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Protective effects of *Rosmarinus officinalis* against trichloroacetic acid -induced hepatotoxicity in mice

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Abstract

The effect of the medicinal herb Rosemary (*Rosmarinus officinalis*) with or without trichloroacetic acid (TCA) on the histological structure of liver of Swiss albino mice and on some liver function tests was investigated.

Eighty female mice 20-26gm were divided into 4 groups; control group, group treated with TCA 500 mg/kg body weight in drinking water, group treated by oral gavage with 0.1 ml aqueous extract of Rosemary 60 mg/kg and group treated with TCA and *Rosmarinus officinalis* for 3 weeks.

Mice treated with TCA showed loss of appetite, loss of body furs and decreased activity. These alterations decreased in mice administrated *Rosmarinus officinalis* and TCA.

Final body weight of mice treated with TCA exhibited a significant decrease in comparison with control group. An improvement in weight was recorded in mice treated with *Rosmarinus officinalis* and TCA.

Treatment with TCA, led to a significant increases in serum alanin aminotransferas (ALT) and insignificant increase in aspartate aminotransferase (AST) and total protein. Administration of *Rosmarinus officinalis* with TCA inhibited the elevation of ALT but not the elevation of AST or total protein.

Liver sections of TCA treated group illustrated congestion of central veins with hemolysis and loss of normal architecture with prominent Kupffer cells. Some hepatocytes exhibited slight hypertrophy. Focal necrotic areas associated with inflammatory cells infiltration and vacuolation of hepatocytes were obvious. Histochemical examination revealed increased reactivity of hepatocytes with periodic acid Schiff technique (PAS). In mice treated with *Rosmarinus officinalis* and TCA; most pathological lesions disappeared and hepatocytes showed reactivity to PAS close to that of control group.

Key words: *Rosmarinus officinalis*, trichloroacetic acid, hepatotoxicity histopathological changes, histochemical.

Introduction

Rosemary (*Rosmarinus officinalis*) belonging to the family Lamiaceae, is best appreciated as a medicinal plant. Traditionally, Rosemary has been used as a tonic and stimulant, analgesic, antirheumatic, carminative, diuretic, expectorant, anti-epileptic, anti-spasmodic in renal colic, dysmenorrhoea, relieving respiratory

disorders effects and for effects on human fertility (Al-Sereiti et al., 1999). It was cultivated in Mediterranean first, then transplanted to China in Dynasty. Now, it is cultivated in all of the world (Hui-Hur et al.,2001). Rosemary plant and oil are commonly used as spice and flavoring agents in food processing for its desirable flavor and high antioxidant activity(Alexandrov et al.,2006). It is a commonly used plant for the remedy of various ailments in many parts of the world(Valenzuela et al., 2004; Katerinopoulos et al., 2005). Rosemary extracts contain active antioxidative factors such as phenolic diterpenes, flavonoids and phenolic acids(Ho et al.,2000). The major constituents of rosemary are caffeic acid and its derivatives such as rosmarinic acid (Herrero et al .,2005).

According to the World Health Organization (WHO) in 2008, about 80% of the world's population relies on traditional medicine for their primary healthcare needs (Pierangeli, 2009). In fact, phytotherpay has been widely used because of the low cost and the easy availability of medicinal plants (Salah et al., 2011).Patients who are self-medicated with herbs for preventive and therapeutic purposes may assume that these products are safe because they are natural. Nevertheless, some of them can cause adverse effects or have the potential to interact with other medications (Erog˘lu et al., 2009).

TCA is a colorless to white crystalline solid with a sharp, pungent odor (NIOSH, 2003). It is formed from organic material during water chlorination (IPCS, 2000) and has been detected in groundwater, surface water distribution systems, and swimming pool water (US. EPA, 2000). It was also detected in vegetables, fruits, and grains. Therefore, human exposure to TCA can also occur via food consumption (Reimann et al., 1996). Oral half lethal dose (LD50) of 4970 mg/kg of body weight for TCA have been reported in mice (Woodard et al., 1941).

Liver is the primary organ of drug metabolism. It plays a key role as detoxification agency in the body. Any damage to this organ may cause serious disorders in the form of various diseases which can be observed in the form of histopathological and biochemical lesions(Soyal et al.,2007).Therefore, this study aimed to evaluate the protection afforded by Rosemary against TCA -induced liver damage in mice.

Materials and Methods

Apparent healthy adult female 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. They were acclimatized for one week prior to the start of experiments.

Rosemarinus officinalis were purchased from a local herb grocery in Algabal Alakhder-Libya. The plant was authenticated by Department of Botany, Faculty of Agriculture, Omar El- Mukhtar University, Al Bayda-Libya. The plant was cleaned and air-dried. Leaves powder of 15 gm were mixed with 100 ml boiling distilled water and steeped in boiled water in a closed vessel for few min. The crude extracts were filtered by a piece of gauze.

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Some mice were given Trichloroacetic acid (TCA) (Sigma Co., Germany) in drinking water; as an example of environmental pollutants, by the dose 500mg/kg for 3 weeks.

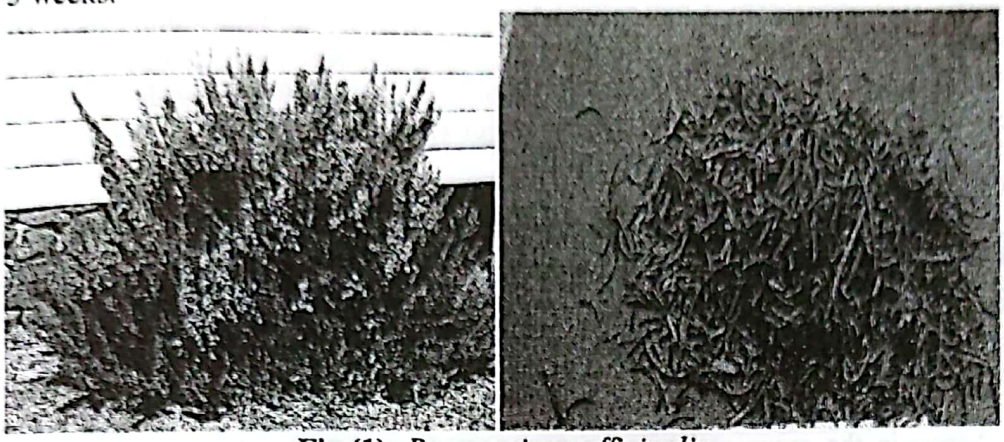

Fig.(1): *Rosemarinus officinalis*

Experimental design:

The experiment included 80 female mice ranged from 20-26gm, divided into 4 groups of 20 mice each. Group I (control group); received distilled water at dose level 4 ml/kg by oral gavage. Group II(TCA treated group); received TCA at dose level of 500 mg/kg body weight in drinking water (Doses were estimated based on default drinking water intake values for mice).Group III (*Rosemarinus officinalis* treated group); treated orally by oral gavage with 0.1 ml aqueous extract of Rosemarins officinalis at dose level 60 mg/kg bw. as used in traditional medicine (equivalent to dose used by a human weighing 70 kg). This dose was determined according to Paget and Barnes(1964). Group IV (*Rosemarinus officinalis* and TCA treated group); treated with TCA and the aqueous extract of *Rosemarinus officinalis* for 3 weeks.

