Ameliorative Effect of Aqueous Leave Extract of *Ocimum Basilicum* on Ccl_4 - Induced Hepatotoxicity and Apoptosis in Albino Rats

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Abstract: Chemical-induced liver injury depends mostly on the oxidative stress. Basil or sweet basil (*Ocimum basilicum*) is known to have numerous pharmacological activities. The present study **aims** to investigate the effect of basil on Ccl_4-induced hepatotoxicity and apoptotic in albino rats. The result showed CCl_4 caused impairment of the normal structural organization of the hepatic lobules, congestion and dilatation of blood vessels, cytoplasmic vacuolization of the hepatocytes, leucocytic infiltrations and fatty degeneration. The biochemical results showed that there was an increase in serum level of ALT , AST, ALP, cholesterol , triglyceride, , LDL and HDL. Moreover, CCl_4 induced hepatic apoptosis. Treating animals with CCl_4 and aqueous leaves extract of *O. basilica* led to an improvement, in both histopathological and biochemical alterations induced by CCl_4. Also, apoptosis was repaired by shared administration with both *O. basilicum* and CCl_4. These results proved that *O. basilica* had an ameliorative effect against liver injury produced by **CCl_4** due to its antioxidant activity.
[Saber A. Sakr, Sabah F. El-Abd, Mohamed Osman, Asmaa M. Kandil, Mona S. Helmy **Ameliorative Effect of Aqueous Leave Extract of *Ocimum Basilicum* on Ccl_4 - Induced Hepatotoxicity and Apoptosis in Albino Rats**. Journal of American Science 2011; 7(8): 116-127]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: CCl_4 – Hepatotoxicity- rat- *O. basilicum* - Apoptosis

1. Introduction:

Oxidative stress has been shown to play a very crucial role in some diseases including liver disease. Free radical that generate inside the body is responsible for oxidative stress and compounds that can scavenge free radicals have great potential in ameliorating these disease processes (**Sies, 1997**). Basil or sweet basil (*Ocimum basilicum)* is a plant that belongs to the family Labiatae and is known as Tulsi in Hindi , Holy Basil in English and Rehan in Egypt. It is known to have numerous pharmacological activities. 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)** investigated the efficacy of the leaf essential oil of *Ocimum gratissimum* Linn. in promoting hair growth in cyclophosphamide-induced hair loss. The results showed that ocimum oil may be 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. **Rupert (2009)** reported that basil or basil oil have agents for prevention and treatment of cardiovascular disease. It has also been shown that OS leaf extracts can protect the liver from heavy metals (**Sharma *et al.*, 2002**) and prevent isoproterenal induced myocardial necrosis in rats (**Sood *et al.*, 2005**).

Chemical-induced liver injury depends mostly on the oxidative stress in hepatic tissue. Carbon tetrachloride (CCl4)-induced liver damage is the best characterized system of xenobiotic-induced hepatotoxicity and is a commonly screening model to evaluate the hepatoprotective potential of drugs with antioxidant properties. Administration of CCl_4 causes extensive changes in liver morphology including steatosis, inflammation and necrosis (**Qiu *et al.*, 2005**). It induced liver fibrosis, cirrhosis, enhanced lipid peroxidation, increases ALT and causes collagen deposition in liver tissue (**Nan *et al.*, 2002, Campo *et al.*, 2004**). **SuYanga *et al.* (2008)** reported that single oral dose of CCl_4 produced significantly elevated levels of serum ALT, AST activities and extensive liver necrosis and fatty changes .Carbon tetrachloride was metabolized in

the liver by cytochrome P450 of the endoplasmic reticulum with the formation of a highly toxic trichlorometheyl radical (CCl_3) (**Conner *et al.*, 1990**).

2. Material and Methods

Animals

Male albino Wistar rats weighing 100 ± 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 twice a week for 6 weeks.

Group3. Rats were injected intraperitonealy with 1.0ml/kg b.w of 10% CCl_4 dissolved in olive oil twice a week for 6 weeks (Sakr et al.2010).

Group4. Rats were injected with CCl_4 (1.0 ml/kg) followed by oral administration with aqueous O. *basilicum* extract at a dose level of 20ml/kg twice a week for 6 weeks.

Histological examination

The treated animals and their controls were sacrificed by decapitation after 2, 4 and 6 weeks of treatment. Liver 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.

Biochemical assays

For biochemical assays blood was collected and centrifuged at 3000 rpm for 10 minutes and stored at -20 ^{0}C .Liver function enzymes ALT and AST were determined in serum according to the method of **Gella *et al.* (1985)**.The activity of alkaline phosphatase was assayed by the method of **El-Aaser and El-Marzabani (1975)**. Cholesterol and triglycerides were measured using the methods of **Zlaktis *et al.* (1953),** and **Fassati and Prencipe (1982)**, respectively.

