animals with deltamethrin and aqueous extract of basil led to an improvement in histological and biochemical alterations induced by deltamethrin. The biochemical results showed that creatinine and urea appeared within normal level. Reduction in the level of MDA (lipid peroxidation marker) and increase in the activities of SOD and CAT was recorded. It was concluded from this study that basil aqueous extract has a beneficial impact on deltamethrin-induced nephrotoxicity in albino rats by its antioxidant effect.

Keywords: Deltamethrin, Nephrotoxicity, Basil, Oxidative stress.

INTRODUCTION

The use of plants in medicine is an age-long practice in various parts of the globe for both preventive and curative. Today, it is estimated that about 80% of the world population relies on botanical preparations as medicine to meet their health needs (Ogbera *et al.,* 2010).Basil or sweet basil (*Ocimum basilicum)* is a plant that belongs to the family Labiatae and has shown its potential to be therapeutic in averting several diseases in various countries. Many studies have established that basil leaves extracts have potent antioxidant , anti-aging , anticancer , antiviral , and antimicrobial properties (Chiang *et al.,* 2005 ; Bozin *et al.,* 2006 ; Manosroi *et al.,* 2006 ; Almeida *et al.,* 2007, Akujobi *et al.,* 2010).

Orafidiya *et al.,* (2006) reported that ocimum oil was capable of enhancing normal hair growth and promoting follicular proliferation in cyclophosphamide-induced hair loss. Sethi (2003) reported that leaves of ocimum sanctum possess good antioxidant as well as antistress potentials in experimental animals. Consumption of basil or basil oil has been associated with a reduction in total cholesterol**,** low-density lipoprotein and triglyceride (Hicham *et al.,* 2009). Batra and Gupta (2006) indicated that Ocimum sanctum leaf supplementation reduced the severity of hydropericardium, hepatitis, myocarditis accompanied with haemorrhages, oedema in lungs, lymphocytic depletion in lymphoid organs and focal interstitial nephritis. Ocimum leaf extracts were found to protect the liver from heavy metals (Sharma *et al*., 2002) and prevent isoproterenal induced myocardial necrosis in rats (Sood *et al.,* 2005). Rupert (2009) reported that basil or basil oil can be used in prevention and treatment of cardiovascular disease. Free radicals and reactive oxygen species (ROS) play a crucial role in development of different diseases. The enhanced production of free radicals can be induced by a variety of xenobiotics. Deltamethrin is a pyrethroid insecticide used extensively in pest control. Deltamethrin intoxication is one of the chemicals which cause free radical generation (Manna *et al.,* 2005). Deltamethrin is metabolized in the liver through hydrolytic ester cleavage by cytochrome P450's and the oxidative route (Eraslan *et al*., 2007). A number of studies have showed different toxic effects of deltamethrin in in mammalian animals. Treating pregnant rats from day 6 to day 15 of pregnancy with deltamethrin caused retardation of growth, hypoplasia of the lungs, dilation of the renal pelvis and increase in placental weight (Abdel-Khalik *et al*., 1993). Lukowicz-Ratajczak and Krechniak (1992) reported that deltamethrin suppress immune system in Balb/c mice. It inhibited the mitotic index and increased the frequency of chromosomal aberrations in the bone marrow of rats (Agarwal *et al*., 1994). Deltamethrin was found to induce histological alterations in liver, kidney and lungs (Manna *et al.,* 2005; Erdogan *et al.,* 2006, Shona *et al.,* 2010). The increasing use of pyrethroids interested the researchers in reducing the damages caused for this class of pesticides in the environment. This stimulated us to examine the potential protective effect of *Ocimum basilicum* extract on deltamethrin-induced nephrotoxicity in albino rats.

MATERIALS AND METHODS

Animals

Male albino Wistar rats weighting 130 ± 5 g were kept in the laboratory under constant conditions of temperature (24 ± 2 ^{o}C) for at least one week before and through the experimental work, being maintained on a standard diet composed of composed of 20% casein, 15% corn oil, 55% corn starch, 5% salt mixture and 5% vitaminzed starch. Water was available *ad-libitum*.

