Role of *Capparis spinosa* in ameliorating trichloroacetic acid induced toxicity in liver of Swiss albino mice

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Abstract: The hepatoprotective effect of the mixture of leaves powder of the medicinal plant; *Capparis spinosa* and honey against some biochemical and histological alterations induced in Swiss albino mice intoxicated with trichloroacetic acid (TCA) was investigated. 120 male mice 20-26gm were divided into 6 groups. Group I was the control group; group II treated orally with honey (40 mg/kg body weight for 3 weeks), group III treated orally with a mixture of *Capparis spinosa* leaves powder and honey(40 mg/kg for 3 weeks), group IV Given orally aqueous extract of *Capparis spinosa* leaves powder for 3 weeks, group V treated with TCA in drinking water (500mg/kg for 3 and 6 weeks, then left for 3 weeks for recovery and group VI (Regeneration group) received TCA for 6 weeks then treated with a mixture of *Capparis spinosa* and honey for 3 weeks. The treatment with TCA for 6 weeks induced 6% deaths in the experimental animals. The mortality increased to 14% in the group left for recovery without treatment whereas, in the group intoxicated with TCA then treated with the mixture of *Capparis spinosa* and honey the mortality reached 6% only. TCA induced a significant decrease in the final body weight comparing to the control group. Administration of the mixture of *Capparis spinosa* and honey showed an insignificant increase in the final body weight comparing to TCA alone treated group. Results of the biochemical studies showed abnormal levels of serum enzyme activities in TCA treated animals. Whereas, animals intoxicated with TCA and treated with the mixture of *Capparis* spinosa and honey showed an improvement in the levels of these serum markers. Histological examination revealed that administration of TCA induced various pathological lesions in liver tissues; included stenosis of hepatic sinusoids, dilatation and congestion of central veins, hemolysis and focal inflammatory cells infiltration, hyperplasia of Kupffer cells, hypertrophied hepatocytes and necrotic hepatocytes with vacuolated cytoplasm were frequently observed. Sever reactivity of most hepatocytes to periodic acid Schiff (PAS) was also noticed. Intoxicated mice treated with the mixture of *Capparis spinosa* leaves powder and honey showed an improvement in the histological structure.

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Key words: *Capparis spinosa*, trichloroacetic acid, histopathological liver, liver functions, mice*(Mus-musculus*)

1. Introduction

Herbal medicine is still the main stream of about 75 to 80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents **(Acharya *et al.*, 2008).** *Capparis spinosa* L. family Capparidaceae is one of the most common aromatic plants growing in wild in the dry regions around the west or central Asia and the Mediterranean basin. *Capparis spinosa* is well known with its common name 'Capers' in different countries **(Azaizeh *et al.*, 2003 and Tlili *et al.*, 2011)**. It had been known for centuries in traditional phytomedicine **(Benzidane *et al.*, 2013)**. In Libya and many other countries, *Capparis spinosa* was found to be used traditionally for treatment of a variety of diseases and cancer **(Kulisic-Bilusic *et al.*, 2012)**. *Capparis spinosa* considered as a very important source of medicine for antidiabetic **(Ziyyat *et al.*, 1997)**, antihepatotoxic **(Gadgoli and Mishra, 1999)**, antifungal **(Ali-Shtayeh *et al.*, 1999)**, diuretic, antihypertensive and poultice **(Çaliş *et al.*, 1999)**, antihyperlipidemic **(Eddouks *et al.*, 2005)** and antihelminthic properties **(Mustafa, 2012)**. Other activities included chondrocyte protective **(Panico *et al.*, 2005)**, as well as inhibitory effect on fibroblast proliferation and type I collagen production in progressive systemic sclerosis **(Cao *et al.*, 2008)**. The presence of several quercetin and kaempferol glycosides, as well as of hydroxycinnamic acids, had been demonstrated in capers **(Bonina *et al.*, 2002)**.

Trichloroacetic acid (TCA) (CC13COOH) is a colorless to white crystalline solid with a sharp, pungent odor **(NIOSH, 2003)**. It is formed from organic material during water chlorination **(Coleman *et al.*, 1980 and IPCS, 2000)** and had been detected in groundwater, surface water distribution systems, and swimming pool water. TCA was detected in vegetables, fruits, and grains **(Reimann *et al.*, 1996)** and can be taken up into foodstuffs from the cooking water **(U.S. EPA, 2005)**. Therefore, human exposure

to TCA can also occur via food consumption. TCA is mainly used in the production of its sodium salt, which is used in many industries; as a herbicide, etching agent and antiseptic **(Lin *et al*., 2005)**. Oral half lethal dose (LD_{50}) of 4970 mg/kg of body weight for TCA have been reported in mice **(Woodard *et al*., 1941).**

Laboratory mouse is an animal most commonly used in mammalian biological studies and in the human disease modeling, due to the following factors: easy breeding, availability of inbred strains, short generation time, refined map of the genome and an extensive knowledge of biological and immunological properties **(Wirth-Dzięciołowska *et al*., 2009).**

Liver diseases remain to be serious health problems and the management of liver disease is still a challenge to the modern medicine. Liver plays an essential role in regulation of physiological processes, involved in several vital functions such as storage, secretion and metabolism. It also detoxifies a variety of drugs and xenobiotics and plays a central role in transforming and clearing the chemicals and is susceptible to the toxicity from these agents **(Pal and Manoj, 2011).** However, till now no much is known about the dose-related toxicity of medicinal plants, particularly at the histological side **(Kulisic-Bilusic *et al*., 2012)**.Therefore, the present study aimed to study the possible protective role of *Capparis spinosa* leaves as used in traditional medicine in Libya on biochemical and histopathological alterations of the liver induced in an animal model intoxicated with trichloroacetic acid**.**

2. Materials and Methods

Experimental animals

Healthy adult male Swiss *albino mice (Mus-musculus*) 8 to 10 weeks old and weighing 22 ±4 gm were obtained from the Animal Breeding House of faculty of Veterinary Medicine, Omar El- mukhtar University, Albayda, Libya. They were housed in the laboratory animal room in clean plastic cages under controlled conditions of temperature (20 ± 2°C) and photoperiod (14h light: 10h dark) cycle. The animals were maintained on standard commercial pellet diet and clean drinking water *ad libitum*. Mice were acclimatized for 1 week prior to the start of experiments.

Materials used:

Fresh plants of *Capparis spinosa* were collected from Blgray region Algabal Alakhder in Al Bayda-Libya between March and April 2012. The plant was authenticated by Department of Botany, Faculty of Agriculture, Omar El mukhtar university, Al Bayda-Libya. Only the leaves were used. They were cleaned, air-dried and then powdered mechanically.

Honey sample

Natural bees honey **(**vehicle**)** used in this study was purchased from the local honey market in Al Bayda - Libya. The honey was collected form beehives built on Algabal Alakhder - Libya. This honey is also locally known as Seder honey. It was filtered to remove solid particles.

