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Effect of *Capparis spinosa* leaves on some haematological and histopathological changes associated with exposure of mice to trichloroacetic acid.

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Abstract

The present work was conducted to study the possible protective role of *Capparis spinosa* leaves and their efficacy against hematological and histological alterations resulted in an animal model intoxicated with trichloroacetic acid(TCA).

Hundred male mice 20-26 gm were divided into 5 groups; control group, group II treated orally with honey (40 mg/kg body weight for 3 weeks), groupIII treated orally with a mixture of Capparis spinosa leaves powder and honey(40 mg/kg for 3 weeks), groupIV treated with TCA in drinking water (500mg/kg for 3 and 6 weeks, then left for 3 weeks for recovery) and groupV (Regeneration group) treated with TCA for 6 weeks then treated with a mixture of Capparis and honey (40 mg/kg for 3 weeks).

Marked disturbance in the hematological parameters was demonstrated in mice treated with TCA for 3 and 6 weeks. Mice treated with TCA then with a mixture of *Capparis* spinosa leaves powder and honey showed an improvement in total leukocytic count and percentage of lymphocytes comparing to TCA treated group. Also, significant improvement in the platelets count was demonstrated.

Histological examination of spleen sections of mice treated with TCA revealed obvious pathological findings including disorganization of lymphoid follicles, hyperplasia in white pulp, depletion of lymphocyts in red pulp with sub capsular edema, some necrotic cells in white and red pulp, increasing megakaryocytes, haemosiderosis and fibrosis in red pulp and in some lymphoid follicles. Administration of a mixture of *Capparis spinosa* leaves powder and honey lessened most of the pathological lesions in mice intoxicated with TCA.

Key words: *Capparis spinosa*, trichloroacetic acid , hematological, histopathological changes, spleen, mice (*Mus musculus*).

Introduction

Herbal medicine is a complementary therapy that uses plants to treat disorders. In various countries throughout the world, a large number of plants have been used as therapeutic agents in the traditional medicine (Kumar et al., 2012), but there are no enough documents in the literature about their probable toxic effects (Monfared,2013). 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).

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,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) activities 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, has also been demonstrated in capers (Bonina et al. 2002).

Trichloroacetic acid (TCA) (CC13COOH) is mainly used in the production of its sodium salt, which is used in many industries; as an herbicide, etching agent and antiseptic (Lin et al., 2005). TCA 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 has 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. Oral half lethal dose (LD50) of 4970 mg/kg of body weight for TCA have been reported in mice (Woodard et al., 1941).

The spleen is the largest secondary lymphoid organ, is considered the draining site for compounds that are administered intravenously, and is therefore, considered an important organ to evaluate for treatment-related lesions. Due to the presence of B and T lymphocytes, the immunotoxic effects of xenobiotics or their metabolites on these cell populations may be reflected in the spleen. Therefore, it is one of the recommended organs to evaluate for enhanced histopathology of the immune system (Elmore, 2006). The present work aimed to study the possible protective role of *Capparis spinosa* leaves and their efficacy as used in traditional medicine in Libya on heamatological parameters and histopathological alterations of the spleen induced in an animal model intoxicated with trichloroacetic acid.

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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 *adlibitum*. Mice were acclimatized for 1 week prior to the start of experiments.

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) .

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 40 mg/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)

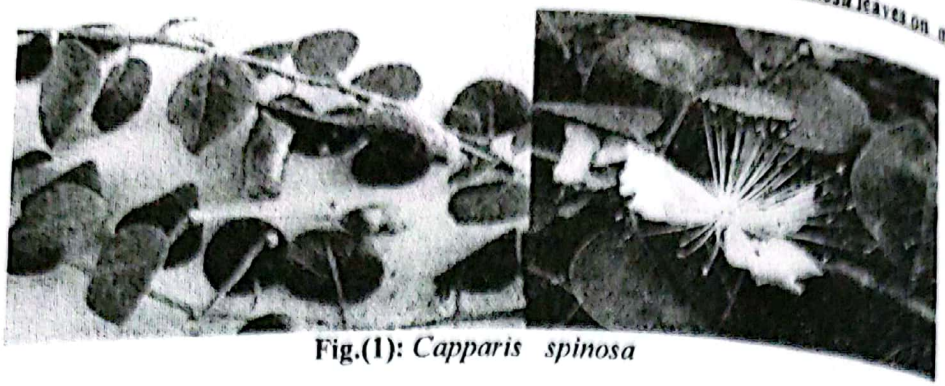

Fig.(1): *Capparis spinosa*

Experimental design:

100 healthy adult male mice were divided into 5 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 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 successive weeks.

Group IV: 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 V: 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**.

Acute toxicity studies:

The acute toxicity study for the aqueous extract of *Capparis spinosa* was performed using Swiss albino mice. The animals were fasted overnight prior to the experiment and maintained under standard conditions. The extract were administered orally in increasing doses (600, 1200, 2400 and 4800 mg/kg by oral route) and found safe up to dose of 4000 mg/kg body weight.

Clinical signs:

During the whole experimental period clinical signs were noted and recorded daily to recognize the behavior, depression, food and water consumption, signs of difficult breathing, salivation, diarrhea, eye color, muscular weakness, body furs feces , activities and all signs of toxicity.

Body weight:

Body weights of mice in all groups were measured at the beginning and at 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).

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Hematological studies:

Twenty four hours after the end of experimental period, unanesthetized mice from both control and experimental groups were sacrificed by slaughtering(cervical dislocation). Blood samples were collected from the neck blood vessels into clean sterile containers containing (Ethylene diamine tetra acetic acid) EDTA (1mg/ml fresh blood).Red blood corpuscles count(RBCs), hemoglobin concentration(Hb), packed cell volume (PCV)or hematocrite(HCT), total and differential leucocytic count, mean corpuscular volume (MCV), mean corpuscular hemoglobin(MCH), mean corpuscular hemoglobin concentration (MCHC) and platelets count were measured and calculated according to Lewis et al. (2001) using an automatic hematocyte analyzer DLAGON LTd Auto Hematology Analyzer D.Cell 60.

Histopathological studies:

For the light microscopic examination, the spleen was carefully dissected out and quickly fixed in 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 program was used for analysis the results and drawing the figures.

Results and Discussion

Acute toxicity observation:

The aqueous extract of *Capparis spinosa* did not show any sign or symptoms of toxicity and mortality up to the dose 4000 mg/ kg body weight.

Clinical observations:

No behavioral changes or abnormal signs in the external features of mice treated orally with honey only or with a mixture of *Capparis spinosa* leaves powder and honey for 3 weeks during the experimental period comparing to the control group. However, some animals treated with this mixture showed less activities during the first week of administration. Also, no obvious changes in both the behavior and the external features in mice treated with TCA for 3 or 6 weeks. Daily observations revealed that there was no unusual behavior or change in the external features of animals in both recovery and regeneration groups. Some authors had 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).

Body weight:

Our data of body weights were illustrated in Table 1 and Figure 2. Statistical analysis revealed that the body weight increased gradually in control and honey treated groups through the experimental period. The final body weight gain was increased by 6% and 5.6% above the initial body weight of mice in the control and honey treated groups respectively. A slight insignificant changes were recorded in the final body weight gain in mice treated with the mixture of *Capparis spinosa* leaves powder and honey comparing to initial and final body weights of the control 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 (1000-3000 mg/kg) when compared with control groups. Sofowora (1993) speculated that the presence of tannins and other phenolics in Capperis interferes with absorption of nutrient resulting in weight loss.

In the current study administration of TCA alone induced a marked, time depended, decrease in the mean body weight gain. The final body weight decreased by – 9.4 % above initial body weight .It was also found that the final body weight of mice treated with TCA showed significant decrease compared to the control group. This was found consistent with other studies 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 (Acharya et al.,1995). Decreased body weight were 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). 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 combined action of cholinergic and 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 concentration of 5 g/litre (about 312 mg/kg of body weight per day) for 10, 20 or 30 days(Parnell et al., 1988).

In the present work a slight and insignificant increase in the final body weight was demonstrated in mice intoxicated with TCA in drinking water at dose level 500 mg/kg-day for 6 weeks and received a mixture of capparis *spinosa* and honey comparing to TCA alone treated group. The final body weight decreased by – 5.2 % above initial body weight in mice treated with the mixture of leaves powder of *Capparis spinosa* and honey with TCA in drinking water at dose level 500 mg/kg-day for 6 weeks. 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. It was previously seen that the mechanism by which *Capparis spinosa* exert its protective action against CCl4 induced alternations in the liver might be due to the antioxidant effect of the plant extract (Aghel et al.,2007). 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) .

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Table (1): Effect of *Capparis spinosa* with and without trichloroacetic acid (TCA) on body weight gain of mice.

