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Original Article

Hepatoprotective effect of *Ocimum basilicum* extract against the toxicity of diazinon in albino rats: Histopathological and immunohistochemical evaluation

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ABSTRACT

Recent studies showed that the Basil (*Ocmuim basilicum*) is known to have numerous pharmacological activities. The present study aims to investigate the efficacy of *Ocmium basilicum* extract, natural herb, against hepatotoxicity induced in rats by diazinon which is one of the most common insecticides. The intoxicated rats showed many pathological changes, including impairment of neutral structural organization of the hepatic lobule, leucocytic infiltration, cytoplasmic vacuolization of the hepatocytes and fatty degeneration. The immunohistochemical result showed increase cell proliferation as reflected by an increase in PCNA expression, whereas the increase in apoptotic rate was associated with an increase in the bax expression. Moreover the biochemical results showed that there was an increase in transaminases(AST, ALT).Concomitant treatment with aqueous extract of Basil led to an improvement in histological and immunohistochemical changes induced by diazinon and this attributed to basil antioxidant activity and free radicals scavenging properties.

Key words. Diazinon, *Ocmuim basilicum, Rat, histology. Immunohistochemistry*

INTRODUCTION

The use of herbal medicines has increased considerably, because they are becoming a popular alternative treatment in different countries. Plants constitute an important source of active natural products with different biological properties. Various phytochemical components, especially polyphenols (such as flavonoids, phyenyl propanoids, phenolic acids, tannins, etc) are known to be responsible for the free radical scavenging and antioxidant activities of plants [1].

Ocimum basilicum (Basil) is an annual herb of the Lamiaceae family, which is widely cultivated in different regions of the world. *O. basilicum* was found to have numerous pharmacological activities. Basil leaves extracts have potent antioxidant, anti-aging, anticancer, antiviral, and antimicrobial properties [2, 3, 4]. It also possesses good antioxidant as well as antistress potentials in experimental animals [5]. Batra and Gupta [6] reported that supplementation of *O sanctum* leaf reduced the severity of hydropericardium, hepatitis, myocarditis accompanied with haemorrhages, oedema in lungs, lymphocytic depletion in lymphoid organs and focal interstitial nephritis. Sakr *et al*. [7] reported that *O. basilicum* extract improved hepatotoxicity and apoptosis induced by CCl_4 in rats.

Organophosphorous (OP) pesticides are applied to numerous crops, including wheat and corn. Diazinon [phosphoric acid, O, O-diethyl O (2-isopropyl-6-methyl-4-pyridinyl)] phosphorothioate is an organophosphorus insecticide widely used in agricultural practice throughout the world to control flies, lice, and other insect pests of ornamental plants and food crops [8]. Residual amounts of diazinon have been detected in food products [9]. The toxicity of diazinon was reported by various investigators. Treating rats with diazinon caused alterations in glucose and testosterone levels [10] and caused histopathological changes in liver [11]. Kalender *et al*.[12] reported that diazinon causes changes in liver enzymes and biochemical indices and ultrastructure changes in hepatocytes . It also resulted in decrease in splenic T-dependent antibody response to DNP fecal and thymus atrophy in mice [13].Treatment with diazinon induced significant increase in lipid peroxidation

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and decreased total antioxidant capacity in rat liver and muscle [14]. The present work aims to study the hepatoprotective effect of *Ocemium basiculum* extract against histological and immunohistochemical alterations induced by diazinon in liver of rats.

MATERIALS AND METHODS

Diazinon: Diazinon (Nasr-Cidol, 60%EC) was obtained from El-Nasr Mediate chemical Co.,Egypt. It was prepared in distilled water before use.

Ocimum extract: Fresh leaves of *Ocimum basilicum* were collected from a garden within Faculty of Science, Menoufia University, Shebin El-kom, Egypt. The leaves were rinsed with clean water to remove any foreign matter. Leaves were dried in the shade and ground to a fine powder using a laboratory mixer. One hundred grams of leaf powder was refluxed with 750ml of double distilled water for one hour and concentrated using rotary evaporator.The extract was kept in -20^{0}C until used for experiment. The aqueous extract was used at a dose level of 20 mg/kg *O. basilicum* [7].