Preparation of the mixture of *Capparis spinosa* and honey:

Leaves powder of *Capparis spinosa* (400mg) were well mixed with 40 gm of Seder honey and used at dose level 40mg/kg body weight (0.1ml/mouse) (equivalent to dose used by a human weighing 70 kg in traditional medicine).The mixture of *Capparis spinosa* leaves powder and honey was prepared according to the prescriptions given by traditional healers. The dose was determined according to **Paget and Barnes (1964).**

Trichloroacetic acid (TCA) was purchased from (Sigma Co, Germany). TCA was chosen because it had been reported to increase liver growth, cell proliferation, and induce cancer and tumor in kidney and liver of mice **(Bull *et al*., 1990; Pereira, 1996; Pereira & Phelps, 1996; Channel *et al*., 1998 and Pereira *et al*., 2001).**

Fig.(1): *Capparis spinosa*

Experimental Design

A total of 120 apparent healthy adult male mice were divided into 6 groups of 20 mice each and subjected to the following treatments:

Group I: Is the **control group;** it received distilled water at dose level 4 ml/kg by oral gavage for 3 and 6 successive weeks.

Group II: Received honey by oral gavage at dose level 4 ml/kg for 3 successive weeks.

Group III: Treated orally by oral gavage with a mixture of *Capparis spinosa* leaves powder and honey at dose level 40 mg/kg body weight suspended in 0.1ml honey once per day for 3 weeks.
Group IV: Given orally by oral gavage aqueous extract of *Capparis spinosa* leaves powder at dose level 40 mg/kg body weight once per day for 3 successive weeks.
Group V: Treated with TCA at dose level 500 mg/kg body weight in drinking water for 3 and 6 successive weeks (Doses were estimated based on default drinking water intake values for mice). After the end of the experimental period the animals in this group left for recovered and known as **recovery group**.
Group VI: Received TCA at dose level 500 mg/kg body weight in drinking water for 6 successive weeks then treated orally by oral gavage with a mixture of Capparis *spinosa* and honey at dose level 40 mg/kg body weight once per day for 3 successive weeks and known as **regeneration group**.

Clinical signs:

Animals were observed daily to note and record any changes in the behavior, depression, food intake and signs of difficult breathing, salivation, diarrhea, muscular weakness and any signs of toxicity.

Body weight

Body weights of mice in all groups were measured at the beginning and the end of the experiment. Body weights were also recorded at weekly intervals using electronic balance. Weight gains and the body weight changes (%) were calculated according to **Tütüncü *et al.* (2010)**.

Biochemical studies:

Twenty four hours after the end of experimental period, un-anesthetized mice from both control and experimental groups were sacrificed by slaughtering (cervical dislocation). Peripheral blood samples were collected from the neck blood vessels into free anticoagulated containers and centrifuged at 3000 rpm for 10 minutes and the supernatant serum was collected in Eppendorf. Serum activities of alanine aminotransferas (ALT) and asparatate aminotransferease (AST) were determined calorimetrically according to the method recommended by **Reitman and Frankel (1957).** Alkaline phosphatase (ALP) was assayed according to **Babson *et al.* (1966).** Total protein was measured according to **Lowry *et al.*, (1951).** Determinations of parameters were performed using an automated biochemical analyzer (Chemistry analyzer photometer by used commercial available kits from Analyticon Biotechnologies (Germany).

Histopathological studies:

For the light microscopic examination, liver was carefully dissected out and quickly fixed in aqueous Bouin's fluid, dehydrated in ascending grades of ethyl alcohol, cleared in xylene, impregnated in paraffin wax and sections of 5–7 μm thickness were taken. The deparaffined sections were stained with Harri's haematoxylin and eosin (H&E) and periodic acid Schiff (PAS) according to **Bancroft& Gamble (2008)**. Histological sections were examined by light microscope with digital camera (Nikon Eclipse E400).

Statistical Analysis:

All values were expressed as mean ± SEM. Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Duncan's test. *P* values < 0.05 were considered to be statistically significant. Excel programs was used for analysis the results and draw the figures.

3. Results

Clinical signs and mortality

No obvious changes in the behavior and external features were observed in control and treated animals. However, some animals treated with the mixture of *Capparis spinosa* and honey for 3 weeks showed less activities during the first week of administration. Also, no lethality were recorded during the experiment period in control, honey treated group, mice treated with mixture of *Capparis spinosa* leaves powder and honey, aqueous extract of *Capparis spinosa* leaves powder as well as, mice treated with TCA for 3 weeks. While, 6% deaths was recorded in mice treated with TCA for 6 weeks, which increased to 14% in recovery group. On the other hand, the repeated oral administration of the mixture of *Capparis spinosa* and honey after TCA; in the regeneration group, the percentage of mortality reached 6% only comparing to the recovery group.

Body weight:

A slight insignificant changes in the final body weight gain in honey treated group, mice treated with mixture of *Capparis spinosa* leaves powder and honey and aqueous extract of *Capparis spinosa* leaves powder comparing to control group were recorded. On the other hand the treatment with TCA only induced significant decrease in the final body weight compared to control group. Mice intoxicated with TCA for 6 weeks and administrated the mixture of *Capparis spinosa* and honey showed a slight and insignificant increase in the final body weight comparing to TCA alone treated group.

Biochemical studies:-

Results of the biochemical analysis revealed insignificant decrease in the values of ALT, AST, ALP and total protein in mice treated with honey only, mixture of *Capparis spinosa* leaves powder and honey as well as, aqueous extract of *Capparis spinosa* compared to control group. Although, a slight insignificant increase in the value of ALP in mixture of *Capparis spinosa* leaves powder and honey treated

group was recorded. Animals treated with TCA alone either for 3 or 6 weeks showed insignificant decrease in the value of ALT compared to control group. However, the value of ALT was still lower in recovery group (insignificant decrease) than that of the control. Treatment with the mixture of *Capparis spinosa* leaves powder and honey after stoppage of TCA treatment (Regeneration group) caused a significant increase in the value of ALT compared to the control group (Fig.2).

A decrease in the values of AST in mice intoxicated with TCA for 3 weeks (decrease insignificantly) or 6 weeks and recovery group (decrease significantly) were recorded compared to control group. However, AST value was still lower (insignificant decrease) than that of the control (Fig.3).

The mice intoxicated with TCA for 3 or 6 weeks and recovery group showed significant increase in the values of ALP which was more pronounced in TCA for 6 weeks treated group. While, an improvement (decrease) in the value of ALP was recorded in mice treated with the mixture of *Capparis spinosa* leaves powder and honey after stoppage of TCA treatment compared to TCA only treated group. However, the value of ALP was still higher (insignificant) than the value of the control group(Fig.4).

Total protein was increased significantly in mice treated with TCA for 6 weeks and recovery groups. While, insignificant alterations in the values of total protein in other groups were recorded(Fig.5).