Groups / Time	Control	Honey	Capparis + Honey	TCA Only	TCA with capparis and honey
Mean of Initial body weight (gm)	25 ± 0.3^{a}	25 ± 0.3^{a}	25 ± 0.5^{a}	25.1 ± 0.3^{a}	25 ± 0.2^{ac}
Mean of body weight (gm) after1 week	25.8 ± 0.3^{a}	25.3 ± 0.3^{a}	24.6 ± 0.4^{a}	24.3 ± 0.3^{ab}	24.2 ± 0.3^{ac}
Mean of body weight (gm) after 2 week	25.9 ± 0.2^{a}	25.6 ± 0.3^{ac}	24.7 ± 0.4^{ab}	23.7 ± 0.2^{b}	24 ± 0.2^{bc}
Mean of body weight (gm) after3 week	26.2 ± 0.3^{a}	25.6 ± 0.3^{ad}	24.6 ± 0.5^{ab}	23.7 ± 0.3^{bd}	23.5 ± 0.1^{bc}
Mean of body weight (gm) after4 week	26.2 ± 0.2^{a}	25.7 ± 0.3^{a}	24.4 ± 0.3^{ab}	23.4 ± 0.4^{b}	23.5 ± 0.2^{bc}
Mean of body weight (gm) after5 week	26.4 ± 0.2^{a}	$26 \pm 0.2a$	24.8 ± 0.3^{ab}	23.6 ± 0.3^{b}	23.5 ± 0.1^{bc}
Mean of body weight (gm) after6 week	26.5 ± 0.1^{a}	26.2 ± 0.2^{a}	24.9 ± 0.2^{ab}	23.5 ± 0.4^{b}	23.7 ± 0.1^{bc}
Mean of final body weight (gm) weeks				22.8 ± 0.8^{b}	23.7 ± 0.2^{bc}
The mean of change in the body weight (%)	6 %	5.6 %	-0.4 %	-9.4 %	-5.2 %

Each value represent the mean ±S.E. of body weight of survival animals in each group. Values ,within raw and column with no common superscripts are statistically significant at $P \leq 0.05$.

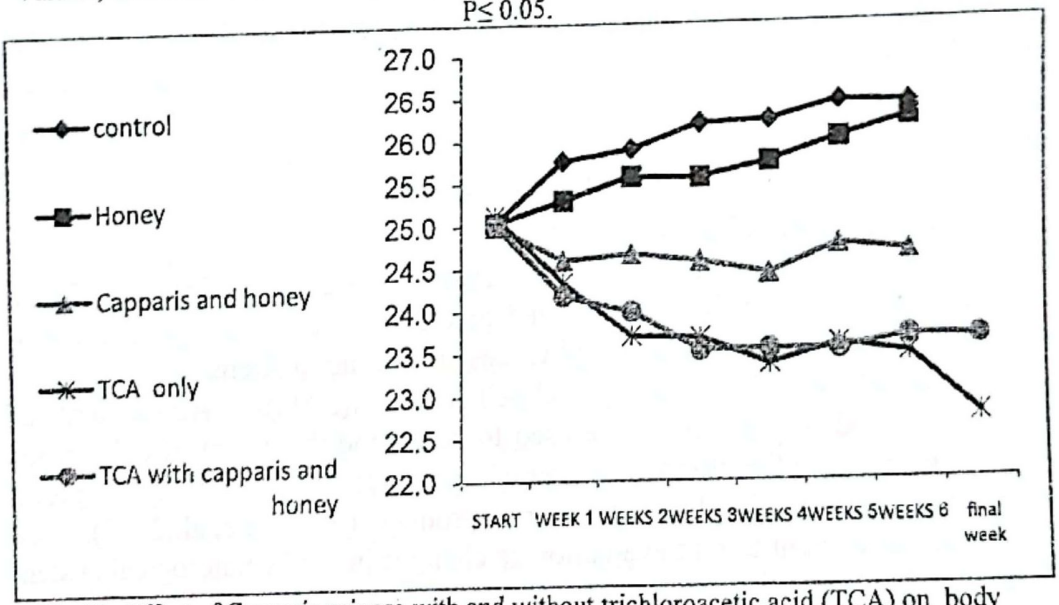

Fig.(2): Effect of Capparis spinosa with and without trichloroacetic acid (TCA) on body weight gain (gm) of mice.

Mortality:

No deaths were recorded during the experiment period in mice of control and honey treated group. Also, treatment with the mixture of leaves powder of *Capparis spinosa* and honey did not induce deaths in the experimental animals. Similarly, no visible changes or lethality were observed in mice treated with different extracts of *Capparis zeylanica* . Acute toxicity results showed that the LD50 was greater than 5000 mg/kg (Karanayil et al., 2011). Similar finding had been previously 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. Also, 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 that treatment with TCA at dose 500 mg/kg body weight for 6 weeks induced 6% deaths in the experimental animals. The mortality increased to 14% in the recovery group. Furthermore, 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. Our results clearly demonstrated that repeated oral administration of the mixture of *Capparis spinosa* and honey after TCA reduced the percentage of mortality in the regeneration group comparing to the recovery group. The percentage of mortality reached 14% in the recovery group and 6% in the regeneration group. Phytochemicals studies have shown the presence of many beneficial compounds in *Capparis spinosa* such as spermidine, rutin, quercetin, kaempferol, stigmasterol, campesterol, tocopherols, and carotenoids. Biological studies revealed important anti-oxidative, anti-inflammatory and immunomodulatory properties (Tlili et al., 2011).

Hematological results:

The results of hematological parameters are shown in Table 2 and Figures 3 – 13. Our findings revealed that the hematological parameters of control group were within normal values according to Aleman et al. (2000), Feldman et al. (2000) and Schnell *et al* .(2002). Assessment of hematological parameters can be used to determine the extent of deleterious effect of plant on the blood of an animal. It can also be used to explain blood relating functions of a plant extract or its products (Yakubu et al.,2007). Such analysis is relevant to risk evaluation as changes in the hematological system have higher predictive value for human toxicity, when the data are translated from animal studies (Olson et al.,2000).

In the present work, the hematological parameters in mice treated with honey or the mixture of *Capparis spinosa* leaves powder and honey

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showed no statistical difference from control group in the values of hemograme (Hb, RBCs, PCV, MCV, MCH, (MCHC). Treatment with honey or the mixture of *Capparis spinosa* leaves powder and honey induced insignificant decrease in the total WBCs count and insignificant alterations in the percentage of lymphocytes and monocytes and, insignificant increase in the granulocytes. Additionally, insignificant increase in the platelets count was demonstrated. Our data may be supported by the findings of Haque and Haque (2011) who reported that the changes in RBC, WBC, differential count of WBC, platelets count and Hb % were statistically not significant in rats treated with the chloroform extract of the roots of *Capparis zeylanica* Linn at a dose of 300 mg/rat/day for days.

Administration of TCA for 3 weeks caused disturbance in hematological parameters in male mice. It induced significant decrease in Hb. RBCs were increased significantly but insignificant decrease in PCV was detected. MCV and MCH were significantly decreased comparing to control group, while MCHC showed insignificant decrease. Significant decrease in WBCs accompanied by significant increase in granulocytes and marked but insignificant decrease in lymphocyte and monocytes percentage were demonstrated. Whereas, significant elevation in the platelets count was noticed. However, the treatment with TCA for 6 weeks induced insignificant increase in Hb, RBCs and PCV whereas, insignificant decrease in MCV, MCH and MCHC were noticed. Our data revealed insignificant decrease in WBCs accompanied by insignificant increase in granulocytes and significant decrease in lymphocytes. Insignificant increase in monocytes percentage was also demonstrated together with a significant elevation in the platelets count.

The disturbance in hematological parameters by TCA was previously reported by Poon et al. (2002) and Celik and Temur (2009). The last authors showed that oral administration of TCA 2000 ppm to rats in drinking water for 52 days caused a significant decrease in RBCs, MCV, MCH, MCHC, Hb and HCT levels while, platelet counts was found to be increased. These observations led us to conclude that TCA administration at sublethal dosage possessed hematotoxic effect. Celik and Temur (2009) suggested that the decrease in RBC, Hb and HCT, might be due to the effect of TCA on blood-forming organs causing the anemic condition of the treated animals. Anemia may be due to the inhibition of erythropoiesis and hemosynthesis and to an increase in the rate of erythrocytes destruction in hematopoietic organs (Savithri et al.,2010). However, Ancheva et al. (2003) illustrated that toxic substance causes damage to the erythrocytes membrane resulting in hemolysis or decrease of blood iron level which may be the cause of decreased concentration of Hb and PCV. This hematological alterations might be also due to the effect of TCA on the activity of δ-aminolevulinic acid dehydrogenase, key enzyme of heam synthesis. Furthermore, the toxic

action produced by TCA might be attributed to its ability to generate reactive oxygen species which induce oxidative damage of the circulating blood cells leading to their breakdown, shortening of life span and/or suppression of blood forming cells (Ivaicoli et al., 2003). Another reason for lower count of RBCs and PCV was described by Othman et al. (2004) who demonstrated lower level of erythropoietin; an essential hormone for red cells production, in TCA treated mice .The decrease in Hb can be related to reduction in size of RBC, impaired biosynthesis of heam in bone marrow or due to reduction in the rate of formation of RBCS (Mahmoud and Elbessoumy, 2013). A depression in the hematocrit value can be attributed to total cell depletion in peripheral blood aided by disturbances in steady state mechanisms in blood forming organs as well as an increase in plasma volume (Garima and Goyal ,2007).

