Assessment of the effects of *Capparis spinosa* on the testes and epididymis of albino mice intoxicated with trichloroacetic acid

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Abstract: The present investigation was undertaken to evaluate the effectof *Capparis spinosa* leaves on the testicular tissue and epididymis in normal and trichloroacetic acid(TCA) intoxication mice.Healthy male mice (20-26gm body weight, 8 to 10 weeks old) were divided into 6 groups. Group I was the control group; group II treated orally with honey (40 mg/kg body weight for 3 weeks), group III treated orally with a mixture of *Capparis spinosa* leaves powder and honey(40 mg/kg for 3 weeks), groupIV treated orally with aqueous extract of *Capparis spinosa* leaves powder(40 mg/kg body weight for 3 weeks), groupV treated with TCA in drinking water (500mg/kg for 3 and 6 weeks, then left for 3 weeks for recovery) and groupVI (Regeneration group) was given TCA for 6 weeks then treated with a mixture of *Capparis spinosa* leaves powder and honey (40 mg/kg for 3 weeks).Histological sections of testis of mice administrated with mixture of leavespowderof *Capparis spinosa* and honey or aqueous extract of *Capparis spinosa* showed mild abnormalities in some seminiferous tubules including disorganization or proliferation of germ cells lead to obscure their lumen, slight decrease in the spermatozoa in some seminiferous, clumped interstitial cells and congestion of blood vessels in interstitial tissue. Slight degeneration in interstitial tissue was also seen. Administrated of TCA for 3 and 6 weeks showed severe and variable lesions in testicular tissue including disorganization and decrease of germ cells in some seminiferous tubules, sloughing of germ cells, desquamation of germ cells with accumulation of the necrotic debris in the tubular lumen. Some of the seminiferous tubules were completely devoid of mature sperms and absence of spermiogenesis. Marked atrophy of seminiferous tubules associated with edema of the intertubular tissue and decrease interstitial cells were commonly observed. Such lesions in the testicular tissues were most pronounced in TCA recovery group.Animal intoxicated with TCA and treated with the mixture of leavespowderof *Capparis spinosa* and honey showed noticeable decrease of the testicular lesions. There were no significant lesions detected in the epididymal sections in mixture of leavespowderof *Capparis spinosa* and honey treated groups. The treatment with TCA caused many and sever degenerated changes including epididymal ductus with abnormal shape and many showed destructed and decrease in stereocilia of epididymal tubules, exfoliated, disorganized and necrotic epithelial cells as evident by the presence of pyknosis of nuclei, vacuolization in the epithelium, with accumulation of the necrotic debris in the tubular lumen, as well as, reduction in sperm density. Hyperplasia mononuclear cells infiltration and edema in the intertubular connective tissue and intertubular haemorrhage were seen in most of the examined specimens. These histopathological alterations became more pronounced in TCA recovery group. Such alterations were less prominent in mice intoxicated with TCA and treated with mixture of *Capparis spinosa* and honey.
[Aglal A. Alzergy and Saad M.S. Elgharbawy. **Assessment of the effects of *Capparis spinosa* on the testes and epididymis of albino mice intoxicated with trichloroacetic acid.** *J Am Sci* 2015;11(8):28-42]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 6

Key words:*Capparis spinosa*, trichloroaceticacid,histopathological changes,testicular tissue and epididymis, mice.

1. Introduction

Herbal medicine is a complementary therapy that uses plants to treat disordersIn various countries throughout the world, a large number of plants have been used as therapeutic agents in the traditional medicine **(Kumar *et al*., 2012).** *Capparis spinosa* L. family Capparidaceae is one of the most common aromatic plants growing in wild in the dry regions around the west or central Asia and the Mediterranean basin. *Capparis spinosa* is well known with its common name Caper in different countries **and** used in traditional medicines to cure various illnesses **(Azaizeh *et al*., 2003 and Tlili *et al*., 2011).** Caper is very good sources of glucosinolates (glucocapparin, glucoiberin, sinigrin, glucobrassicin), flavonoids (rutin, kaempferol), phenolic acids, alkaloids, which are known to provide health-improving benefits due to their various biological activities (antioxidant, anticancerogenic, antimicrobial, antimutagenic) **(Kulisic-Bilusic *et al*., 2012).** The caper has been known for centuries in traditional phytomedicine, it was found to possess various important medicinal properties and used in phytomedicine as antifungal **(Ali-Shtayeh and Abu Ghdeib 1999)**, anti-inflammatory **(Al-Said *et al.* 1988)**, antihyperlipidemic **(Eddouks *et al*., 2005)**, diuretic, antihypertensive,