Body weight:

Body weights of mice in all groups were measured weekly and at the beginning and the end of the experimental period using electronic balance. The mean gain in body weights between the successive intervals were estimated according to Tütüncü *et al* (2010).

Biochemical studies:

Twenty four hours after the end of experimental period, unanesthetized mice from both control and experimental groups were sacrificed by slaughtering. Peripheral blood samples were collected from the neck blood vessels into free anticoagulated containers, 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 methods of Reitmans and Frankel (1957). Total protein was measured according to Lowry et al. (1951) using commercial available

kits from Analyticon Biotechnologies(Germany) and automated biochemical analyzer photometer.

Histological studies:

After slaughtering, liver was removed immediately. Pieces of the liver were fixed in 10% neutral formalin and Bouin's solution, processed and embedded in paraffin wax. Sections of 5-7 um thick were cut with the rotary microtome (Leica RM 2125) and stained with Harries haematoxyline 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 data were expressed as Mean ± S.E.M. The results were analyzed statistically by one-way analysis of variance (ANOVA) followed by Duncan's test. Excel programs were used for analysis the results and draw the figures.

Results

From daily observations, it was found that there were no unusual behavior or change in the external features of animals treated with *Rosemarinus officinalis*. However, slight decrease in food intake was recorded. On the other hand, mice treated with TCA showed loss of appetite, loss of body furs and rough coat. In addition, decreased activity in some individuals was noticed. These alterations decreased in mice administrated *Rosemarinus officinalis* and TCA. However, No deaths were recorded during the 3 weeks of the experiment period in the control or the treated animals.

Statistical analysis revealed that there was insignificant changes in the initial body weight of control and all experimental groups. The body weight increased gradually in the control group through the experimental period. The final body weight gain of mice in control group increased by 15.4% above the initial body weight. Administration of TCA alone induced marked decrease in the mean body weight gain. The final body weight decreased by 1.4 % from the initial body weight. It was also found that the final body weight showed significant decrease in comparison with the control group. An improvement in the final body weight was noticed in mice treated with aqueous extract of *Rosemarinus officinalis* and TCA comparing to TCA only treated group. The final body weight increased by 0.98 % above the initial body weight in mice treated with *Rosemarinus officinalis* and TCA. A slight and insignificant increase in the final body weight was demonstrated in mice received *Rosemarinus officinalis* only. The final body weight increased by 3.5 % above the initial body weight in mice treated with *Rosemarinus officinalis* only. It was found that *Rosemarinus officinalis* only inhibited the significant decrease in the mean of final body weight gain comparing to control group (Table 1) and represented in (Fig.2).

Table (1): Effect of aqueous extract of *Rosemarinus officinalis* with and without TCA on body weight gain of mice.

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Groups \ Time	Mean of Initial body weight (gm)	Mean body weight after one week	Mean body weight after two weeks	Mean of final body weight (gm) after three weeks	The mean of change in body weight (%)
Control	20.1 ± 0.1^{a}	21.9 ± 0.4^{b}	23.1 ± 0.5^{b}	23.2 ± 0.6^{b}	15.4 %
TCA only	20.9 ± 0.2^{a}	20.7 ± 0.4^{ab}	21.1 ± 0.6^{a}	20.1 ± 0.5^{a}	-1.4 %
Aqueous extract of *Rosemarins officinalis*	20.0 ± 0.2^{a}	20.0 ± 0.6^{a}	20.7 ± 0.9^{a}	20.7 ± 0.9^{a}	3.5 %
Aqueous extract of *Rosemarins officinalis* & TCA	20.4 ± 0.3^{a}	21.0 ± 0.8^{ab}	20.1 ± 0.5^{a}	20.6 ± 0.6^{a}	0.98 %

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

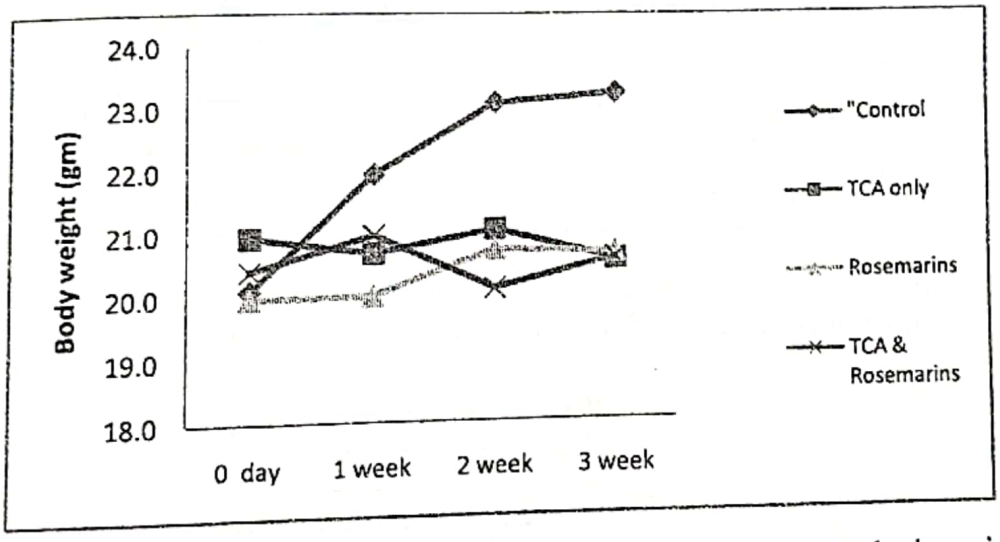

Fig.(2): Effect of *Rosemarinus officinalis* with and without TCA on body weight gain of mice.

Physiological and biochemical studies:-

Treatment with TCA alone or with Rosemarinus officinalis alone led to a significant ($p< 0.05$) increase in serum alanin aminotransferas (ALT). While, administration of Rosemarinus with TCA inhibited the elevation of ALT (Table 2) and (Fig.3).However, insignificant ($p> 0.05$) increase in serum Aspartate aminotransferase (AST) activity and total protein occurred in animals treated with TCA only, Rosemarinus alone and those animals treated with Rosemarinus and TCA(Table 2) and (Figs.4 and 5).

