Hepatoprotective effects of *Juniperus phoenicea* L. on trichloroacetic acid induced toxicity in mice: Histological, Ultrastructure and Biochemical Studies

Aglal A. Alzergy[1] and Saad M.S. Elgharbawy[1,2]

[1]Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Omar Al Mukhtar University, AL Beida Libya.
[2]Department of Cytology and Histology, Faculty of Veterinary Medicine Cairo University.
E-mail: aglalalzergy@yahoo.com- drsaadelgharbawy@yahoo.com

Abstract: The present study was conducted to evaluate the possible ameliorative effect of aqueous extract of *Juniperus phoenicea* leaves on clinical, histological, ultra structural, and biochemical parameters against exposure to trichloroacetic acid (TCA) induced oxidative stress and liver toxicity in Swiss albino mice. Eighty female mice 20-26gm were divided into 4 groups. Group I was kept as a control, group II treated orally with TCA 500 mg/kg body weight in drinking water, group III treated orally with aqueous extract of *J. phoenicea* (40mg/kg body weight.) once daily for 3 successive weeks and group IV treated with TCA and *J. phoenicea*. Mice were examined for signs of toxicity and weekly body weight changes. Blood and serum samples obtained from sacrificed animals at the end of the study were used to evaluate some biomarker liver functions (ALT, AST and total protein). Specimens of livers were processed for histological studies by light microscopy. Some liver specimens were also processed to be studied by transmission electron microscopy. Neither clinical signs nor abnormalities in behavior and external features were observed in mice treated with aqueous extract of *J. phoenicea*. Mice treated with TCA showed loss of appetite, loss of body furs and decreased activity. These alterations decreased in mice administrated aqueous extract of *J. phoenicea* and TCA. Administration of *J. phoenicea* had protective effect against TCA induce significant decline in the final body weight gain of female mice. Biochemical analysis revealed that intoxicated mice with TCA, led to a significant increases in serum alanin aminotransferas (ALT) and insignificant increase in aspartate aminotransferase (AST). Significant amelioration in these parameters was found in mice treated with *J. phoenicea* and TCA. Serum total protein in all treated groups were not found to be significantly different from the control. Histological and ultrastructure examinations also confirmed the protective efficacy of *J. phoenicea*. Administrated of TCA showed many severe pathological lesions include prominent vacuolated hepatocytes, dilatation and congestion of blood vessels with intravascular hemolysis of numerous red blood corpuscles, loss of normal histological architecture with stenosis of hepatic sinusoids and hyperplasia of Kupffer cells. Moreover, some hepatocytes exhibited abnormal division. Also, necrosis of some hepatocytes with pyknotic or karyolitic nuclei were noticed. However, focal necrotic areas associated with inflammatory cells infiltration were frequently observed. Furthermore, most hepatocytes revealed severe reactivity with periodic acid Schiff technique (PAS). Mice treated with TCA and *J. phoenicea* showed marked tissue repair and disappearance of most pathological changes. Moderate reactivity of most hepatocytes with PAS stain were frequently noticed. Electron microscopic examination of liver of mice treated with TCA showed abnormal nuclear features with decrease and abnormal heterochromatin distribution and increase nucleoli. Crowded cytoplasm of hepatocytes with small electron dens granules represented lysosomes and mitochondria with indistinct details beside, few dilated rough endoplasmic reticulum with indistinct attached ribosomes and accumulated lipid droplets of variable size were also recorded. In addition, congested blood sinusoids and hypertrophied endothelial lining cells with few and poorly identifiable organelles were seen. Also, necrotic Kupffer cells with irregular fragmented nuclei were detected. No obvious ultrastucture changes were observed in hepatocytes of mice treated with *J. phoenicea*. However, few vacuoles, slight increase in glycogen content and dispersed cytoplasm contained clumps of intact organelles in many hepatocytes were demonstrated. Genertally, administration of *J. phoenicea* lessened most sever alterastructure changes in hepatocytes of TCA intoxicated mice.
[Aglal A. Alzergy and Saad M.S. Elgharbawy. **Hepatoprotective effects of *Juniperus phoenicea* L. on trichloroacetic acid induced toxicity in mice: Histological, Ultrastructure and Biochemical Studies.** *J Am Sci* 2017;13(12):41-61]. ISSN 1545-1003 (print); ISSN 2375-7264 (online). http://www.jofamericanscience.org. 7. doi:10.7537/marsjas131217.07.

Key words: *Juniperus phoenicea*, trichloroacetic acid, biochemical histopathological and ultrastructure liver mice *(Mus-musculus)*.

1. Introduction

Juniperus phoenicea L. tree (Family Cupressaceae) is one of medicinal plants, known locally as" Araar" and signify all that is wondrous in nature, for each part of the tree have been used as traditional medicine for household remedy against

various human ailments from antiquity **(Correll and Johnston, 1970 and Chatterjee and Pakrashi, 1994)**. It has almost 70 species throughout the world and are mostly distributed in the northern hemisphere **(Topc *et al*., 1999)**. *J. phoenicea* L. trees are considered to be one of the most important constituents of the vegetation of Al-Jabel Akhdar, EL Beida Libya. It constitutes about 80% of the total number of the trees and evergreen shrubs that exist in Al-Jabel Al-Akhdar area **(Anonymous, 2005)**. It is widely growing on the rocky soils of the Mediterranean regions **(Shkukani *et al*.,2008)**. *Juniperus* L. species had been used to cure various inflammatory and infectious diseases in folk medicine **(Akkol *et al.,* 2009)**. *Juniperus* are used in aromatherapy, through inhalation, massage, bathing, or ingestion to create good health and beauty **(El-Sawi *et al*., 2007)**. The phytochemical investigation of the methanolic extract of *J. phoenicea* revealed the presence of four flavonoid compounds namely, myricitrin, quercetin, cosmosin, quercitrin and two phenolic compounds; ρ-coumaric acid and caffeic acid (**Harborne**,**1993; Di Carlo *et al*.,1999; Aboul-Ela *et al*., 2005)**. Traditionally, *juniper* had been taken by mouth to treat conditions of the gastrointestinal tract, such as gas indigestion and poor appetite **(Bayazit, 2004)**. Their oils also help to increase the flow of digestive fluids, improve digestion and eliminate gas and stomach cramping **(Uphof, 1968)**. *Juniperus* were utilized in medicine as an antihelmintic, antiseptic and for wound healing **(Dudareva *et al.,* 2006)**. *Juniperus* genera are mainly used as diuretic, stimulant, antiseptic and for common cold **(Tumen *et al.,* 2012)**.

Trichloroacetic acid (TCA) is a colorless to white crystalline solid with a sharp, pungent odor **(NIOSH, 2003)**. TCA is formed from organic material during water chlorination **(Coleman *et al.,* 1980 and IPCS, 2000)**. TCA is known to be contaminants in drinking water **(Acharya *et al*., 1997)** and had been detected in groundwater, surface water distribution systems, and swimming pool water. Human exposure to TCA occurs directly through the consumption and use of tap water disinfected with chlorine-releasing disinfectants **(U.S. EPA, 2005)**. It was also detected in vegetables, fruits, and grains. Therefore, human exposure to TCA can also occur via food consumption **(Reimann *et al*., 1996)**. TCA 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)**.

Liver is an important organ responsible for metabolism, bile secretion, elimination of many substances, blood detoxification, synthesis and regulation of essential hormones. Liver diseases have become a worldwide problem and are associated with significant morbidity and mortality (**Singh *et al*.,2014**). However, 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)**. 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)**. However, Liver is very important organs for detoxification of xenobiotics and also is very sensitive to damage, especially against free radicals **(Stickel *et al.,* 2017)**.

An alternative approach to the use of chemically synthesized drugs for the treatment of disorders is the use of natural plant extracts **(Ali *et al*.,2010)**. In fact, phytotherpay has been widely used because of the low cost and the easy availability of medicinal plants **(Salah *et al*., 2011)**. Medicinal plants are the source of a large number of bioactive compounds, exploited for natural product based drug development program for the treatment of many diseases **(Lata *et al*., 2014**). Herbal medicines had been reported to show protective effects from liver fibrosis and injury **(Al-Attar and Shawush, 2015)**. Thus, many hepatoprotective herbal preparations had been recommended in alternative systems of medicine for the treatment of hepatic disorders. *J.phoenicea* leaves were widely used in traditional medicine to treat various types of ailments. Therefore, the objectives of this study are to evaluate the possible ameliorative effect of aqueous extract of *J. phoenicea* leaves on clinical, histological, ultrastructural, and biochemical parameters against prolonged exposure to TCA induced oxidative stress and liver toxicity in Swiss albino mice. As well as, the present work aimed to illustrate the effects of aqueous extract of *J. phoenicea* administration on normal liver tissue.

2. Materials and Methods

Experimental animals:

Healthy adult male Swiss albino female *(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 Al Mukhtar University, EL Beida, Libya. They were housed in the laboratory animal room in clean plastic cages (10 mice/ cage) under controlled conditions of temperature (20 ± 2)°C and photoperiod (12h light: 12h dark) cycle. Animals were maintained on standard commercial pellet diet and clear drinking water *ad*

libitum, mice were acclimatized for 1 week prior to the start of experiments.

Materials used:

Herbal Medicine used (Plant materials):

Fresh plants **Fig. (1)** purchased from a local herb grocery in Algabal Alakhder -Libya. The plant was authenticated by Department of Botany, Faculty of Agriculture, Omar Al Mukhtar University, EL Beida Libya. All unwanted materials like stems, flowers, roots or stones were removed from the leaves. The plants were cleaned, air-dried and then powdered mechanically to prepare aqueous extract of the plant as used in traditional medicine.

Preparation of the aqueous extracts of *Juniperus phoenicea*:

Leaves powder of (500mg) *J. phoenicea* were mixed with 50 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 and the filtrates were freshly prepared and left a few minutes before administration and each mouse received orally 0.1ml/mouse at dose level 40 mg/kg body weight. The aqueous extracts of *J. phoenicea* was prepared according to the prescriptions given by traditional healers. A dose was determined according to **Paget and Barnes (1964)**.

Trichloroacetic acid (TCA) was purchased from (Sigma Co, Germany). Mice were given TCA in drinking as 500mg/kg for 3 weeks. 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): *Juniperus phoenicea*

Experimental Design:

Eighty apparent healthy adult female mice were divided into 4 groups of 20 mice each and subjected to the following treatments:

Group I (control group): Mice received distilled water at dose level 4 ml/kg by oral gavage for 3 successive weeks and served as negative control (untreated control group).

Group II (TCA treated group): Mice received TCA at dose level 500 mg/kg body weight in drinking water for 3 successive weeks (and served as positive control) (Doses were estimated based on default drinking water intake values for mice).

Group III (Aqueous extract of *Juniperus phoenicea* treated group): Mice were given orally by oral gavage 0.1 ml aqueous extract of *J. phoenicea* at dose level 40 mg/kg body weight once per day for 3 successive weeks.

Group IV (Aqueous extract of *J. phoenicea* and TCA treated group): Mice received TCA at dose level 500 mg/kg body weight in drinking water for 3 successive weeks and treated orally by oral gavage with 0.1 ml aqueous extract of *J.phoenicea* at dose level 40 mg/kg body weight once per day for 3 successive weeks.

I- Clinical signs studies:

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 or mortality. Also, 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 gain and the body weight changes (%) were calculated according to **Tütüncü *et al.* (2010)**.

II -Biochemical studies:

Twenty four hours after the end of experimental period, unanesthetized mice from both control and experimental groups were sacrificed by cervical

dislocation. Peripheral blood samples were collected from the neck blood vessels.

For biochemical parameters, blood samples were collected into free anticoagulated containers and centrifuged at 3000 rpm for 10 minutes and the supernated serum was collected in eppendorf and utilized for estimation of various biochemical parameters. Serum activities of alanine aminotransferas (ALT or SGPT) and asparatate aminotransferease (AST or SGOT) were determined colorimetrically according to the method recommended by **Reitman and Frankel (1957).** Total protein was measured according to **Lowry *et al.* (1951)**. Determinations of parameters were performed using an automated biochemical analyzer (Chemistry analyzer photometer by using commercial available kits from Analyticon Biotechnologies (Germany).

II- Histological studies:

Preparation of the tissue for light microscopy:

Portions of liver were fixed in 10% neutral formalin, Bouin's solution, Susa and zenker formol fluids, dehydrated through graded concentrations of ethanol, cleared in xylene and embedded in paraffin wax (melting point between 56°C and 58°C). Paraffin sections of 5-7 um thickness were cut with rotary microtome (Leica RM 2125) and stained with Harries haematoxyline and eosin (H & E) as well as, Periodic Acid Schiff (PAS) stains according to **Bancroft and Gamble (2008)**. The slides were covered by Canada balsam and cover slides. Histological sections were examined by light microscope with digital camera (Nikon Eclipse E400) and histopathological changes were recognized and photographed.