poultice **(Çaliş *et al*., 1999)** and anti-hepatotoxic agent **(Gadgoli and Mishra, 1999**).

Honey is a natural source of antioxidants and it also contains vitamins, minerals, enzymes, acids and sugar **(Frankel *et al*., 1998; Taormina *et al*., 2001 and Chepulis *et al*., 2009).** It is used in traditional and Islamic medication as one of the most important and as a valuable remedy **(Sarahroodi, 2012).** Honey is traditionally consumed as a nutrient, as well as for the enhancement of fertility **(Abu-Zinadah *et al*., 2013)**. Honey was also found to reduces inflammation, edema, and exudation and stimulates tissue regeneration that promotes healing **(Al-Waili and Saloom, 1999).** This protective effect of honey may be attributed to the biologically active compounds such as vitamins, flavonoids, and antioxidants that work together to scavenge free radicals. Therefore, bees' honey can be used to protect animals and humans against the adverse effects of melamine toxicity **(El Rabey *et al*., 2013).** In honey (20 mg/ kg body weight/day for 4 weeks) treated rats, normal and regular seminiferous tubules were clearly appears. The germinal cells were greatly regulated with normal diameter and mild dilatation of the somniferous tubules with normal complete spermatogenic series **(Abu-Zinadah *et al*., 2013).**

Widespread use of Trichloroethylene (TCE) as resulted in the contamination of surface water, ground water, and hazardous waste disposal sites **(Coleman *et al*., 1984).** Dichloroacetic acid (DCA) and trichloroacetic acid (TCA) are major metabolites of Trichloroethylene (TCE) (**Bruckner *et al*., 1989; IARC, 1995).** Trichloroaceticacid (TCA) is a colorless to white crystalline solid with a sharp, pungent odor **(NIOSH, 2003).** TCA is formed from organic material during water chlorination **(IPCS, 2000; Coleman *et al*., 1980)**. Trichloroaceticacid (TCA) are known to be contaminants in drinking water **(Acharya *et al*., 1997)** and has 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).** TCA was detected in vegetables, fruits, and grains **(Reimann *et al*., 1996).** Therefore, human exposure to TCA can also occur via food consumption.TCA is mainly used in the production of its sodium salt, which is used in many industries (e.g., as an herbicide, etching agent and antiseptic). An increase in incidence of benign and malignant liver tumors was observed in mice orally administered trichloroethylene. Also, increased incidences of testicular tumors and renal-cell tumors were observed in rats exposed to trichloroethylene by inhalation or ingestion **(IARC, 1995).**

The use of histopathological evaluations of animal tissue is of prominent role in male reproductive risk assessment. It provides information on the severity of the toxicity and cellular site of the damage as well as the possible potential for recovery **(USEPA, 1996).** Mouse testes have been established as a useful model for studies reproductive toxicology **(Xie *et al*., 2014).** Testis is the major organ for male sexual development and fertility **(Wilhelm, *et al*., 2007).** It secretes hormones to promote male-specific traits and produces sperm for reproduction. The epididymis is an important part of the male reproductive system. It provides the favorable milieu for acquisition of fertilizing ability, motility, storage and survival of spermatozoa **(Brooks, 1981).** The information regarding the effect of the mixture of leaves powder of *Capparis spinosa* and honey which used traditionally in Libya for treatment many disorder on the histology of testis and epididymis is nearly lacking which forms the stimulus for the present study.