Table (2): Influence of aqueous extract of *Rosemarinus officinalis* with and without TCA on some liver functions tests of mice.

Items \ Groups	Control	TCA only	Aqueous extract of *Rosemarins officinalis*	TCA & Aqueous extract of *Rosemarins officinalis*
ALT (IU/L)	23.3 ±3.3[a]	62.3±8.4[c]	53±10.1[b]	46±6.4[a]
AST (IU/L)	54.3± 24.5[a]	57±22.5[a]	71.3±4.3[a]	122±41.6[a]
Total protein (g/dl)	4.9±0.3[a]	5.7±0.2[a]	6.3±0.1[a]	5.5±0.3[a]

Each value represent the mean ±S.E. of 5 animals in each group. Values ,within raw with no common superscripts are statistically significant at $P \leq 0.05$.
Alanin aminotransferas (ALT), Aspartate aminotransferase (AST).

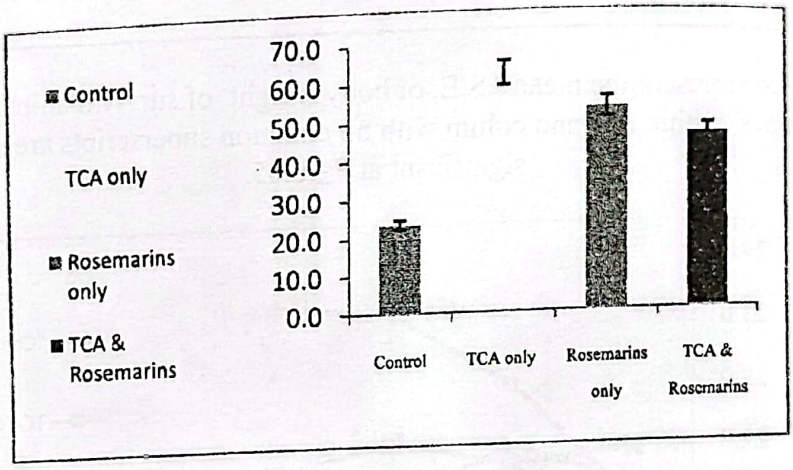

Fig.(3): Influence of aqueous extract of *Rosemarinus officinalis* with and without trichloroacetic acid(TCA) on Alanin aminotransferas ALT (IU/L).

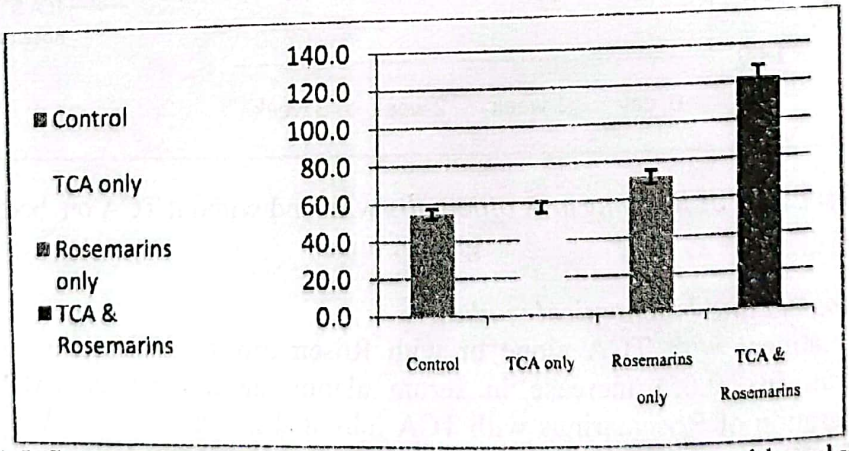

Fig.(4): Influence of aqueous extract of *Rosemarinus officinalis* with and without trichloroacetic acid(TCA) on Aspartate aminotransferase AST (IU/L)

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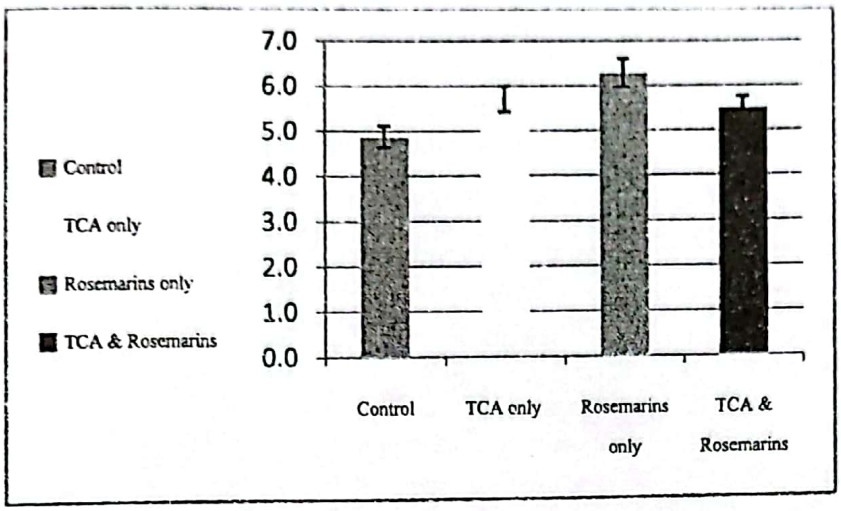

Fig.(5): Influence of aqueous extract of *Rosemarinus officinalis* with and without trichloroacetic acid(TCA) on total protein (g/dl).

Histological and histochemical observations:

Sections of liver of control mice showed normal lobular architecture with central vein and radiating cords or plates of hepatocytes, separated by hepatic sinusoids (Fig.6). On staining of the hepatic tissues with periodic acid Schiff (PAS) technique, there was a moderate reactivity of some hepatocytes and weak reactivity of others (fig.7).

In mice given TCA, many liver sections showed congestion of central veins with intravascular hemolysis of red blood corpuscles. Loss of normal histological architecture with stenosis of hepatic sinusoids in some areas associated with prominent Kupffer cells were noticed. Dilatation of sinusoids in other regions were also observed. Moreover, some hepatocytes were characterized by more acidophilic cytoplasm, while, other hepatocytes exhibited slight hypertrophy. In addition to these hepatocellular alterations, vacuolation of hepatocytes became a prominent feature in this group. Necrosis of some hepatocytes with pyknosis of their nuclei and karyorrhexis or karryolysis of other nuclei were also noticed(figs8and.9). However, in some sections, the area of focal necrosis was associated with infiltration of inflammatory cells and more dilated hepatic sinusoids (fig.10). Furthermore, the histochemical examination of liver sections of mice treated with TCA revealed that most hepatocytes were filled with cytoplasmic granules that severely reacted with PAS technique (fig.11).