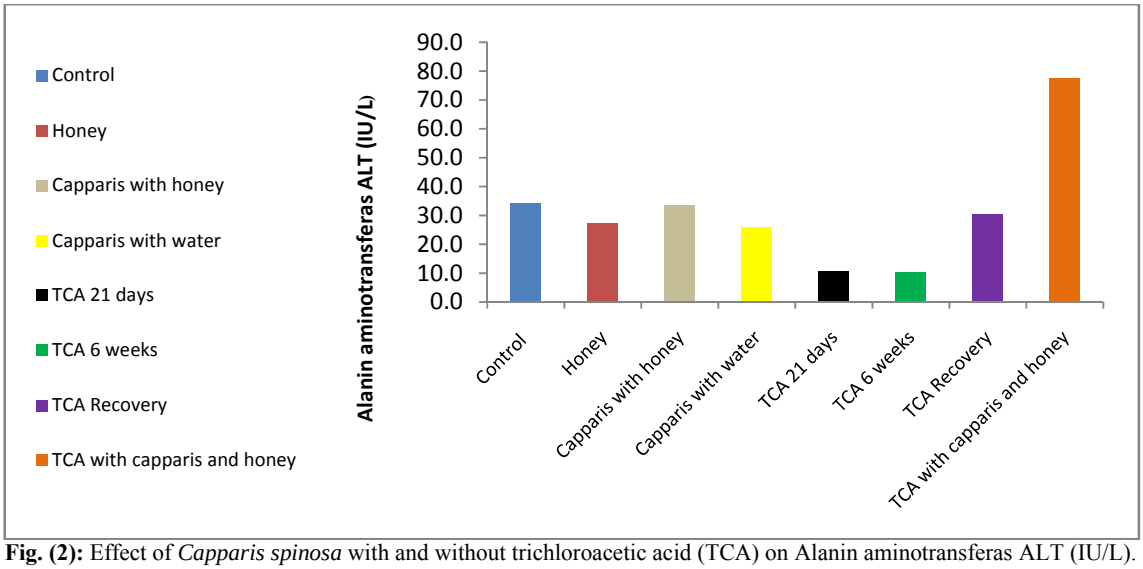

Fig. (2): Effect of *Capparis spinosa* with and without trichloroacetic acid (TCA) on Alanin aminotransferas ALT (IU/L).

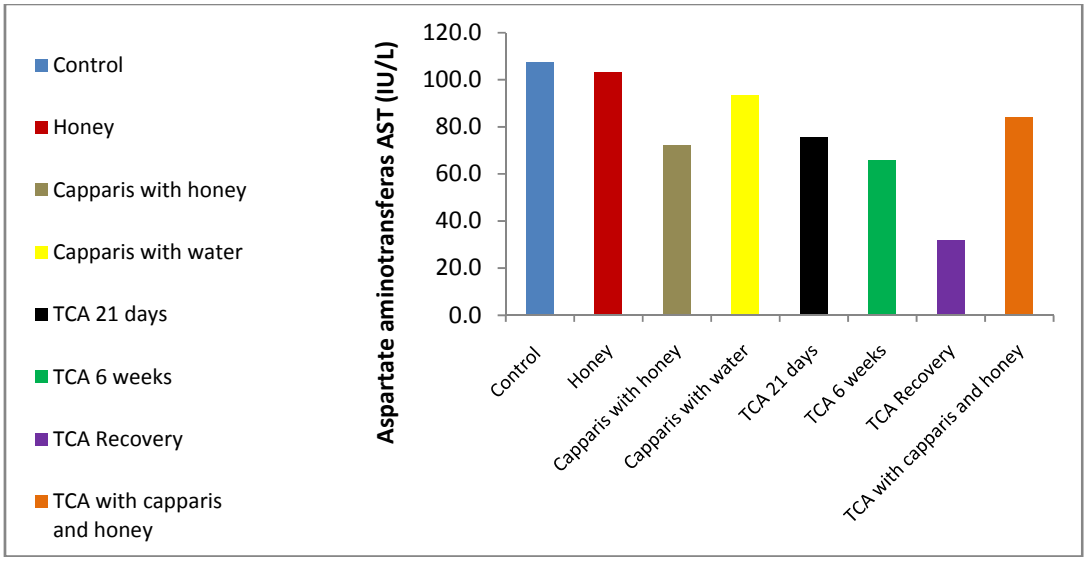

Fig. (3): Effect of *Capparis spinosa* with and without trichloroacetic acid (TCA) on Aspartate aminotransferase AST (IU/L).

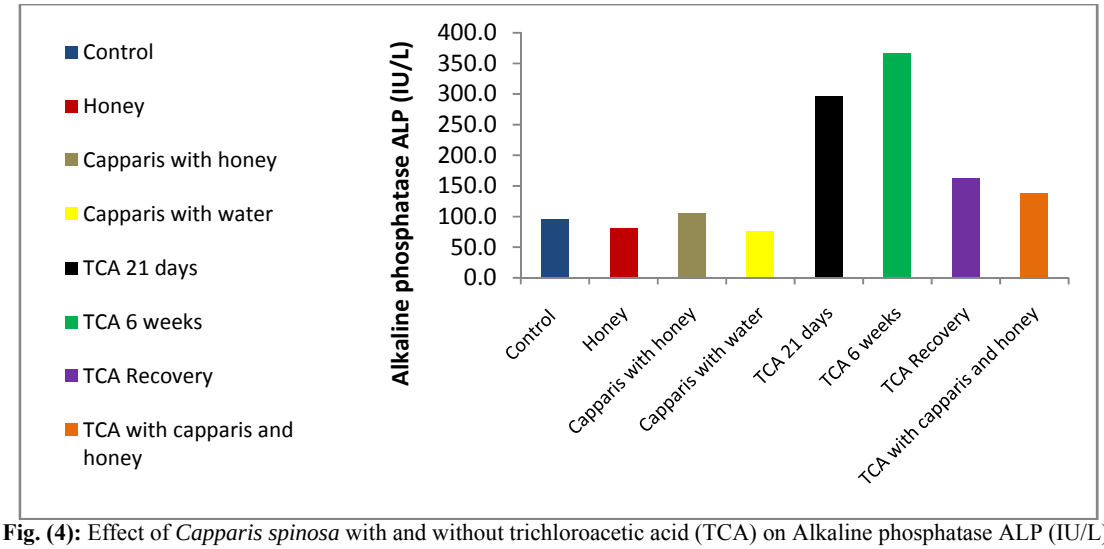

Fig. (4): Effect of *Capparis spinosa* with and without trichloroacetic acid (TCA) on Alkaline phosphatase ALP (IU/L).

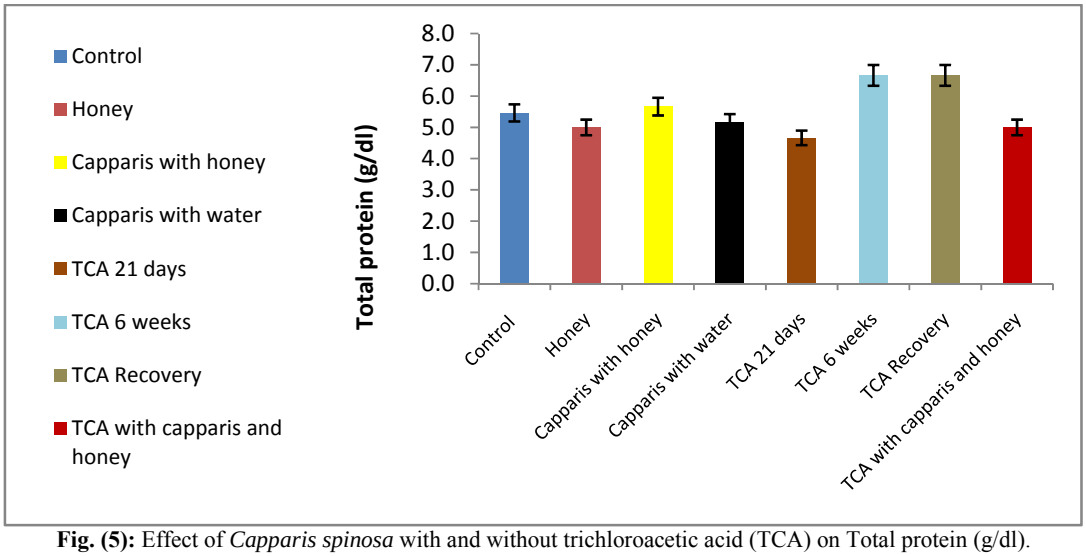

Fig. (5): Effect of *Capparis spinosa* with and without trichloroacetic acid (TCA) on Total protein (g/dl).