Therefore, the present work aimed to study the possible protective role of *Capparis spinosa* leaves which used in traditional medicine in Libya for treatment of a variety of diseases including cancer on histological structure of the testis and epididymis of mice intoxicated with trichloroacetic acid. The objective of this study is also to detect whether *Capparis spinosa* leaves have deleterious side effects on normal tissuestructure or no.

2. Material and Methods

Experimental animals

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

Materials used:

Fresh plants of *Capparis spinosa* were collected from Blgray region Algabal Alakhder in Albayda - Libya between March and April 2012. The plant was authenticated by Department of Botany, Faculty of Agriculture, Omar El mukhtar university, Albayda - 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.

Honey sample:

Natural bees honey **(**vehicle**)** used in this study was purchased from the local honey market in Al Bayda - Libya. The honey was collected form beehives

built on Algabal Alakhder - Libya. This honey is also locally known as Seder honey. It was filtered to remove solid particles.

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

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

Preparation of the aqueous extracts of *Capparis spinosa*:

Leaves powder of *Capparis spinosa* (400mg) were mixed with 40 ml boiling water and steeped in boiled water in a closed vessel for few minutes. The crude extracts were filtered by a piece of gauze and the freshly prepared filtrates were left for a few minutes before administration. Each mouse received orally 0.1ml at dose level 40mg/kg body weight.

Trichloroacetic acid (TCA) was purchased from (Sigma Co, Germany). TCA was chosen because it had been reported to 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).**

Experimental design

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

Group I (control group): It received distilled water at dose level 4 ml/kg by oral gavage for 3 and 6 successive weeks and served as negative control (untreated control group).

Group II (vehicle group): Given orally by oral gavage natural bees honey at dose level 4 ml/kg for 3 successive weeks.

Group III (*Capparis spinosa* and honey treated group): Treated orally by oral gavage a mixture of *Capparis spinosa* leaves powder and honey at dose level 40 mg/kg body weight suspended in 0.1ml honey once per day for 3 successive weeks.

Group IV (*Capparis spinosa* water treated group): Given orally by oral gavage aqueousextract of *Capparis spinosa* leaves powder at dose level 40 mg/kg body weight once per day for 3 successive weeks.

Group V (TCA treated group): Treated with TCA at dose level 500 mg/kg body weight in drinking water for 3 and 6 successive weeks (Doses were estimated based on default drinking water intake values for mice). After the end of the experimental period the animals in this group left for recovered and known as **recovery group.**

Group VI (*Capparis spinosa* and honey after TCA treated group) (Regeneration group): Received TCA at dose level 500 mg/kg body weight in drinking water for 6 successive weeks then treated orally by oral gavage with a mixture of *Capparis spinosa* leaves powder and honey at dose level 40 mg/kg body weight once per day for 3 successive weeks.

Histopathological studies:

Mice were sacrificed by cervical dislocation, testes and epididymis were carefully excised. The tissues were immediately immersed in Bouin's fixative for 24 hours, dehydrated in ascending grades of ethyl alcohol, cleared in xylene, impregnated in paraffin wax and sections of 5–7 µm thickness were taken. The deparaffined sections were stained with Harri's haematoxylin and eosin (H&E) and Crossmon's trichrome stain according to **Bancroft & Gamble (2008)**. The slides were covered by Canada balsam and cover slide. Histological sections were examined by light microscope with digital camera (Nikon Eclipse E400) and histopathological changes were recognized and photographed.

3. Results

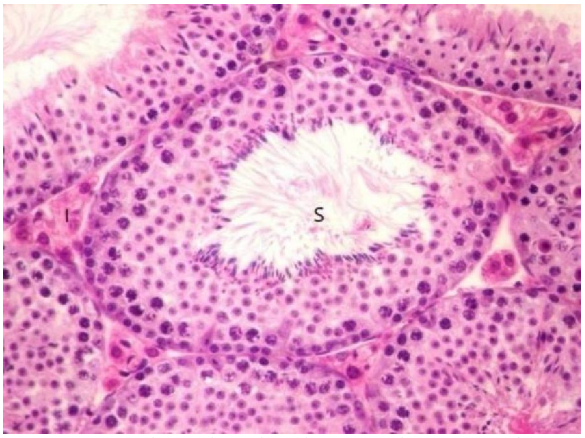


Fig.(1): A section of testis of mouse from control group showing normal architecture of testicular tissue, Seminiferous tubule (S) interstitial tissue (I) (H&E stain, X200).