Analysis of total WBCs count and differential leucocytes count in our study revealed decrease in WBCs and lymphopenia in TCA treated group which may be due to the direct toxic action of TCA on leucopoiesis in lymphoid organs. The decrease in total WBCs count is directly related with either their decreased production from the germinal center of lymphoid organs or increased lyses due to presence of toxicity in the body (Avadheshkumar and Singh ,1998). However, stress and/or infection can cause a decrease in lymphocytes count. In addition, higher proportions of neutrophils and lower lymphocytes suggesting stimulated immune and oxidative stresses in the intoxicated animals(Abd El Kader et al.,2012).

Hematological examination in the current study also revealed that mice treated with TCA for 6 weeks then left for 3 weeks for recovery exhibited insignificant alterations in most hemograme parameters comparing with the control group and with mice treated with TCA for 6 weeks. While significant elevation in MCHC value was demonstrated. Our data revealed insignificant decrease in WBCs accompanied by insignificant increase in granulocytes and insignificant decrease in lymphocyte and monocytes percentage. This decrement in WBCs may indicate a detrimental effect on the body's immune system. Whereas the platelets count was increased significantly in the recovery group.

Mice received TCA for 6 weeks then treated with the mixture of leaves powder of Capparis *spinosa* and honey for 3 weeks (Regeneration group) showed no ameliorated changes in hemograme (Hb, RBCs ,PCV ,MCV, MCH and MCHC). Decrease in RBCS count may be returned to the reduction in erythropoiesis in bone marrow and faster rate of destruction of peripheral RBC in the spleen (Coles 1986). Whereas an improvement in total leucocytes count and the percentage of lymphocytes comparing to mice treated with TCA only may indicate a potential effect of the mixture of Capparis *spinosa* and honey to inhibit the immunotoxicity. Generally, the increase in WBCs count indicates the activation of defense mechanism and immune system (Whitby et al., 1980). Also, a significant improvement in the

platelets count comparing to mice treated with TCA only was observed. It was previously seen that oral administration of methanol extract of *Capparis sepiaria* at the doses of 200 and 400 mg/kg body weight per day for 14 days converted altered hematological parameters (Hb , RBCs , PCV,WBCs, the percent of neutrophils ,eosinophile, lymphocytes, monocytes) more or less to the normal values and increased antioxidant levels in Dalton's ascites lymphoma tumor bearing mice (Sreenivas et al.,2012).Fiorani et al. (2006) corroborate the honey as an antianemic and immunostimulant agent. Consequently, it is also possible that administration of the mixture of leaves powder of *Capparis spinosa* and honey as used in traditional medicine could ameliorate the deleterious effect of TCA. However, the protective effect of honey might be attributed to the biologically active compounds such as vitamins, flavonoids, and antioxidants that work together to scavenge free radicals (El Rabey et al.,2013). Therefore, *capparis spinosa* can be used in combination with bees' honey to protect animals and human against the adverse effects of toxicity.

Table (2): Effect of *Capparis spinosa* with and without trichloroacetic acid (TCA) on the peripheral blood of mice.

Groups / Parameters	Control	Honey	Capparis + Honey	TCA Only 21 days	TCA only 6 weeks	TCA recovery group	TCA with capparis and honey (Regeneration group)
Hb (g/dl)	15.1 ± 0.4^{ab}	14.8 ± 0.5^{ab}	15.1 ± 0.3^{ab}	13.6 ± 1.2^{b}	15.4 ± 0.0^{a}	15.6 ± 0.8^{a}	14.7 ± 0.6^{ab}
RBCS (10^{12}/L)	10.2 ± 0.3^{ac}	11.5 ± 0.2^{ad}	10.9 ± 0.4^{ac}	11.6 ± 0.3^{de}	11.5 ± 0.2^{ae}	10.6 ± 0.7^{ace}	9.5 ± 0.6^{bc}
PCV (%)	47.2 ± 0.6^{a}	47.5 ± 1.1^{a}	45.6 ± 1.6^{a}	46.8 ± 2.3^{a}	47.8 ± 1.6^{a}	44 ± 1.5^{ab}	39.7 ± 2.9^{b}
WBCs (10^{9}/L)	9.9 ± 1.4^{ac}	8.9 ± 1.6^{ac}	6.4 ± 0.8^{ad}	3.6 ± 0.4^{d}	5.9 ± 0.5^{ad}	7.1 ± 2.1^{ad}	12.3 ± 2.3^{bc}
Granulocytes (%)	10.5 ± 1^{a}	16.4 ± 2.1^{ac}	18.6 ± 1.3^{ac}	27 ± 2.7^{c}	22.9 ± 10.5^{ac}	24 ± 6.1^{ac}	26.8 ± 2^{c}
Lymphocytes (%)	79.6 ± 1.6^{a}	79.5 ± 0.9^{a}	76.1 ± 1.6^{ab}	68.9 ± 2.2^{ab}	66.1 ± 8.9^{b}	71.7 ± 4.4^{ab}	74.3 ± 1.8^{ab}
Monocytes (%)	5.8 ± 4^{a}	5.8 ± 0.2^{a}	5.6 ± 0.7^{a}	4.2 ± 2^{a}	6.4 ± 1.1^{a}	6.3 ± 0.9^{a}	4.8 ± 0.6^{a}
Platelets(10^{9}/L)	278.3 ± 36.4^{a}	306.3 ± 65.5^{a}	331.3 ± 58.8^{ac}	737.3 ± 60.8^{b}	753.3 ± 63.2^{b}	816.3 ± 88.4^{b}	492 ± 10.1^{c}
MCV (fl)	45 ± 0.0^{a}	41.3 ± 0.7^{ac}	41.7 ± 0.3^{ac}	40 ± 1^{c}	41.3 ± 1.9^{ac}	41.7 ± 2.2^{ac}	41.7 ± 1.9^{ac}
MCH (pg)	14.7 ± 0.1^{a}	13.6 ± 0.2^{ac}	13.7 ± 0.2^{ac}	13 ± 0.1^{c}	13.6 ± 0.3^{a}	14.7 ± 0.4^{a}	14.8 ± 0.6^{a}
MCHC (g/dl)	32.7 ± 0.0^{ac}	31.2 ± 0.3^{a}	33 ± 0.4^{ac}	32.5 ± 0.4^{a}	32.3 ± 1^{a}	35.3 ± 0.9^{bc}	37.4 ± 2^{b}

Each value represent the mean ±S.E. of 5 animals in each group. Values , within raw with no common superscripts are statistically significant at P≤ 0.05 Hemoglobin concentration (Hb). Red blood corpuscles (RBCs). Packed cell volume (PCV). White blood cells count (WBCs) Mean corpuscular volume (MCV). Mean corpuscular hemoglobin (MCH). Mean corpuscular hemoglobin concentration (MCHC).

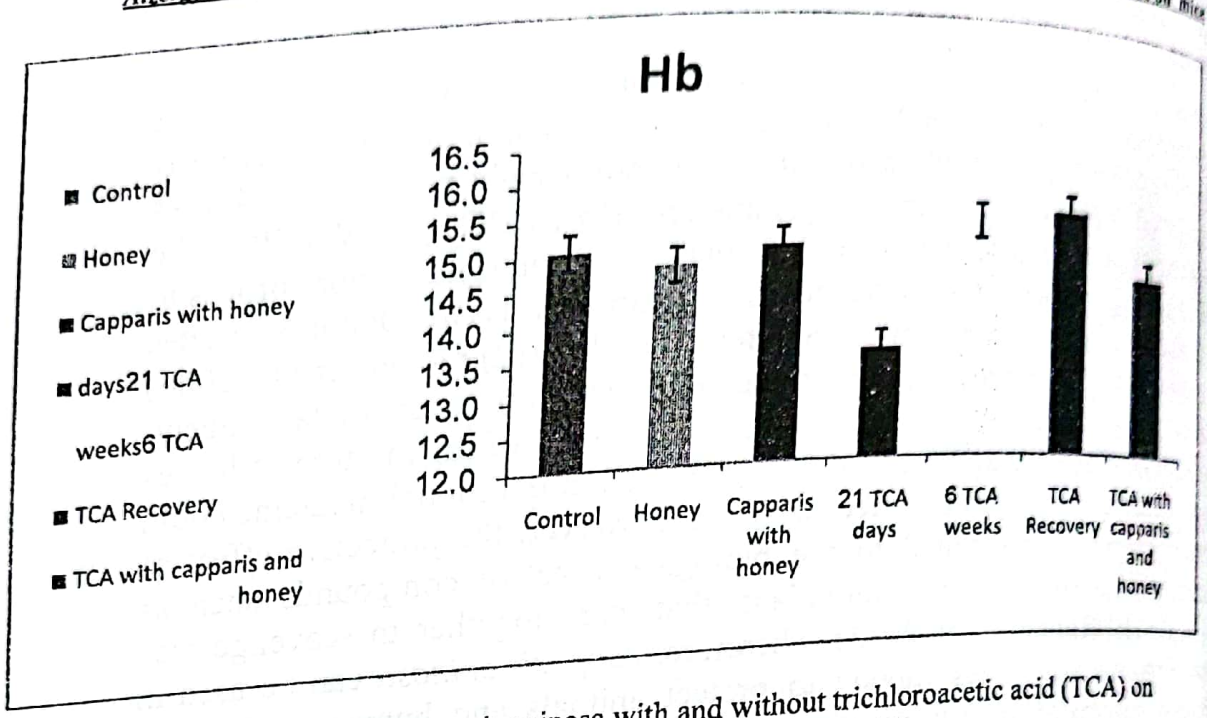

Fig.(3): Effect of Capparis spinosa with and without trichloroacetic acid (TCA) on Hemoglobin concentration Hb (g/dl).