Preparation of the tissue for transmission electron microscopy (TEM):

Small pieces of fresh specimens of liver were removed and fixed by immersing them immediately in about 2 ml of 4F1G buffered with 0.1 M phosphate buffer (pH7.3) for 24 hours. Specimens were then postfixed in 2% OsO4 at 4°C for 2 hours, dehydrated in graded series of ethanol and embedded in Epon-araldite mixture in labeled beam capsules. LKB ultramicrotome was used to obtain ultrathin sections (50 nm thick) which were picked upon 200 mesh naked copper grids. Grids were double stained with uranyl acetate for ½ h and lead citrate for 20-30 min **(Reynolds,1963).** Scoping the grids was achieved by using Jeol 100 CX TEM.

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. Also, excel programs was used for analysis the results and draw the figures.

3. Results

Clinical signs studies:

From daily observation, it was found that there were no mortality, unusual behavior or external features in animals treated with aqueous extract of *J. phoenicea* comparing to control group. On the other hand, mice treated with TCA in drinking water showed decrease in food intake, loss body furs, and rough coat. In addition, hypoactivities in some individuals were noticed. It was observed that the aqueous extract of *J. phoenicea* lessened the undesired behavior and external features resulted from TCA administration.

Administration of TCA induced marked decrease in the mean body weight gain. The final body weight decreased by 1.4 % above initial body weight. It was also found that TCA only exhibited significant decline in the final body weight gain compared to control group. The final body weight gain of mice treated with aqueous extract of *J.phoenicea* increased by 13.3% above initial body weight. Administration of aqueous extract of *J.phoenicea* induced insignificant alterations in the final body weight comparing to control group. A significant improvement in the final body weight was demonstrated in mice intoxicated with TCA and received aqueous extract of *J.phoenicea*. The final body weight increased by 8.3 % above initial body weight (Table 1 and Fig 2).

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

Time Groups	**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^{bc}	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 *Juniperus phoenicea*	20.9 ± 0.3^{a}	21.4 ± 0.7^{ab}	23.0 ± 1.3^{b}	23.7 ± 1.7^{b}	13.3 %
Aqueous extract of *Juniperus phoenicea* & TCA	20.4 ± 0.3^{a}	20.6 ± 0.5^{ab}	22.3 ± 0.3^{bc}	22.1 ± 0.4^{c}	8.3 %

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

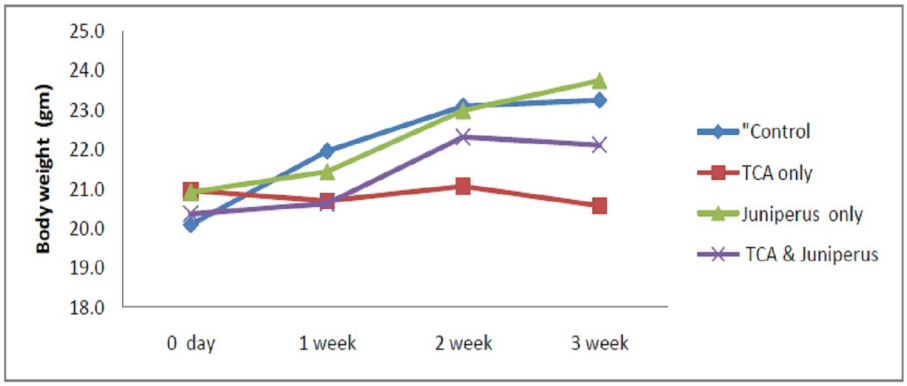

Fig. (2): Effect of aqueous extract of *J.phoenicea* with and without TCA on body weight gain of mice.

Physiological and biochemical studies:

Result of biochemical parameters are shown in (Table 2) and (Figs. 3 -5). Treatment with aqueous extract of *J. phoenicea* cause slight insignificant elevation in Alanin aminotransferas (ALT), and insignificant decrease in Aspartate aminotransferase (AST) concentrations compared to control group. While, significant ($p< 0.05$) increase of serum ALT concentrations and insignificant increase in AST concentrations were found in TCA intoxicated mice group. Biochemical analysis revealed that administration of aqueous extract of *J.phoenicea* with TCA exhibited reduction in serum ALT and AST concentrations compared to TCA only treated group. It was found that the ALT and AST concentrations increased insignificantly comparing to control group. Serum total protein in all treated groups were not affected and showed slight alterations compared to control value.

Table (2): Influence of aqueous extract of *Juniperus phoenicea* with and without TCA on liver functions tests in mice.

Groups Items	Control	TCA only	Aqueous extract of Juniperus phoenicea only	TCA & Aqueous extract of Juniperus phoenicea
ALT (IU/L)	23.3 ±3.3[a]	62.3±8.4[b]	26.3±4.7[a]	30.7±1.2[a]
AST (IU/L)	54.3± 24.5[a]	57±22.5[a]	29.3±7.4[a]	29.7±5.4[a]
Total protein (g/dl)	4.9±0.3[a]	5.7±0.2[a]	5.8±0.2[a]	5.5±0.4[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≤ 0.05
Alanin aminotransferas (ALT), Aspartate aminotransferase (AST)

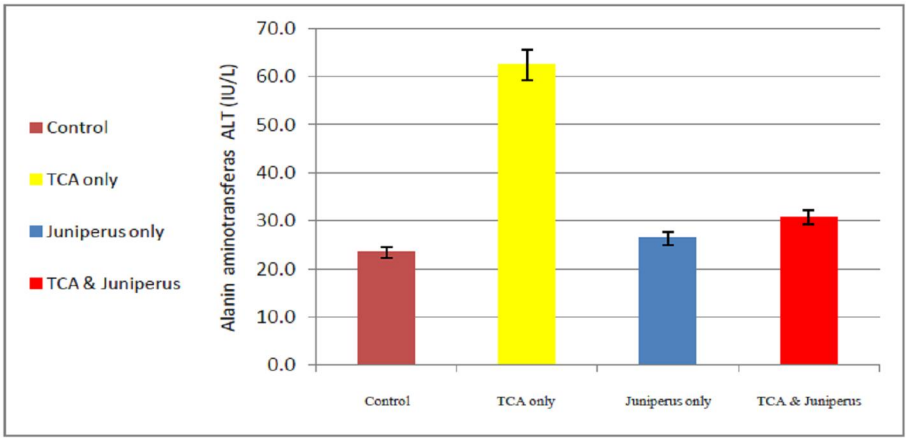

Fig. (3): Influence of aqueous extract of *J. phoenicea* with and without TCA on ALT (IU/L).

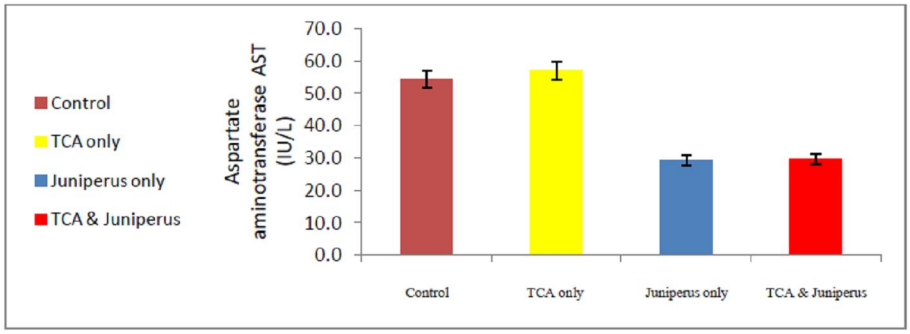

Fig. (4): Influence of aqueous extract of *J.phoenicea* with and without TCA on AST (IU/L).

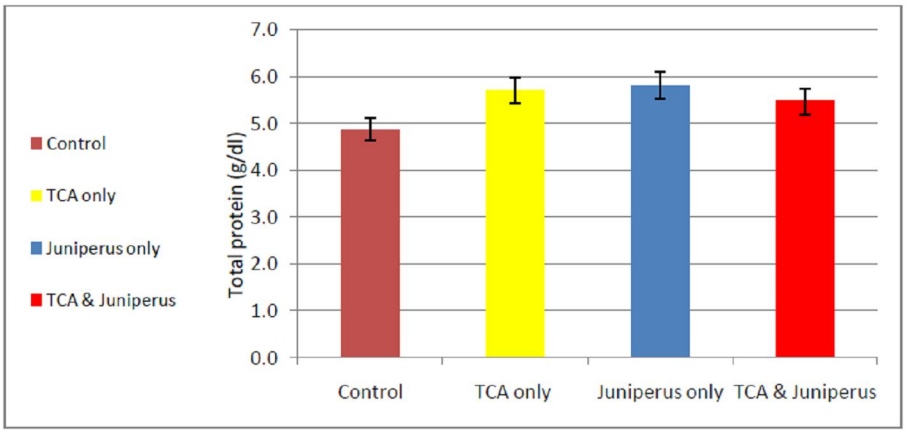

Fig. (5): Influence of aqueous extract of *Juniperus phoenicea* with and without TCA on total protein (g/dl).

Histological and ultrastracture studies:

Histological examination of the liver sections of the control group showed normal lobular architecture with central vein and radiating cords of hepatocytes, separated with distinct hepatic sinusoids lined with endothelial and Kupffer cells. Hepatocytes were polygonal in shape with slightly acidophilic, granular cytoplasm. They had large, or sometimes two, rounded centrally located nuclei with prominent nucleoli (Fig.6). Moderate reactivity of some hepatocytes with periodic acid Schiff technique (PAS) and weak reactivity of others were observed (Fig.7). Examination of the histological sections of liver of aqueous extract of *J. phoenicea* treated mice clearly revealed intracytoplasmic vacuoles in some liver cells and certain stenosis of the hepatic sinusoids (Fig.8) in comparison with the control group. Also, moderate reactivity of the hepatocytes with PAS stain was noticed (Fig.9).

Liver sections of mice given TCA showed severe and many pathological lesions including prominent vacuolated hepatocytes, dilatation and congestion of blood vessels with intravascular hemolysis of numerous red blood corpuscles, loss of normal histological architecture with stenosis of hepatic sinusoids and hyperplasia of Kupffer cells. Moreover, some hepatocytes exhibited abnormal division which caused hyperplastic hepatic lesions. These hepatocytes characterized by more acidophilic cytoplasm with deep basophilic granularity and slight hypertrophy. Also, necrosis of some hepatocytes with pyknotic or karryolysis of nuclei were noticed. However, area of focal necrosis associated with inflammatory cells infiltration were frequently observed (Figs.10 -12). Furthermore, the histochemical examination of liver sections of mice treated with TCA revealed severe reactivity of most hepatocytes with PAS stain (Figs.13). Mice treated with TCA and aqueous extract of *J. phoenicea* showed a marked tissue repair and disappearance of most pathological changes, although the persistence of necrosis of some hepatocytes and the intracytoplasmic vacuoles remained distributed in the liver cells with a reduction in their quantity and size (Fig.14). Most hepatocytes showed a moderate reactivity and weak reactivity of others to PAS stain (Fig.15).

Transmission electron microscopic examination of the liver of the control mice revealed that the liver consisted of two major cell types, parenchymal cells (hepatocytes) and non- hepatocytes (Kupffer cells and endothelial cells). Each hepatocyte had polygonal shape exhibited a round large centrally located nucleus with regular smooth surface and clearly observed nucleolus. The nucleus surrounded by an

envelope of two membranes interrupted by nuclear pores. The nucleus possessed light euchromatin and dark heterochromatin. The latter was typically concentrated as small irregular clumps along the inner surface of nuclear envelope, associated with the nucleolus and few heterochromatin aggregations scattered in the nucleoplasm. The cytoplasm of hepatocytes contained numerous organelles particularly; oval mitochondria with distinct cristae, lysosomes, parallel cisternae of rough endoplasmic reticulum (rER) with distinct attached ribosomes. Also, aggregated glycogen granules (rosette shape) and few small lipid droplets were seen. Hepatocytes separated by blood sinusoids lined by spindle shaped endothelium and Kuppfer cells with large oval or triangular nuclei and more cytoplasmic organelles. Bile canaliculi between parenchymal hepatocytes were noticed. The sinusoidal surface of hepatocytes displayed microvilli (Figs.16-18).