Preparation of ocimum extract

Fresh leaves of *Ocimum basilicum* were collected from a garden within Genetic Engineering and Biotechnology Research Institute, Menoufia University, Sadat City, Egypt.The leaves were rinsed with clean water to remove any foreign matter. Leaves were blended with distilled water. The mixture was strained, the merc pressed and the mixture was filtrated using filter paper. The aqueous extract was used at a dose level of 20 ml/kg O. *basilicum* (Offiah and Chikwendu,1999).

Experimental design

All the experiments were done in compliance with the guide for the care and use of laboratory animals. Animals were divided into four groups:

Group1. Animals were fed on the standard diet and were served as control group.
Group2. Animals of this group were administrated with oral aqueous O. *basilicum* extract at a dose level of 20 ml/kg daily for 6 weeks.
Group3. Animal of this group were orally given deltamethrin at a dose level of $1/_{10}$ LD_{50} (0.6 mg/kg body weight) (Oda *et al*., 2011) in corn oil, daily for 6 weeks.
Group4. Rats were given deltamethrin (0.6 mg/kg body weight) followed by oral administration with aqueous O. *basilicum* extract at a dose level of 20ml/kg daily for 6 weeks.

Histological examination

The treated animals and their controls were sacrificed by decapitation after 4 and 6 weeks of treatment. Kidney was removed and fixed in Bouin's fluid. Fixed materials were embedded in paraffin wax and sections of 5 micrmetres thickness were cut. Slides were stained with haematoxylin and eosin for histological examination. The number of the affected glomeruli was calculated and was expressed as a percentage in relation to the total number of the glomeruli in 10 non overlapping microscopic fields and their mean values were obtained.

Biochemical assays

For biochemical assays, blood samples were collected from animals after 4 and 6 weeks of treatment. Sera were obtained by centrifugation of the blood sample and stored at -20°C. Urea and creatinine were measured using a fully automated Hitachi 911 analyzer (Tokyo, Japan). A commercial randox kits (Randox Laboratories, LTD, Ardomre, Crumlin, United Kingdom) were used in these analysis. Lipid peroxidation was estimated in the renal tissue as the concentration of thiobarbituric acid reactive product (malondialdhyde) according to (Ohkawa *et al.,* 1979). Superoxide dismutase activity was measured using the methods of Rest and Spitznagel (1977). Catalase activity was determined from the rate of decomposition of H_2O_2 (Aebi *et al.,* 1974)**.**

Statistical analysis

The results were expressed as mean ± SD of different groups. The differences between the mean values were evaluated by ANOVA followed by Student's "t" test using Minitab 12 computer program (Minitab Inc., State Collage, P.A).

RESULTS

Histological observations

Kidney sections of control rats showed normal renal tubules and renal corpuscles. The bowman capsule and the glomeruli appeared to be prominent and normal (Fig.1A).

There were no histopathological changes observed in animals given O. *basilicum* extract. Treating animals with deltamethrin for 4 weeks revealed many histological alterations. The renal veins were enlarged and congested with blood and the renal tubules showed wide lumen and separation of the epithelial cells from its membrane (Fig.1B). Intertubular leucocytic infiltrations were abundant (Fig.1C). Such alterations became severe in animals given deltamethrin for 6 weeks. Distortion of the renal architecture and atrophy of glomeruli was observed (Fig.2A). The renal tubules were degenerated and showed intraluminal exfoliation with granular cast formation as well as pyknosis of the nuclei (Fig.2B). Leucocytic inflammatory cells were spread in large areas of the intertubular tissue (Fig.3A). However, treatment with O. *basilicum* extract ameliorated these histopathological alterations, but the tubules still appeared with wide lumens (Fig.3B). Figure (4) revealed that the percentage of the affected glomeruli was significantly decreased in rats treated with deltamethrin and O. *basilicum* extract in comparison with deltamethrin group.