Histological sections of livers of mice treated with the aqueous extract of *Rosemarinus officinalis* exhibited some intracytoplasmic vacuoles in some hepatocytes (fig.12).In addition, dilatation of a branch of the portal vein were also recognized. However, the same group showed moderate reactivity of some liver cells and weak reactivity of others with PAS stain especially in the periportal area (fig.13).

In the group of mice treated with *Rosemarinus officinalis* along with TCA; most of the pathological lesions which previously appeared in the group intoxicated

with TCA were disappeared. These lesions included; congestion of blood vessels, intravascular hemolysis, hypertrophy of hepatocytes, focal necrosis as well as inflammatory cells infiltration. On the other hand, vacuolation of hepatocytes was persisted. Moreover, loss of histological architecture with stenosis of hepatic sinusoids became obvious in this group(fig.14). However, in PAS stained sections, some hepatocytes exhibited a moderate reactivity while others showed weak reactivity(fig.15); which were nearly similar to that of the control group.

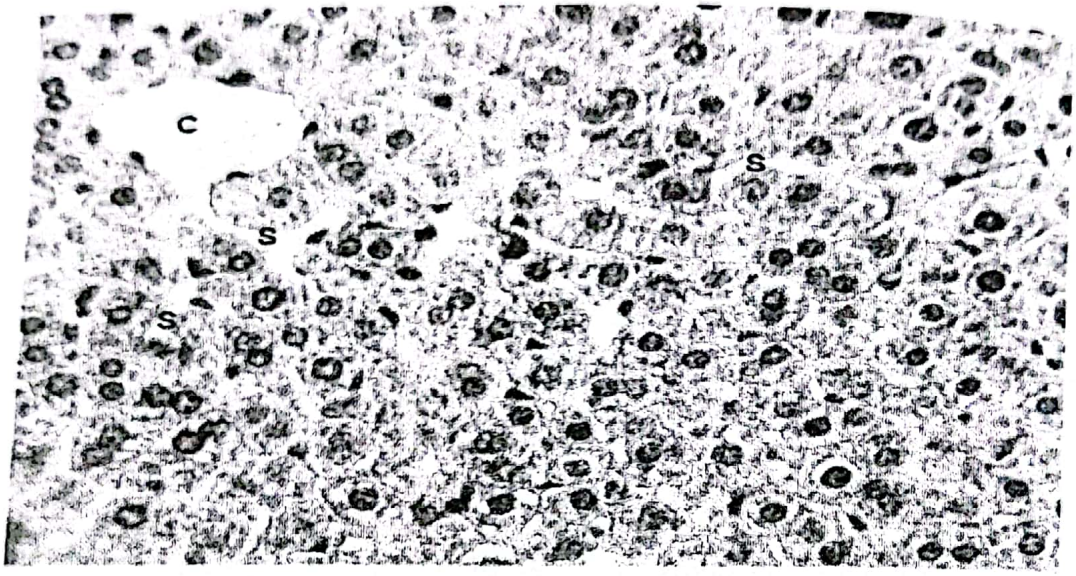

Fig.(6):A section of liver of female mouse of the control group showing normal architecture of liver tissues with central vein(C) and radiating plates of hepatocytes, separated by hepatic sinusoids(S) (H&E stain, x400).

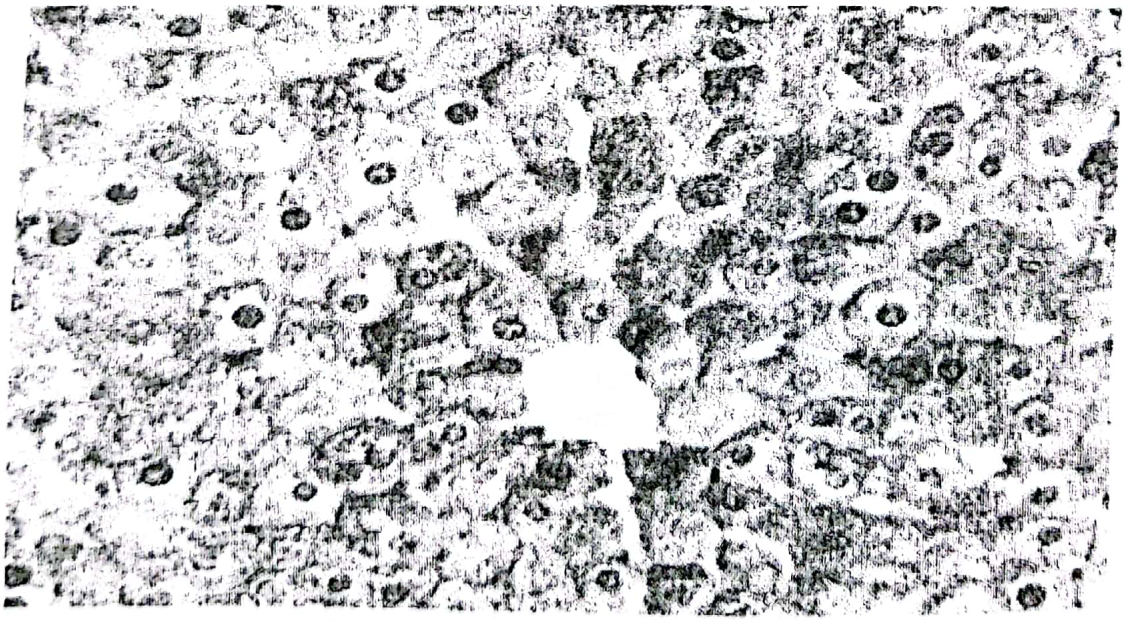

Fig.(7):A section of liver of female mouse of control group showing moderate reactivity of some hepatocytes and weak reactivity of others with periodic acid Schiff (PAS stain, x400).