Sections of liver of mice treated with TCA showed that the hepatocytes appeared with prominent destroyed nuclei which reflected a remarkable degree of deterioration. Such nuclei showed abnormal nuclear features with decreased and abnormal heterochromatin distribution and increase nucleoli. Many nucleoli with sever abnormal alterations were seen. Also, cytoplasm of hepatocytes crowded with small electron dens granules represented lysosomes and mitochondria with indistinct details besides few dilated rough endoplasmic reticulum with indistinct attached ribosomes were prominent. In addition, congested blood sinusoids and hypertrophied blood sinusoidal lining cells with few and poorly identifiable organelles were seen. Also, necrotic Kupffer cells with irregular fragmented nuclei were detected. Moreover, fatty changes indicated by accumulated numerous lipid droplets of variable size were noticed (Figs. 19- 24).

No obvious ultrastructure changes were observed in the hepatocytes of mice treated with aqueous extract of *J. phoenicea*. Most hepatocytes showed normal nuclei with clear nucleoli and normal chromatin distribution. In hepatocytes cytoplasm mitochondria with preserved cristae and moderate electron density could be seen. Also, parallel intact rough endoplasmic reticulum with normal attached ribosomes, few vacuoles and moderate to slight increase in glycogen content were demonstrated. No congested blood sinusoids and sinusoidal lining cells with normal nuclei and well preserved organelles were seen. However, many hepatocytes showed dispersed cytoplasm contained clumps of intact organelles and many glycogen rosettes (Figs.25 and 26).

Administration of aqueous extract of *J. phoenicea* lessened most sever ulterastructure changes in hepatocytes of TCA intoxicated mice. However, some hepatocytes showed nuclei with obvious nucleoli associated with heterochromatin and slight dilated nuclear envelope. Also the cytoplasm was found to contain intact organelles including small mitochondria with distinct cristae, lysosomes and vesicles of Golgi apparatus. However, slight dilated rough endoplasmic reticulum with less attached ribosomes were noticed. Accumulation of some lipid droplets of variable size, in addition to, congested blood sinusoids, blood sinusoidal lining cells with normal nuclei and few organelles were seen. The sinusoidal surface of hepatocytes with few destructed microvilli was also noticed) Figs. 27 and 28).

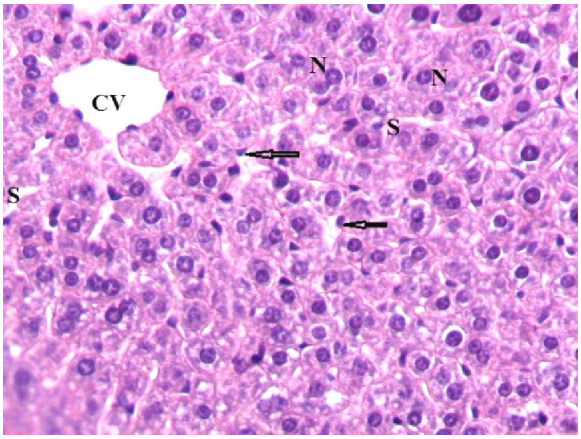

Fig. (6): A section of liver of female mouse of control group showing normal architecture with central vein (CV), hepatic sinusoids **(S)** hepatocytes with distinct rounded nuclei (N). Kupffer cells (Arrows) (H & E stain, x400).

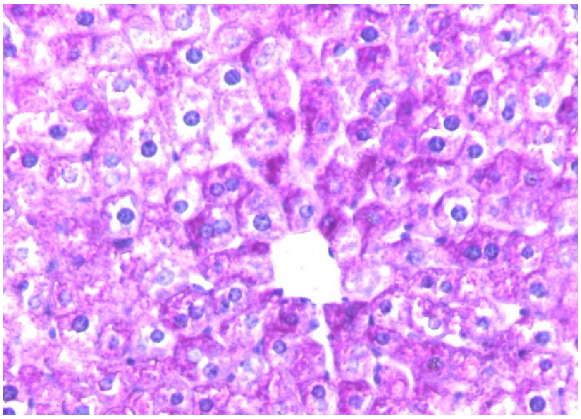

Fig. (7): A section of liver of female mouse of control group showing moderate reactivity of some hepatocytes and weak reactivity of others with periodic acid Schiff (PAS stain, x400).

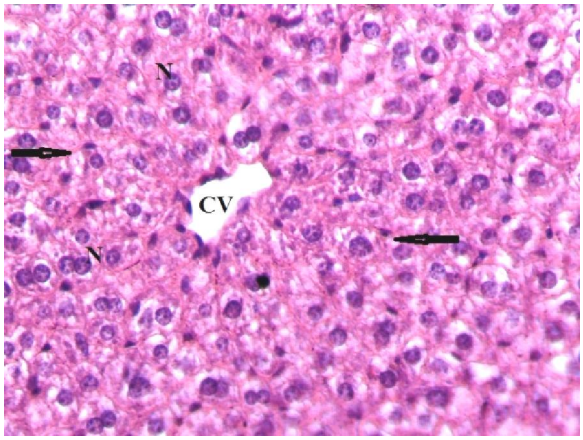

Fig. (8): A section of liver of mouse treated with aqueous extract of *Juniperus phoenicea* showing hepatocytes with normal nuclei (N) central vein (CV). Notice, hepatic sinusoids (Arrows) (H & E stain, x 400).

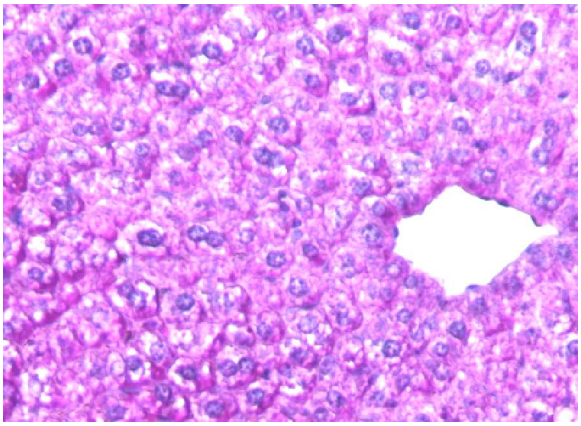

Fig. (9): A section of liver of mouse treated with aqueous extract of *Juniperus phoenicea* showing moderate reactivity of hepatocytes with periodic acid Schiff (PAS stain, x400).

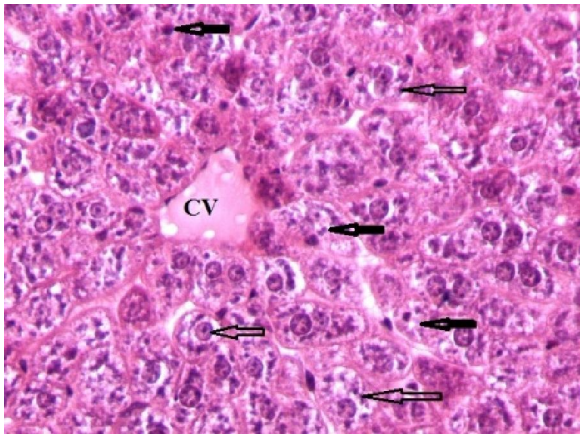

Fig. (10): A section of liver of mouse treated with TCA showing congestion of central vein (CV) with hemolysis of red blood corpuscles, hypertrophied hepatocytes with vacuolated cytoplasm (Arrows) and necrotic hepatocytes (Thick Arrows) (H & E stain, x400.

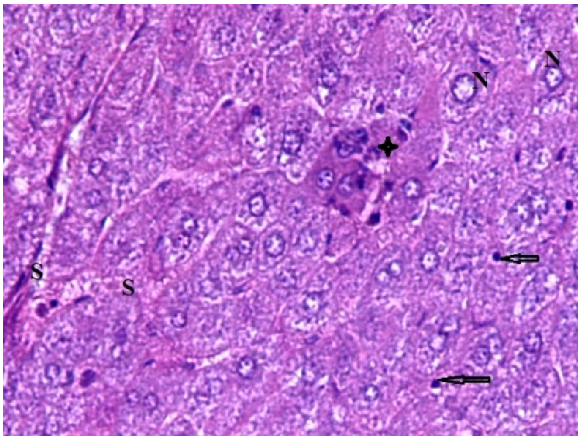

Fig. (11): A section of Liver of mouse treated with TCA indicating loss of hepatic architecture, hypertrophy of hepatocytes with abnormal nuclear feature (N), congestion and stenosis of hepatic sinusoids (S) and hyperplasia of Kupffer cells (Arrows). Notice the abnormal division of hepatocytes (Star) (H & E stain, x400).

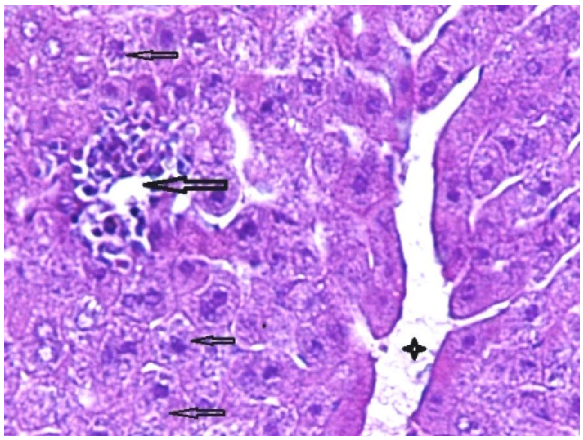

Fig. (12): A section of Liver of mouse treated with TCA elucidating focal necrotic area associated with inflammatory cells infiltration (Thick Arrows) and vacuolated hepatocytes (Arrows), dilated hepatic sinusoids and blood vessels (Star) (H & E stain, x400).

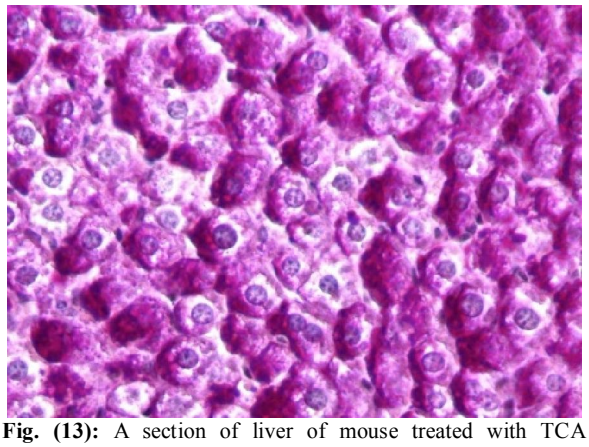

Fig. (13): A section of liver of mouse treated with TCA showing sever reactivity of most hepatocytes with periodic acid Schiff stain (PAS stain, x400).

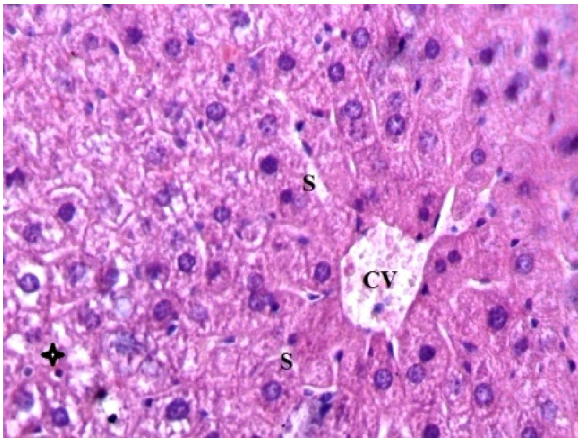

Fig. (14): A section of Liver of mouse treated with TCA and *Juniperus phoenicea* indicating that hepatic sinusoids (S) returned to normal histological architecture. Decrease in hepatocytic vacuolation although, the persistence of some necrotic hepatocytes (Star), central vein (CV) (H & E stain, x400).

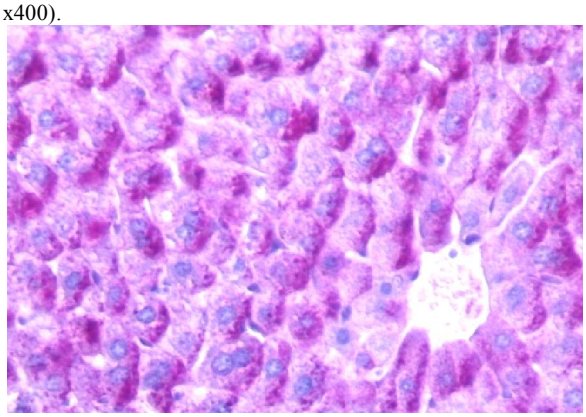

Fig. (15): A section of Liver of mouse treated with TCA and *Juniperus phoenicea* showing moderate reactivity of most hepatocytes and weak reactivity of others with periodic acid Schiff stain (PAS stain x400).