Histological and histochemical observations:

Livers of control mice exhibited normal histological architecture. Cords of hepatocytes radiated from the central vein and separated by hepatic sinusoids. Most hepatocytes had one, or sometimes two round to slightly oval nuclei. Blood sinusoids were lined with endothelial and Kupffer cells (Fig.6). However, there was a normal positive reactivity of hepatocytes with periodic acid Schiff (PAS) technique (Fig.7).

Many liver sections of mice treated with honey (Fig.8) or with the mixture of *Capparis spinosa* leaves powder and honey for 3 weeks showed no obvious histopathological changes. Histochemical examination showed mild decreased reactivity of liver cells to PAS stain (Fig. 9). However, some liver sections of mice treated with the mixture of *Capparis spinosa* leaves powder and honey for 3 weeks revealed stenosis and congestion of hepatic sinusoids, necrotic hepatocytes with vacuolated cytoplasm and pyknotic or karryolysis nuclei (Fig.10). In addition, apoptosis in some hepatocytes in the form of destructed cytoplasm and fragmented nuclei were also noticed (Fig.11). Sections of mice treated with aqueous extract of *Capparis spinosa* showed also normal architecture, however, mild congestion of central veins in some sections and hyperplasia of Kupffer cells were recognized (Fig.12). Histochemical examination of liver section from both mice treated with the mixture of *Capparis spinosa* leaves powder and honey (Fig.13) and with aqueous extract of *Capparis spinosa* exhibited mild decreased reactivity of liver cells to PAS stain.

The liver sections of mice treated with TCA showed many histopathological alterations including loss of normal histological architecture with stenosis of hepatic sinusoids associated with hyperplasia of

Kupffer cells, dilatation and congestion of central veins, with intravascular hemolysis of red blood corpuscles. Focal inflammatory cells infiltration, and hypertrophied hepatocytes with large nuclei. In addition, necrotic hepatocytes with vacuolated cytoplasm and pyknotic or karryolysis of their nuclei were frequently observed (Figs.14 and 15). These pathological changes were more obvious in mice treated with TCA for 6 weeks (Figs.16 and 17).

Beside the above alterations liver sections of mice received TCA for 6 weeks then left for 3 weeks for recovery (recovery group) showed many hepatocytes with abnormal nuclear features (Fig.18). In other sections, increased connective tissue around blood vessels were noticed (Fig19). Sever reactivity of most hepatocytes to PAS appeared in liver sections of mice treated with TCA for 3 and 6 weeks as well as, in recovery group (Fig.20). On the other hand, liver sections of mice intoxicated with TCA then treated with the mixture of leaves powder of *Capparis spinosa* and honey for 3 weeks (Regeneration group) revealed disappearance of most pathological changes although, focal inflammatory cells infiltration, hyperplasia of Kupffer cells and vacuolated hepatocytes were persisted. While, an improvement in nuclear features and hepatic sinusoids were demonstrated. Congestion in some hepatic sinusoids and central vein were also detected (Fig.21). However, moderate reactivity of most hepatocytes and weak of others to PAS occurred (Fig.22).

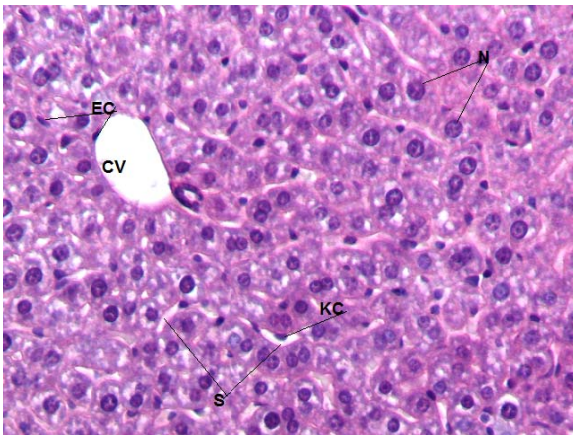

Fig. (6): A section of liver of mouse of the control group showing hepatic cords radiating from the central vein (CV) and separated by hepatic sinusoids(S) lined by endothelial cells (EC) and Kupffer cells (KC). Hepatocytes nuclei(N) (H&E stain, X400).

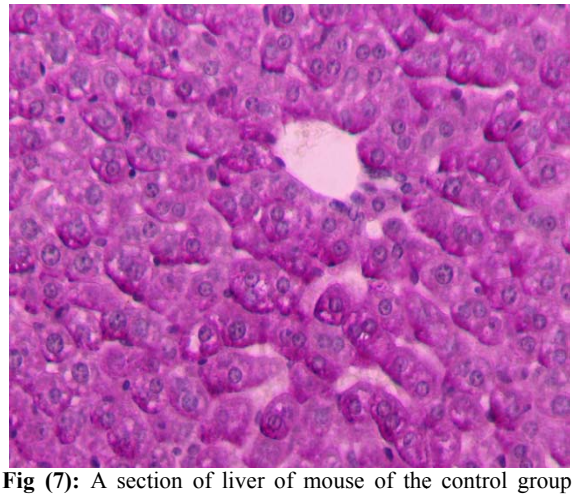

Fig (7): A section of liver of mouse of the control group showing normal positive reaction of hepatocytes with periodic acid Schiff (PAS stain, X400).

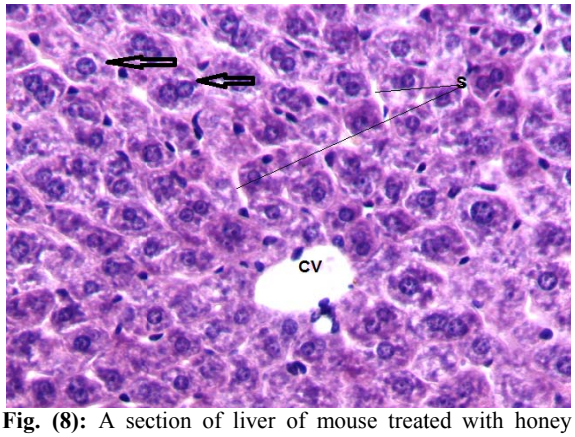

Fig. (8): A section of liver of mouse treated with honey showing normal architecture, hepatocytes (Arrows), central vein (CV), hepatic sinusoid(S) (H&E stain, X 400).

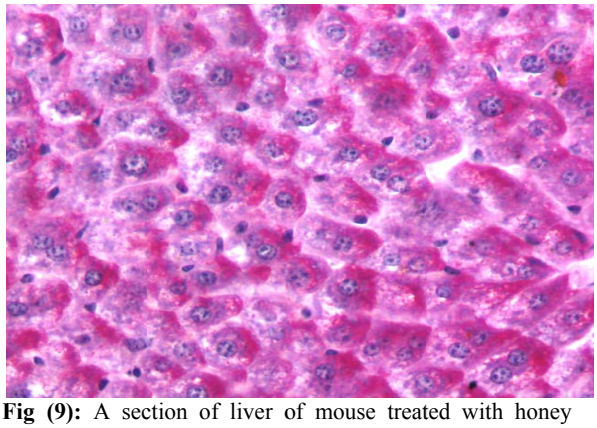

Fig (9): A section of liver of mouse treated with honey showing normal positive reaction of hepatocytes with periodic acid Schiff (PAS stain, X 400).