Animals: Healthy male Wister rats weighting 150 ± 5 g were kept in the animal house under constant conditions of temperature (24 ± 2 ^{0}C) for at least one week before and through the experimental work, being maintained on a standard diet composed of 20% casein, 15% corn oil, 55% corn starch, 5% salt mixture and 5% vitamins. Water was available *ad-libitum*. 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 (10 rats) were fed on the standard diet and were served as a control group.**Group2**: Animals (10 rats) of this group were orally given aqueous O. *basilicum* extract at a dose level of 20 mg/kg 5 days / week for 6 weeks.**Group3**: Animals (10 rats) of this group were orally given diazinon at a dose of 20mg/kg.b.wt. [15], 5 days / week for 6 weeks. **Group4**:10 rats were given diazinon (20mg/kg b.wt) followed by oral administration with aqueous O. *basilicum* extract (20 mg/kg) 5 days/ week for 6 weeks.

Histological Study: Animals were dissected and their livers were removed. For histological preparations, the liver was fixed in Bouin's fluid, dehydrated, cleared and embedded in paraffin wax. Paraffin sections of 5 micron thickness were prepared and stained with Ehrlich's haematoxylin and eosin [16].

Immunohistochemical study: For immunohistochemical localization of PCNA and bax,formalin fixed sections were stained using the avidin-biotin peroxidase method[17].Formalin fixed paraffin embedded tissue sections were deparaffinized and endogenous peroxidase was blocked with H_2O in methanol, the sections were heated in 0.01 mol/l citrate buffer in a microwave pressure cooker for 20 minutes. The slides were allowed to cool to room temperature, and nonspecific binding was blocked with normal horse serum for 20 minutes at room temperature.Monoclonal antibody was used for detection of nuclear PCNA, a marker of proliferating cells (code no: M7187, dilution 1:40, DAKO). Anti-bax (Dako, Cambridge, UK) monoclonal antibodies were used for detection of bax. Counterstaining was done using Mayer's hematoxylin (BioGenex, Cat. No.94585).

Biochemical study: For the biochemical study,sera were obtained by centrifuging the blood samples and storing them at 20℃ until the assays could be completed. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined by colorimetric method according to Gella *et al.*[18]

Statistical Analysis: Data were expressed as mean values ± SD. All statistical analyses were performed using SPSS statistical version 16 software package (SPSS® 4 Inc., USA).

RESULTS

Histological results: Figure (1a) showed a histological section in liver of a control rat. The figure showed a normal liver picture in which the central vein lies at the center of the lobule surrounded by the hepatocytes with strongly eosinophilic granulated cytoplasm and distinct nuclei. In addition between the strands of hepatocytes the hepatic sinusoids are exhibited. Examination of liver sections prepared from rats 3 weeks following the application of diazinon has displayed apparent signs OD degenerative changes. Some of the central veins were dilated, congested with blood and their endothelial lining were eroded (Fig.1b). Inflammatory leucocytic infiltration comprised of lymphocytes and sparse eosinophils were observed (Fig.1c). Among the conspicuous signs of injury encountered was the cytoplasmic vacuolization of the hepatocytes which so extensive in some cells to the extent that only alight remnants of the cytoplasmic mass was left in those cells (Fig.1d).

Degenerative symptoms became more conspicuous and widely spread in liver of animals given diazinon for six weeks. Marked distortion and disorganization of the liver tissue were noticed.Congested blood vessels and leucocytic infiltrations were obviously a common phenomenon in the impaired tissue (Fig.2a). It was also evident that most of the hepatocytes were manifesting fatty degeneration (Fig.2b). Animals treated with diazinon and O. *basilicum* extract showed an obvious degree of improvement. These specimens revealed that the liver structure had started to regain its usual disposition and the hepatic cells appeared aligned together (Fig.2c).

Immunohistochemical observations: Liver cells of control rats showed negative expression of PCNA (Fg.3a). The number of the PCNApositive staining cells increased in liver cells of rats treated with diazinon (Fig.3b).A decrease of PCNA was recorded in animals given diazinon and O. *basilicum* extract (Fig.3c). Immunohistochemical examination of liver for expression of bax showed negative expression in hepatocytes of control rats (Fig.4a). Treating animals with diazinon caused marked elevation in expression of bax (Fig.4b).The hepatocytes of rats given diazinon and O. *basilicum* extractshowed a decrease in expression of bax and few cells appeared with positive staining (Fig.4c).