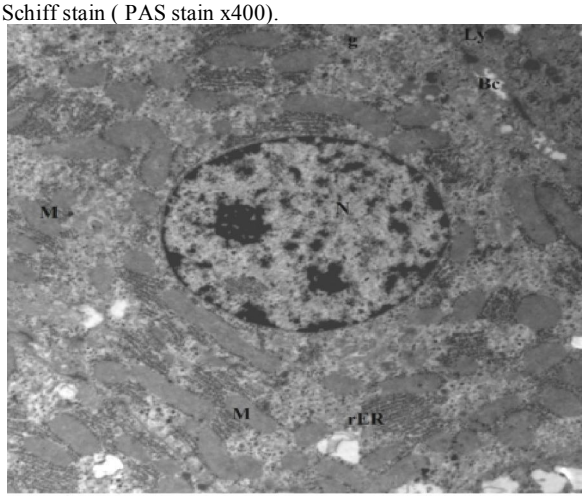

Fig. (16): An electron micrograph of liver of mouse from control group showing normal hepatocyte with round nucleus (N) and cytoplasm with many organelles particularly rough endoplasmic reticulum (rER), mitochondria (M) and lysosomes (Ly). Notice, bile canaliculi (Bc). (Uranyl acetate and Lead citrate stain, X 27500).

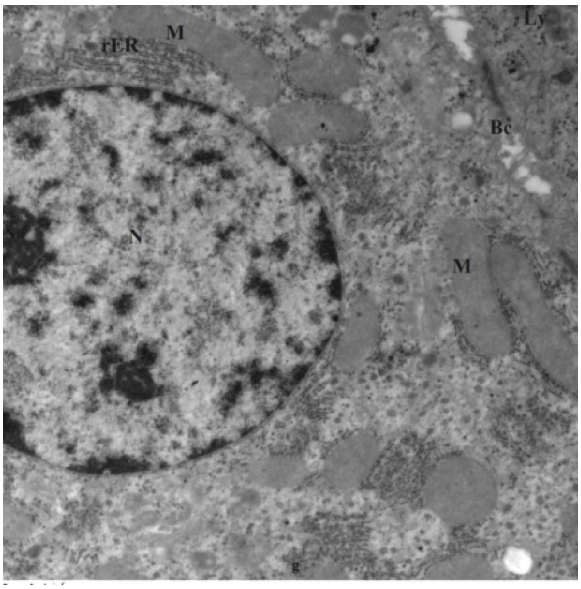

Fig. (17): Higher magnification of fig. (16) showing normal hepatocyte with round nucleus (N) and cytoplasm with many organelles particularly rough endoplasmic reticulum (rER), glycogen granules (g), mitochondria (M) and lysosomes (Ly). Notice, bile canaliculi (Bc) (Uranyl acetate and Lead citrate stain, X 41300).

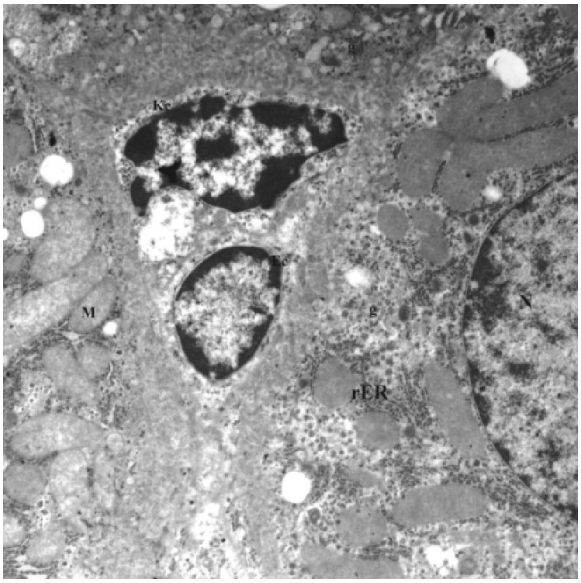

Fig. (18): An electron micrograph of liver of mouse from control group showing normal hepatocyte with round nucleus (N), rough endoplasmic reticulum (rER), mitochondria (M). Notice, endothelial cell (Ec) and Kupffer cell (Kc) (Uranyl acetate and Lead citrate stain, X 34400).

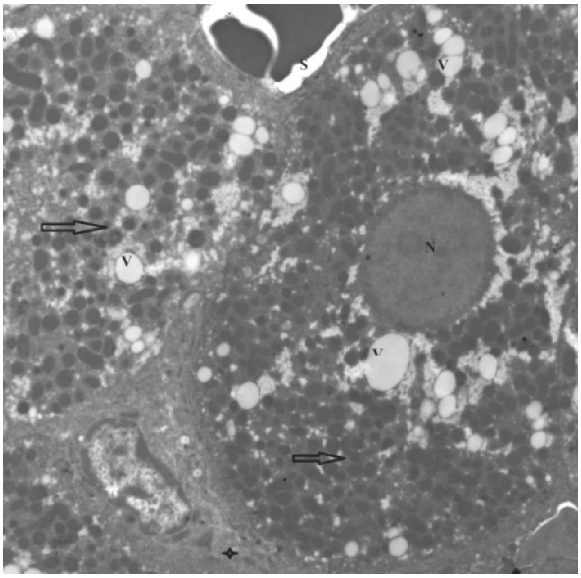

Fig. (19): An electron micrograph of liver of mouse treated with TCA showing hepatocyte with abnormal nuclear features (N), cytoplasm crowded with electron dens granules and indistinct organelles (Arrows), congested blood sinusoids (S), many vacuoles (V), hypertrophied blood sinusoidal lining with poorly identifiable organelles (Star) (Uranyl acetate and Lead citrate stain, X 20600).

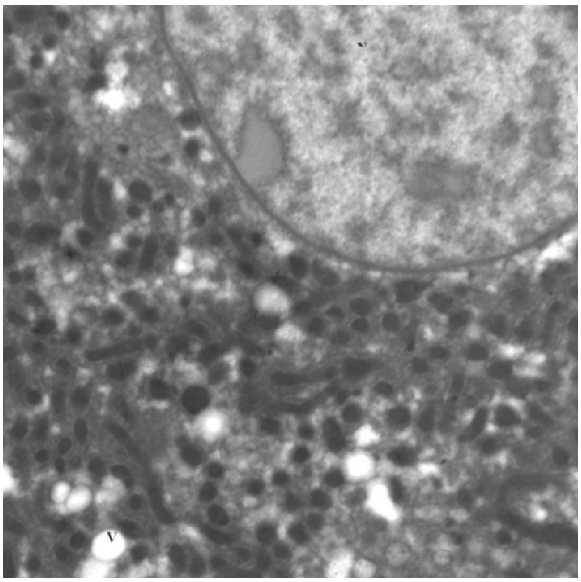

Fig. (20): An electron micrograph of liver of mouse treated with TCA showing hepatocyte with abnormal nuclear features (N), cytoplasm crowded with electron dens granules and indistinct organelles, many vacuoles (V) (Uranyl acetate and Lead citrate stain, X 41300).

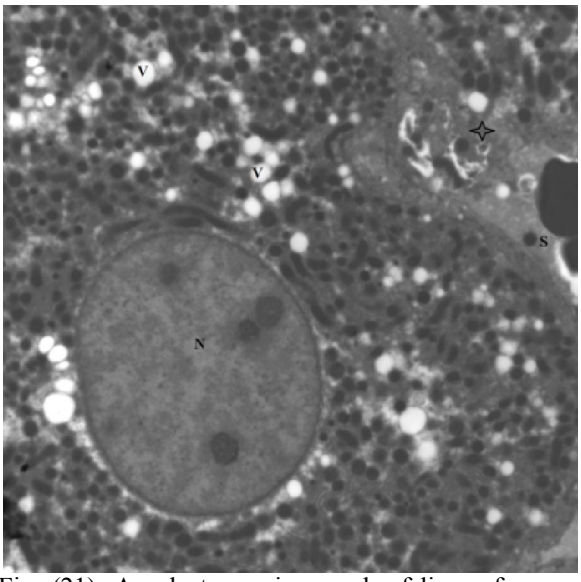

Fig. (21): An electron micrograph of liver of mouse treated with TCA showing hepatocyte with abnormal nuclear features (N) and increase nucleolei, cytoplasm crowded with electron dens granules and with indistinct organelles, congested blood sinusoids (S), many vacuoles (V). Note necrotic Kupffer cell (Star) (Uranyl acetate and Lead citrate stain, X 20600).

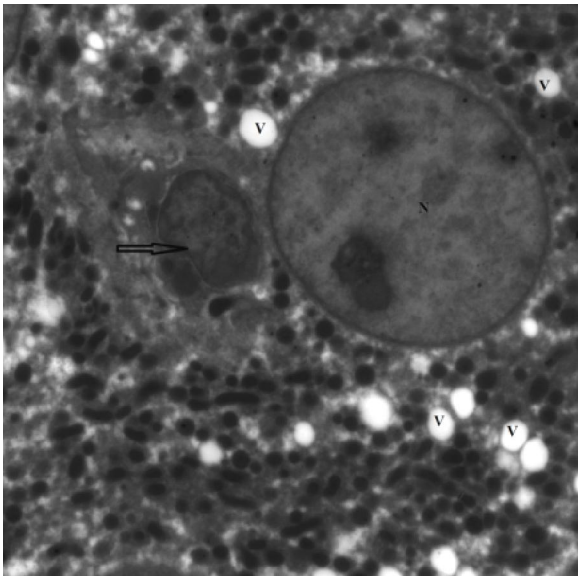

Fig. (22): An electron micrograph of liver of mouse treated with TCA showing hepatocyte with abnormal nuclear features (N) and increase nucleolei, cytoplasm crowded with electron dens granules and indistinct organelles, many vacuoles (V). Note necrotic cell with irregular fragmented nucleus (Arrow) (Uranyl acetate and Lead citrate stain, X 27500).

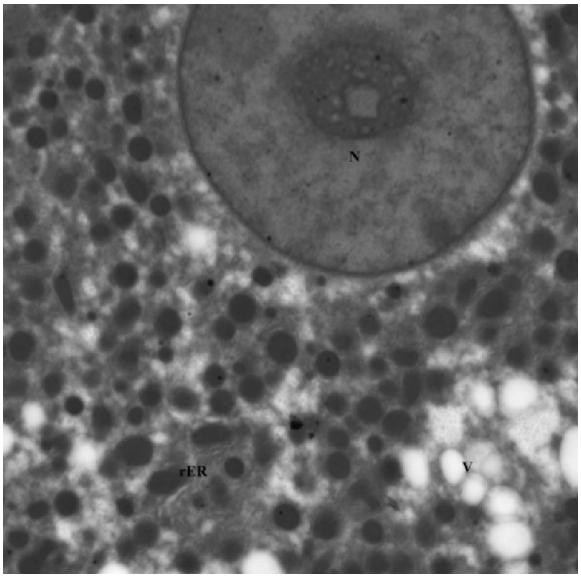

Fig. (23): An electron micrograph of liver of mouse treated with TCA showing hepatocyte with abnormal nuclear features (N), cytoplasm crowded with electron dens granules and indistinct organelles, few dilated rough endoplasmic reticulum (rER) with indistinct attached ribosomes, vacuoles (V) (Uranyl acetate and Lead citrate stain, X 34400).

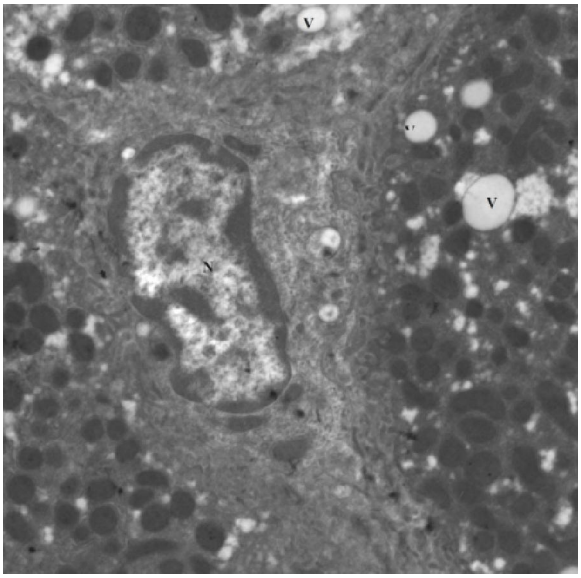

Fig. (24): An electron micrograph of liver of mouse treated with TCA showing hepatocyte with abnormal nuclear features (N), cytoplasm crowded with electron dens granules and indistinct organelles, vacuoles (V), hypertrophied blood sinusoidal lining cells with few and poorly identifiable organelles (Uranyl acetate and Lead citrate stain, X 41300).