Sections of testes of control group revealed that the testis consisted of seminiferous tubules in a compact arrangement with the interstitium tissue stroma, containing clumps of interstitial Leydig cells. Seminiferous tubules had well definite tubular basement membrane and lined by germ cells epithelium reflected different stages of the spermatogenic cycles and supporting Sertoli cells. The spermatogenic cells include the successive stages of spermatogenesis; spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa. Seminiferous tubules has a lumen which may contain some spermatozoa (Figs.1 & 2).

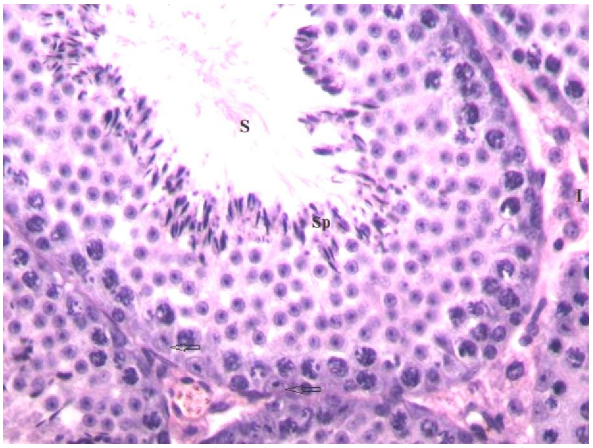


Fig.(2): A section of testis of mouse from control group showing normal architecture of testicular tissue, Seminiferous tubule (S)with intact spermatogenic cycle and mature spermatozoa (Sp), interstitial tissue(I), Sertoli cells (Arrows) (H&E stain, X400).

Histological examination of the testis of mice from honey treated group revealed normal testicular architecture with complete normal spermatogenic layers and well developed sperm. Interstitial Leydig cells showed no structural abnormalities (Fig.3).

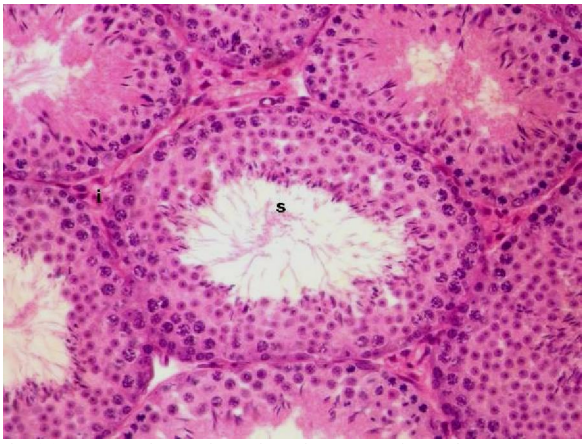
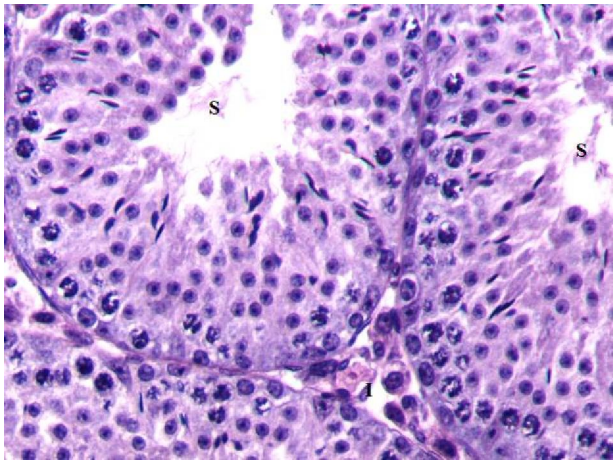


Fig.(3): A section of testis of mouse from honey treated group showing normal architecture, Seminiferous tubule(S) and interstitial tissue(I) (H&E stain,X200).