DNA Fragmentation Assay

As a measure of apoptotic DNA fragmentation, the presence of DNA ladder was determined according to **Wlodek *et al.* (1991).** Extraction of DNA was done according to method of Hassab El- Nabi (2009) and Aljanabi S. M. (1997), 10 mg of liver tissue in eppendorf tubes were lysed with 600 microlitre buffer (50 mM NaCl, 1 mM Na_2EDTA, 0.5% SDS, PH 8.3) and gently shaked. The mixure was incubated overnight at 37 C^{O} then, 20 microlitre of saturated NaCl was added the sample, shacked and centrifuged at 12,000 rpm for 10 min. the supernatant was transferred to new Eppendorf tubes and then DNA precipitated by 600 microlitre cold isoproprnol. The mix was inverted several times till fine fibers appear, and then centrifuged for 5 min. at 12,000 rpm. The supernatant is removed and the pellets were washed with 500 microlitre 70% ethyl alcohol centrifuged at 12,000 rpm for 5 min. After centrifugation the alcohol was decanted or tipped out and the tubes plotted on Whatman paper to be dry. The pellets were resuspended in 50 microlitre or appropriate volume of TE buffer (10 mM Tris, 1 mM EDTA, PH 8). The resuspended DNA was incubated for 30 - 60 min with loading mix (Rnase + loading buffer) and then loaded into the gel wells.

Agarose gel electrophoresis

A gel was prepared with 2% agarose containing 0.1% ethidium bromide (200 ug/ml). The DNA samples were mixed with loading buffer (0.25% bromophenol blue, 0.25% xylene cyanole FF and 30% glycerol) and loaded into the wells (2 ug of DNA/lane) with a standard molecular- sized ladder marker (Pharmacia Biotech., USA). The gel was electrophoresed at a current of 50 mA for 2.5 h using the submarine gel electrophoresis machine. The DNA was visualized and photographed with illumination under UV light using a photodocumentation hood (Fisher Scientific, Pittsburgh, PA, USA) equipped with a Polaroid 667 film with an orange filter (Kodak, Rochester, NY, USA).

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).

3. Results

Histological observations

Figure (1) showed the histological structure of the liver of control rat. Liver of animals administered with O. *basilicum* appeared with normal structure. On the other hand, Liver of rats treated with CCl_4 for two weeks showed impairment of the normal structural organization of the hepatic lobules and sinusoidal spaces were enlarged. Intrahepatic veins, central and portal were dilated and congested (Fig. 2). Leucocytic infiltrations were observed (Fig.3). Liver sections prepared from rats 4 weeks post-treatment with CCl_4 revealed that a considerable number of hepatic cells were damaged and lost their characteristic appearance while others showed marked cytoplasmic vacuolization which was so extensive in some cells to the extent that only slight remnants of the cytoplasmic mass cells - frequently forming a narrow peripheral rim was left (Fig.4). The nuclei of these cells were pyknotic. In addition, congestion of the intrahepatic blood vessels and inflammatory leucocytic infiltrations were observed. The histopathological changes of the liver were more increased after 6 weeks and the liver cells were degenerated and suffered from micro and macrovesicular steatosis (Fig.5). Examination of liver sections obtained from rats treated with both CCl_4 and O. *basilicum* for 2 and 4 weeks revealed gradual restoration of the normal structure of liver tissues. A rare leucocytic infiltration was observed, but the central as well as the portal veins were congested (Fig.6). A large number of binucleated hepatocytes were observed. After 6 weeks, the liver tissue appeared normal and fatty infiltrations was absent in the examined specimens (Fig.7).