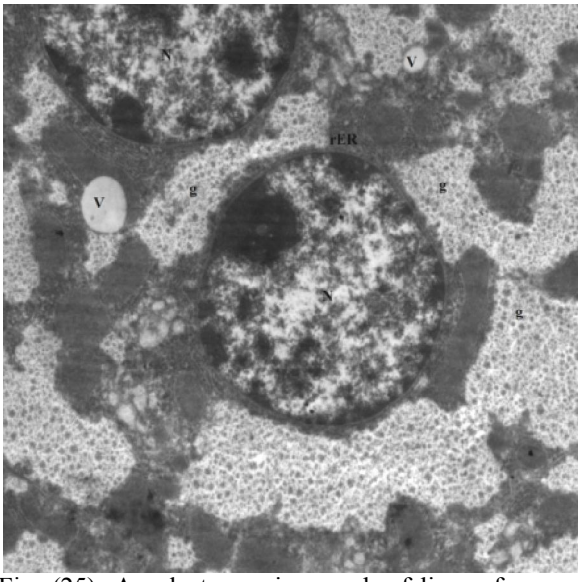

Fig. (25): An electron micrograph of liver of mouse treated with aqueous extract of *Juniperus phoenicea* showing hepatocyte with normal nuclear features (N), cytoplasm with intact organelles, vacuoles (V), many glycogen rosettes (g), rough endoplasmic reticulum (rER) (Uranyl acetate and Lead citrate stain, X 41300).

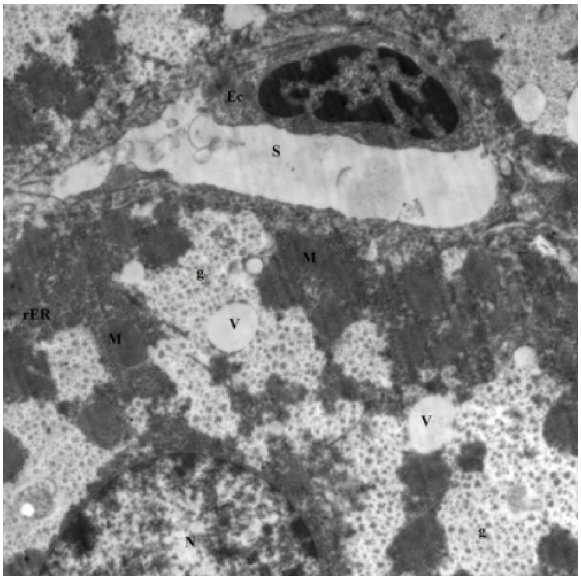

Fig. (26): An electron micrograph of liver of mouse treated with aqueous extract of *J.phoenicea* showing hepatocyte with normal nuclear features (N), cytoplasm with intact organelles, mitochondria (M) with distinct cristae, rough endoplasmic reticulum (rER), vacuoles (V), many glycogen rosettes (g), blood sinusoid (S), endothelium (Ec) (Uranyl acetate and Lead citrate stain, X 41300).

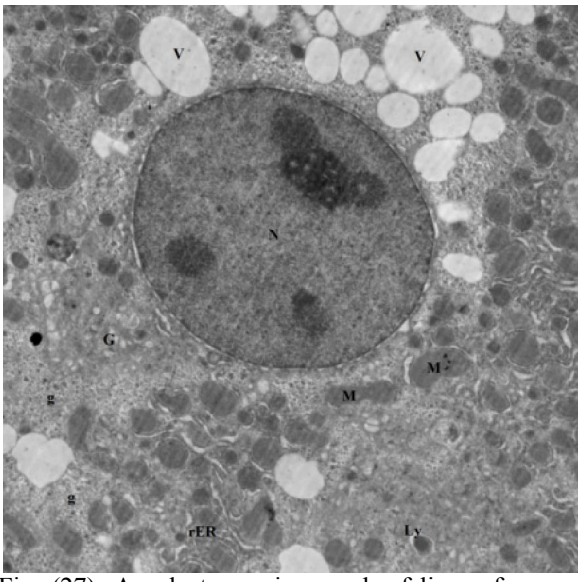

Fig. (27): An electron micrograph of liver of mouse treated with TCA and *J. phoenicea* showing hepatocyte nucleus (N) with obvious nucleolus associated with heterochromatin and slight dilated nuclear envelope, small mitochondria (M), lysosomes (Ly), vesicles of Golgi apparatus (G), slight dilated rough endoplasmic reticulum with less attached ribosomes (rER), lipid droplets (V), glycogen (g) (Uranyl acetate and Lead citrate stain, X 34400).

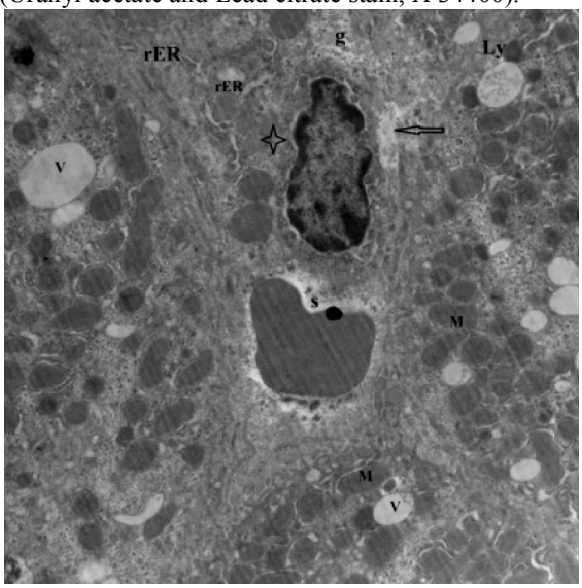

Fig. (28): An electron micrograph of liver of mouse treated with TCA and *J. phoenicea* showing small mitochondria (M), lysosomes (Ly), slight dilated rough endoplasmic reticulum (rER) with less attached ribosomes, lipid droplets (V), congested blood sinusoids (S), blood sinusoidal lining cell (Star), The sinusoidal surface of hepatocytes with few destructed microvilli (Arrow) (Uranyl acetate and Lead citrate stain, X 34400).

4. Discussion

In the present study no mortality and abnormalities in behavioral and external features or significant differences in food and water consumption were noted in mice treated with aqueous extract of *J. phoenicea*. On the contrary, **Thekra (2007)** observed some levels of toxicity in rats treated with *J. phoenicea* as evident by diarrhea suffered by the rats. Therefore, It can be concluded that *J. phoenicea* extracts at dose level used in the present work,40 mg/ kg body weight for 3 weeks have a low risk of toxicity. *J.phoenicea* L. leaves were found to contain active components due to the antioxidant properties owing to its content of flavonoid and phenolic compounds (**Ibrahim and Risk, 2005).** On the other hand, mice treated with TCA in drinking water showed decrease in feed intake, loss body furs, and rough coat. In addition, hypoactivities in some individuals were noticed. It was found that the aqueous extract of *J.phoenicea* lessend the undesired behavior and external features resulting from TCA administration.

The present investigation showed that the final body weight gain of mice treated with aqueous extract of *J.phoenicea* increased by 13.3% above initial body weight. Similar findings were recorded by **Al-Attar *et al*. (2016)** who found that, supplementation with J. procera leaves extract in normal mice showed a remarkable increase in the percentage change of body weight. Also, the present study proved that, administration of aqueous extract of *J. phoenicea* had a protective effect against TCA induced significant decline in the final body weight gain of mice compared to control group. This was found to be 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 trichloroacetate in drinking-water at dose level 32.5 mg/kg of body weight per day for 2 years **(DeAngelo *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 gains may be due to the combined action of cholinergic and oxidative stress **(Mansour and Mossa,2010 and Saafi *et al*.,2011)** and/or due to the increased degradation of lipids and proteins as a direct effect of toxic compound exposure **(Goel *et al*., 2005; Heikal and Soliman, 2010 and Mossa *et al*., 2011)**.

Mice treated with TCA developed marked hepatic deleterious as observed from significant increase of serum level of ALT enzyme and

insignificant increase in AST concentration as well as slight alteration in serum total protein. Intoxicated mice showed an increase in the activities of serum ALT and AST enzymes. The increase in these enzymes agreed with previous study of **De Angelo *et al*. (1997)** who recorded an increase in liver serum enzyme activity in rats exposed to trichloroacetate in drinking-water at dose level 32.5 mg/kg of body weight per day for 2 years. Meanwhile, intraperitoneal administration of TCA 200 mg/kg caused significant increases in AST but no significant differences in the level of ALT **(Demür and Elük, 2006).** it was also reported that TCA causes changes in biochemical parameters on rats following drinking water exposure **(Poon *et al*., 2000 and Celik and Temur, 2009).** On the other hand, **Bull *et al*. (1990)** found that administered TCA orally; 2000 ppm for 52 days caused a significant decrease in protein. **Kaneko (1985) and Bush (1991)** suggested that the increase in the serum levels of AST and ALT was associated with liver damage. Elevated serum levels of liver-specific enzymes as well as alterations in several other liver parameters and reduction in the levels of serum total proteins may indicate liver or kidney disease **(Sharpe *et al*.,1996)**. 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)**. **Celik (2007)** concluded that elevated serum enzymes probably resulted from damage of liver cells by TCA and subsequent leakage of the enzymes into plasma. The same authors added that, TCA treatment caused different effects on antioxidant defense system and lipid peroxidation in various tissues of rats at the end of the TCA treatment. Moreover, liver is a major organ attacked by reactive oxygen species **(Sanchez-Valle *et al*.,2012)**.

However, biochemical analysis revealed that administration of aqueous extract of *J. phoenicea* with TCA in the present study inhibited the elevation of serum liver function markers; ALT, AST and total protein concentrations compared to TCA only treated group. Similarly, rats administered *J.phoenicea* methanolic extract at dose 300 mg/kg body weight three times per week orally for one and half month caused more pronounced improvement in serum markers of liver in rats intoxicated with carbon tetra chloride **(Rizk *et al*., 2007).** This was also in accordance with the work done by **Ali *et al*. (2010)** who noticed that the protected group with *J. phoenicea* showed an even more remarkable effect with values close to those of the control group compared to the carbon tetra chloride intoxicated group. The same authors speculated that, the role of these extracts in restoring different enzymatic activities and in ameliorating the toxic and hazardous disorders induced on the liver may be due to a high antioxidant activity for the flavonoids. Also, **Alqasoumi *et al*. (3013)** found that, hinokiflavone isolated from aerial parts of *J. phoenicea* showed significant activity in reducing the elevated levels of liver enzymes; ALT and AST in carbon tetrarchloride intoxicated rats. **Gianni *et al*. (2005) and Ibrahim and Risk (2005)** Confirmed that, this improvement with natural extracts revealed the antioxidant effect of *J.phoenicea.* This is supported by the view that serum levels of transminases return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes **(Thabrew *et al*., 1987)**. The improvements in the level of total protein after treatment with *J. phoenicea* extract may be due to the promotion of ribosome assembly on endoplasmic reticulum which facilitates uninterrupted protein biosynthesis (**Rajesh and Latha, 2004**).

Histopathological methods are commonly used for detecting and evaluating organ-specific effects related to chemical exposure **(Travlos *et al*., 1996 and Crissman *et al*., 2004).** Our results are in agreement with **Acharya *et al*. (1997)** who observed that liver of rats exposed to TCA in drinking water at dose level 3.8 mg/kg-day for 10 weeks led to histological alterations in the liver such as loss of hepatic architecture. Also, **De Angelo *et al*. (2008)** confirmed that TCA caused loss of hepatic architecture. Inflammatory cells infiltration observed herein was also in accordance with **(US EPA,2011).** Also **De Angelo *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. The liver had consistently been identified as a target organ for TCA toxicity in short-term **(Goldsworthy and Popp, 1987** and **De Angelo *et al*., 1989)** and longer-term **(Bull *et al*., 1990 and Mather *et al*., 1990)**. **Bull *et al*. (1990)** suggested that TCA appears to increase lipid peroxidation, and the production of free radicals may be responsible for its effect.