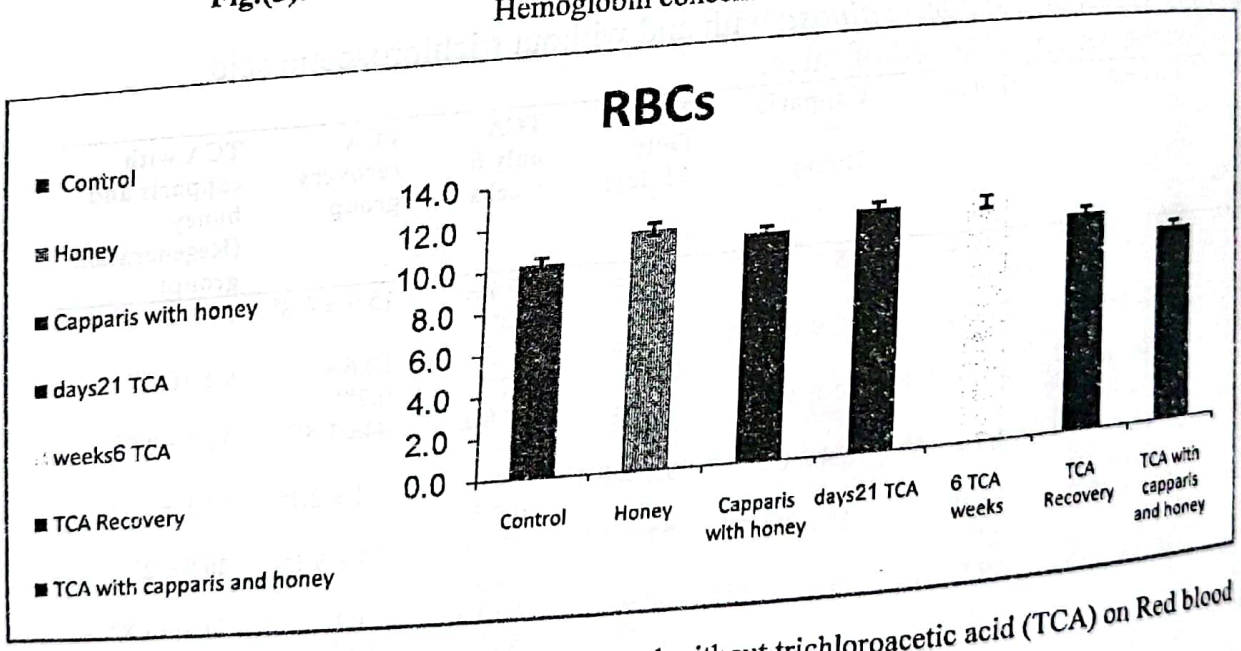

Fig.(4): Effect of Capparis spinosa with and without trichloroacetic acid (TCA) on Red blood corpuscles RBCS (10^{12}/L).

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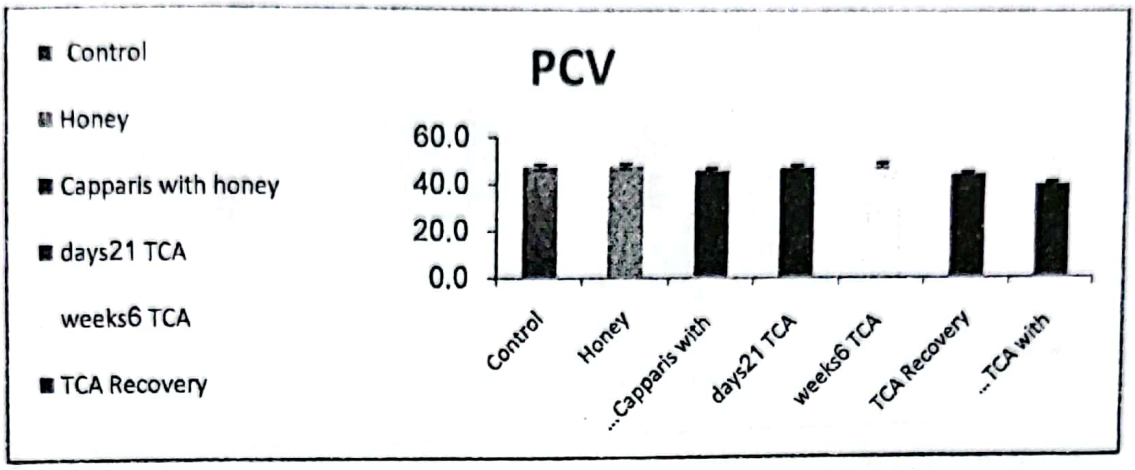

Fig.(5): Effect of Capparis spinosa with and without trichloroacetic acid (TCA) on Packed cell volume PCV (%).

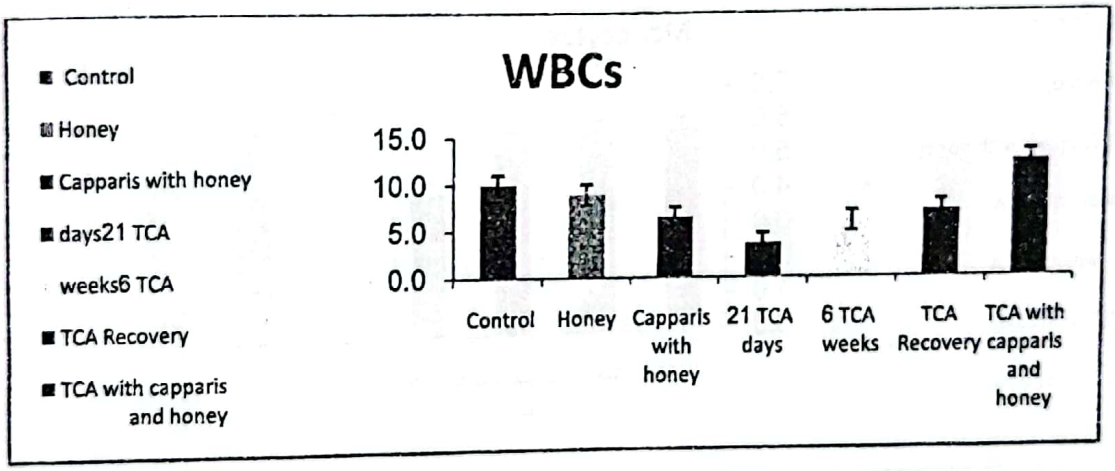

Fig. (6): Effect of Capparis spinosa with and without trichloroacetic acid (TCA) on total white blood cells count WBCs (10^9/L).

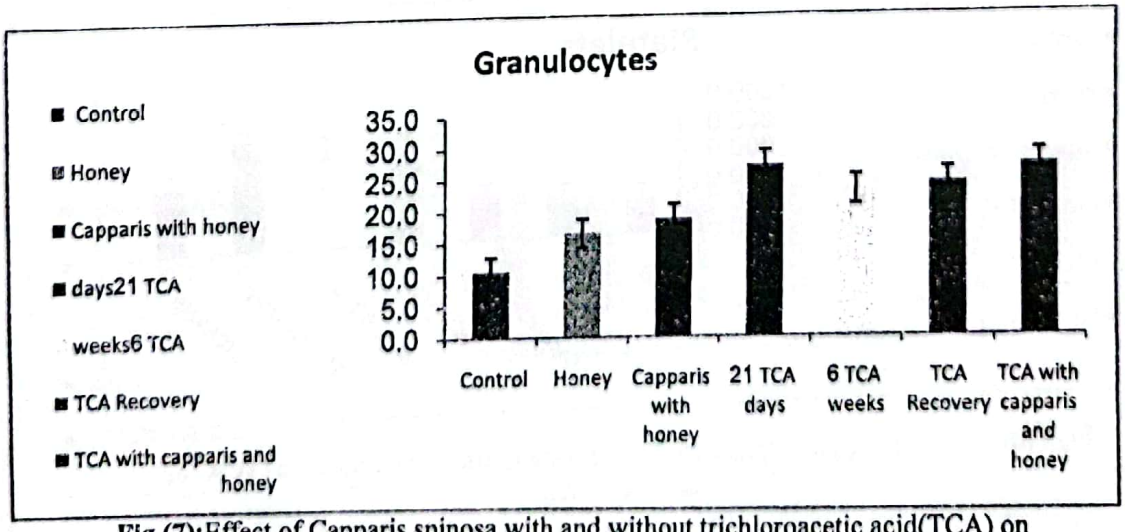

Fig.(7): Effect of Capparis spinosa with and without trichloroacetic acid(TCA) on Granulocytes (%)