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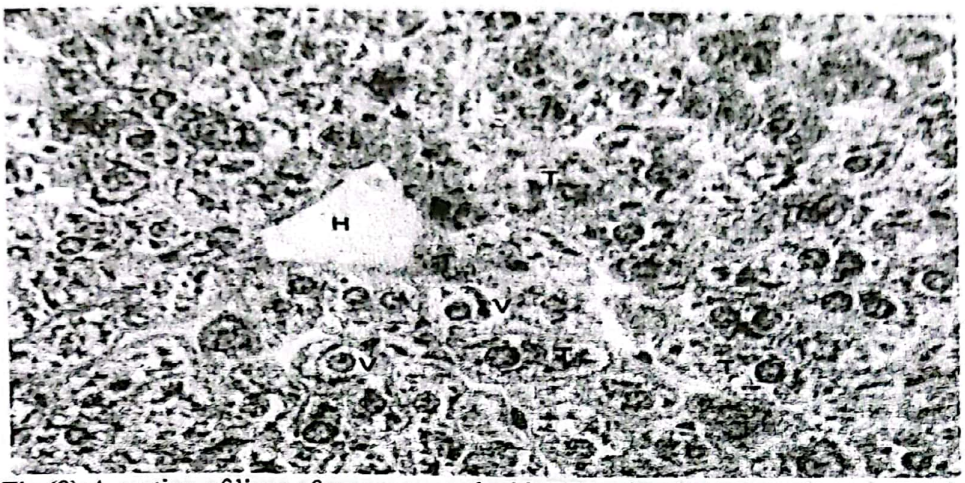

Fig.(8):A section of liver of mouse treated with TCA showing congestion of central vein with certain hemolysis (H), vacuolation of hepatocytes(V) hypertrophied hepatocytes(T) and necrosis(N) of hepatocytes (H&E stain, x400).

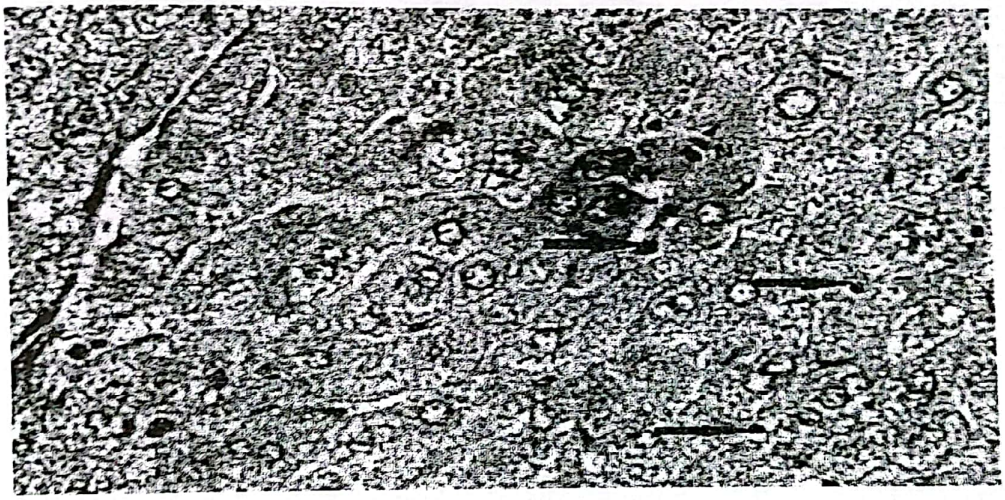

Fig.(9):A section of liver of mouse treated with TCA indicating loss of hepatic architecture and necrosis(N) of hepatocytes and stenosis of sinusoids with prominent Kupffer cells(Arrows) (H&E stain, x400)

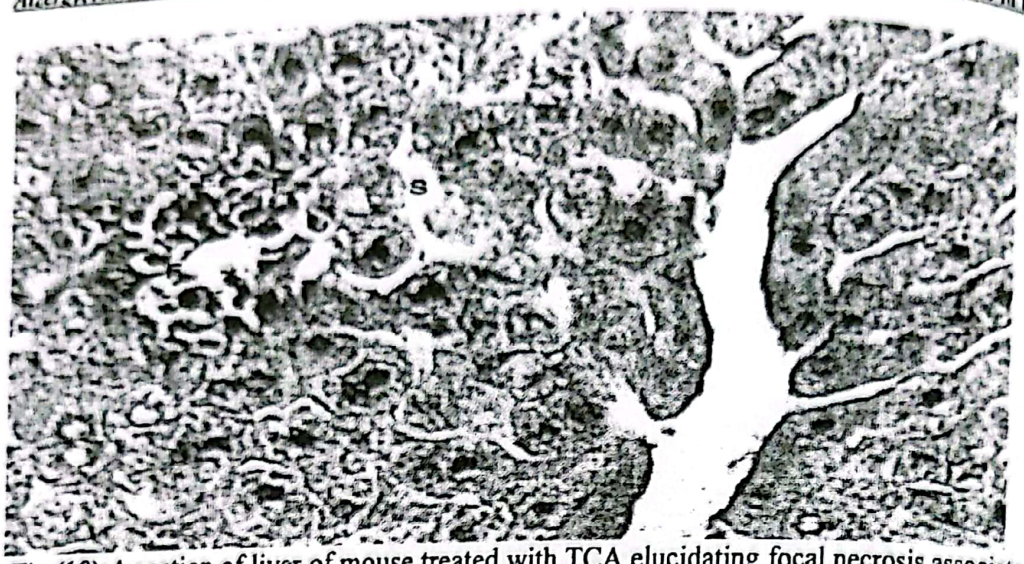

Fig.(10):A section of liver of mouse treated with TCA elucidating focal necrosis associated with inflammatory cells infiltration(F) and dilated hepatic sinusoids(S) (H&E stain, x400).

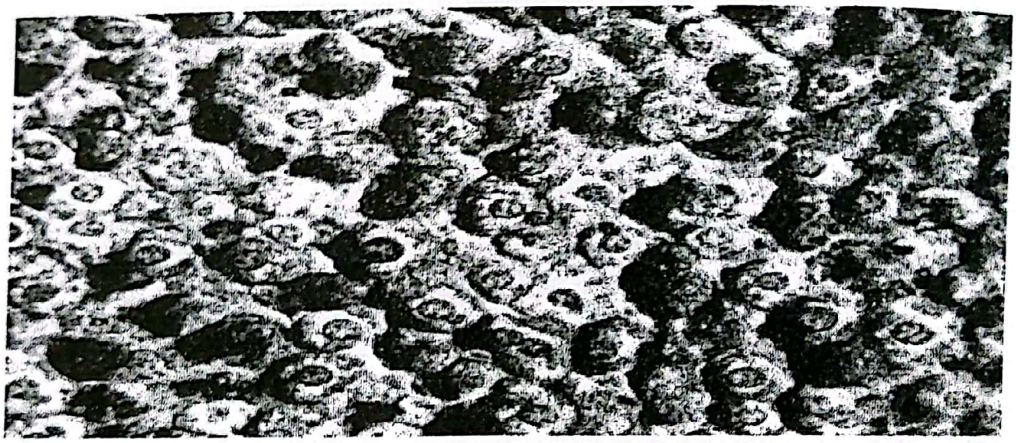

Fig.(11):A section of Liver of mouse treated with TCA showing sever reactivity of most hepatocytes with periodic acid Schiff (PAS stain, x400).