Biochemical Results: Biochemical analysis revealed that diazinon treatment was accompanied by a significant increase in activity of ALT and AST in compared with control group. On the other hand, treatment with diazinon and O. *basilicum* extract showed a reduction in the activity of these two enzymes (Figs.5&6).

DISCUSSION

Organophosphorus insecticides are used throughout the world for control of agricultural and domestic insect pests. On the other hand, contact with organophosphorus pesticides is produced health problem for agricultural workers [19]. The present results showed that diazinon caused many histopathological alterations in liver of rats. The most marked signs of tissue impairment were congestion of blood vessels, leucocytic infiltrations, cytoplasmic vaculation of the hepatocytes and fatty degeneration. The magnitude of such changes was time-dependent. In agreement with these results, Hassan *et al*. [20] reported that hyperplasia of hepatocytes, necrosis, lymphocytic infiltrations and steatosis were observed in rats treated with 1/20 LC_{50} of diazinon. Anthony *et al*. [21] reported that rats chronically treated withsub-lethal doses of diazinon showed lipid accumulation in the liver. El-Shenawy *et al*. [22] reported that intoxicated mice with diazinon resulted in hydropic degeneration, necrosis and focal microvascular steatosis in liver. Elevation of ALT and AST activities was recorded in sera of rats treated with diazinon. Similarly, Ahmed [23] found an increase in transaminases (AST, ALT) in sera of rats treated with 1/30 LD_{50} diazinon for 3 weeks. Kalender *et al*.[24] recorded an elevation in ALT, AST, ALP, total cholesterol, and triglyceride levels in rats treated with diazinon. Gokcimen *et al.* [25] observed histopathological alterations in liver and pancreas of rats exposed to diazinon. They added that there was statistically significant difference between the control and diazinon given groups by means of serum amylase, lipase,and ALT and AST activities. It was reported that when liver is injured or damaged, additional AST and ALT are released into the blood stream and the increase levels of these two enzymes in sera is an indication of hepatocellular injury. Thus, the histological results together with elevation in ALT and AST recorded in the present work indicated the hepatotoxicity of diazinon.

Concerning the immunohistochemical results, an increase in expression of bax, the proapototic protein, was observed in hepatocytes of diazinon-treated animals. This result indicates that diazinon induced apoptosis in liver of rats. In this concern, Lari*et al*.[26] reported that diazinon induced hepatic apoptosis through activation of caspases-9 and -3 and increasing Bax/Bcl-2 ratio.Diazinon was also found to induced apoptosis through elevation of Bax/Bcl2 ratio (both protein and mRNA levels), cytochrome *c* release to the cytosol and activation caspase 3 in cardiac tissue [27] .NTera2/D1 cell line exposed to diazinon showed a time-dependent enhancement of cell death.The cell death caused by the exposures showed a number of features characteristic of apoptosis, including membrane and mitochondrial potential changes [28]. Rush *et al.*[29] studied the neurotoxic mechanism of chlorpyrifos and diazinon in primary cortical cultures. Their results showed that diazinon toxicity was not affected by glutamate receptor antagonists, but was attenuated by the caspase inhibitor ZVAD. They added that diazinoninduced chromatin condensation characteristic of apoptosis.Over expression of the proliferating cell nuclear antigen (PCNA) were recorded in liver of diazinon-treated animals. Proliferating cell nuclear antigen (PCNA) is an essential regulator of the cell cycle and serves as a co-factor for DNA polymerase delta in S-phase and is involved in DNA repair during DNA synthesis [30]. The temporal pattern of PCNA expression makes it a useful tool to study cell proliferation. It starts to accumulate in the G1 phase of the cell cycle, reaches the highest level

during the S phase and decreases during the G2/M phase[31].

The toxic effects of organophosphate insecticides occur through the generation of reactive oxygen species (ROS) causing damage to the various membranous components of cell [32]. Theimbalance between the formation of ROS andmechanism of enzymatic and nonenzymatic antioxidants as a body defense system can lead to oxidative stress. Oxidative stress has been reported to be the primary mechanism of diazinon toxicity. Shah and Iqbal [33] reported that diazinon enhances renal lipid peroxidation and decreased the activities of renal antioxidant enzymes (e.g. catalase, glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase, glutathione S-transferase).Abdou and ElMazoudy [34] reported that diazinon induced significant increases in the level of serum malondialdehyde and the activity of lactate dehydrogenase in female rats.