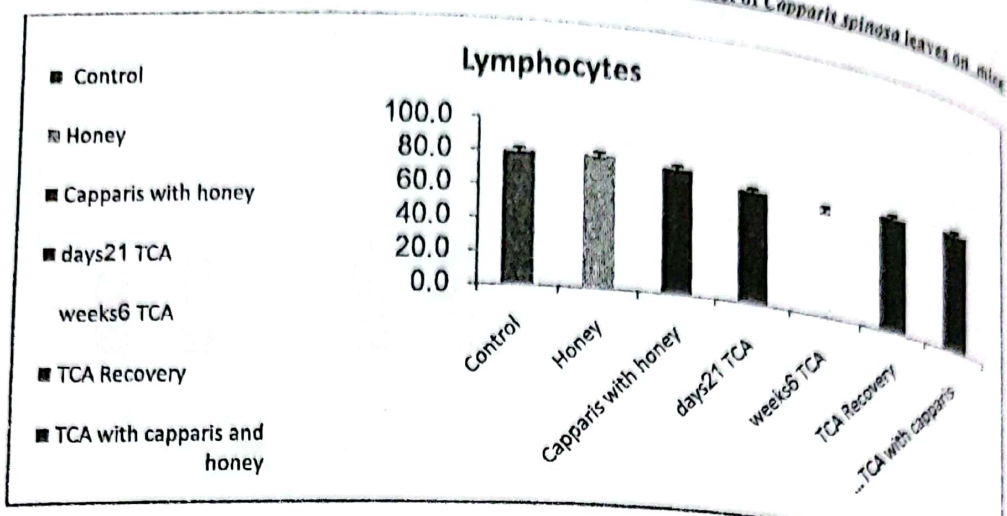

Fig.(8):Effect of *Capparis spinosa* with and without trichloroacetic acid(TCA)on Lymphocytes (%)

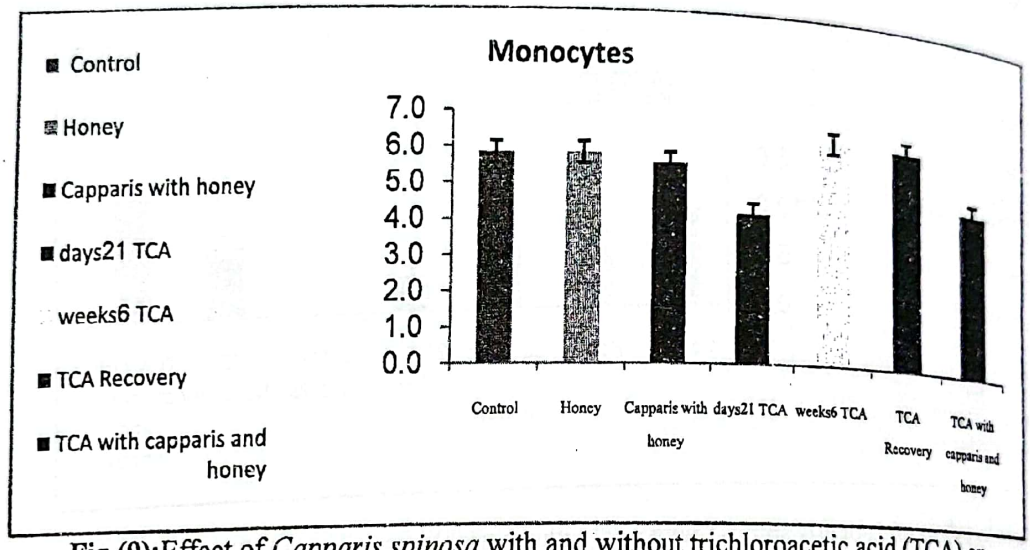

Fig.(9):Effect of *Capparis spinosa* with and without trichloroacetic acid (TCA) on Monocytes (%).

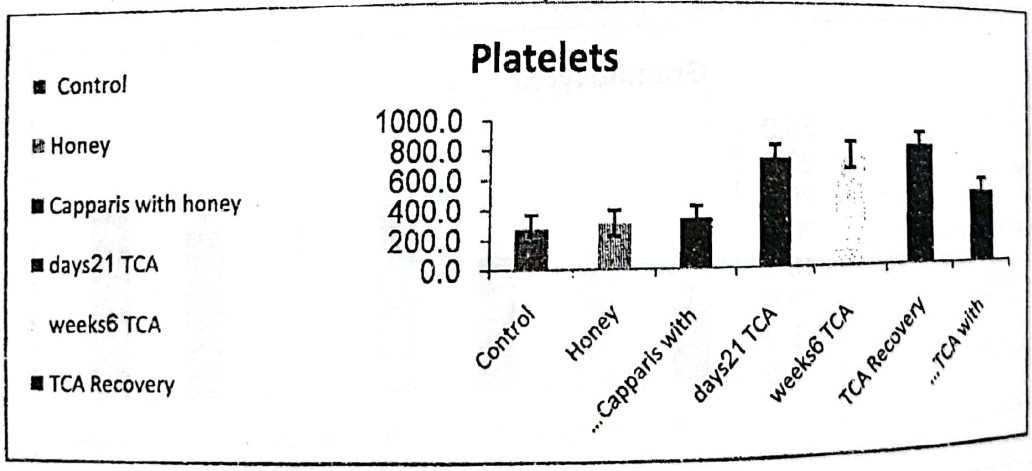

Fig.(10):Effect of Capparis spinosa with and without trichloroacetic acid (TCA) on Platelets(10^9/L).

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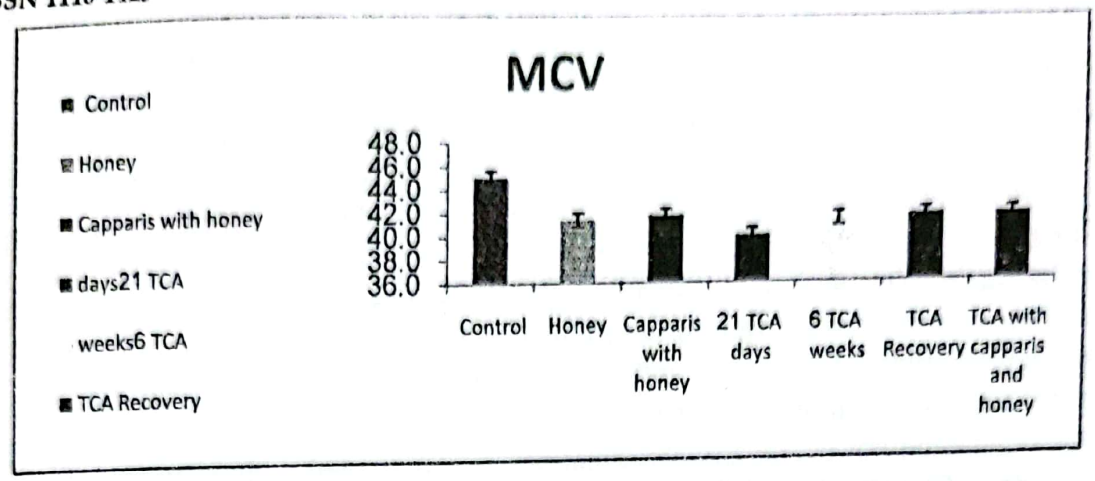

Fig.(11): Effect of Capparis spinosa with and without trichloroacetic acid (TCA) on Mean corpuscular volume MCV (fl).

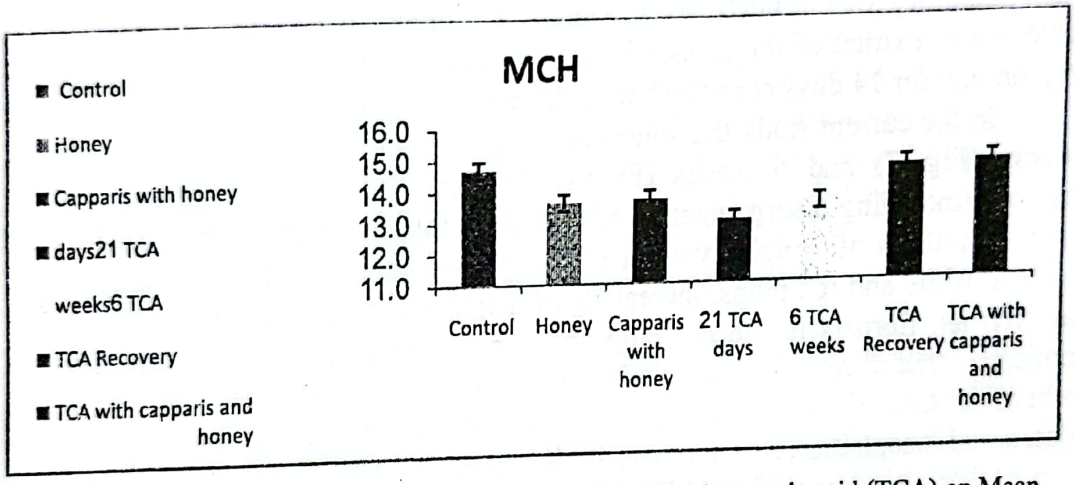

Fig.(12): Effect of Capparis spinosa with and without trichloroacetic acid (TCA) on Mean corpuscular hemoglobin MCH (pg).