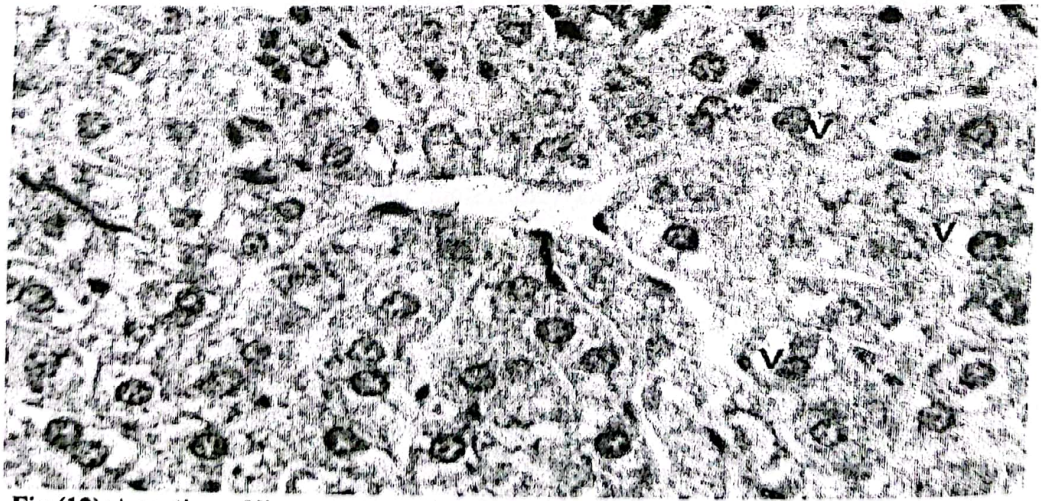

Fig.(12):A section of liver of mouse treated with the aqueous extract of *Rosemarinus officinalis* indicating some vacuolation(V) of hepatocytes (Arrows) (H&E stain, x400).

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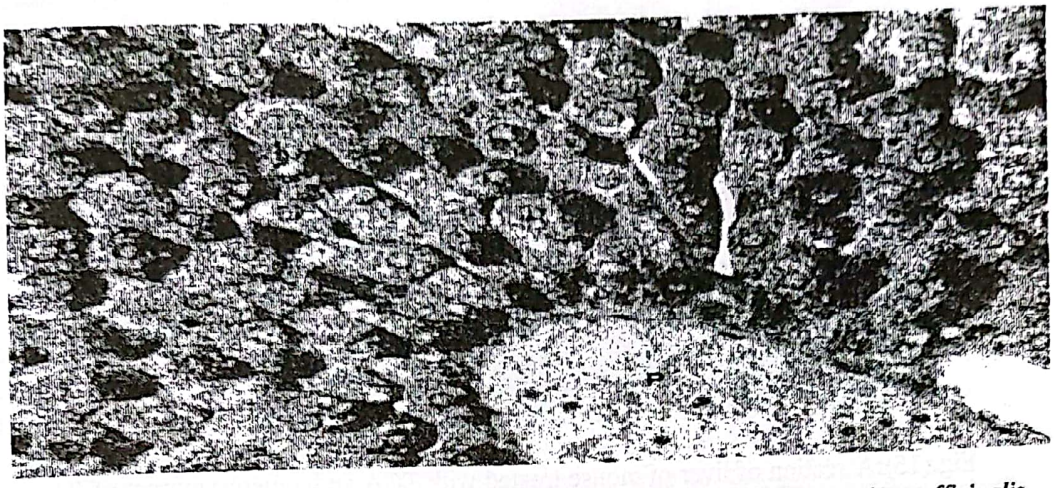

Fig.(13):A section of liver of mouse treated with aqueous extract of *Rosemarinus officinalis* showing moderate reactivity of some liver cells and weak reactivity of others with periodic acid Schiff. Notice the dilatation of a branch of the portal vein (P) (PAS stain, x400).

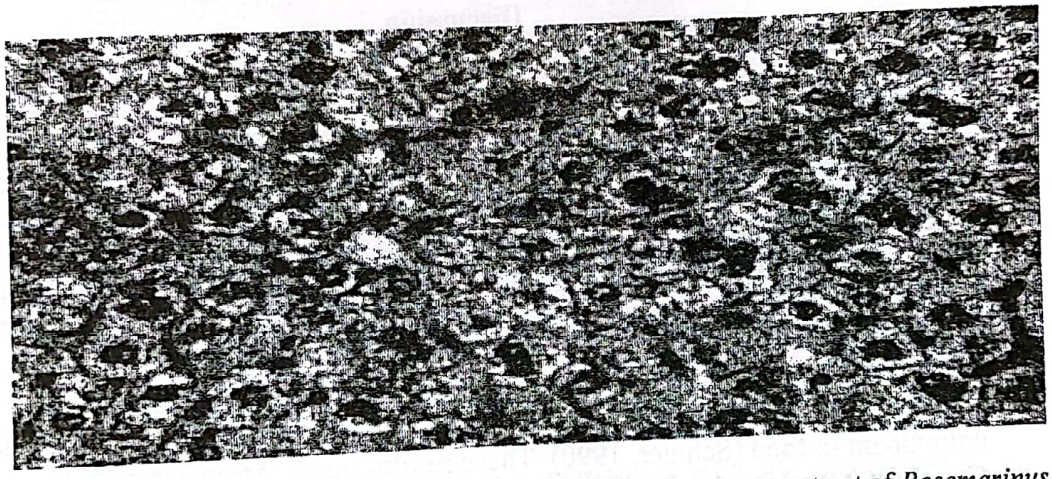

Fig.(14):A section of liver of mouse treated with TCA and aqueous extract of *Rosemarinus officinalis* indicating a quite improvement of liver tissues exposed to TCA. but there is still persistent hepatocytic vacuolation (H&E stain, x400).