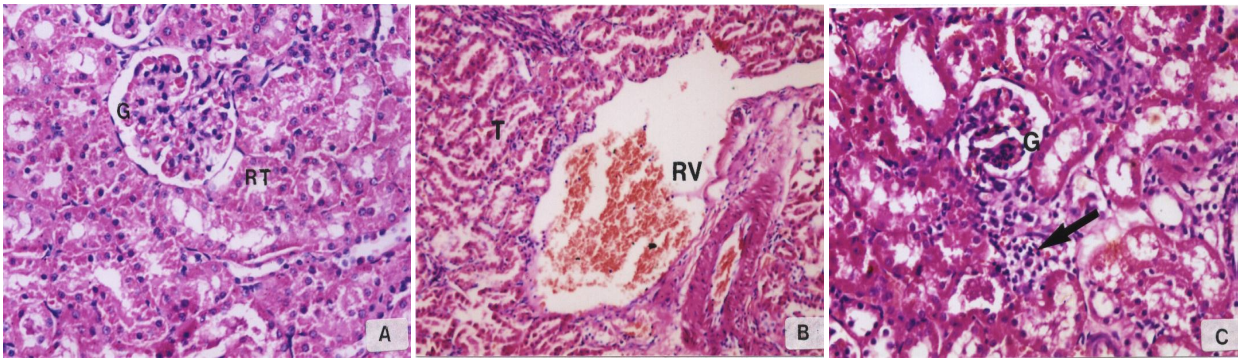


Fig. 1 Sections in the kidney cortex of **(A)** control rat showing a glomerulus (G) and renal tubules (RT); **(B-C)** 4 weeks after deltamethrin treatment showing enlarged and congested renal vein (RV), tubules with wide lumens (T), leucocytic infiltrations (arrow) and fragmented glomerulus (G), (X300).

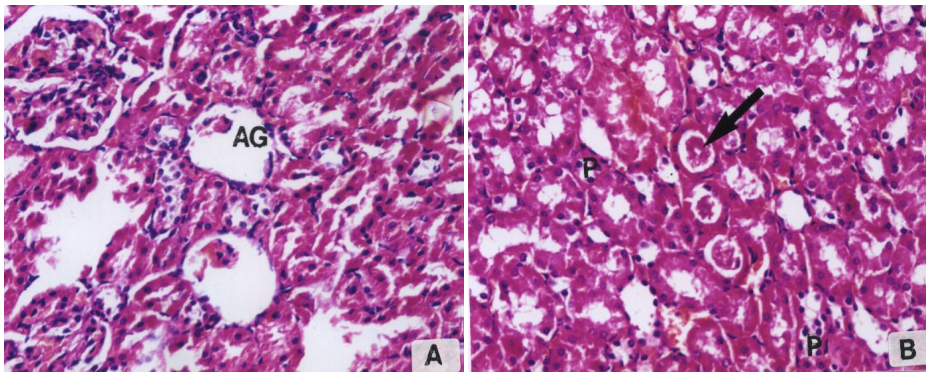


Fig.2 (A) Section in kidney cortex 6 weeks after deltamethrin treatment showing atrophied glomeruli (AG) and degenerated tubules. **(B)** proteinaceous casts in the lumen of renal tubules (arrow) and tubular epithelial cells with pyknotic nuclei (P), (X300),