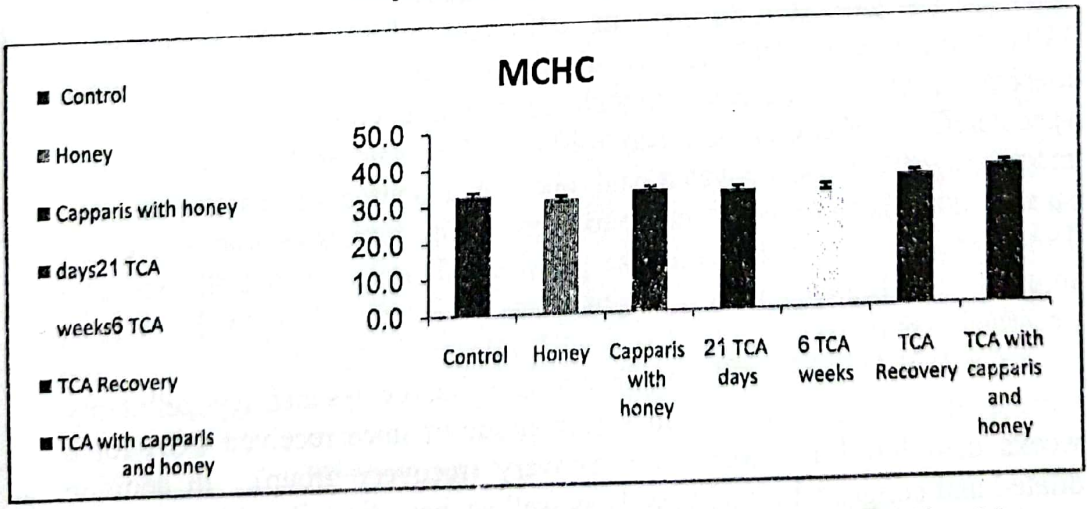

Fig.(13): Effect of Capparis spinosa with and without trichloroacetic acid (TCA) on Mean corpuscular hemoglobin concentration MCHC (g/dl).

Histopathological study:

Examination of the spleen sections of control mice showed normal architecture. It was composed of white and red pulps surrounded by a capsule of dense connective tissue. White pulp was consisted of lymphoid nodules with the central artery located eccentrically. Lymphoid nodules of white pulp separated from red pulp with well visible marginal zone. Red pulp was composed of splenic cords and sinusoids, Megakaryocytes with an irregularly lobulated nucleus were visible among the cells of red pulp(Fig.14).No obvious histopathological changes was detected in the spleen sections of mice treated with honey only(Fig.15) or with the mixture of leaves powder of *Capparis spinosa* and honey(Fig.16). Our findings are in agreement with Sini et al.(2010) who found that histological 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 heart, liver, 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.

In the current study the spleen sections of mice treated with TCA for 3 weeks (Fig.17) and 6 weeks (Figs.18-20) revealed obvious pathological findings including disorganization of lymphoid follicles, hyperplasia in white pulp, depletion of lymphocytes in red pulp with edema, and some necrotic cells in white and red pulps. Increasing of megakaryocytes and hemosiderosis as well as, fibrosis in the red pulp and some lymphoid follicles were also noticed. therefore, the cellularity of spleen was affected by TCA administration. However, splenic immunosuppression may attributed to the decreased lymphatic cells numbers in the spleen as well as in other immune organs(Monfared et al.,2014). TCA has the ability to induce oxidative-stress responses, such as lipid peroxidation and oxidative DNA damage following acute or short-term TCA dosing in mice (Larson and Bull, 1992; Austin et al., 1995; Parrish et al., 1996 and Austin et al., 1996). Moreover, a potential mechanism of TCA-induced oxidative stress via macrophage activation was speculated by Hassoun and Ray (2003).Other studies have shown that macrophages can be activated and become a source of reactive oxygen species that may produce damage to surrounding tissues (Karnovsky et al., 1988; Briggs et al., 1986). Menezes et al.(2005) reported that all extensive injuries were repaired with collagen fibers which may lead to the fibrosis observed here in.

Obvious increase in the number of megakaryocytes and hypocellularity were evident in the red pulp in spleen tissue of mice received TCA for 6 weeks then left for 3 weeks for recovery (recovery group). In addition dilated and congested blood vessels as well as, necrotic cells with condensed nuclei were noticed (Fig.21).On the other hand, Administration of the mixture of *Capparis spinosa* and honey (Figs.22and23) after stoppage of the

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treatment with TCA; lessened most of the aforementioned pathological lesions. This may confirm that the treatment of mice with the mixture of Capparis and honey has a better effect in attenuating the adverse effects of toxicity induced by TCA than the animals left for recovered without treatment. Similarly, administration of honey has 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). Duman et al . (2013) suggested that combination of capparis with honey may have additive effect on decreasing the oxidative damage and tissue toxicity. Therefore, it is plausible to suggest that the effect of *capparis spinosa* with honey in attenuating the toxic effect induced by TCA in this study could be partly mediated by their combined counteraction on oxidative stress within the organs via their antioxidant properties.

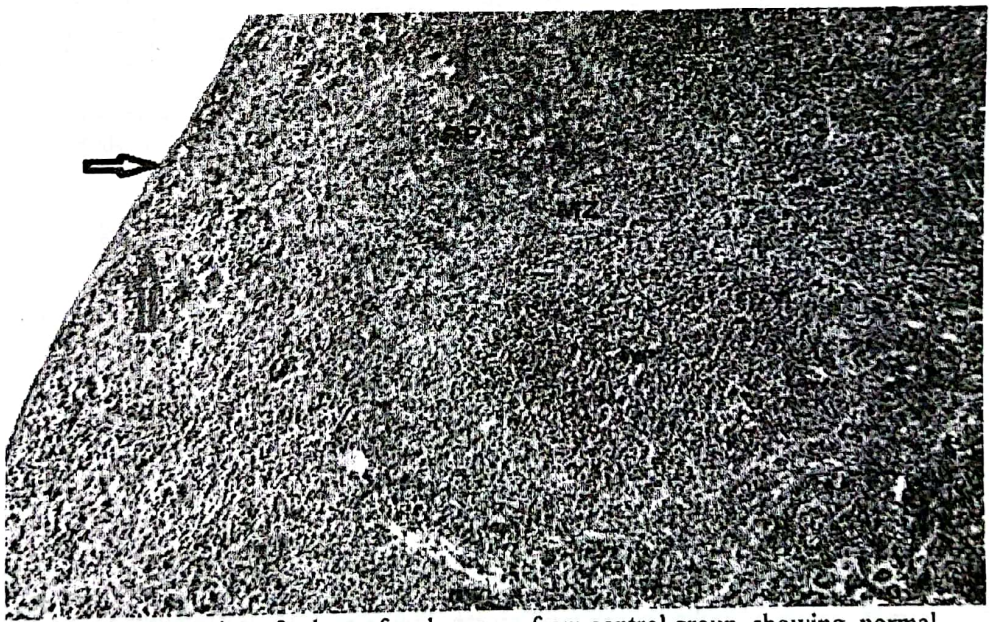

Fig.(14):A section of spleen of male mouse from control group showing normal architecture of spleen, white pulp(WP),Red pulp(RP),capsule(Arrow),marginal zone (MZ), trabeculae (Red Arrow) (H&E stain, X200).

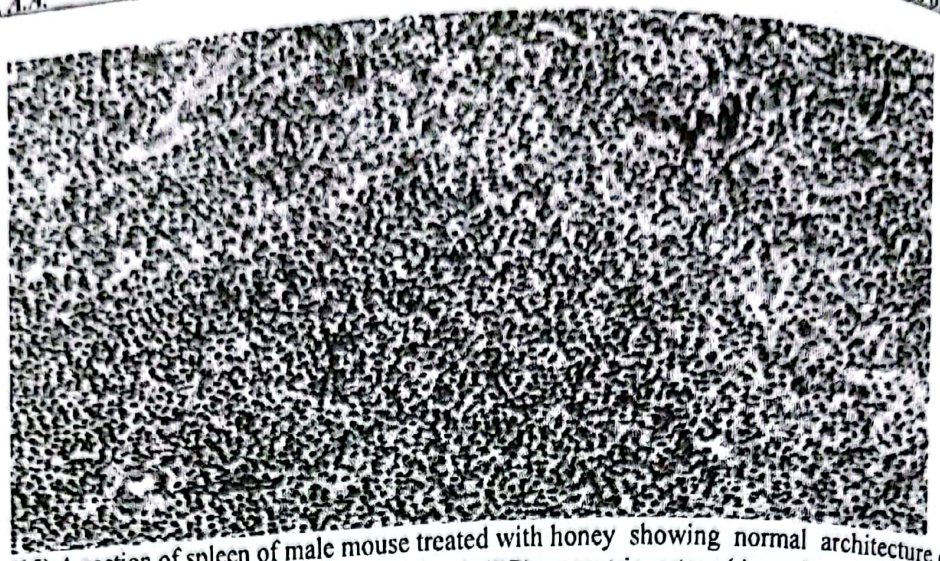

Fig.(15):A section of spleen of male mouse treated with honey showing normal architecture of spleen, white pulp(WP), Red pulp(RP), eccentric artery (Arrow) (H&E stain , X200).