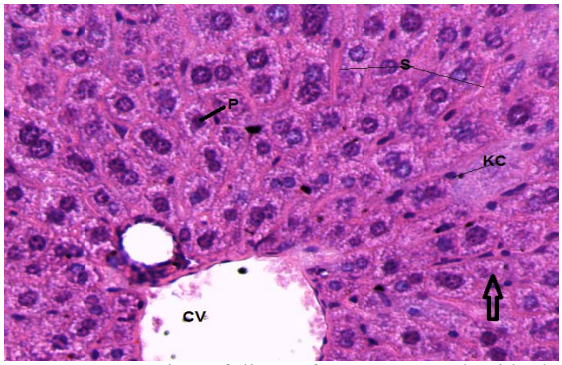

Fig (10): A section of liver of mouse treated with the mixture of *Capparis spinosa* leaves powder and honey illustrating few vacuolar degeneration of hepatocytes with pyknotic (p), or karyolitic (Arrow) nuclei, central vein (CV), mild stenosis of hepatic sinusoid(S), Kupffer cells (KC) (H&E stain, X400).

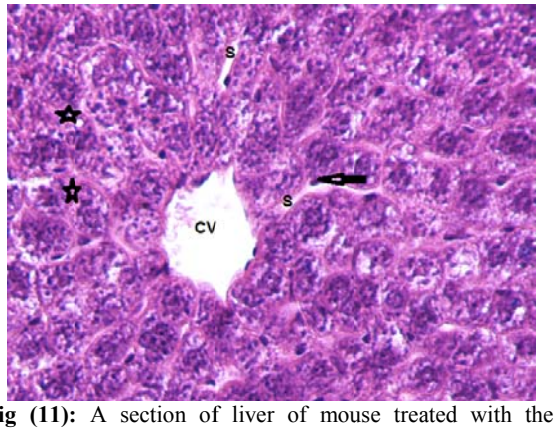

Fig (11): A section of liver of mouse treated with the mixture of *Capparis spinosa* leaves powder and honey showing some hepatocytes with apoptosis in the form of destructed cytoplasm and fragmented nuclei (Stars),blood sinusoids(S), Kupffer cells (Arrow), central vein (CV) (H&E stain, X400).

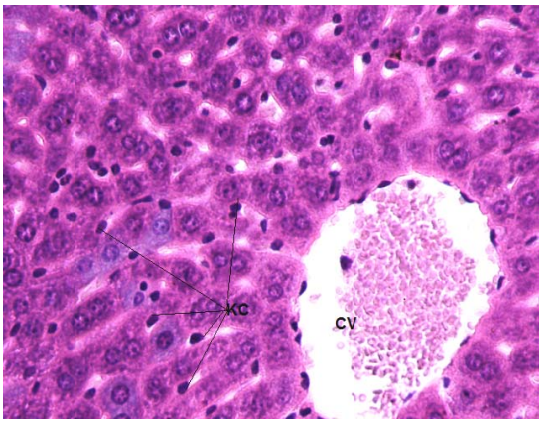

Fig (12): A section of liver of mouse treated with the aqueous extract of *Capparis spinosa* showing normal architecture, mild congestion of central vein(CV) and hyperplasia of Kupffer cells(KC) (H&E stain, X400).

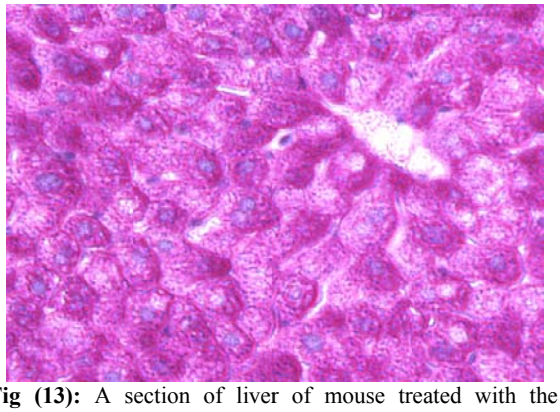

Fig (13): A section of liver of mouse treated with the mixture of *Capparis spinosa* leaves powder and honey exhibiting mild decreased reactivity of some hepatocytes with periodic acid Schiff (PAS stain, X400).

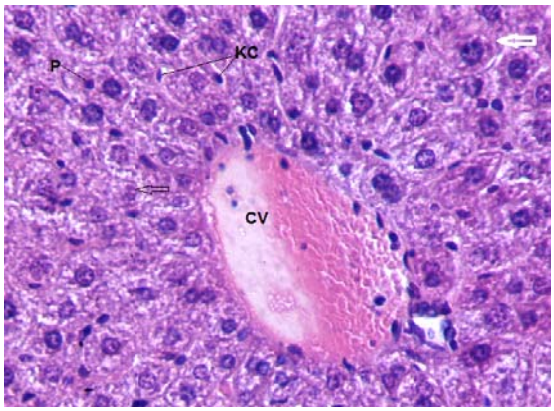

Fig. (14): A section of liver of mouse treated with TCA for 3 weeks showing congestion of central vein (CV) with hemolysis, hypertrophied hepatocyte with large nucleus (White arrow), pyknotic (p), karryolysis (Arrow) nuclei, Kupffer cells (KC). Note stenosis of hepatic sinusoids (H&E stain, X400).

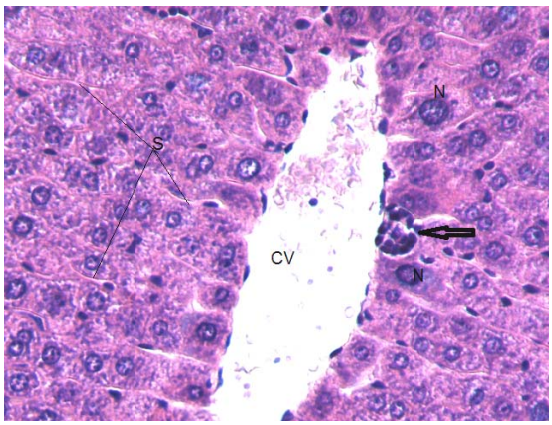

Fig. (15): A section of liver of mouse treated with TCA for 3 weeks showing focal inflammatory cells infiltration(Arrow) hypertrophy of hepatocyte with large nuclei(N), central vein (CV) and stenosis hepatic sinusoids (S), (H&E stain, X400).

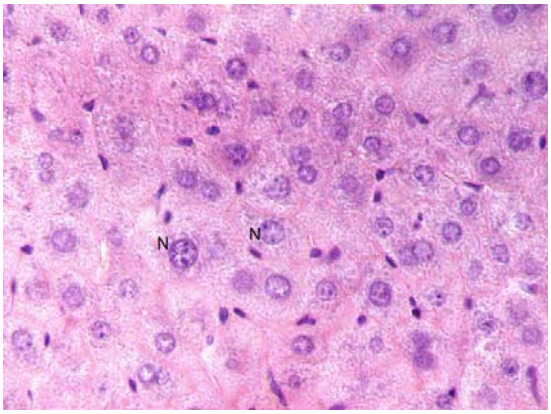

Fig. (16): A section of liver of mouse treated with TCA for 6 weeks showingloss of normal histological architecture with stenosis of hepatic sinusoids hypertrophied and hepatocytes with large nuclei(N) (H&E stain, X400).