However, vacuolation and necrosis of hepatocytes observed in our study were also observed by **Acharya *et al*. (1997) and US EPA (2011)**. Our TEM studies revealed accumulated lipid droplets of variable sizes. In addition, congested blood sinusoids and hypertrophied blood sinusoidal lining cells with few and poorly identifiable organelles were seen. Also, necrotic Kupffer cells with irregular fragmented nuclei were detected. It was also observed that TCA caused centrilobular necrosis and vacuolation in hepatocytes as recorded by **De Angelo *et al*. (2008)**. 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 and Mc Gavin,1995)**. Other liver effects noted in laboratory animals exposed to trichloroethylene include necrosis **(DEFRA and EA, 2004)**. **Robbins and Angell (1976)** regarded such vacuolation to represent primary morphologic response to many forms of cell injury. They also attributed it to the noxious effects of treatment on the cell membranes, both structurally and functionally, causing marked disturbances in its permeability system. This presumably leads to enhanced imbibitions of water into the cells. When it sufficiently accumulates in the cells, such intracellular water produced clear cytoplasmic vacuoles indication. The occurrence of these pathologic symptoms commonly referred to as hydropic degeneration or fatty degeneration caused by lipid abundance in such instance **(Albasha and Aza, 2014)**. The increase in fat was also explained by **Mori (1983)** that; the increased lipid in the hepatocytes after exposure to drugs or toxins could be due to impaired synthesis of lipoproteins or due to the abnormal transport of lipoproteins via Golgi apparatus and its secretory vacuoles. **Cervato *et al*. (2000)** confirmed that, Lipid peroxidation is a multistep reaction that can result in destruction of cellular membranes. Moreover, TCA has the ability to induce oxidative-stress responses, such as lipid peroxidation and oxidative DNA damage following administration of a single oral dose **(Parrish *et al*., 1996 and Hassoun and Ray, 2003)**. Furthermore, **Celik (2007)** found that TCA treatments caused different effects on antioxidant defense system and lipid peroxidation in various tissues of rats. The role of oxidative stress had been documented in the pathophysiology of numerous disorders (**Emami *et al*.,2011)**. The resultant intracellular stress may lead to cell death caused by either cell shrinkage and nuclear disassembly; apoptosis, or swelling and lyses; necrosis **(Singh *et al*.,2011)**.

Regarding the ultrastructure alterations of the cytoplasmic organelles; hepatocytes of mice treated with TCA revealed sever and abnormal features on cytoplasmic level with small electron dens granules represented lysosomes and mitochondria with indistinct details besides few dilated rER with indistinct attached ribosomes. It was previous reported that the endoplasmic reticulum stress is suggested to be an important mechanism in the pathogenesis of chronic liver diseases including drug induced liver injury and ischemia/reperfusion injury **(Dara *et al*., 2011). Kendry and Laszlo (1975)** concluded that the alteration of rER constitutes the main adverse effect of drug and toxins affecting the liver due to its important role in protein synthesis. Moreover, the destruction and decreased number of tubular arrays of the rER and mitochondrial- rER associates can be considered as an indication of impaired protein synthesis in the affected hepatocytes (**Hasan,2011**). Mitochondrial dysfunction is a common observation in several acute and chronic liver diseases such as drug-induced hepatotoxicity, hepatocellular carcinoma and ischemia/reperfusion injury **(Serviddio *et al*.,2010)**. Mitochondria play an essential role in regulating the intrinsic pathway of hepatocyte apoptosis as well as hepatocyte necrosis **(Kroemer and Reed, 2000; Schoemaker and Moshage, 2004 and Sola *et al*., 2007)**. However, mitochondrial function is important in the regulation of cellular life and death, including disease states. Moreover, the disturbance in mitochondrial function and distribution can be accompanied by significant morphological alterations **(Mumcuoglu *et al*.,2012).** The increase in the lysosomes number might also reflect an increase of the synthesis of hydrolytic and detoxifying enzymes **(Spit *et al*.,1981)**. The presence of several lysosomal bodies in any cell might be indicating the degenerative activity **(Farber,1984 and Kamble *et al*.,2013).**

TEM studies of hepatocytes of mice treated with TCA showed sever and abnormal nuclear features with abnormal and irregular nuclear shapes, as well as, decrease and abnormal heterochromatin distribution and increase nucleoli. However, the nucleus is one of the most prominent cellular organelles within eukaryotic cells, and altered nuclei shape is considered to be important for cell function **(Webster *et al*.,2009)**. It had been speculated that changes in nuclei shape might lead to changes in chromosome organization, which in turn can affect gene expression **(He *et al*.,2008)**. The condensation of nuclei is believed to be one of the two major morphological features of apoptosis; the cell suicide program **(Wang *et al*.,2011)**. Also, the hepatotoxic effects of chemical agents may involve different mechanisms of cytolethality. These mechanisms may either direct effect on organelles like mitochondria, endoplasmic reticulum, cytoskeleton, microtubules and nucleus or indirect effect on cellular organelles through the activation and inhibition of signalling kinases, transcription factors and gene-expression profiles **(Kedderis, 1996 and Kaplowitz, 2004)**.

The hypertrophy of hepatocytes in mice treated with TCA with abnormal mitotic division features were 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)**. Also**,** hypertrophy of hepatocytes was a characteristic feature in liver of TCA treated rats at dose level 3.8 mg/kg-day for 10 weeks **(Acharya *et al*.,1997)**. TCA increased cell proliferation in liver of female mice treated with 2, 6.67, or 20 mmol/L TCA in drinking water for 5 days **(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)**. Other liver effects noted in laboratory animals exposed to trichloroethylene include necrosis and cell hypertrophy **(DEFRA and EA, 2004)**. Ultrastructure nuclear changes in hepatocytes similar to those exhibited in malignant cells as reported by **Fortoul *et al*. (2008)**. Oxidative stress is thought to underlie carcinogenesis in humans induced by chemical exposure **(Klaunig, 2011 and Kryston, 2011).** On the other hand, an abnormal nuclei shape is also associated with cancer **(Zink *et al*.,2004)**.

The severe reactivity of most hepatocytes with PAS technique 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 in liver cells as assessed by PAS staining in a 52-week study of mice exposed to 1 or 2 g/L in drinking water. Also **Mather *et al.* (1990)** 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 glycogen accumulation. Furthermore, **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 and Mc Gavin (1995)** confirmed that glycogen degeneration or **glycogen** storage disease is characterized by excessive hepatic accumulation of glycogen.

Sections of liver of *J. phoenicea* treated mice showed that the central vein and hepatic cells were almost normal and there was a moderate reactivity of the hepatocytes with PAS in comparison with liver of the control group. No obvious ultrastucture changes were also observed in hepatocytes of mice treated with *J. phoenicea*. Most hepathocytes showed nuclei with normal nuclear features and well preserved organelles. These results are in agreement with **Ali *et al*. (2010)** who observed that the histological structure of the liver of *J. phoenicea* treated rats is almost normal. On the other hand, the results of the present study revealed the presence of some intracytoplasmic vacuoles in some liver cells as well as, certain stenosis of the hepatic sinusoids in livers of *J. phoenicea* treated mice. Moreover, TEM studies revealed that, many hepatocytes showed dispersed cytoplasm contained clumps of intact organelles and many glycogen rosettes. **Erog˘lu *et al.* (2009)** concluded that 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. **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 and Idodo-Umeh and Ogbeibu, 2010)**. 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)**. This may explain some pathological alterations in the liver of few animals treated with *J. phoenicea* in the present work.

The group of mice treated herein with TCA and *J. phoenicea* showed a marked tissue repair and a quite improvement of Liver tissues and disappearance of most pathological changes. Administration of aqueous extract of *J. phoenicea* also, lessened most sever ulterastructure changes in hepatocytes of TCA intoxicated mice. Moreover, hepatocytes showed reactivity to PAS close to that of the control group. These results were supported by the conclusion of **Rizk *et al*. (2007)** that *J. phoenicea* can be used efficiently for reducing severe liver disorders due to their potent antioxidant and antihepatotoxic activities. *J.phoenicea* L. leaves were found to contain antioxidant properties due to its content of flavonoid and phenolic compounds (**Ibrahim and Risk, 2005)**. Similarly, **Ali *et al.* (2010)** showed that methanolic extracts of *J. phoenicea* L have preventive action on carbon tetrachloride induced liver toxicity. The same authors attributed the role of these extracts in ameliorating the toxic and hazardous disorders induced in the liver to the high antioxidant activity of the flavonoid compounds as well as the phenolic compounds. However, **Di Carlo *et al.* (1999)** confirmed that these flavonoid and phenolic compounds possess significant antioxidant and thereby anti-hepatotoxic properties. **Alqasoumi *et al.* (3013)** showed that treatment with of hinokiflavone isolated from aerial parts of *J. phoenicea* showed great activity in restoring normal appearance of

hepatocytes and resulted in normal lobular pattern as well as, good recovery with absence of necrosis and fatty depositions with only mild congestion and minimal portal inflammation in rats received hepatotoxic agent carbon tetrarchloride. **Al-Attar *et al*. (2016)** reported that, *J.* procera leaves extracts illustrated a pronounced attenuation in thioacetamide-induced structural damage and hepatic cirrhosis in mice. They also, suggested that the supplementation of these extracts may act as antioxidant agents and could be an excellent adjuvant support in the therapy of hepatic damage. Furthermore, **Gdoura *et al*. (2013)** demonstrated a potential and beneficial effect of *J.Phoenicea* in attenuating oxidative stress and enhancing the body's own anti-oxidant defences in oxonate-treated rats. However**,** as peroxidative processes are involved in many degenerative physiopathologic events as aging, cancer and diabetes, a good pro-/antioxidant balance is very important for health **(Gutteridge,1995)**. The antioxidant could also stop chain reaction propagation promoted by lipidic radicals **(Cervato *et al*.,2000)**. The protective role of plants is due to the presence of antioxidative constituents like phenolics, flavonoids, tannins which are able to delay or inhibit the oxidative stress **(Lata *et al*., 2014**). The potency of the extracts will open new areas for the development of safe and cheap hepatoprotective drugs from natural wealth for treatment of a wide range of liver diseases **(Giulia *et al*.,1999 and El-Banhawey *et al*.,2007)**.

Inflammatory cells infiltration observed in the group treated with TCA was also disappeared in the group treated with TCA and aqueous extract of *J.phoenicea*. According to **Tumen *et al.* (2012)**, the experimental studies revealed that *J. phoenicea* display remarkable anti-inflammatory activities, which support the folkloric use of the plant**. Akkol *et al*. (2009)** confirmed that, Juniperus L. species have been used to cure various inflammatory and infectious diseases in folk medicine. However, Inflammation is a physiopathological response of living tissues to injuries that lead to the local accumulation of plasmatic fluid and white blood cells. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases. Therefore, the use of anti-inflammatory agents is helpful in the therapeutic treatment of these pathologies **(Gdoura *et al*., 2013).**

This study revealed that, the hypertrophy of hepatocytes with abnormal mitotic divisions which may indicate the carcinogenicity of TCA disappeared in mice treated with TCA and *J. phoenicea*. In the same respect, *J. phoenicea* L. leaves were found to contain active components and due to these components; they show anti-proliferative activity against abroad range of human tumors **(Rizk *et al*., 2007)** and it may have some effects against certain kinds of cancer **(Bayazit, 2004).** Moreover, ether extract of *J. phoenicea* L. was highly active against liver carcinoma cell line **(El-Sawi and Motawe 2008)**. Furthermore, flavonoid contents of *J. phoenicea* inhibit the tumor growth by interfering with some phases of the cell cycle **(Salucci *et al*., 2002)**.

Conclusion

The present investigation demonstrates that the damage found in the group received TCA was still detected in the group received both TCA and *J.phoenicea* but to a lesser extent. From the present work we concluded that, aqueous extract of *J. phoenicea* leaves at dose consumed in the traditional medicine (40 mg/kg body weight) for 3 weeks can be used efficiently for reducing severe liver disorders and led to an improvement in biochemical parameters as well as, histology and ultrastructure of the liver but did not completely improve the damage caused by TCA. This effect may be related to the potent antioxidant constituents and antihepatotoxic activities of this plant. Although administration of *J. phoenicea* alone did not cause any lethality or changes in the general behavior, it causes some histopathological alterations. Therefore, medicinal plants should not be taken haphazard for long periods and must be taken under medical supervision. Further studies concerned with *J. phoenicea* as used in folk medicine and its effects on the liver and also studies including human hepatocytes will be warranted to increase the validity of the work.

Acknowledgements

This work was supported financially by Libyan authority for research science and technology. Also, the authors appreciate all who help us to complete the present work.