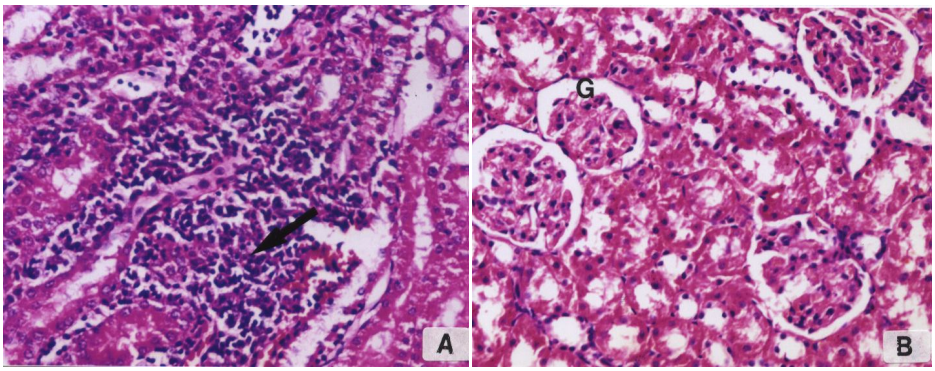


Fig.3 (A) Section in kidney cortex 6 weeks after deltamethrin treatment showing severe leucocytic infiltrations (arrow). **(B)** Section in kidney of a rat treated with deltamethrin and showing normal glomeruli (G) and renal tubules with somewhat wide lumen, (X 300).

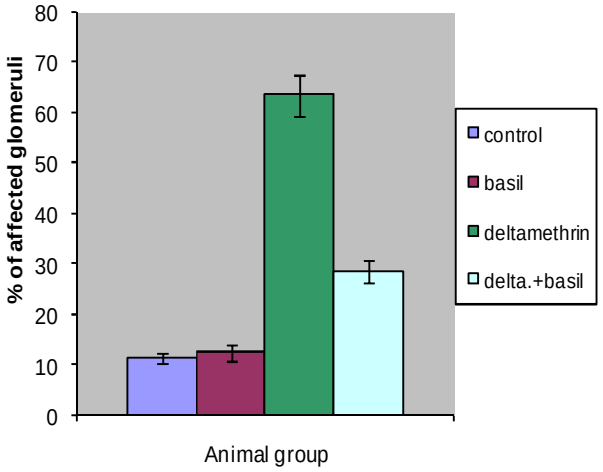
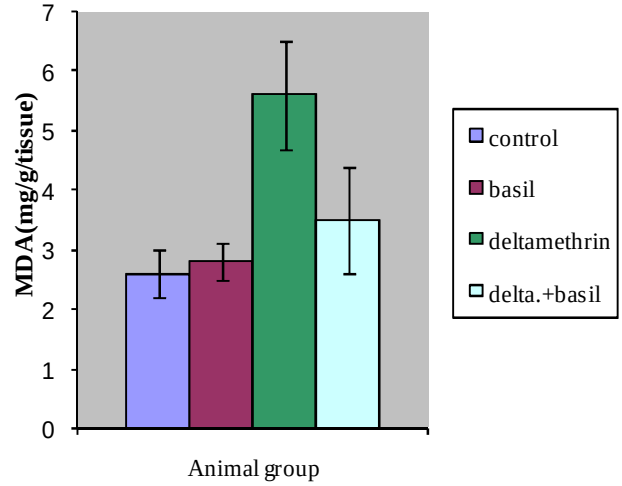


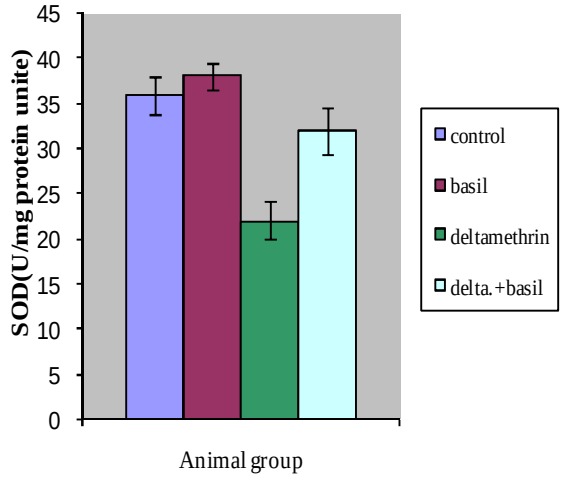
Fig 4. The mean percentage of the affected glomeruli in kidney of different groups.