Biochemical results

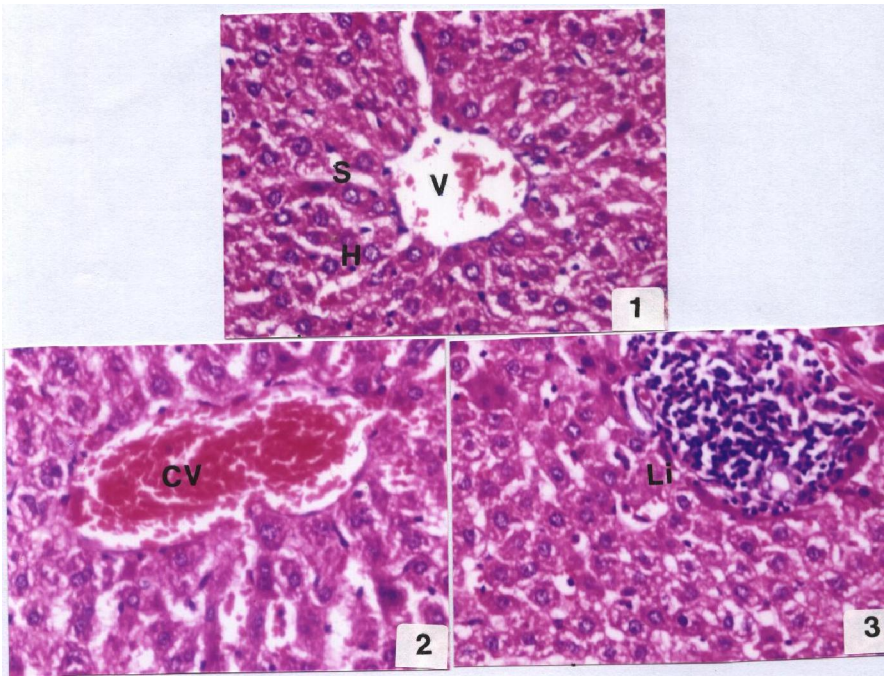
Treatment with CCl_4 for 6 weeks caused a highly significant elevation ($P<0.05$) in the activity of ALT, AST and ALP as compared to those of the control animals. All these parameters were restored to near normal values in rats treated CCl_4 and *O. basilicum* (Figs.8-10).Both control and animals given *O. basilicum* showed no significant differences in serum activity of ALT, AST and ALP. Administration of CCl_4 to rats caused significant increase in cholesterol and triglycerides compared with animals of control groups. Animals treated with both CCl_4 and *O. basilicum* extract showed reduction in their sera level of cholesterol and triglycerides in comparison of those given CCl_4 (Figs.11&12). Figures 13 and 14 showed that treating animals with CCl_4 induced significant increase in serum HDL and LDL concentrations after 4 and 6 weeks post - treatment compared with control group. On the other hand, animals treated with CCl_4 and *O. basilicum* extract had a noticeable increase in the concentration of these parameters compared with animals received CCl_4 alone.

Biochemical features of apoptosis

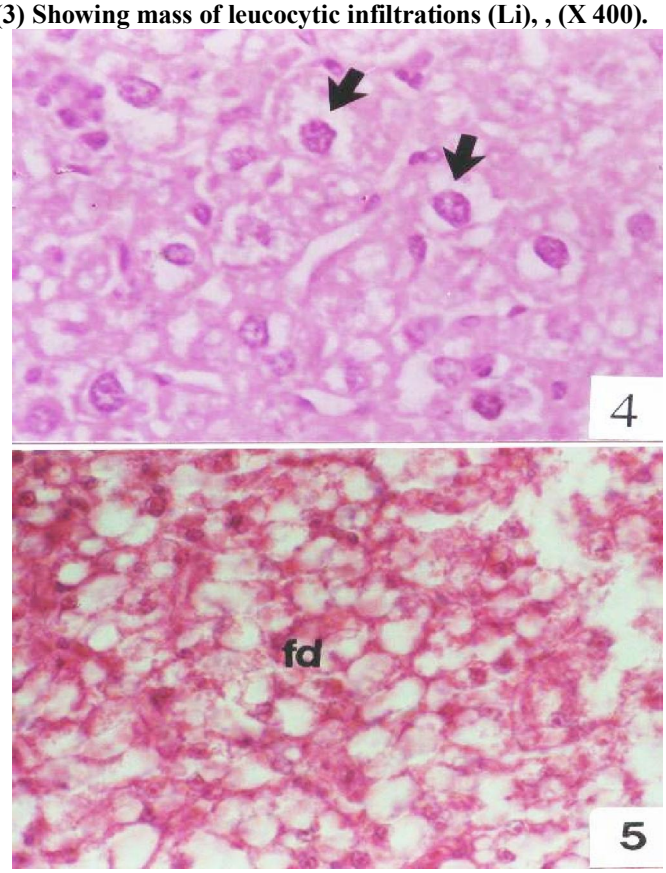
Administration of carbon tetrachloride for 6 weeks induced fragmentation of DNA in rat livers (Fig.15). The total optical density of released DNA was 128 when compared with controls (table 1). Animals treated with *O. basilicum* were not display any increased in fragmented DNA and the total optical density was in normal range.Fragmentation of DNA was repaired by shared administration with both *O. basilicum* and carbon tetrachloride for 6 weeks as rosemary significantly decreased the total optical density of released DNA with value of 25 when compared with CCl_4 treated group.

4. Discussion

Results obtained in the present work indicated that CCl_4 induced histological and biochemical alterations in liver of albino rats. Concerning the histological effects, liver of CCl_4-treated animals showed many degenerative changes including cytoplasmic vacuolization of the hepatocytes, fatty infiltrations, leucocytic infiltrations, congestion of blood vessels, and fibrosis. Similar results were obtained by **Sakr *et al.* *(2010)*** in albino rats intoxicated with CCl_4. Moreover, the current results are in accordance with those of **Sreelatha *et al.*(2009)** and **Lodhi *et al.*(2009)** who reported that liver injury including marked alteration of the entire liver structures with degenerative changes were observed after CCl_4 administration. Fatty infiltrations were observed in liver of CCl_4 treated rats. In agreement with this result **Qiu *et al.* (2005)** and **Panovska *et al.* (2008)** reported that CCl_4 caused extensive liver necrosis and fatty changes .**Brody *et al* .(1961)** attributed the fatty changes in the liver to excessive mobilization of free fatty acids from the fat depots induced by the lipolytic effects of the increased circulating catecholamines and the centrilobular necrosis to the catecholamines-induced decrease in hepatic flow. Liver fibrosis was observed after 6 weeks of treating rats with CCl_4. **Qiu *et al.* (2005)** reported that CCl_4 caused centrilobular necrosis followed by fibrosis.**Nan *et al.*(2002)** mentioned that CCl_4 is the most widely used chemical for inducing liver fibrosis.