References

1. Aboul-Ela, M.; El-Shaer, N. and Abd El-Azim, T. (2005). Chemical constituents and antihepatotoxic effect of the berries of Juniperus phoenicea part II. Nat. Prod. Sci., 11: 240-247.
2. Acharya, S; Mehta, K; Rodrigues, S; Pereira, J; Krishnan, S and Rao, C. (1995). Administration of subtoxic doses of t-butyl alcohol and trichloroacetic acid to male Wistar rats to study the interactive toxicity. Toxicol Lett 80:97-104.
3. Acharya, S.; Mehta, K.; Rodriguez, S.; Pereira, J.; Krishnan, S. and Rao. C.V. (1997). A histopathological study of liver and kidney in male Wistar rats treated with subtoxic doses of t-

butyl alcohol and trichloroacetic acid. Exp Toxicol Pathol.,49(5):369-73.
4. Akkol, E.K.; Güvenc, A.; Yesilada, E. (2009). A comparative study on the antinociceptive and anti-inflammatory activities of five *Juniperus* taxa. J. Ethnopharmacol. 125:30-336.
5. Albasha, M.O. and Aza, A.E. (2014). Effect of cadmium on the liver and amelioration by aqueous extracts of fenugreek seeds, rosemary, and cinnamon in guinea pigs: Histological and biochemical study. Cell Biology, 2(2): 34-44.
6. Ali, S.; Rizk, M; Ibrahim, N; Abdallah, M; Sharara, H. and Moustafa, M. (2010). Protective role of Juniperus phoenicae and cupressus sempervirens against CCL4. World J. Gastrointest. Pharmacol. Ther., 1 (6): 123-131.
7. Anonymous, (2005). Study and Evaluation of Natural Vegetation in Al-Jabel Al-Akhdar area. Study and evaluation of natural variation committee. South of Al-Jabel Al-Akhdar project, Libya, pp: 945.
8. Austin, E.W.; Parrish, J.M.; Kinder, D.H. and Bull, R.J. (1996). Lipid peroxidation and formation of 8-hydroxydeoxyguanosine from acute doses of halogenated acetic acids. Fundam Appl Toxicol., 31:77-82.
9. Al-Attar, A.M.; Alrobai, A.A. and Almalki, D.A. (2016). Effect of *Olea oleaster* and *Juniperus procera* leaves extracts on thioacetamide induced hepatic cirrhosis in male albino mice. Saudi Journal of Biological Sciences, 23(3): 363–371.
10. Al-Attar, A.M. and Shawush, N.A. (2015). Influence of olive and rosemary leaves extracts on chemically induced liver cirrhosis in male rats. Saudi J. Biol. Sci., 22: 157–163.
11. Alqasoumi, S.I; Farraj, A.I. and Abdel-Kader,M.S. (2013). Study of the hepatoprotective effect of *Juniperus phoenicea* constituents. Park J. Pharm. Sci.,26(5):999-1008.
12. Bancroft, J.D. and Gamble, M. (2008). Theory and practice of histological techniques.6th ed. Churchill Livingston Edinburgh, London and New York.
13. Bayazit, V. (2004). Cytotoxic effects of some animal and vegetable extracts and some chemicals on liver and colon carcinoma and myosarcoma. Saudi Med. J.,25:1329-1332.
14. Bogusz, M.J.; Al Tufail, M. and Hassan, H. (2002). How natural are 'natural herbal remedies'? A Saudi perspective. Adverse Drug React Toxicol Rev., 21: 219- 229.
15. Bull, R.J.; Sanchez, I.M.; Nelson, M. A.; Larson, J. L. and Lansing, A. J. (1990). Liver tumor induction in B6C3F1 mice by dichloroacetate and trichloroacetate Toxicol.,63(3): 341-359.
16. Bush, B. M. (1991). Interpretation of Laboratory results for small animal clinicians. Blackwell Scientific Publications London.
17. Carlton, W.W. and McGavin, M.D. (1995). Special veterinary pathology. second ed. Philadelphia, New York.
18. Celik, I. (2007). Determination of toxicity of trichloroacetic acid in rats: 50 days drinking water study. Pestic. Biochem. Physiol., 89:39-45.
19. Celik I. and Temur A. (2009). Determination hematotoxic and hepatotoxic effects of trichloroacetic acid at sublethal dosage in rats. Food and Chemical Toxicology 47(6): 1324–1326.
20. Cervato, G.; Carabelli, M.; Gervasio, S.; Cittera, A.; Cazzola, R. and Cestaro, A. (2000). Antioxidant properties of extracts of oregano (*Origanum vulgare*) leaf extracts. J. Food Biochem., 24: 453-465.
21. Chan, K. (2003). Some aspects of toxic contaminants in herbal medicines. Chemosphere, 52: 1361-1371.
22. Channel, S.R.; Latendresse, J.R.;. Kidney, J.K.; Grabau, J.H.;. Lane, J.W.; Steel-Goodwin, L. and. Gothaus, M. C. (1998). A subchronic exposure to trichloroethylene causes lipid peroxidation and hepatocellular proliferation in male B6C3F1 mouse liver. Toxicol Sci., 43: 145-54.
23. Chatterjee, A. and Pakrashi, S. (1994). The treatise on Indian medicinal plants. New Delhi: Publications and Information Directorate, pp 76.
24. Coleman, W.; Melton, R.; Kopfler, F.; Barone, K.; Aurand, T. and Jellison, M. (1980). Identification of organic compounds in a mutagenic extract of a surface drinking water by a computerized gas chromatography/mass spectrometry system (GC/MS/COM). Environ Sci Technol., 14:576-588.
25. Correll, D. and Johnston, M. (1970). Manualmof the vascular Plant of Texas. Texas Research Foundation. Renner, Texas; pp: 1881.
26. Crissman, J.W.; Goodman, D.G.; Hildebrandt, P.K.; Maronpot, R.R.; Prater, D.A.; Riley, J.H.; Seaman W.J. and Thake, D.C. (2004). Best practice guideline: toxicologic histopathology. Toxicol. Pathol.,32:126–131.
27. Dara, L.; Ji, C. and Kaplowitz, N. (2011).The contribution of endoplasmic reticulum stress to liver diseases. Hepatology, 53: 1752-1763.
28. De Angelo, A.; Daniel, F.; Most, B.and Olson, G. (1997). Failure of monochloroacetic acid and trichloroacetic acid administered in the drinking water to produce liver cancer in male F344/N rats. J Toxicol Environ Health,52:425-445.

29. DeAngelo, A.; Daniel, F.; Wong, D.; George, M. (2008). The induction of hepatocellular neoplasia by trichloroacetic acid administered in the drinking water of the male B6C3F1 mouse. J Toxicol Environ Health A 71:1056-1068.
30. DeAngelo, A; Daniel, F; McMillan, L; Wernsing, P; Savage, R, Jr. (1989). Species and strain sensitivity to the induction of peroxisome proliferation by chloroacetic acids. Toxicol Appl Pharmacol 101:285-298.
31. Demür, H. and Elük, Ü. (2006). Acute Effects of Trichloroacetic acid on Serum Enzyme Levels and Erythrocyte Carbonic Anhydrase Activity in Rats. Turk J Biol., 30: 121-125.
32. Department for Environment Food and Rural Affairs (DEFRA) and Environment Agency (EA) (2004). Contaminants in soil: Collation of toxicological data and intake values for humans. Trichloroethylene. Environment Agency. Bristol.
33. Di Carlo, G.; Mascolo, N.; Izzo, A.A. and Capasso, F. (1999). Flavonoids: old and new aspects of a class of natural therapeutic drugs. Life Sci., 65: 337-353.
34. Dudareva, N.; Negre, F.; Nagegowda, D. and Orlova, I. (2006). Plant volatiles: Recent advances and future perspectives. Crit. Rev. Plant Sci.; 25, 417-40.
35. El-Banhawey, M.A.; Ashry, M.A.; El-Ansary, A.K. and Aly, S.A. (2007). Effectof Curcuma longa or parziquantel on Schistosoma mansoni infected mice liver--histological and histochemical study. Indian J Exp Biol., 45: 877-889.
36. El-Sawi, S. A.; Motawae, H. M. and Alib, A. M. (2007). Chemical composition, cytotoxic activity and antimicrobial activity of essential oils of leaves and berries of *Juniperus phoenicae* L. grown in Egypt. Afr. J. Trad. CAM., 4 (4): 417 – 426.
37. El-Sawi, S. A. and Motawe, H.M. (2008). Labdane, Pimarane and Abietane diterpenes from the fruits of Juniperus phoenicae L. grown in Egypt and their Activities against Human Liver Carcinoma. Canadian Journal of pure and applied sciences., 2 (1): 115-122.
38. Emami, S.A.; Asgary, S.; Naderi, G, A.; Shams Ardekani, M.R.; Kasher, T.; Aslani, S.; Airin, A. and Sahebkar, A. (2011). Antioxidant activities of Juniperus foetidissima essential oils against several oxidative systems. Brazilian Journal of Pharmacognosy, 21(4): 627-634.
39. Erog˘lu, H. E.; Aksoy, A.; Hamzaog˘lu, E.; Budak, U. and Albayrak, S. (2009). Cytogenetic effects of nine Helichrysum taxa in human lymphocytes culture. Cytotechnology, 59:65–72.
40. Farber, E. (1984) Cellular biochemistry of the stepwise development of cancer with chemicals: G. H. A. Clowes memorial lecture. Cancer Res., 44: 5463-5474.
41. Fortoul, T.I.; Piñón-Zarate, G.; Diaz-Bech, M.E.; González-Villalva, A.; Mussali- Galante, P. et al. (2008) Spleen and bone marrow megakaryocytes as targets for inhaled vanadium. Histol Histopathol 23: 1321-1326.
42. Gdoura, N.; Murat, J.; Abdelmouleh, A. and Elfekl, A. (2013). Effects of *Juniperus phoenicea* extract on uricemia and activity of antioxidant enzymes in liver, erythrocyte and testis of hyperuricemic (oxonate-treated) rats. African Journal of Pharmacy and Pharmacology, 7(8):416-425.
43. Gianni, S.; Silvia, M.; Marivaittoria, M.; Martina, S.; Stefano, M.; Matteo, R. and Renato, B. (2005).Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals, antimicrobial in food, J. food Chem., 91:621-632.
44. Giulia, D.; Nicola, M.; Angelo, A.; Izzo, A. and Francessco, C. (1999). Flavonoids: Old and new aspects of class of natural therapeutic drugs. Life Sci.,65: 337-353.
45. Goel, A.; Dani, V. and Dhawan, D.K. (2005). Protective effects of zinc on lipid peroxidation, antioxidant enzymes and hepatic histoarchitecture in chlorpyrifosinduced toxicity. Chem Biol Interact.,156: 131–140.
46. Goldsworthy, T and Popp, J. (1987). Chlorinated hydrocarbon-induced peroxisomal enzyme activity in relation to species and organ carcinogenicity. Toxicol Appl Pharmacol., 88:225-233.
47. Gutteridge, J.M.C. (1995). Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin.Chem., 41: 1819-1828.
48. Harborne, J.B. (1993).The flavonoids advance in research. London: Chapman and Hall; pp. 460–461.
49. Hasan, S.M. (2011): Effect of lead toxicity on liver of male albino mice ultrastructural study. Kufa Med. Journal,14(1):214-220.
50. Hassoun, E. and Ray, S. (2003). The induction of oxidative stress and cellular death by the drinking water disinfection by-products, dichloroacetate and trichloroacetate in J774. A1 cells. Comp Biochem Physiol C Toxicol Pharmacol., 135:119-128.
51. He, S.; Dunn, K. L.; Espino, P. S.; Drobic, B.; Li, L.; Yu, J.; Sun, J. M.; Chen, H. Y.; Pritchard, S. and Davie, J. R. (2008): Chromatin organization and nuclei microenvironments in cancer cells. J. Cell Biochem. 104:2004-2015.