Seminiferous tubules with nearly normal cycle of spermatogenesis, well preserved Sertoli cells and well delineated tubular basement membrane were seen in *testis* of mice treated with mixture of leaves powder of *Capparis spinosa* and honey. While, disorganization and proliferation of germ cells in some seminiferous tubules leading to obscure their lumina were observed. Also, as light decrease in the spermatozoa was evident in some seminiferous tubules. In addition, clumped interstitial cells and congestion of blood vessels in the interstitial tissue were observed in most of the examined specimens. However, degenerated interstitial tissue and slight decrease of intestinal cells in few animals were noticed (Figs.4 & 5).



Fig(4): A section of testis of mouse from *Capparis spinosa* and honey treated group showing intact Seminiferous tubule (S) with slight decrease in spermatozoa, interstitial Leydig cells (I) (H&E stain, X400).

Most seminiferous tubules with normal features and intact spermatogenesis cycle were noticed in histological sections of testes of mice treated with aqueous extract of *Capparis spinosa*. While, disorganization and proliferation of germ cells in few Seminiferous tubules leading to obscure their lumina were evident. Slight degeneration in interstitial tissue was also seen. In addition, congestion and dilation of blood vessels in interstitial tissue were observed in most examined specimens (Figs.6 & 7).

Lesions observed in TCA treated groups were severe and variable. Microscopic examination of testis sections of male Swiss albino mice treated with TCA alone for 3 and 6 weeks showed disorganization and decrease of germ cells in some seminiferous tubules. Sloughing of germ cells with accumulation of necrotic debris in the tubular lumina were also observed. Some of the seminiferous tubules were completely devoid of mature sperms and absence of spermiogenesis in the majority of the seminiferous tubules were noticed. Marked atrophy of seminiferous tubules associated with edema of the intertubular tissue and decrease interstitial cells were commonly observed(Figs.8& 9). Such lesions in the testicular tissues were more pronounced in recovery group (Fig.10).

Animals intoxicated with TCA and treated with the mixture of leavespowderof *Capparis spinosa* and honey (Regeneration group) showed noticeable decrease of the testicular lesions. The seminiferous tubules appeared with slight irregular tubular basement membrane and slight decreased germ cells. However, mature spermatozoa in seminiferous tubules were

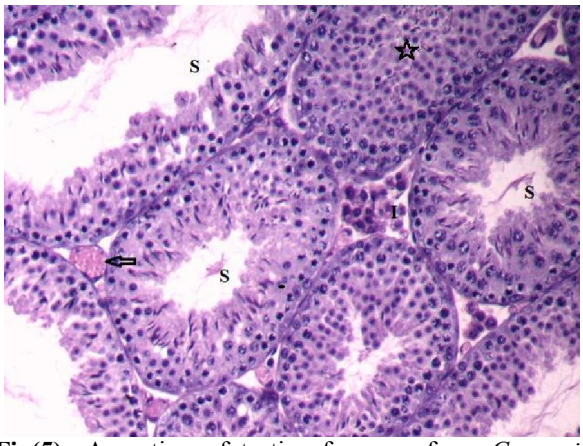
demonstrated. Also, an improvement of interstitium tissue was seen (Fig.11).

Epididymis of control mice showed normal histological architecture; lined by pseudostratified epithelia composed of basal and columnar cells with stereocilia. The thickness of the epididymal epithelium varied from the thickest portion in the proximal caput to the thinnest in the caudal region. The ductus epididymis surrounded by a connective tissue sheath containing smooth muscle fibers and blood vessels. The lumina of epididymal duct contained stored spermatozoa (Figs.12,13 & 14).