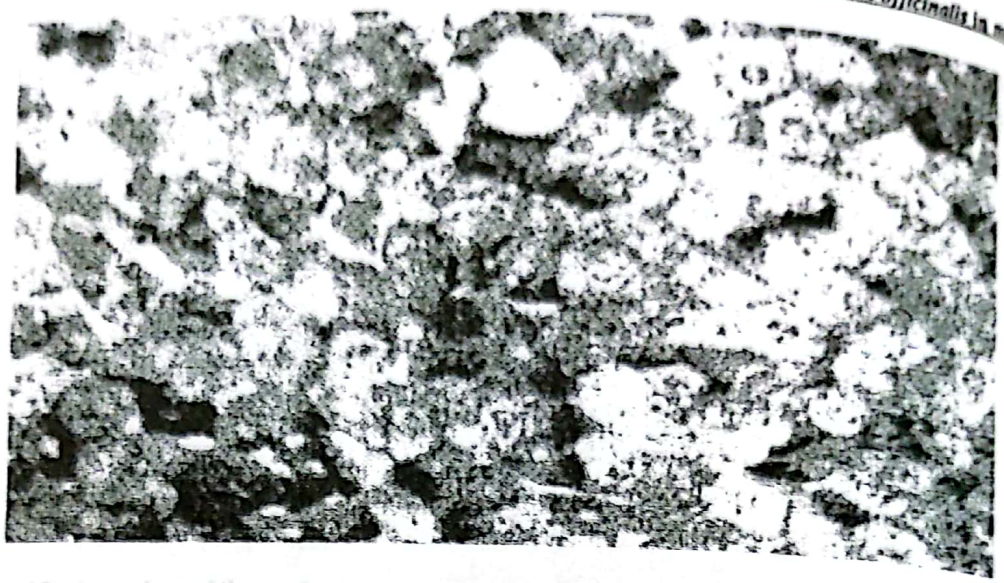

Fig.(15):A section of liver of mouse treated with TCA and aqueous extract of *Rosemarinus officinalis* showing moderate reactivity of some hepatocytes and weak reactivity of others with periodic acid Schiff (PAS stain, x400).

Discussion

The present study showed that there were no unusual behavior or changes in the external features of animals treated with *Rosemarinus officinalis*, but a slight decrease in food intake was recorded. On the other hand, mice treated with TCA showed loss of appetite, loss of body furs, rough coat and decreased activity in some individuals. In general it was found that *Rosemarinus officinalis* lessened the undesired behavior and the changes in the external features resulted from TCA administration. Similarly, no abnormal signs, behavioral changes, significant differences in food and water consumption were noted during the 2-week observation period conducted in rats treated orally with *Rosemarinus officinalis* extracts at a single dosage of 2.000 mg/kg of body weight (Anadon et al.,2008). This plant has been shown to be safe in toxicity studies in animal models, when added as antioxidant to food (Schuler, 1990). These results are in agreement with Garima and Goyal (2007) who reported that no toxic effects in terms of sickness were observed in Swiss albino mice treated with Rosmarinus officinalis leaf extract (at a dose of 100, 200, 400, 800, 1000, 1500 and 2000 mg/kg body weight by oral gavage for 5 days), and also these doses did not show any change in urination and defecation pattern. Moreover, there was no changes in the behavior and external features could be detected in rabbits treated with rosemary comparing to control group (Alzergy et al., 2010). On the contrary, Nusier et al. (2007) suggested that the ingestion of *Rosemarinus officinalis* extract at dose level 250 and 500 mg/ kg body wt for 63 days may impose toxic effects on fertility in male rats.

No deaths were recorded during the 3 weeks of the experiment period in the control and treated animals. Similar finding had been previously described by Garima and Goyal (2007) who reported that there was no mortality in Swiss albino mice treated with *Rosemarinus officinalis* leaves extract at different doses up to 2000 mg/kg body weight by oral gavage for 5 days. This was found to be consistent with other studies where no mortality were observed during the 2-week observation period conducted in rats treated orally with *Rosemarinus officinalis* extracts at a

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single dosage of 2000 mg/kg of body weight. Therefore, rosemary extracts appear to have a low risk of acute toxicity according to Anadon et al. (2008). However, the present results of TCA were in agreement with that of DeAngelo et al. (2008) who stated that no decrease in animal survival was found in mice exposed to TCA in drinking water at dose level up to 5g/L for 60 or 104 weeks.

The present study revealed that the administration of TCA induced a marked decrease in the mean body weight gain and the final body weight showed significant decrease in comparison with the control group. De Angelo et al.(2008) recorded a decreased in the body weight in mice exposed to TCA in drinking water at dose level 5g/L for 60 weeks relative to the control. This was consistent with other studies where the body weight was decreased 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). The reduction in body weight gain may be due to the oxidative stress (Mansour and Mossa,2010 and Saafi et al.,2011) and/or due to the increased degradation of lipids and proteins as direct effects of toxic compound exposure (Heikal and Soliman ,2010; Goel et al.,2005 and Mossa et al., 2011).

In the present work an insignificant increase in the final body weight was demonstrated in mice received aqueous extract of *Rosemarinus officinalis*. An improvement in the final body weight was noticed in mice treated with *Rosemarinus officinalis* and TCA comparing to TCA only treated group. However, Garima and Goyal (2007) reported an insignificant differences in the body weight in mice treated with *Rosemarinus officinalis* leaf extract at different doses up to 2000 mg/kg body weight by oral gavage for 5 days. Similar results were recorded by Nusier et al., 2007 in male rats treated orally with *Rosemarinus officinalis* extract at dose level 250 and 500 mg/ kg body weight for 63 days. Moreover, this finding was also supported by Anadon et al.(2008) in rats treated orally with *Rosemarinus officinalis* extracts at a single dosage of 2000 mg/kg of body weight when compared with those of the control group.

Treatment with TCA, led to a significant increase in ALT and insignificant increase in AST and total protein. The increase in the serum levels of AST and ALT was reported to be associated with liver damage (Kaneko, 1985 and Bush, 1991). Moreover, 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).However, administration of Rosemary with TCA in the present study inhibited the elevation of ALT but not the elevation of AST or total protein. Nusier et al. (2007) found that AST, and ALT levels were not altered in male rats fed orally with *Rosmarinus officinalis* at dose level 250 and 500 mg/ kg body wt for 63 days. Abd El Kader et al. (2012) reported that the liver enzymes ALT and AST activities were significantly reduced in rats pre-treated by aqueous extract of rosemary at dose 1000 mg/kg by gastric intubation for 5 days followed by intraperitoneal injection of lead acetate when compared to lead acetate only treated group. The same authors speculated that the pretreatment with aqueous extract of rosemary significantly elevated the activity of antioxidant enzymes in hepatic tissue when compared with lead acetate only treated group.

Our results are in agreement with Acharya et al.(1997) who observed that the liver of rats exposed to TCA in drinking water at dose level 3.8 mg/kg-day for 10 weeks caused histological alterations in the liver such as 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 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.

The hypertrophy of hepatocytes in mice treated with TCA was frequently noticed in this study which may indicate the carcinogenicity of TCA was supported by Mather et al. (1990) who found that male rats received TCA in drinking water at 36.5 or 355 mg/kg of body weight per day 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. 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 was stated by Pereira(1996).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).