52. Heikal, T.M. and Soliman, M.S. (2010). Effect of fish oil supplementation on brain DNA damage and hepatic oxidant/antioxidant status in dimethoate-treated rats. J Egypt Soc Toxicol., 42: 1-9.
53. IARC. International Agency for Research on Cancer (1995). Trichloroethylene. In IARC Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 63, pp 75–158. Lyon, France.
54. Ibrahim, N.A. and Risk, M. (2005). Phytochemical investigation and hepatoprotective activity of *Juniperus phoenocea* leaves growing in Egypt. Bull. Fac. Pharm. Cairo Univ., 43:175-183.
55. Idodo-Umeh, G. and Ogbeibu, A.E. (2010). Bioaccumulation of the heavy metals in cassava tubers and plantain fruits grown in soils impacted with petroleum and non-petroleum activities. Research Journal of Environmental Sciences, 4: 33- 41.
56. IPCS. International Programme on Chemical Safety (2000). Disinfectants and disinfectant by-products. In Environmental Health Criteria, Geneva, Switzerland: World Health Organization.
57. Kamble, P.; Kulkarni, S. and Bhiwgade, D.A. (2013). Ultrastructural and Antioxidant Studies of Etoposide Treated Kidney of Rat. J Cancer Sci Ther., 5(4): 137- 141.
58. Kaneko, J. T. and Cornelius, C. E. (1985). Clinical Biochemistry of domestic animals. Academic Press. NY.
59. Kaplowitz, N. (2004). Drug-induced liver injury. Clin Infect Dis., 38: S44-S48.
60. Kedderis, G.L. (1996). Biochemical basis of hepatocellular injury. Toxicol Pathol., 24: 77-83.
61. Kendry, G. and Laszlo, B. (1975). Morphologic spectrum of drug-induced liver damage. Light and electron microscopic study Mag. Lorv. Arch., 28: 269.
62. Klaunig, J.E.; Wang, Z.; Pu, X. and Zhou, S. (2011). Oxidative stress and oxidative damage in chemical carcinogenesis. Toxicol Appl Pharmacol., 254: 86–99.
63. Kulisic-Bilusic, T.; Schmoller, K.; Schnabele, K.; Siracusa, L. and Ruberto, G. (2012). The anticarcinogenic potential of essential oil and aqueous infusion from caper (*Capparis spinosa* L.). Food Chemistry, 132: 261–267.
64. Kroemer, G. and Reed, J.C. (2000). Mitochondrial control of cell death. Nat Med., 6: 513-519.
65. Kryston, T.B.; Georgiev, A.B.; Pissis, P. and Georgakilas, A.G. (2011). Role of oxidative stress and DNA damage in human carcinogenesis. Mutat Res., 711: 193–201.
66. Larrey,D. (1997). Hepatotoxicity of herbal remedies. J. Hepatol., 26: 47-51.
67. Lata, S.; Singh, S.; Tiwari, K.N. and Upadhyay R. (2014). Evaluation of the antioxidant and hepatoprotective effect of *Phyllanthus fraternus* against a chemotherapeutic drug cyclophosphamide. App Biochem Biotech.,173:2163–2173.
68. Lin, H.; Huang, E.Y.; Chang, H.Y. and Chang Chien, C.C. (2005). Therapeutic effect of tropical applications of trichloroacetic acid for vaginal intraepithelial neoplasia after hysterectomy. Jap J Clin Oncol, 35: 651-654.
69. Lowry, O.H.; Rosebrough, N.J.; Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193(1): 265- 275.
70. Mansour, S.A. and Mossa, A.H. (2010). Adverse effects of lactational exposure to chlorpyrifos in suckling rats. Hum Exper Toxicol.,29: 77-92.
71. Mather, G.G.; Exon, J.H. and Koller, L.D. (1990).Subchronic 90-day toxicity of dichloroacetic and trichloroacetic acid in rats. Toxicology, 64:71–80.
72. Mori, M. (1983). Ultrastructural changes of hepatocyte organells induced by chemicals and their relation of fat accumulation in the liver. Acta Pathol. Jpn., 33: 911–922.
73. Mossa, A. H.; Refaie, A.A. and Ramadan, A. (2011). Effect of exposure to mixture of four organophosphate insecticides at no observed adverse effect level dose on rat liver: The protective role of vitamin C. Res J Envir Toxicol.,5: 323-335.
74. Mumcuoglu, E.U.; Hassanpour, R.; Tasel, S.F.; Perkins, G.; Martone, M.E. and Gurcan, M.N. (2012). Computerized detection and segmentation of mitochondria on electron microscope images. J. Microsc. Journal of Microscopy. 246 (3): 248-265.
75. NIOSH. (National Institute for Occupational Safety and Health). (2003). NIOSH pocket guide to chemical hazards. (97-140). Cincinnati, OH. http://www.cdc.gov/niosh/npg/npgdcas.html.
76. Paget, G.E. and Barnes, J.M. (1964). Evaluation of drug activities. In: Pharmacometrics Laurence DR, Bacharach AL, editors. New York: Academic Press, Pp.161.
77. Parrish, J.; Austin, E.; Stevens, D.; Kinder, D. and Bull, R. (1996). Haloacetate-induced oxidative damage to DNA in the liver of male B6C3F1 mice. Toxicology,110:103-111.
78. Pereira, M.A. (1996). Carcinogenic activity of dichloroacetic acid and trichloroacetic acid in the

liver of female B6C3F1 mice. Fundam Appl Toxicol., 31: 192–199.
79. Pal, R.K. and Manoj, J. (2011). Hepatoprotective activity of alcoholic and aqueous extracts of fruits of *Luffa cylindrica* Linn in rats. Annals of Biological Research, 2 (1): 132-141.
80. Pereira, M.A. and Phelps, J.B. (1996). Promotion by dichloroacetic acid and trichloroacetic acid of N-methyl- N-nitrosourea-initiated cancer in the liver of female B6C3F1 mice. Cancer Lett., 102: 133–141.
81. Pereira, M.A.; Kramer, P.M.; Conran, P.B. and Tao, L. (2001). Effect of chloroform on dichloroacetic acid and trichloroacetic acid-induced hypomethylation and expression of the c-myc gene and on their promotion of liver and kidney tumors in mice. Carcinogenesis, 22: 1511–1519.
82. Poon, R.; Nadeau, B. and Chu, I. (2000). Biochemical effects of chloral hydrate on male rats following 7-day drinking water exposure. J Appl Toxicol., 20: 455-61.
83. Poon, R.; Nakai, J. Yagminas, A. et al. (2002). Subchronic toxicity of chloral hydrate on rats: a drinking water study. J Appl Toxicol., 22: 227-36.
84. Rajesh, M. and Latha, M. (2004). Preliminary evaluation of the antihepatotoxic activity of Kamilari, a polyherbal formulation. J. Ethnopharmacol., 91: 99-104.
85. Reimann, S.; Grob, K. and Frank, H. (1996). Chloroacetic acids in rainwater. Environ Sci Technol., 30:2340-2344.
86. Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of serum glutamate oxaloacetic acid and Pyruic acid transaminases. Am. J. Clin. Path., 28:56-63.
87. Reynolds, E.S. (1963). The use of lead citrate at high pH as electron opaque stain in electron microscopy. J. Cell Biol.,17:208-212.
88. Rizk, M; Abdallah, M; Sharara, H; Ali, S; Ibrahim, N and Mustafa, M. (2007). Efficiency of Cupressus sempiverens L. and Juniperus phoenicea against carbon tetrachloride hepatotoxicity in Rats. Trends Med. Res.,2 (2): 83-94.
89. Robbins, S.L. and Angell, M. (1976): Basic Pathology.2nd ed. W.B. Saunders Company, Phildelphia, London.
90. Saafi, EB.; Louedi, M.; Elfeki, A.; Zakhama, A.and Najjar, M.F. (2011). Protective effect of date palm fruit extract (Phoenix dactylifera L.) on dimethoate inducedoxidative stress in rat liver. Exp Toxicol Pathol., 63: 433–441.
91. Salah, S. H.; Abdou, H. S.; Abd El Azeem, A. S. and Abdel-Rahim, E.A. (2011). The antioxidative effects of some medicinal plants as hypoglycemic agents on chromosomal aberration and abnormal nucleic acids metabolism produced by diabetes stress in male adult albino rats. Journal of Diabetes Mellitus.1(1), 6-14.
92. Salucci, M., Stivala, L. A., Maiani, G., Bugianesi, R., and Vannini, V. (2002). Flavonoids uptake and their effect on cell cycle of human colon adenocarcinoma cells (CaCo2). British Journal of Cancer, 86(10): 1645–1651.
93. Sanchez-Valle, V.; Chavez-Tapia, N.C.; Uribe, M.; Mendez-Sanchez, N. (2012). Role of oxidative stress and molecular changes in liver fibrosis: A review. Curr. Med. Chem., 19:4850–4860.
94. Schoemaker, M.H. and Moshage, H. (2004). Defying death: The hepatocyte's survival kit. Clin Sci (Lond)., 107: 13-25.
95. Serviddio, G.; Bellanti, F.; Sastre, J.; Vendemiale, G. and Altomare, E. (2010). Targeting mitochondria: A new promising approach for the treatment of liver diseases. Curr Med Chem., 17: 2325-2337.
96. Sharpe, P.C.; McBride, R.and Archbold, G.P. (1996). Biochemical markers of alcohol abuse. QJM., 89: 137-144.
97. Shkukani, H.; Salhab, A. and Disi, A. (2008). Antifertility Effect of Ethanolic Extract of Juniperus phoenica (L.) in Male Albino Rats. Journal of Herbal Pharmacotherapy. 7(3-4): 179-189.
98. Singh, A.; Bhat, T.K. and Sharma, O.P. (2011). Clinical Biochemistry of Hepatotoxicity. J Clinic Toxicol., S4:001- 019.
99. Singh, S.; Lata, S. and Tiwari, K. (2014). Aegle marmelos leaves protect Liver against Toxic effects of Cyclophosphamide in mice. New York Science Journal,7(9): 43-53.
100. Sofowora, A. (1993). Medicinal plants and Traditional Medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria; 270- 289.
101. Sola, S.; Aranha, M.M.; Steer, C.J. and Rodrigues, C.M. (2007).Game and players: Mitochondrial apoptosis and the therapeutic potential of ursodeoxycholic acid. Curr Issues Mol Biol., 9: 123-138.
102. Soyal, D.; Jindal, A.; Singh, I. and Goyal, P.K. (2007). Protective capacity of Rosemary extract against radiation induced hepatic injury in mice. Iran. J. Radiat. Res., 4 (4), 161-168.
103. Spit, b.J.; Wibowo, A.A. Feron, V.J. and Zielhuis, R.L. (1981). kidneys of rabbits treated with lead acetate. Arch Toxicol., 49: 85-91.
104. Stickel, F.; Datz, C. Hampe, J. and Bataller, R. (2017). Pathophysiology and management of

alcoholic liver disease: update 2016. Gut Liver; 11(2): 173-188.
105. Thabrew, M.; Joice and Rajatissa, W. (1987). A comparative study of the efficacy of Pavetta indica and Osbekia octandra in the treatment of liver dysfunction. Planta Medica,53:239-241.
106. Thekra, M. (2007). Effet of acetonic extract phoencian junipers fruits (Juniperus phoenicea L.) on some reproductive parameters in albino mice (Balb/c). M. Sc thesis, University of Basrah: 1-40.
107. Topc¸, G.; Erenler, u, R. and Akmak O. C. (1999). Diterpenes from the berries of Juniperus excelsa," Phytochemistry, 50(7):1195–1199.
108. Travlos, G.S.; Morris, R.W.; Elwell, M.R.; Duke, A.; Rosenblum, S. and Thompson, M.B. (1996). Frequency and relationship of clinical chemistry and liver and kidney histopathology findings in 13- week toxicity studies in rats. Toxicology, 107:17–29.
109. Tülüncü, M.; Özbek, H.; Bayram, I.; Cengiz, N.; Özgökce, F. and Him, A. (2010). The effects of diethylether extract of Helichrysum plicatum Dc. Subsp. Plicatum and tanacetum balsamita L. Subsp. Balsamitoides (Sch. Bip.) Grierson (Asteraceae) on the acute liver toxicity in rats. Asian. J. Anim. Vet. Adv.,5(7): 465-471.
110. Tumen, I.; S¨untar, I.; Keles, H. and Akko, E. (2012). A therapeutic approach for wound Healing by using essential oils of cupressus and Juniperus species Growing in turkey. Evidence - based complementary and Alternative medicine.10:1-7.
111. Uphof, J. (1968). Dictionary of Economic Plants, Verlag von Cramer, Germany. p 290.
112. U.S. EPA. (U.S. Environmental Protection Agency). (2005). Drinking water addendum to the criteria document for trichloroacetic acid. (EPA 822-R-05-010). Washington, DC: U.S. Environmental Protection Agency, Office of Water.
113. US. EPA (United States Environmental Protection Agency). (2011). Trichloroacetic acid (TCA). CASRN., 76:03-9.
114. Wang, Y., Liu, F., Wei, Y. and Liu, D. (2011): The effect of exogenous melamine on rat hippocampal neurons. Toxicol. Ind. Health, 27: 571-576.
115. Webster, M.; Witkin, K.L. and Cohen-Fix, O. (2009): Sizing up the nucleus: nuclei shape, size and nuclei-envelope assembly. J. Cell Sci. 122:1477-1486.
116. Zink, D.; Fischer, A.H. and Nickerson, J.A. (2004): Nuclear structure in cancer cells. Nat. Rev. Cancer, 4:677-87.

12/20/2017