Figs. 1-3: (1) Section of liver of a control rat showing hepatocytes (H), central vein (V), sinusoids (S) and Kupffer cells (K), (2) section of liver of ccl_4-treated rat after two weeks showing congested and enlarged central vein (CV), (3) Showing mass of leucocytic infiltrations (Li), , (X 400).



Figs 4-5: (4).Liver section of ccl_4-treated rat for 4 weeks showing cytoplasmic vacuolization of the hepatocytes (arrows).(5), Specimen obtained from a rat treated with ccl_4 for 6 weeks showing fat droplets (fd) of different sizes,(X 400).

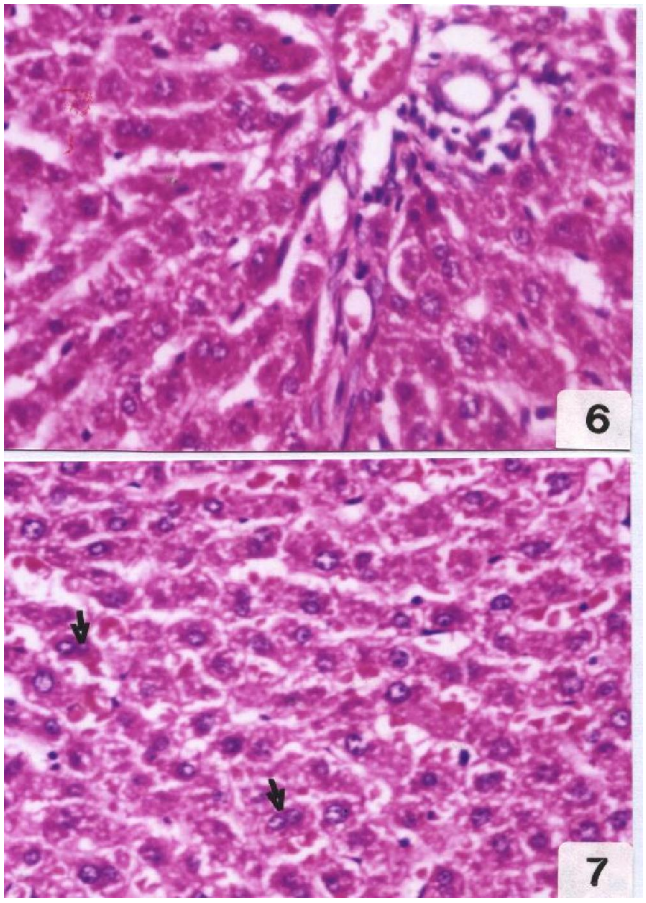


Fig 6-7: (6). Liver section of a rat treated with ccl_4 and ocemium for 4 weeks showing congested portal vein (P), (7) Specimen obtained from a rat treated with ccl_4 and ocemium showing an obvious degree of improvement with large number of binucleated cells (arrows), (X 400).