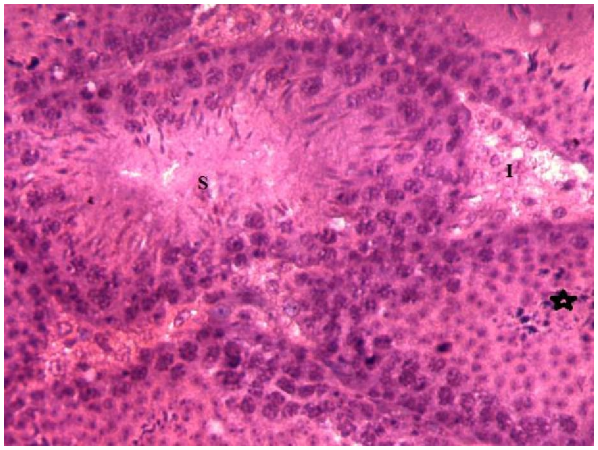
Histological examinations of the epididymis indicated that there were no detectable abnormalities and alterations in epididymal tissue of mice treated with honey in comparison to the control group. There were no obvious lesions detected in the epididymal sections in mixture of leaves powder of *Capparis spinosa* and honey treated groups. However some sections showed a remarkable decrease in spermatozoa. Also, congestion, small hemorrhagic areas and mild edema in the connective tissue surrounding epididymal tube were observed in other sections (Figs.15 & 16).

Treatment with TCA caused many histological changes in the epididymal tissue. In some sections; epididymal tube lost its normal shape and other sections showed destructed and decreased stereocilia. Exfoliated, disorganized and necrotic epithelial cells as evident by the presence of pyknotic nuclei, with accumulation of necrotic debris in the tubular lumen, and reduction in sperm density. Most sections were devoid of sperms and many sections were found to contain debris. Hyperplasia, mononuclear cells infiltration and wide spacing of connective tissue fibers surrounding the epididymal tube were clearly observed suggesting presences of edema with large degenerative areas and hemorrhage in peritubular connective tissue were seen in most of the examined specimens. As well as degeneration, vacuolization in the epithelium and atrophy were detected in some epididymal sections (Fig.17). These histopathological alterations became more pronounced in the recovery group since degenerated epididymal tube and absence of spermatozoa were still observed (Figs.18 & 19).

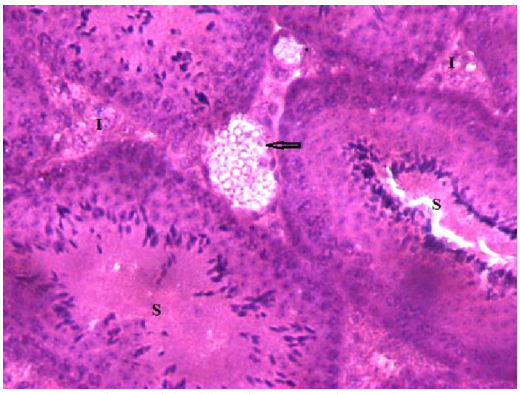
Examination of epididymis of mice treated with TCA for 6 weeksthen treated with the mixture of leaves powder of *Capparis spinosa* and honey for 3 weeks (regeneration group) revealed less prominent histopathological changes when compared with TCA only treated group. In these specimens, the ductus epididymis showed normal epithelial cells with distinct stereocilia and normal features of nuclei. Some recovery in sperm density was also noticed. Treatment with the mixture of *Capparis spinosa* and honey succeeded to decrease the abnormalities of most epipdidymal sections in TCA intoxicated mice although some epididymal sections showed noticeable histopathologicl lesions evidenced by the presence of moderate to severe epididymal degeneration, necrosis and exfoliated of epithelial cells accompanied with edema and accumulation of mononuclear cells infiltration in the connective tissue surrounding epididymal tube. Moreover, in most sections the epididymal tube lumen appeared devoid of the spermatozoa (Figs.20, 21 & 22). However, the incidence and severity of histopathologicl lesions were lower in comparison with those in the recovery group.



Fig(5): A section of testis of mouse from *Capparis spinosa* and honey treated group showing intact Seminiferous tubule (S), Seminiferous tubule with proliferation of germ cells and obscured lumen (Star), interstitial cells (I), congested blood vessel (Arrow) (H&E stain, X200).



Fig(6): A section of testis of mouse fromaqueous extract of *Capparis spinosa* treated group showing intact Seminiferous tubule (S), Seminiferous tubule with proliferation of germ cells and obscured lumen (Star), interstitial cells (I) (H&E stain, X400).