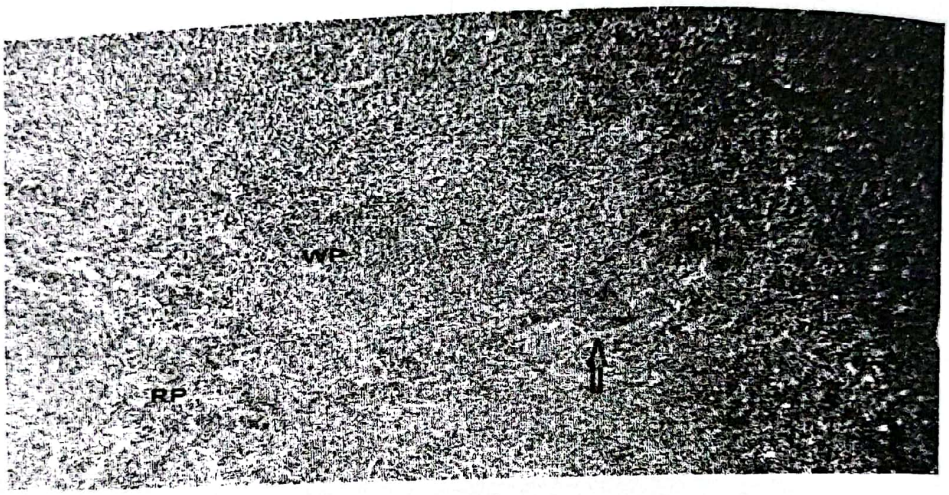

Fig.(16):A section of spleen of male mouse treated with Capper and honey for 3 weeks showing normal histological structure of white pulp(WP), and red pulp(RP), Megakaryocytes (MKS), Trabeculae (Arrow) (H&E stain , X200).

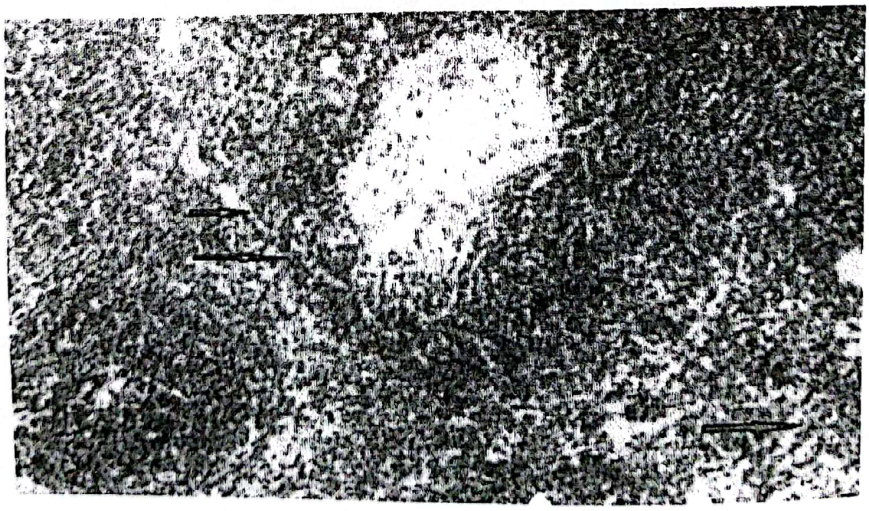

Fig.(17):A section of spleen of male mouse treated with TCA for 3 weeks showing fibrosis and lymphoid depletion and some necrotic cells in white and red pulp, Hemosedrine (Arrows) (H&E stain,X200).

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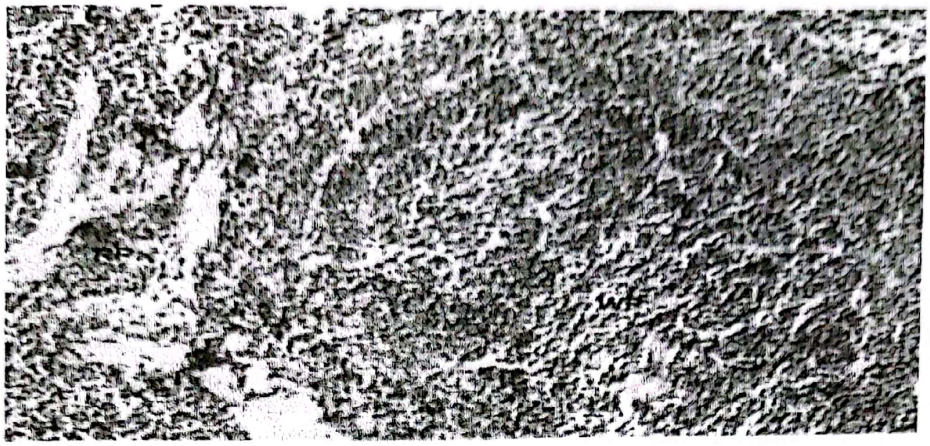

Fig.(18):A section of Spleen of male mouse treated with TCA for 6 weeks illustrating hyperplasia in white pulp (WP); hemosiderosis and fibrosis in red pulp(RP) (H&E stain,X200).

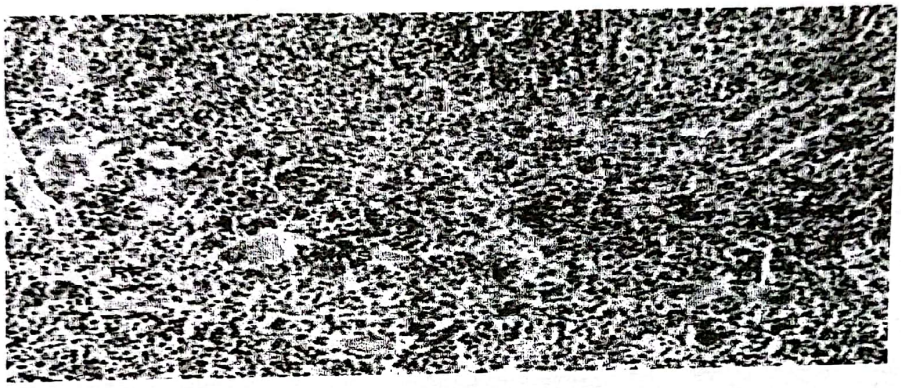

Fig.(19):A section of spleen of male mouse treated with TCA for 6 weeks showing hyperplasia in white pulp (WP) hypocellularity and edema in red pulp (RP), Megakaryocyte (MKS) (H&E stain, X 200).

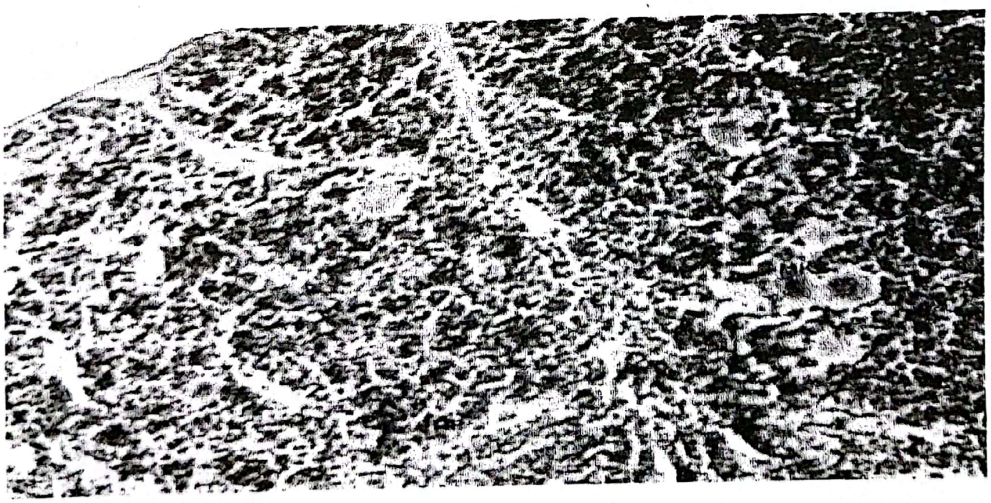

Fig.(20):A section of spleen of male mouse treated with TCA for 6 weeks illustrating hyperplasia in white pulp (WP); hemosiderosis, hypocellularity and edema in red pulp (RP), Megakaryocyte (MKS) (H&E stain, X 200).