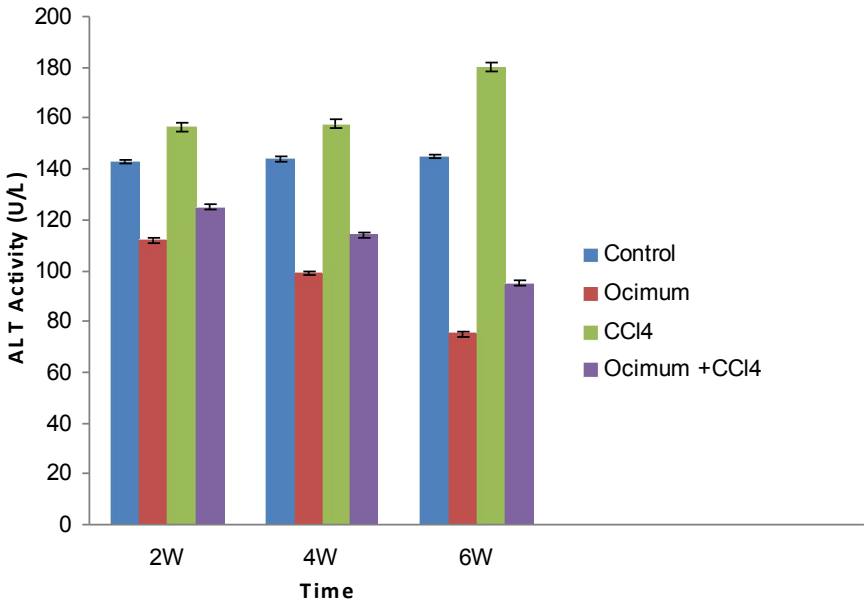


Fig.8. Change in ALT activity in different experimental groups

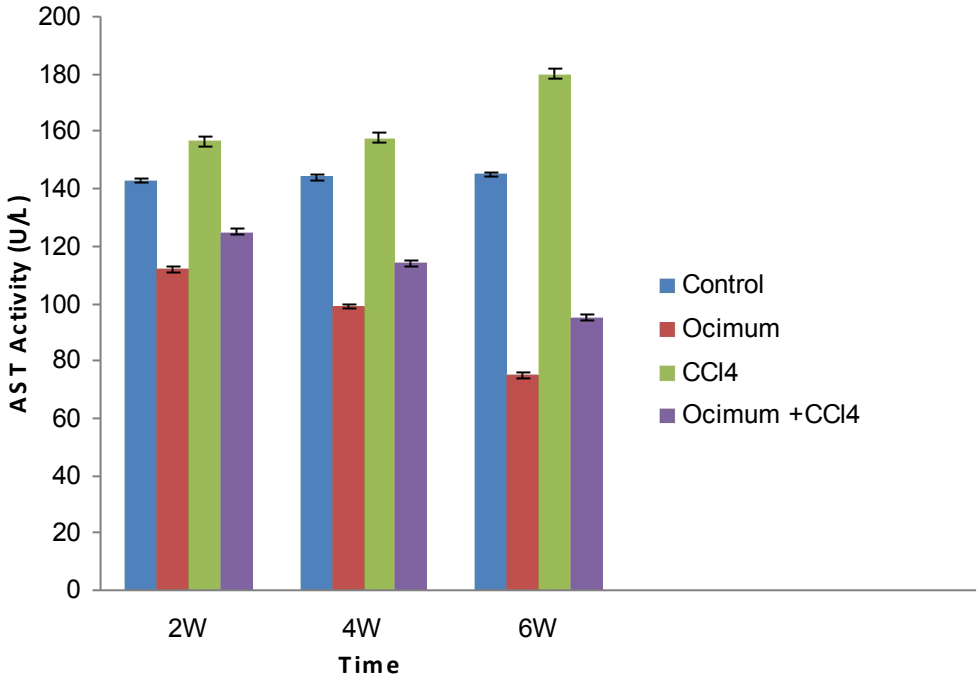


Fig.9. Change in AST activity in different experimental groups

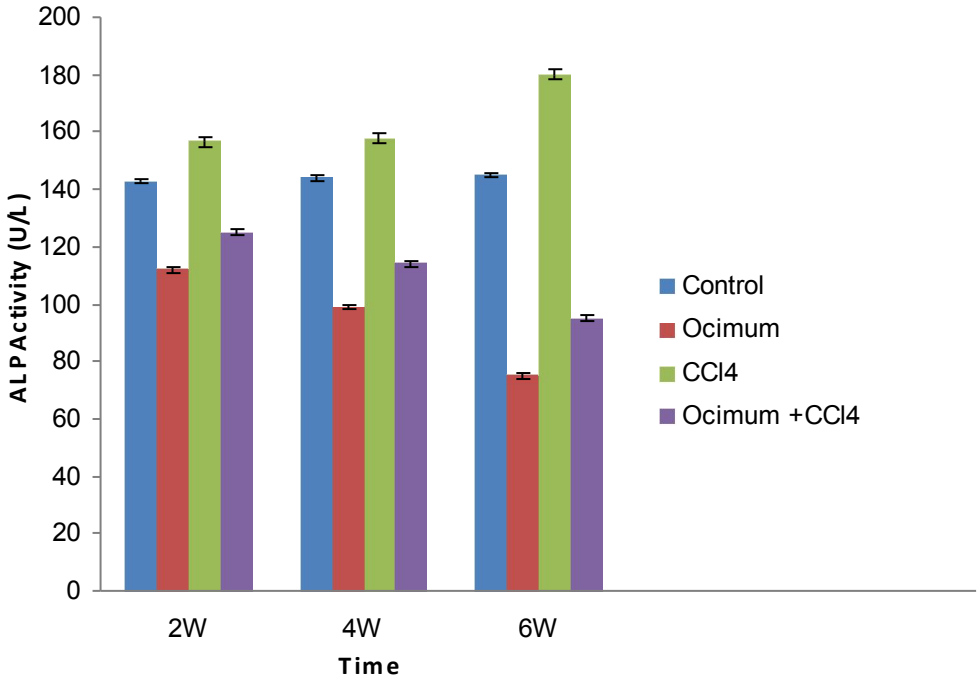


Fig.10. Change in ALP activity in different experimental groups