Biochemical results

Treatment with deltamethrin for 6 weeks caused a highly significant elevation (P<0.05) in the level of serum urea, and creatinine as compared to those of the control animals. On the other hand, these parameters were restored to near normal values in rats treated deltamethrin and *O. basilicum*. Both control and animals given *O. basilicum* showed no significant differences in serum activity of urea and creatinine (table 1).

Administration of deltamethrin to rats caused significant increase (P<0.05) in renal MDA compared with animals of control groups. Animals treated with both deltamethrin and *O. basilicum* extract showed reduction in MDA in comparison of those given deltamethrin (Fig.5A). Figures 5(B,C) showed that the activity of the antioxidant enzymes, SOD and CAT was significantly decreased in kidney of animals given deltamethrin.In contrast, treatment with deltamethrin and *O. basilicum* extract caused a dramatic increase in SOD and CAT activity. There was no significant difference in the renal activities of these enzymes among the control group and *O. basilicum* group.





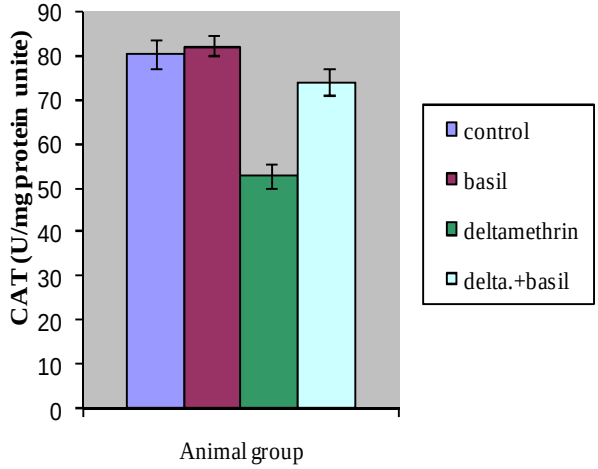


Fig. 5: Effect of deltamethrin and /or basil on (**A**): MDA, (**B**): SOD, (**C**):CAT.

Table. 1: Effect of deltamethrin and /or basil on serum creatinine and urea.

Parameter	Control group	Basil	Deltamethrin	Deltamethrin + basil
Creatinine (mg/dl)	2.6±0.8	2.4±0.5	4.6±1.2*	2.9±0.6
Urea (mg/dl)	33.4±2.2	34.2±2.5	62.0±4.1*	41.2±2.3

n= 5 animals for each group.
(*) Significant increase (p<0.05) in comparison with control animals

DISCUSSION

Deltamethrin is one of the environmental pollutant which showed a broad spectrum toxicological effects and biochemical dysfunctions constituting serious hazards to health. Histological examination of kidney of deltamethrin-intoxicated rats revealed many alterations such as tubular degeneration, atrophy of glomeruli, leucocytic infiltrations and congestion of renal blood vessels. Such observations were reported by some investigators. Shona *et al.*, (2010) observed many histological and ultrastructural changes in kidney of rats exposed to deltamethrin. Tos-Luty *et al.*, (2001) observed hypertrophy of Bowman's capsules and hyaline deposits in renal tubuli after exposing mice to deltamethrin.

Administration of deltamethrin to rats caused cytoplasmic vacuolization of the lining epithelial cells of the renal tubules (Staicu *et al.,* 2007, Shona *et al.,* 2010). The present results showed that deltamethrin caused significant increase in serum urea and creatinine. This indicates diminished ability of the kidneys to filter these waste products from the blood. Similarly, Mongi *et al.,* (2011) reported an increase in the serum urea and creatinine in rats and this toxicity could be attributed to its free radical induced oxidative damage. It was reported that serum concentration of creatinine and urea depends largely on the glomerular infiltration. The change in these two parameters together with the histological results indicate a reduction in the glomerular filtration rate as a result of deltamethrin intoxication.