The severe reactivity of hepatocytes with periodic acid Schiff technique in the present study in the group of mice intoxicated with TCA may indicate that the intracytoplasmic vacuoles resulted from accumulation of neutral mucopolysaccharides which may be glycogen. Bull et al.(1990) noticed that TCA produce small increase in cell size and much a more modest accumulation of glycogen. 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 hepatic swelling and increased glycogen accumulation. However, Carlton & Mc Gavin (1995) confirmed that glycogen degeneration or glycogen storage disease is characterized by excessive hepatic accumulation of glycogen.

Our results are in agreement with Alzergy et al. (2010) who observed that liver of rabbits treated with *Rosemarinus officinalis* showed some intracytoplasmic vacuoles. Moreover, Anadon et al. (2008) reported that *Rosemarinus officinalis* extracts appear to have low acute toxicity. On the other hand, this plant had been shown to be safe in toxicity studies in animal models, when added as antioxidant to food (Schuler, 1990). Moreover, Leaves of *Rosemarinus officinalis* have been shown to be safe and antitoxic in animal tests (Lamaison et al., 1991).

In mice treated with *Rosmarinus officinalis* and TCA; most pathological lesions disappeared and hepatocytes showed reactivity to PAS close to that of the

control group. Similarly, Singletary and Rokusek(1997) indicated that components of rosemary extract have the potential to protect mouse liver from toxic agents. Fahim et al. (1999) found that rosemary ethanolic extract 0.15g/100g b.w. given to rats for 3 weeks showed hepatoprotective effects attributed to the presence of a relatively high percentage of phenolic compounds with high antioxidant activity. Furthermore, *Rosmarinus officinalis* has been also reported to prevent or attenuate decrease in tissue anti-oxidant enzymes in different animal models and to provide cellular protection against oxidative stress (Amin & Hamza, 2006 and Prenesti et al., 2007). *Rosmarinus officinalis* has some therapeutic value in the treatment or prevention of disorders like inflammatory diseases, hepatotoxicity and cancer as stated by Valenzuela et al. (2004) and Katerinopoulos et al.(2005). This plant also, possesses a vasodilator properties of essential oils which can enhance blood flow (Frishman et al., 2004). This character may explain the disappearance of congestion of blood vessels; that previously appeared in the group intoxicated with TCA, after *Rosemarinus officinalis* treatment in the present study.

Conclusion:

The present investigation demonstrates that the aqueous extract of *Rosemarinus officinalis* at dose consumed in the traditional medicine (60 mg/kg body weight) for 3 weeks could ameliorate the toxic effects of trichloroacetic acid which was reflected on the liver structure and function. This effect may be related to the antioxidant constituents in this plant. Although administration of *Rosemarinus officinalis* alone did not cause either any lethality or changes in the general behavior, it causes some histopathological alterations and some adverse effects on the hematological parameters. Therefore, medicinal plants should not be taken haphazard for long periods and must be taken under medical supervision.

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التأثير الوقائي لنبات الإكليل ضد التسمم الكبدي المستحث بحمض الخليك ثلاثي الكلور في الفئران

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أجريت هذه الدراسة لاختبار تأثير العشب الطبي الإكليل مع أو بدون حمض الخليك ثلاثي الكلور على التركيب النسيجي للكبد وكذلك على بعض وظائف الكبد في الفئران السويسرية البيضاء.

اشتملت التجربة على 80 من إناث الفئران بوزن تراوح مابين 20- 26 جرام وتم تقسيمها إلى أربع مجموعات: مجموعة ضابطة، ومجموعة عوملت بحمض الخليك ثلاثي الكلور في ماء الشرب بجرعة 500 مجم/ كجم من وزن الجسم ، ومجموعة تم معاملتها ب0.1 مل من المحلول المائي للإكليل بواسطة الأنبوب الفمي بجرعة 60 مجم/ كجم من وزن الجسم، ومجموعة عوملت بحمض الخليك ثلاثي الكلور والمحلول المائي للإكليل معا لمدة ثلاثة أسابيع.
أدت المعاملة بحمض الخليك ثلاثي الكلور إلى فقدان الشهية وتساقط الفراء مع قله النشاط والحركة. وأدى إعطاء المحلول المائي للإكليل إلى جانب حمض الخليك ثلاثي الكلور إلى تخفيض هذه التغيرات وتحسن في الشكل الخارجي والسلوك.
أظهرت المجموعة المعاملة بحمض الخليك ثلاثي الكلور نقص معنوي في الوزن النهائي للجسم مقارنة بالمجموعة الضابطة. ولكن قيم هذا الوزن قد تحسنت بشكل ملحوظ في الفئران التي تم معاملتها بالمحلول المائي للإكليل وحمض الخليك ثلاثي الكلور مقارنة بالمجموعة المعاملة بحمض الخليك ثلاثي الكلور بمفرده.

سجلت الفئران المعاملة بحمض الخليك ثلاثي الكلور زيادة معنوية في إنزيم الألانين امينوترانس فيريز وزيادة غير معنوية في كلا من إنزيم الاسبارتيت أمينو ترانسفيريز والبروتين الكلي. وأدى العلاج بالمحلول المائي للإكليل إلى جانب حمض الخليك ثلاثي الكلور إلى تثبيط ارتفاع إنزيم الألانين امينوترانس فيريز ولكن لم يؤدي إلى تثبيط ارتفاع إنزيم الاسبارتيت أمينو ترانسفيريز أو البروتين الكلي.

وعلى مستوى الفحص النسيجي أظهرت أكباد الحيوانات المعاملة بحمض الخليك ثلاثي الكلور حدوث احتقان في الأوعية الدموية مع تحلل بعض كريات الدم الحمراء بالإضافة إلى فقد النسيج الكبدي للترتيب والتنظيم الطبيعي وزيادة خلايا كوفر وتضخمها. كما أظهرت بعض الخلايا الكبدية تضخم طفيف، بالإضافة إلى وجود نخر بؤري مصحوب بارتشاح للخلايا الالتهابية مع انتشار فجوات داخل سيتوبلازم الخلايا الكبدية . وأظهر الفحص النسيجي الكيميائي زيادة تفاعل الخلايا الكبدية مع صبغة حمض شيف البيرايو دي. وعند المعاملة بالمحلول المائي للإكليل إلى جانب حمض الخليك ثلاثي الكلور اختفت معظم التغيرات النسيجية المرضية سالفة الذكر. كما أظهرت الخلايا الكبدية تفاعل موجب مع صبغة حمض شيف البيردايوي مقارب لمثيلتها في المجموعة الضابطة.