The main function of antioxidants is to inhibit the initiation or propagation of oxidizing chain reactions by free radicals and reducethe oxidative damage. Many antioxidants compounds are present in natural product. Basil or sweet basil *O. basilicum*isshowed many pharmacological effects. The present results showed that treating rats with diazinon and *O. basilicum*extract improved the histological structure of the liver and caused significant decrease in ALT and AST. These results suggested that *O. basilicum*protects the hepatocytes from injuries and improve liver function. In agreement with these observations,Chiu et al. reported that aqueous extract of *O.gratissium* inhibit CCl4 induced liver injury in rats [35]. *O. sanctum* showed hepatoprotective and immunomodulatory effects on liver injury and immunosuppression induced by isoniazid, rifampicin and pyrazinamide in guinea pig [36].Lahon and Das [37] reported that *O. sanctum* have hepatoprotective effect against paracetamol toxicity in rats. They observed a reduction in sinusoidal congestion, cloudy swelling and fatty changes and regenerative areas of the liver of rats given ethanolic extract of basil. Aluko et al.[38] reported that aqueousextract of O.americanum leaves have significant hepatoprotective ability against paracetamol - induced hepatic damage in rats. They added that there was a significant decrease in the serum levels of ALP, AST, ALT and TBIL with a corresponding increase in the activities of ALP, AST and ALT in the liver of extract treated rats.

Treating rats with diazinon and *O. basilicum*caused a decrease in expression of the antiapoptotic protein,Bax. Similarly, Sakr *et al*.[7] reported that *O. basilicum* ameliorates CCl4-induced hepatotoxicity and apoptosis in rat. Lee *et al*. [39] indicated that aqueous extract of *O. gratissium* leaf may be important in protecting H9c2 cells from H_2O_2-induced cell death by inhibiting the mitochondrial dependent apoptosis pathway. The antioxidant properties of basil were studied in different animal models [5, 40-42]. Chromatographic studies have showed that *O. sanctum* contain various active constituents viz. eugenol, luteolin, ursolic acid, and oleanolic . Eugenol (1-hydroxy-2-methoxy-4- allylbenzene), the active constituent present in *O. sanctum,* has been found to be largely responsible for the therapeutic potentials of basil [43].

CONCLUSION

It is concluded from the present work that the hepatopeotective effect of *O. basilicum* may be attributed to the antioxidant effects of its components.