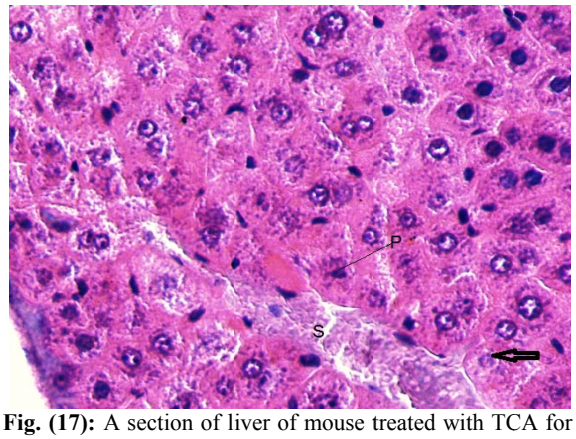

Fig. (17): A section of liver of mouse treated with TCA for 6 weeks showing hepatocytes with vacuolated cytoplasm and pyknotic (p), karyolitic (Arrow) nuclei, congestion of blood vessel **(S)** (H&E stain, X40).

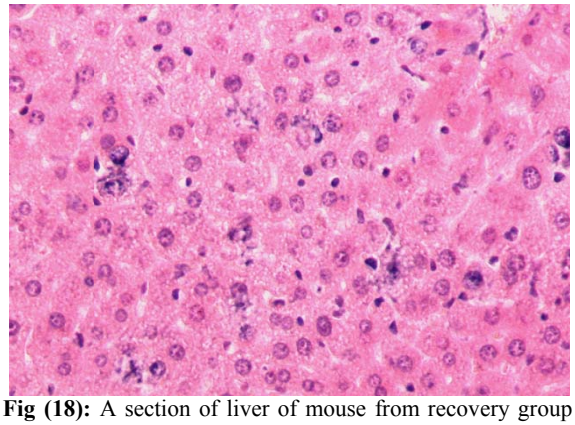

Fig (18): A section of liver of mouse from recovery group illustrating loss of normal histological architecture and vacuolated hepatocytes with abnormal nuclear features (H&E stain, X400).

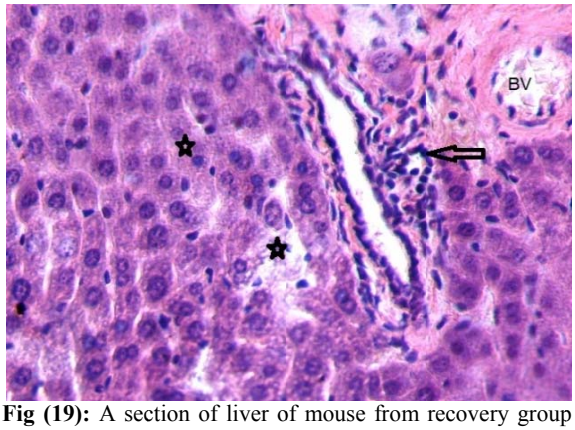

Fig (19): A section of liver of mouse from recovery group showing increased connective tissue around blood vessels (BV), necrotic of hepatocytes (Stars), inflammatory cells infiltration(Arrow) (H&E stain, X400).

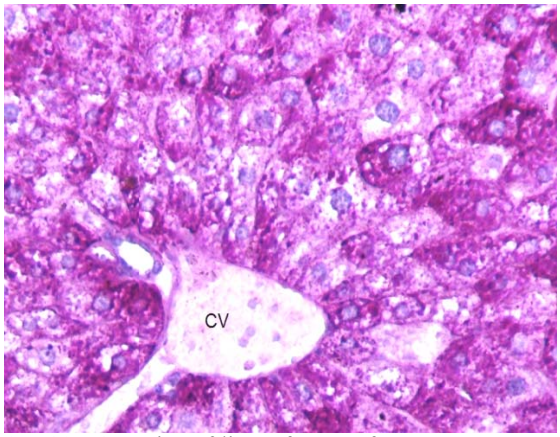

Fig. (20): A section of liver of mouse from recovery group illustrating congestion of central vein (CV) with hemolysis and sever reactivity of most hepatocytes to periodic acid Schiff (PAS stain, X400).

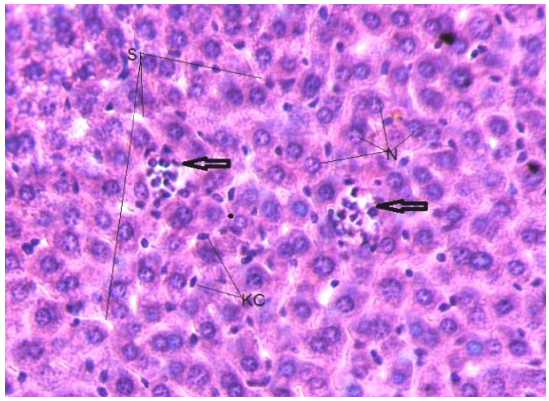

Fig (21): A section of liver of mouse treated with TCA for 6 weeks then treated with a mixture of *Capparis spinosa* and honey showing focal inflammatory cells infiltration (Arrows), hyperplasia of Kupffer cells (KC), an improvement of nuclear features of hepatocytes (N) and hepatic sinusoids (S) (H&E stain, X400).

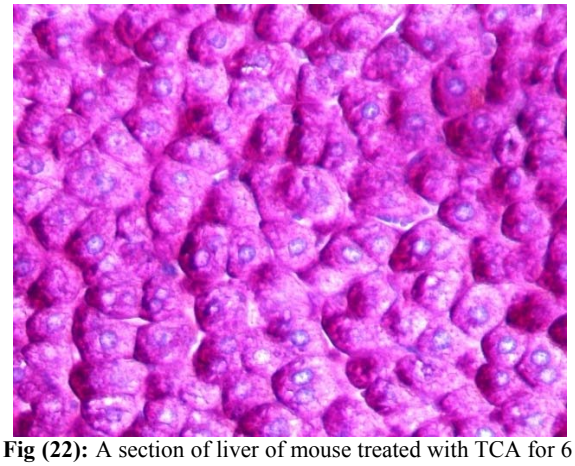

Fig (22): A section of liver of mouse treated with TCA for 6 weeks then treated with a mixture of *Capparis spinosa* and honey illustrating moderate to weak reactivity of most hepatocytes around central veins to periodic acid Schiff (PAS stain, X400).

4. Discussion

In the present work no obvious changes in the behavior or the external features during the experimental period was observed in both the control and treated animals. Similarly, no visible changes were observed in mice treated with different extracts of *Capparis zeylanica* **(Karanayil *et al*., 2011).** It was also reported that *Capparis spinosa* was found to be a safe plant without any toxic manifestations after acute, sub-acute or chronic administration **(Angelini *et al*., 1991).**

Treatment with the mixture of leaves powder of *Capparis spinosa* and honey did not induce deaths in the experimental animals. No lethality was also recorded in mice treated with different extracts of *Capparis zeylanica* and the acute toxicity results showed that the LD50 was greater than 5000 mg/kg **(Karanayil *et al*., 2011).** Similar finding had been described by **Sini *et al.* (2010)** who reported that no death was observed throughout the period of experiment in rats treated with aqueous leaf extract of *Capperis grandiflora* at dose 1000-3000 mg/kg.