Administration of deltamethrin resulted in a significant increase in the renal content of MDA indicating increased lipid peroxidation which implicates the renal oxidative stress. Moreover, deltamethrin caused a significant decrease in the activities of SOD and CAT. Antioxidant enzymes, mainly SOD and CAT are the first line of defense against free radical induced oxidative stress. SOD is responsible for catalytic dismutation of highly reactive and potentially toxic superoxide radicals to hydrogen peroxide, and CAT is responsible for the catalytic decomposition of hydrogen peroxide to molecular oxygen and water (Lee and Choi, 2003). A decrease in the level of antioxidant enzymes and an increase in lipid peroxidation level were recorded after deltamethrin intoxication (Sayeed *et al.,* 2003, Rehman *et al.,* 2006, Mokhtar *et al.,* 2006). Damage of renal tissue observed in the present study may be resulted from the increase in lipid peroxidation and decrease of antioxidant enzymes in the kidney following exposure to deltamethrin.

Administration of *O.basilicum* improved the histological changes induced in the kidney by deltamethrin. Kidney function was also improved as indicated by significant restoration of serum creatinine and urea. In accordance with these results, Sharma *et al.,* (2005) and Karamala *et al.,* (2011) reported that *O.basilicum* has a nephroprotective effect against mercury and lead toxicity. Dietary treatment of *O.basilicum* normalized a high level of serum creatinine in diabetic rats, indicating its protective effect on renal glomerular filtration ability (Suanarunsawat and Songsak, 2005). Makwana and Rathore (2011) reported that ocimum leaf extract suppressed histopathological alterations induced by paracetamol in liver and kidney of rats and restored creatinine, urea as well as liver function enzymes to its normal values. The present results showed that lipid peroxdation was inhibited and the activities of SOD and CAT enzymes were increased after treatment with *O.basilicum*, suggesting the role of *O.basilicum* extract in quenching the ROS formed. A number of studies have reported that *O.basilicum* exhibited protections against xenobiotics-induced oxidative dysfunction**.** Yamamoto *et al.,* (2005) proved that ocimum suppressed hepatic fibrosis and protected liver against parenchymal damage induced by CCL_4. Significant hepatoprotective effects were obtained by ethanolic extract of leaves of O. basilicum against liver damage induced by H_2O_2 and CCl_4 in goat as evidenced by decreased levels of antioxidant enzymes. The extract also showed significant anti lipid peroxidation effects in vitro, besides exhibiting significant activity in superoxide radical and nitric oxide radical scavenging, indicating their potent antioxidant effects (Meera *et al.,* 2009). Dasgupta *et al.,* (2007) found that *O.basilicum* increased the activity of xenobiotic metabolizing phase 1 and phase 11 enzymes, elevating antioxidant-enzyme response by increasing significantly the hepatic glutathione reductase, superoxide dismutase, and catalase activities, increasing glutathione content and decreasing lipid peroxidation and lactate dehydrogenase activity in the liver of mice. Leaves of *O.basilicum* are rich source of flavonoids which have been shown to possess various biological properties related to antioxidant mechanisms. Zhang *et al.,* (2009) reported that the main components of *O. basilicum* were: linalool (29.68%) , (Z)-cinnamic acid methyl ester (21.49%) , cyclohexene (4.41%) , alpha- cadinol (3.99%), 2,4-diisopropenyl-1-methyl-1-vinylcyclohexane (2.27%), 3,5-pyridine-dicarboxylic acid, 2,6-dimethyl-diethyl ester (2.01%), beta-cubebene (1.97%), guaia-1(10),11-diene (1.58%), cadinene (1.41%), (E)-cinnamic acid methyl ester (1.36%) and beta-guaiene (1.30%). It is concluded from the present study that the ameliorative effect of O. *basilicum* against renal toxicity of deltamethrin may be attributed to the antioxidant activity of one or more of its flavonoids.

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