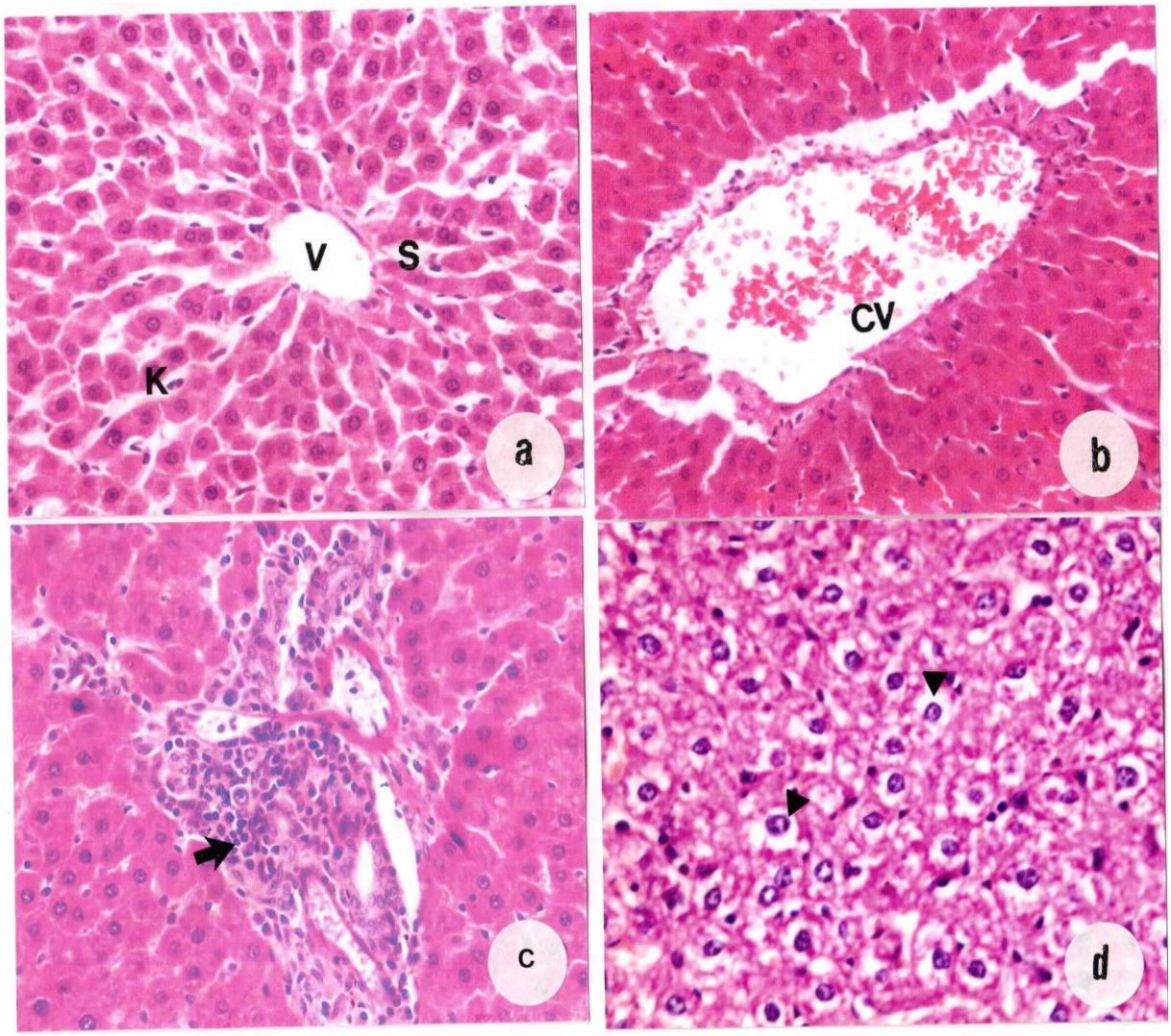

Fig.1. a). Section in liver of a control rat showing central vein (CV),sinusoid (S) and kupffer cell (K) ,b) Diazinon treated rat after 3 weeks showing congested and dilated blood vessel, c). Diazinon treated rat after 3 weeks showing leucocytes infiltration (arrow), d). Diazinon treated rat after 3 weeks showing cytoplasmic vaculation of the hepatocytes(H&E X400).