Our observations reveled no deaths in mice treated with TCA at dose 500 mg/kg body weight for 3 weeks. This result is in agreement with **De Angelo *et al.* (2008)** who reported that no decrease in animal survival was found in mice exposed to TCA in drinking water at dose level 0.5,4. or 5g/L for 60 or 104 week. On the other hand, it was found in this study that treatment with TCA at dose 500 mg/kg body weight for 6 weeks induced 6% deaths in the experimental animals. **Celik (2007)** found that TCA treatments caused different effects on antioxidant defense system and lipid peroxidation in various tissues of rats administered TCA at dose level 300 mg/kg-day in drinking water for 50 days. The mortality increased herein to 14% in the recovery group whereas, in the group intoxicated with TCA then treated with the mixture of *Capparis spinosa* and honey the mortality reached 6% only. Biological studies revealed important anti-oxidative, anti-inflammatory and immunomodulatory properties of *Capparis spinosa* **(Tlili *et al*., 2011).**

The current study revealed that the mixture of leaves powder of *Capparis spinosa* and honey and aqueous extract of leaves powder of *Capparis spinosa* induce a slight insignificant changes in the final body weight gain comparing to control group. On the other hand the treatment with TCA only induced significant decrease in the final body weight. The mice intoxicated with TCA in drinking water for 6 weeks and administrated the mixture of *Capparis spinosa* and honey showed an improvement (A slight and insignificant increase) in the final body weight comparing to TCA alone treated group. This was found to be consistent with **Sini *et al*. (2010)** who reported that no significant changes in body weights of rats treated with aqueous leaf extract of *Capperis grandiflora* with the dose 1000-3000 mg/kg when compared with untreated control groups**. Sofowora (1993)** suggested that the presence of tannins and other phenolics in Capperis interferes with absorption of nutrient resulting in weight loss.

Our results demonstrated that that the final body weight of mice treated with TCA showed a significant decrease compared to the control group. This was in agreement with the study of **Acharya *et al.* (1995)** where the body weight was decreased by approximately 17% in the absence of changes in food consumption in young male rats exposed to TCA in drinking water at dose level 3.8 mg/kg-day for 10 weeks**.** Decreased body weight were also seen in rats exposed to TCA in drinking-water at dose level 32.5 mg/kg of body weight per day for 2 years **(De Angelo *et al*., 1997).** Moreover, Exposure to TCA in drinking water at dose level 0.5,4. or 5g/L for 60 or 104 week decreased body weight by 15% in the high-dose group relative to the control **(DeAngelo *et al*.,2008).** The reduction in body weight gain may be due to the oxidative stress **(Mansour and Mossa,2010 and Saafi *et al*., 2011)**. Also it may be due to the increased degradation of lipids and proteins as a direct effect of toxic compound exposure **(Heikal and Soliman, 2010 and Mossa *et al*., 2011).** On the other hand no treatment-related changes in body weight were found in male rats exposed to TCA in drinking water at a dose level 312 mg/kg body weight per day for 10, 20 or 30 days**(Parnell *et al*., 1988).**

Mice intoxicated with TCA for 6 weeks and administrated the mixture of *Capparis spinosa* and honey showed a slight and insignificant increase in the final body weight comparing to TCA alone treated

group. The protective action against TCA induced alternations in mice body weight may be attributed to the antioxidant effect present in the mixture of *Capparis spinosa* and honey. In addition the protective effect of honey may be attributed to the biologically active compounds such as vitamins, flavonoids, and antioxidants that work together to scavenge free radicals. Therefore, bees' honey can be used to protect animals and humans against the adverse effects of toxicity **(El Rabey *et al*.,2013).**

Total protein was, increased significantly in mice treated with TCA for 6 weeks. However, hepatocytes are involved in protein synthesis, protein storage and transformation of carbohydrates **(Hamel *et al*.,2006).** Mice intoxicated with TCA for 3 or 6 weeks also showed a significant increase in the values of ALP which was more pronounced in TCA for 6 weeks treated group. Increased liver serum enzyme activity was seen in rats exposed to TCA in drinking-water at dose level 32.5 mg/kg of body weight for 2 years **(DeAngelo *et al*., 1997).** It is conceivable that TCA, as a toxicological agent, might interact primarily with liver tissue cell membranes, resulting in structural damage and changes in metabolism of the constituents **(Demür and Elük, 2006)**. ALP may be elevated if bile excretion is inhibited by liver damage. Hepatotoxicity leads to elevation of the normal values due to the body's inability to excrete it through bile due to the congestion or obstruction of the biliary tract, which may occur within the liver **(Singh *et al*.,2011)**. An improvement; decrease, in the value of ALP was recorded in mice treated with the mixture of leaves powder of *Capparis spinosa* and honey after stoppage of TCA comparing to TCA only treated groups and comparing to mice treated with TCA and left for recovery without treatment. It was showed that admenstration of *Capparis spinosa* root bark extract orally 100, 200 & 400 mg/kg for 4 days possess hepatoprotective activity as evidenced by the significant inhibition in the elevated levels of serum enzyme activities in CCl4-intoxicated mice due to the antioxidant effect of the plant extract **(Aghel *et al*., 2007).** Also, honey, like other antioxidant agents, does protect against damage or injury. This protective effect of honey is partly mediated via amelioration of oxidative stress in tissues **(Erejuwa *et al*.,2010 and Kassim *et al*.,2010).** On the contrary, **Martey *et al*. (2013)** reported that *Capparis erythrocarpus* chronic administration at 18 and 180 mg /kg body weight for 6 months in male rats had no effect on serum biochemical parameters.

Results of the biochemical analysis revealed insignificant decrease in the values of ALT and AST in mice treated with TCA either for 3 or 6 weeks as well as in recovery group. It is conceivable that TCA effect might, resulting in decreased enzymes activities by the way of increased reactive oxygen radicals as a result of stress condition in the rat. The superoxide radicals by themselves or after their transformation to H2O2 cause an oxidation of the cysteine in the enzyme and decrease enzymes activity**(Celik *et al*., 2010).** The decrease in activity of AST and ALT was also noticed in prolong exposure of mice to pesticides **(Ambali *et al*.,2007 and Ambali *et al*.,2011)**. **Acharya *et al.* (1995)** in male rats exposed to water containing TCA at dose 3.8 mg/kg b.w for 10 weeks reported that no significant changes were detected in ALT, AST or ALP.

Examination of the liver sections of mice treated with TCA revealed many histopathological alterations. Similar histological changes were noticed by **Acharya *et al.* (1997)** who found that the liver of rats exposed to TCA in drinking water at dose level 3.8 mg/kg-day for 10 weeks led to the loss of hepatic architecture. It was also observed that TCA caused histological alterations in the liver such as centrilobular necrosis, vacuolation in hepatocytes and loss of hepatic architecture as recorded by **De Angelo *et al.* (2008)**. However, vacuolation and necrosis of hepatocytes observed in our study was also observed by **Acharya *et al.* (1997) and US EPA (2011)**. It has been investigated that, vacuolation of hepatocytes may point to fatty changes, hydropic degeneration or glycogen degeneration. Moreover, congestion leads to hypoxia and because of oxygen and nutrient deprivation hepatocytes degenerate or eventually may undergo necrosis **(Carlton & Mc Gavin,1995)**. Inflammatory cells infiltration observed herein was also in accordance with **(US EPA, 2011). DeAngelo *et al.* (2008)** noticed significant increase in the severity of inflammation in the liver of male mice exposed to TCA in drinking water at dose level 5g/L for 60 weeks. **Bull *et al.* (1990)** suggested that TCA appears to increase lipid peroxidation, and the production of free radicals may be responsible for its effects. Moreover, **Hassoun and Ray (2003)** investigated that TCA induced both lipid peroxidation and oxidative DNA damage following administration of a single oral dose. Furthermore, **Celik (2007)** found that TCA treatments caused different effects on antioxidant defense system and lipid peroxidation in various tissues of rats.