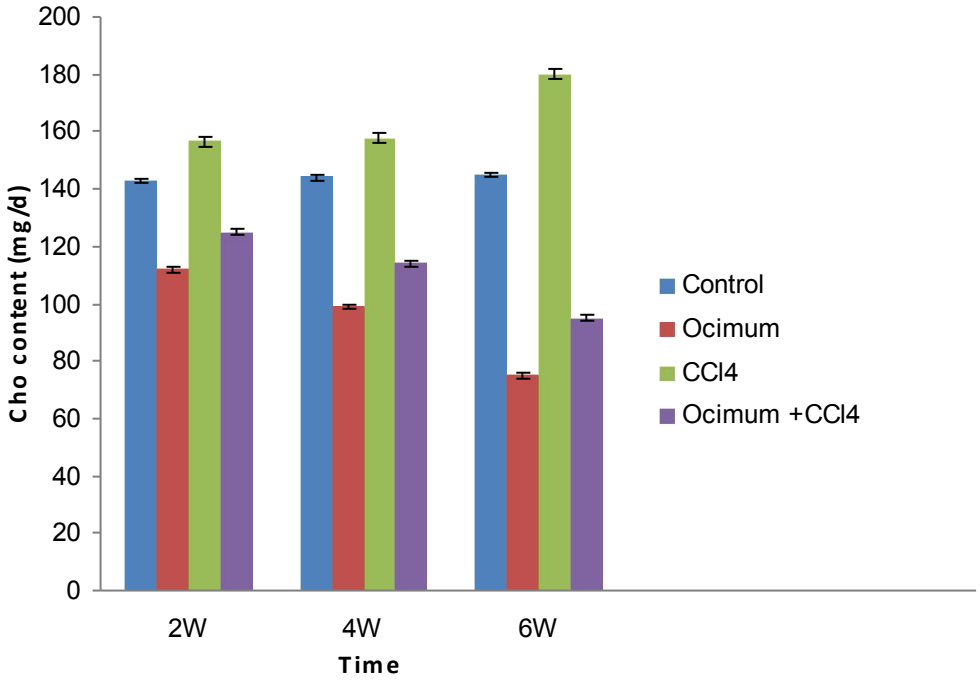


Fig.11. Change in serum cholesterol in different experimental groups

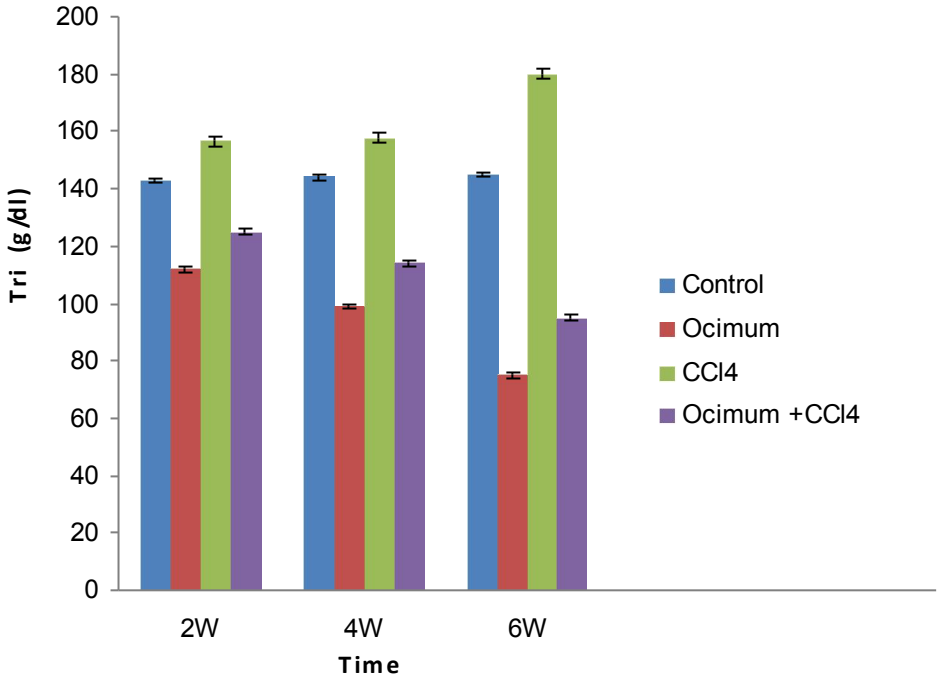


Fig.12. Change in serum triglycerides in different experimental groups

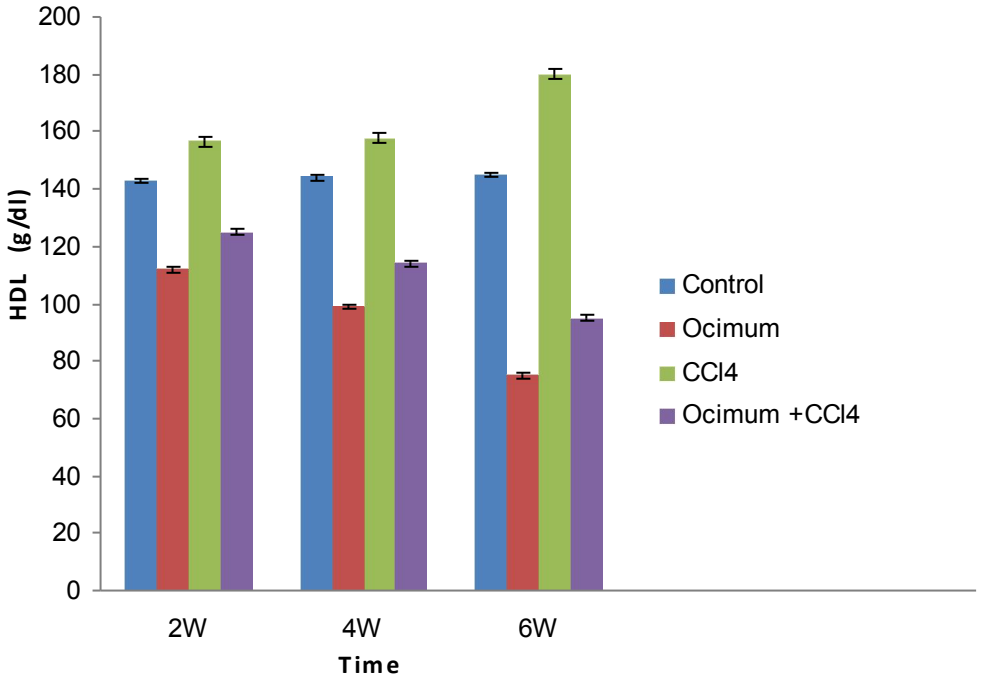


Fig.13. Change in HDL in different experimental groups