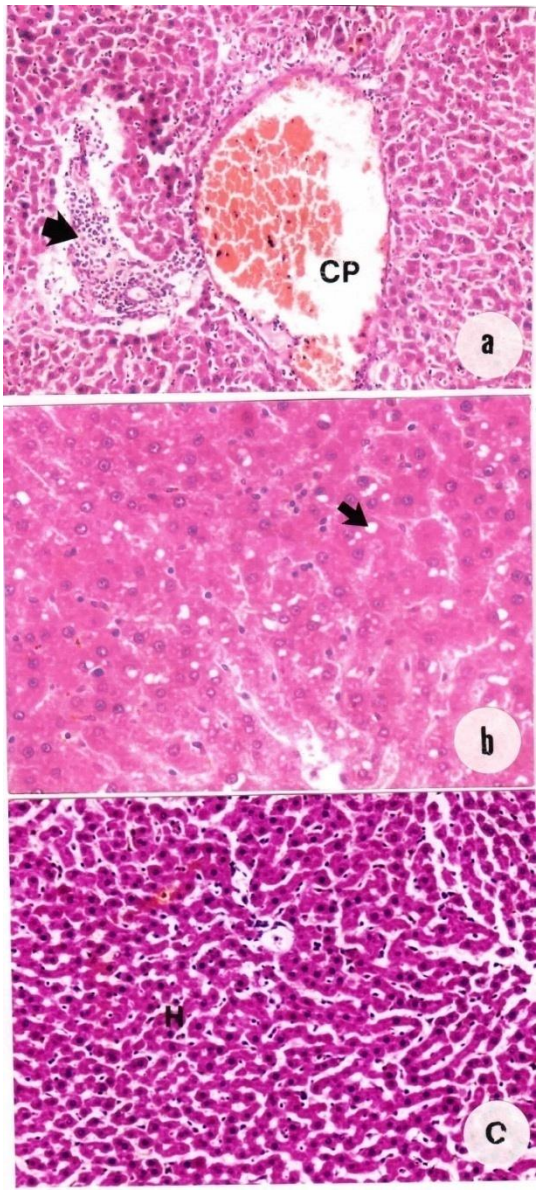

Fig.2. a). Section in liver of a rat treated with diazinon for 6 weeks showing congested portal vein (C P), b).Diazinon treated rat after 6 weeks showing fatty infiltrations (arrow), c). Liver of a rat treated with diazinon and *O. basilicum* showing an improvement in the liver structure (H&E X200).

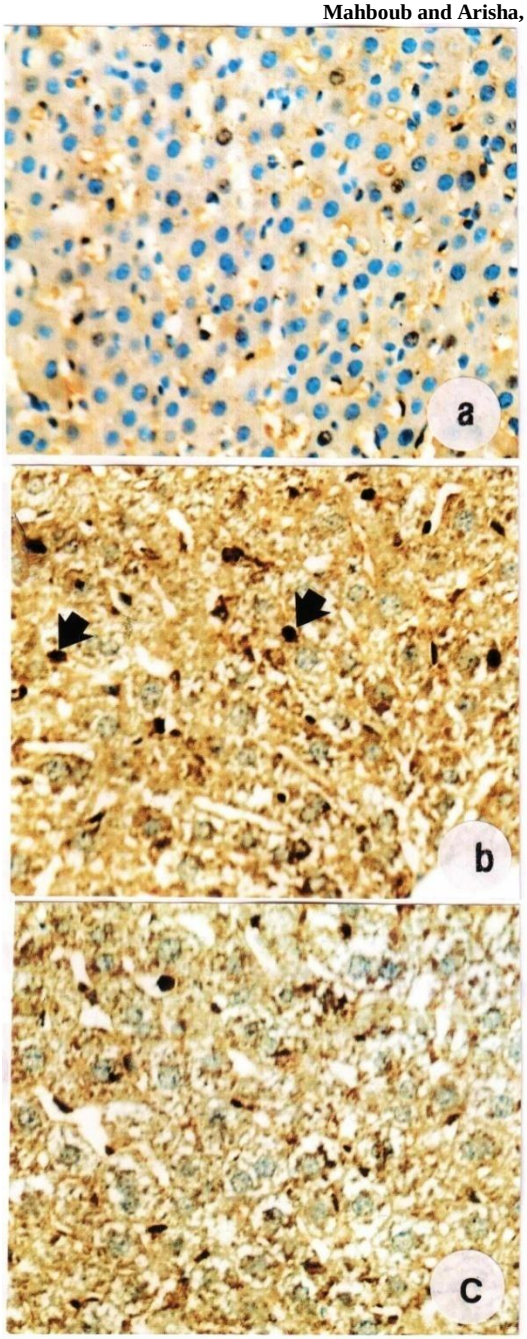

Fig.3. a). Section of liver a control rat, b) Diazinon treated rat showing PCNA positive cells (arrow), (c) liver of a rat treated with diazinon and *O. basilicum* showing decrease of PCNA-positive staining cells(arrow) (Immunostaining X400).