Fig(7): A section of testis of mouse fromaqueous extract of *Capparis spinosa* treated group showing intact Seminiferous tubule (S), interstitial cells (I), congested and dilated blood vessel (Arrow) (H&E stain, X400).

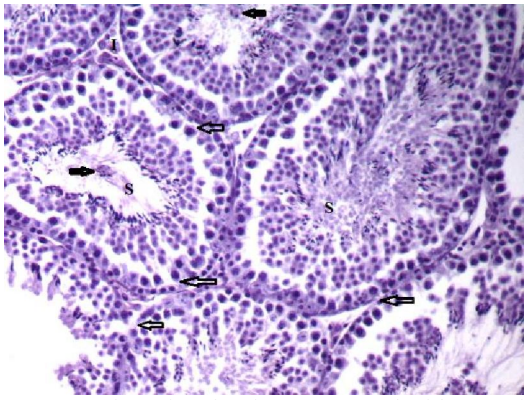


Fig.(8): A section of testis of mouse treated with TCA for 3 weeks illustrating seminiferous tubules with disorganization and decrease of germ cells(S), sloughing of germ cells (Arrows), accumulation of the necrotic debris in the tubular lumina (Thick Arrows), interstitial cells (I) (H&E stain, X400).

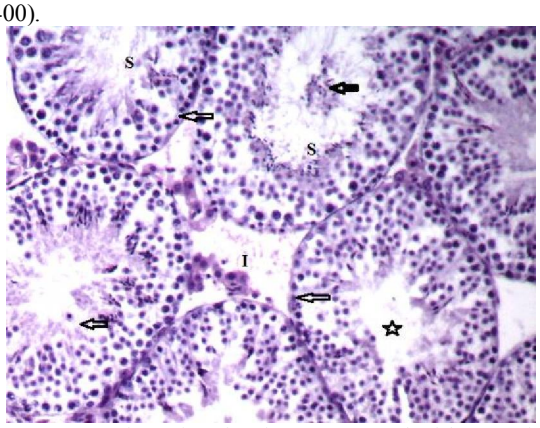


Fig. (9): A section of testis of mouse treated with TCA for 6 weeks illustrating seminiferous tubules with disorganization and decrease of germ cells(S), sloughing of germ cells (Arrows), accumulation of necrotic debris in the tubular lumen (Thick Arrow), complete absence of mature sperms (Stars). Atrophy of seminiferous tubules with edema of the intertubular tissue and decrease interstitial cells (I) (H&E stain, X400).

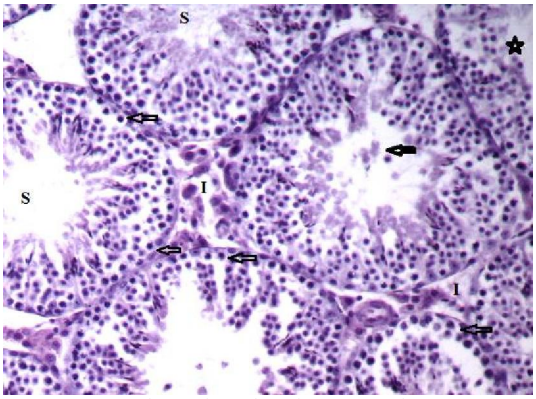
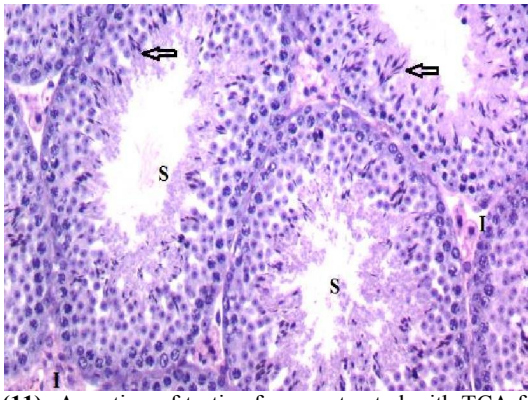


Fig. (10): A section of testis of mouse of recovery group illustrating seminiferous tubules with disorganization and decrease of germ cells(S), sloughing of germ cells (Arrows), accumulation of the necrotic debris in the tubular lumen (Thick Arrow), complete absence of mature sperms (Stars). Atrophy of seminiferous tubules with edema of the intertubular tissue and decrease interstitial cells (I) (H&E stain, X400).