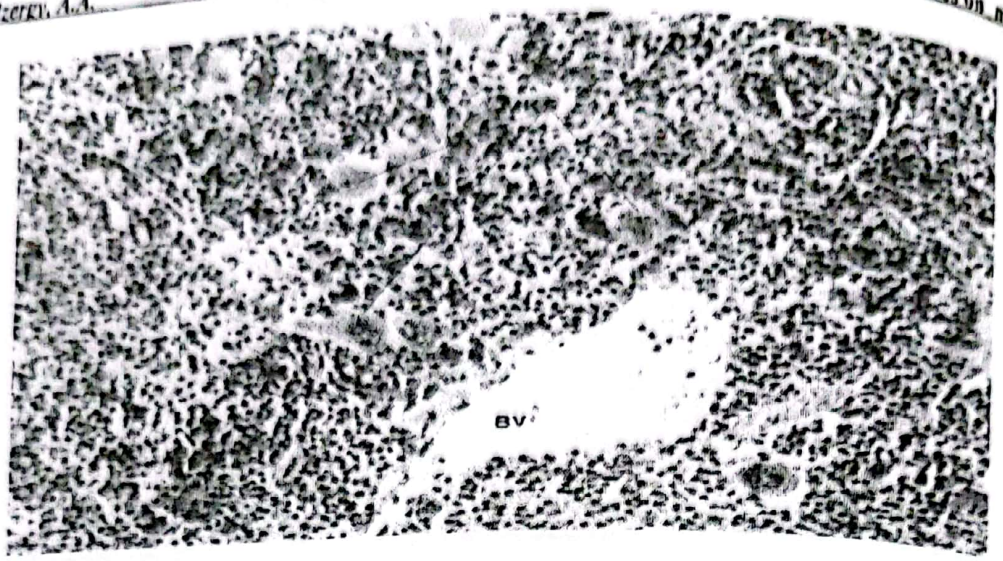

Fig.(21):A section of spleen of male mouse treated with TCA for 6 weeks then left for 3 weeks for recovery showing dilatation and congestion of blood vessels (BV) ,Megakaryocytes (MKS). Note necrotic cells with dens nuclei (H&E stain,X200).

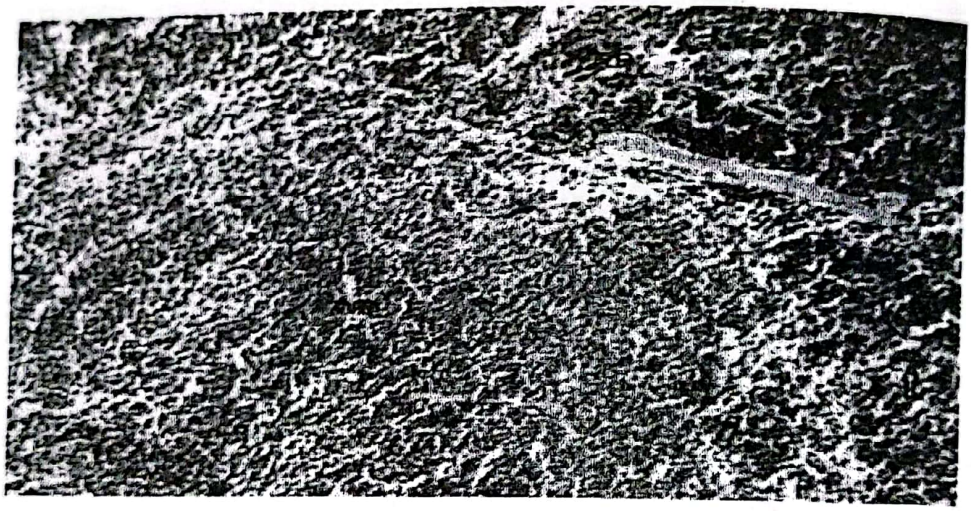

Fig.(22):A section of spleen of male mouse treated with TCA for 6 weeks then treated with a mixture of *Capparis spinosa* and honey(regeneration group) showing red pulp(RP) with few hemosiderosis (Arrow) and white pulp(Wp)with less fibrosis and nearly normal architecture (H&E stain,X200).

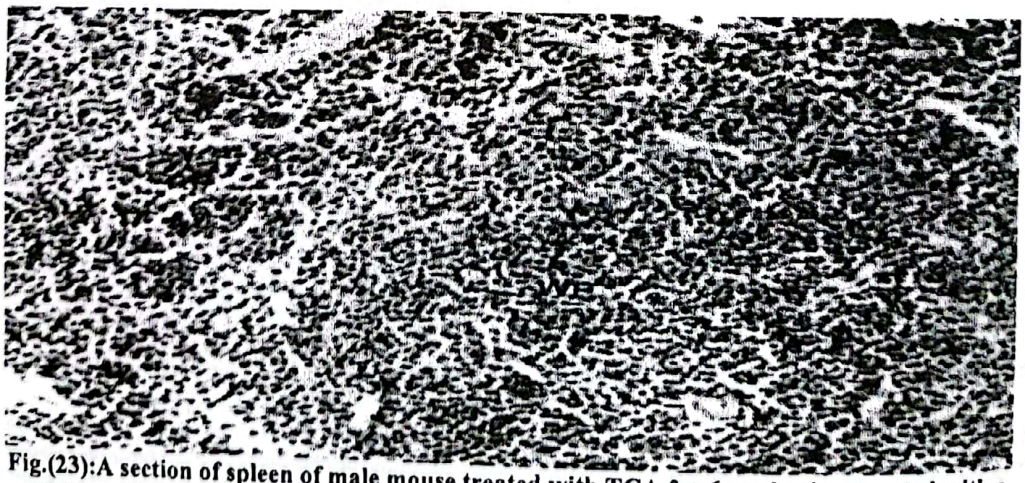

Fig.(23):A section of spleen of male mouse treated with TCA for 6 weeks then treated with a mixture of *Capparis spinosa* and honey showing white pulp(Wp) and red pulp (RP) with lymphoid depletion and few hemosiderosis (Arrow), (H&E stain,X200).

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Conclusion

It was demonstrated that mixture of leaves powder of *Capparis spinosa* and honey (40mg/kg bw.) could produce protective effect in male mice intoxicated with trichloroacetic acid .This response was reflected on the blood and spleen. This may probably occur, in a way or another, to human individuals subjected to environmental pollution. The present investigation demonstrated that at doses consumed in the traditional medicine, mixture of leaves powder of *Capparis spinosa* and honey (40mg/kg bw.) for 3 weeks may be considered as relatively safe, as it did not cause either lethality or changes in the general behavior. Also there was no toxicity on the hematological and histological levels. this effect may be related to its flavonoids and other antioxidant constituents in this plant. Further investigations are needed to elucidate the protective role or side effects of this plant on other organs and system to suggest using of this medicinal plant in therapy.

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تأثير أوراق عشبة الكبار على بعض التغيرات الدموية والنسيجية المرتبطة بتعرض الفئران لحمض الخليك ثلاثي الكلور

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تم إجراء هذا العمل لدراسة الدور الوقائي المحتمل لأوراق عشبة الكبار ضد التغيرات الدموية والنسيجية الناتجة عن تسمم الفئران البيضاء؛ كنموذج حيواني، بحمض الخليك ثلاثي الكلور.

اشتملت التجربة على مائة من ذكور الفئران بوزن تراوح مابين 20–26 جم، وتم تقسيمها إلى خمسة مجموعات: مجموعة ضابطة، والمجموعة الثانية عوملت بعسل السدر عن طريق الفم بجرعة 40 مل / كجم من وزن الجسم لمدة ثلاثة أسابيع. والمجموعة الثالثة عوملت بمخلوط أوراق الكبار والعسل عن طريق الفم بجرعة 40 مل / كجم لمدة ثلاثة أسابيع. والمجموعة الرابعة تم معاملتها بحمض الخليك ثلاثي الكلور في ماء الشرب بجرعة 500 مجم/ كجم وتم تجميع العينات من بعض الحيوانات بعد ثلاثة أسابيع وبعد ستة أسابيع وتركت باقي الحيوانات بدون علاج للتعافي. والمجموعة الخامسة عوملت بحمض الخليك ثلاثي الكلور لمدة ستة أسابيع ثم عوملت بمخلوط الكبار والعسل بجرعة 40 مل / كجم لمدة ثلاثة أسابيع.

أدت المعاملة بحمض الخليك ثلاثي الكلور لمدة ثلاثة أسابيع وستة أسابيع إلى تغيرات ملحوظة في معايير الدم. وأدى علاج الفئران بمخلوط الكبار والعسل بعد إيقاف معاملتها بحمض الخليك ثلاثي الكلور إلى تحسن في كلا من عدد خلايا الدم البيضاء ونسبة الخلايا اللمفاوية مقارنة بالمجموعة المعاملة بحمض الخليك ثلاثي الكلور بمفرده، وحدث أيضا تحسن معنوي في عدد الصفائح الدموية.

وعلى مستوى الفحص النسيجي أظهر طحال الحيوانات المعاملة بحمض الخليك ثلاثي الكلور حدوث تغيرات نسيجية مرضية واضحة؛ اشتملت على عدم انتظام العقيدات اللمفاوية وفرط تنسج اللب الأبيض و هزال لمفاوي في اللب الأحمر مع وذمة تحت المحفظة . علاوة على وجود نخر في بعض الخلايا في كلا من اللب الأبيض واللب الأحمر وزيادة الخلايا النواء وصبغ الهيموسدرين وتليف اللب الأحمر وبعض العقيدات اللمفاوية. وأدى إعطاء الفئران مخلوط الكبار والعسل بعد إيقاف معاملتها بحمض الخليك ثلاثي الكلور إلى تخفيض معظم التغيرات النسيجية المرضية المحدثة نتيجة التسمم بحمض الخليك ثلاثي الكلور.