The hypertrophy of hepatocytes in mice treated with TCA was frequently noticed in this study which may indicate the carcinogenicity of TCA. This was supported by **Mather *et al.* (1990)** who found that male rats received TCA in drinking water at 36.5 or 355 mg/kg body weight for 90 days showed focal hepatocellular enlargement and intracellular hepatic swelling. **Bull *et al.* (1990)** confirmed that TCA is capable of inducing hepatic tumors in mice. Moreover, an increase in incidence of benign and

malignant liver tumors was observed in mice orally administered TCA **(IARC, 1995). Acharya *et al.* (1997)** also mentioned that, hypertrophy of hepatocytes was a characteristic feature in the liver of TCA treated rats in drinking water at dose level 3.8 mg/kg-day for 10 weeks. Also, **Pereira (1996)** reported that TCA increased cell proliferation in the liver of female mice treated with 2, 6.6, 7 or 20 mmol/L TCA in drinking water for 5 days**.** Moreover, hepatocellular neoplasia was noticed in male mice exposed to TCA in drinking water at dose level 5g/L for 60 week **(De Angelo *et al*., 2008).** In the present study apoptosis was noticed in some hepatocytes of mice administrated the mixture of *Capparis spinosa* and honey. However, apoptosis is widely recognized as the major mode of cell death; facilitating the precise regulation of cell numbers. It also serves as a defense mechanism, eliminating potentially dangerous cells such as those exposed to toxins or other adverse environmental conditions **(White, 1996 and Salganik *et al*., 2000).** Moreover, stimulation of apoptosis is one of the mechanisms by which cancer proliferation and progression can be affected **(Surh, 2003).** *Capparis* aqueous infusion is a good source of flavonoids, which are known to be antiproliferative for colon cancer cells **(Kulisic-Bilusic *et al*., 2012)**. Furthermore, flavonoids inhibit the tumor growth by interfering with some phases of the cell cycle **(Salucci *et al*., 2002).**

In mice treated with the mixture of leaves powder of *Capparis spinosa* and honey after stoppage of the treatment with TCA; regeneration group, most pathological lesions disappeared. In contrast, in the recovery group all pathological lesions persisted beside the appearance of many hepatocytes with abnormal nuclear features and increased connective tissue around blood vessels. This may confirm that the treatment of mice with the mixture of *Capparis spinosa* and honey has a better effect in attenuating the adverse effects of toxicity induced by TCA than the animals left for recovered without treatment. However, administration of honey had significantly attenuated the detrimental effect of poisonous materials on different organs of the rat; as it provides anti-inflammatory, immune-stimulant, antiulcer and regenerative effects **(Fiorani *et al*., 2006).** In addition, Honey possesses some biological properties such as antioxidant **(Perez *et al.,* 2006)** and immunomodulatory effects **(Timm *et al.,* 2008).** Furthermore, it is important for the treatment of acute and chronic free radical mediated toxicity **(Abdel-Moneim and Ghafeer 2007).** Also, all parts of *Capparis spinosa* possess antioxidant effects with certain correlation with their polyphenols and flavonoids contents **(Arrar *et al*., 2013)**. Biological studies revealed important, anti-oxidative, anti-inflammatory and immunomodulatory properties of *Capparis* **(Tlili *et al*., 2011).** Thus, it could be suggested that the effect of *Capparis spinosa* with honey may have additive effect in attenuating the oxidative damage and the toxic effect induced by TCA. This could be partly mediated by their combined counteraction on oxidative stress within the organs via their antioxidant properties.

Histological examination revealed that administration of mixture of the *Capparis spinosa* and honey or the aqueous extract of *Capparis spinosa* induced some pathological alterations in the liver of few animals. **Sofowora (1993)** reported that flavonoids are thought to have both proxidant and antioxidant effects on the body. While the antioxidant protects the tissues and organs, the proxidant damages the tissues and organs. However, consumption of herbal remedies in developing countries are generally recognized as safe and effective but some of these herbal remedies have been found to contain hepatotoxic constituents **(Larrey, 1997).** Furthermore, herbal remedies may be contaminated with excessive amount of banned pesticides, microbial contaminants, heavy metals, chemical toxins adulteration with synthetic drugs **(Bogusz *et al*.,2002; Chan,2003 & Idodo-Umeh and Ogbeibu, 2010).** This may explain some alterations in the liver of mice treated with mixture of *Capparis spinosa* and honey and aqueous extract of leaves powder of *Capparis spinosa* in the present work. On the other hand, **Sini *et al*. (2010)** found that the histopathological examination of the organs did not reveal any abnormalities in rats treated with aqueous leaf extract of *Capperis grandiflora* by the dose 1000 -3000 mg/kg. According to **Haque and Haque (2011)** no detectable abnormalities were found in the histopathology of the liver, heart, kidney, or lungs in rats treated with the chloroform extract of the roots of *Capparis zeylanica* Linn at a dose of 300 mg/rat/day for 14 days compared with the control group.

Histochemical examination showed severe reactivity of most liver cells with PAS in mice intoxicated with TCA and in the recovery group as well. Such observations may indicate that the intracytoplasmic vacuoles resulted from accumulation of neutral mucopolysaccharides which may be glycogen. On the other hand, in the liver of mice treated with the mixture of *Capparis spinosa* and honey after TCA most hepatocytes showed moderate reactivity to PAS close to that of the control group. **Bull *et al.* (1990)** noticed that TCA caused a much a more accumulation of glycogen in liver cells. **Mather *et al.* (1990)** also recorded that male rats received TCA in drinking water at 36.5 or 355 mg/kg of body weight per day for 90 days showed increased glycogen accumulation. **Acharya *et al.* (1995)** reported that

glycogen levels increased approximately eight times in male rats exposed to water containing TCA at dose 3.8 mg/kg body weight for 10 weeks. However, **Carlton & Mc Gavin (1995)** confirmed that glycogen degeneration or glycogen storage disease is characterized by excessive hepatic accumulation of glycogen.

Conclusion

The mixture of leaves powder of *Capparis spinosa* and honey at dose consumed in the traditional medicine (40mg/kg body weight) for 3 weeks could ameliorate the toxic effects of trichloroacetic acid and led to an improvement in both biochemical parameters and histopathological lesions, as well as, decreased percentage of mortality. This effect may be related to the flavonoids and other antioxidant constituents in this plant. Although the administration of either the aqueous extract of leaves powder of *Capparis spinosa* or the mixture of *Capparis spinosa* and honey did not cause any lethality or changes in the general behavior, it causes some histopathological alterations and some adverse effects on the biochemical parameters. Therefore, medicinal plants should not be taken haphazard for long periods and must be taken under medical supervision.

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