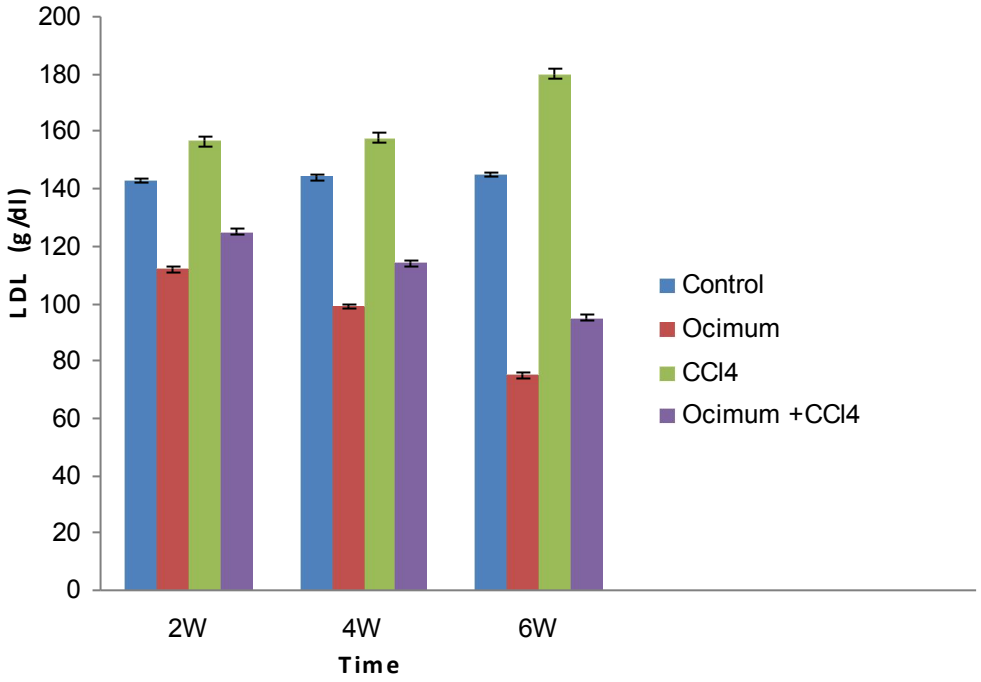


Fig.14. Change in LDL in different experimental groups

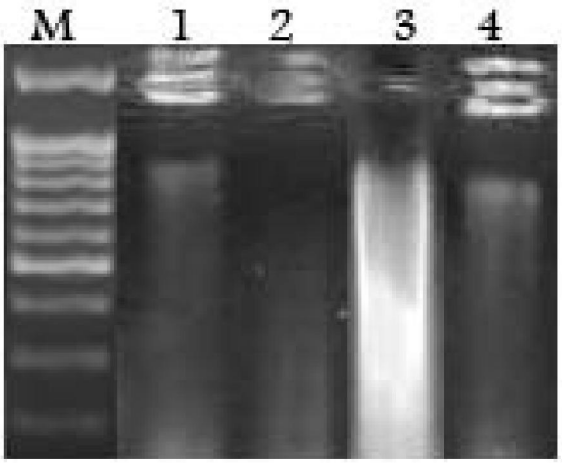


Fig.15. Gel electrophoresis of hepatic DNA M: standard lane, Lane 1: control, lane 2 ocemium, Lane 3: ccl_4 group,, lane 4: ccl_4 and ocemium group.