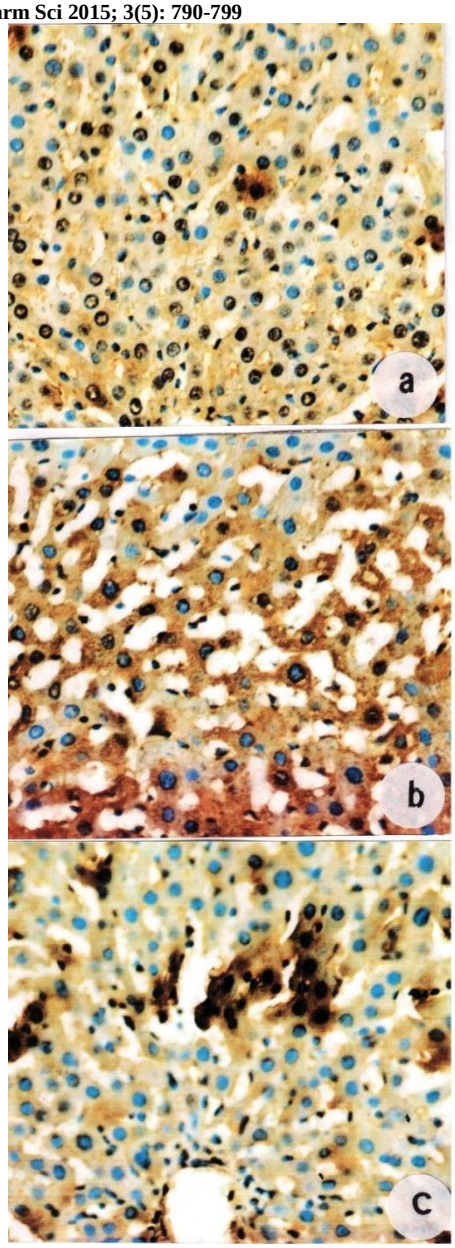

Fig.4.a). Section of liver a control rat, b) Diazinon treated rat showing large number of bax- positive cells (c) liver of a rat treated with diazinon and *O. basilicum* showing decrease of bax-positive staining cells (Immunostaining X400).

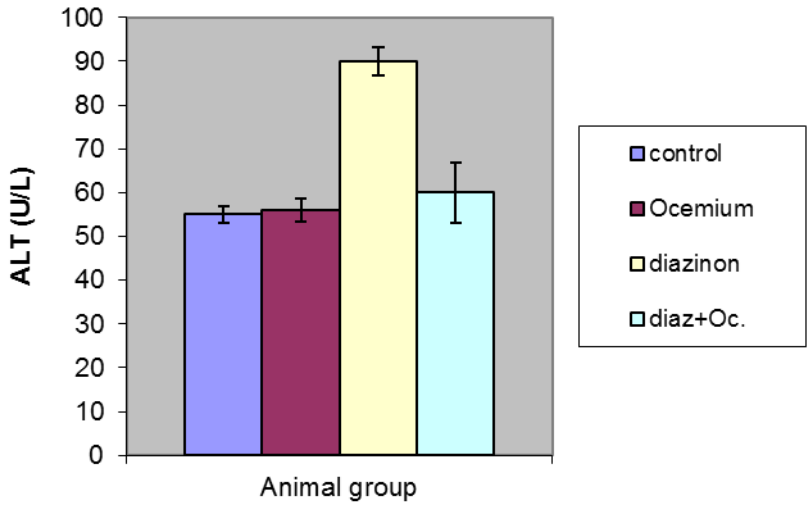

Fig.5. Change in ALT in control and treated groups

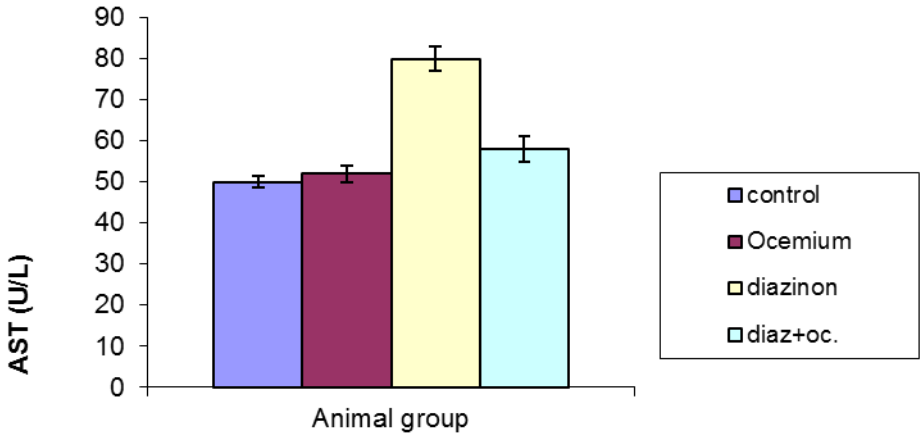

Fig.6. Change in ALT in control and treated groups.

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