Table (1): Changes in values of the total optical density of both intact and fragmented DNA induced in liver of rat.

Total optical density	Control group.	Ocimum group	CCL4 group	CCL4+ Ocimum group
Intact DNA	61	58	32	53
Fragmented DNA	**5**	**7**	128	25

CCl_4 was found to induce apoptosis as represented by DNA fragmentation. This result came in agreement with **Castro *et al.* (1993)** who reported that CCl_4 induced necrosis and DNA fragmentation in Sprague-Dawley male. Rats. **Shi *et al.*(1998)** proved that carbon tetrachloride poisoning induced DNA fragmentation, apoptosis and necrosis in rat liver by immunohistochemical labeling of nuclear DNA fragmentation, flow cytometry and gel electrophoresis .

Significant increase in ALT, AST and ALP levels of sera of CCl_4 treated rats was recorded in the present study. In agreement of this result, **Wang *et al.* (1996)** observed that a single i.p. injection of CCl_4 caused an increase in ALT and AST. Pablo and **Yesenia (2003)** found that liver injury induced by CCl_4 in Wistar rats was accompanied by elevation in serum level of ALT,AST and ALP. Increase in triglycerides and cholesterol were recorded in sera after exposure to CCl_4. Similarly, **Torres-Duran *et al.* (1999)** reported that CCl_4 caused elevation in LDL, HDL, triglycerides and cholesterol.

Oxidative stress is a state of redox imbalance caused by increased reactive oxygen species (ROS) generation and decreased antioxidant capacity. Administration of CCl_4 is an established experimental model of severe toxic liver injury involving generation of oxidative stress. It has been reported that exposure to CCl_4 induces oxidative stress in rats (**Sharma *et al.*,1994**). Oxidative damage primarily occurs through production of reactive oxygen species, including CCl_3 and CCl_3O_2 radicals that subsequently react with biological molecules as well as causing damage to membranes (**Singh *et al.*,1998).** A decrease in the level of antioxidant enzymes and an increase in lipid peroxidation level were recorded after CCl_4 administration (**Campo *et al.*, 2004**).The increase in lipid peroxidation in the liver following exposure to CCl_4 may lead to membrane damage resulting in damage of liver cells. The increase in ALT, AST and ALP is the end results of this phenomenon.

The present findings demonstrated that *O.basilicum* improve the histological changes and increased liver function enzyme activity induced by CCl_4. This indicated the effectiveness of *O.basilicum* in prevention of CCl_4 hepatotoxicity. The hepatoprotective effects of *O.basilicum* have been

shown in studies on experimental liver damage. **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**). **Adhvaryu *et al.*, (2007)** reported that *O. sanctum* have hepatoprotective and immunomodulatory effects on liver injury and immunosuppression induced by Isoniazid , Rifampicin and Pyrazinamide in guinea pig. It has been shown that 2% of dried *O. sanctum* leaf powder supplemented in the diet can lower serum lipid profile and partially protect the liver in diabetic rats (**Suanarunsawat and Songsak, 2005**). It has also been shown that *O. sanctum* leaf extracts can protect the liver from heavy metals (**Sharma *et al.*, 2002**) and prevent isoproterenal induced myocardial necrosis in rats (**Sood *et al.*,2005**). O.basilicum treatment attenuated serum lipid profile. This may be due to the anti-hyperlipidemic action of components of *O.basilicum* leaves. **Suanarunsawat *et al.* (2009)** mentioned that the anti-hyperlipidemic activity of *O.basilicum* may be due to the suppression of liver lipid synthesis.

Zhang *et al.*(2009) reported that the main components of *O. basilicum* are : 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%). **Lee and Scagel (2009)** reported that the presence of chicoric acid (dicaffeoyltartaric acid) was the major phenolic compound, in basil leaves. *O.basilicum* is rich source of flavonoids which have been shown to possess various biological properties related to antioxidant mechanisms. **Dasgupta *et al.*, (2007)** reported 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. **Chinnasamy *et al.*, (2007)** oreported that the protective action of ocimum was attributed to its antioxidant action. They added that this protection may be also due to anti-inflammatory property of ocimum which reduces formation, release, and activity of inflammatory mediators such as cytokines, histamine, prostaglandins, and leukotrienes. **Suanarunsawat *et al.* (2009)** reported that *O. sanctum* leaf have lipidlilowering effect and antioxidant activity in rats fed with a high cholesterol diet. It is concluded from the present work that the hepatoprotective of O. *basilicum* may be attributed to the antioxidant activity of its flavonoids.

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7/12/2011