Fig(11): A section of testis of mouse treated with TCA for 6 weeks then treated with a mixture of *Capparis spinosa* and honey exhibiting seminiferous tubules with slight irregular basement membrane and slight decrease of germ cells(S). Note mature spermatozoa in seminiferous tubules (Arrows), an improvement of interstitial tissue(I) (H&E stain, X400).

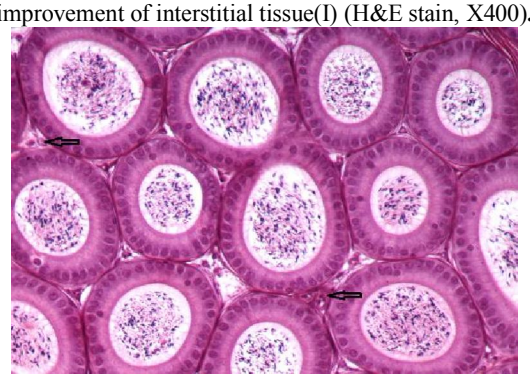


Fig.(12): A section of epididymis of mouse from control group showing epididymal tube lined by pseudostratified epithelia with stereocilia and contained numerous spermatozoa in their lumen. Connective tissue surrounding epididymal tube (Arrows) (H&E stain, X200).

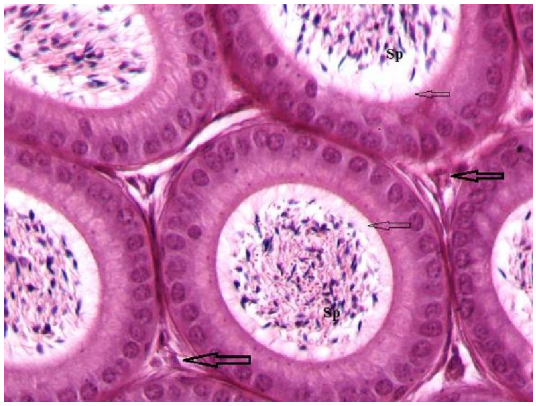


Fig.(13): A section of epididymis of control mouse showing epididymal tube lined by pseudostratified epithelia with stereocilia (Arrows) and contained numerous spermatozoa in their lumen (Sp). Connective tissue surrounding epididymal tube (Thick Arrows) (H&E stain, X400).

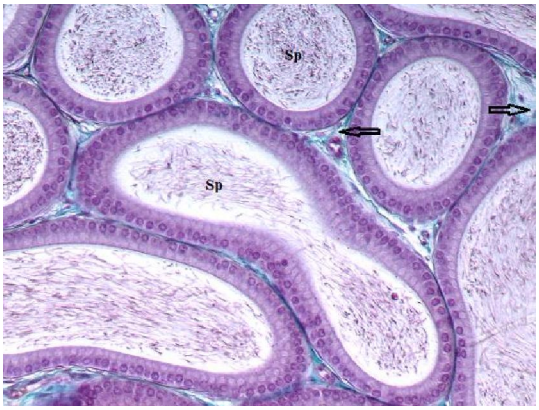
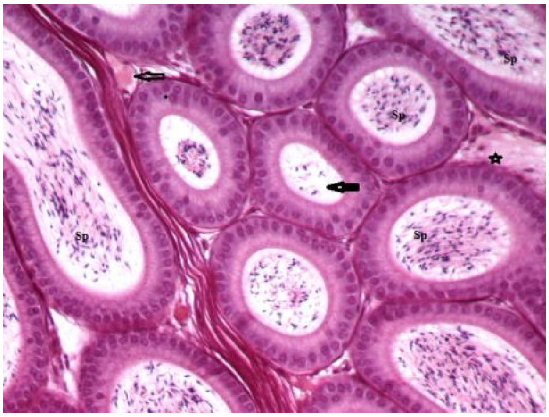
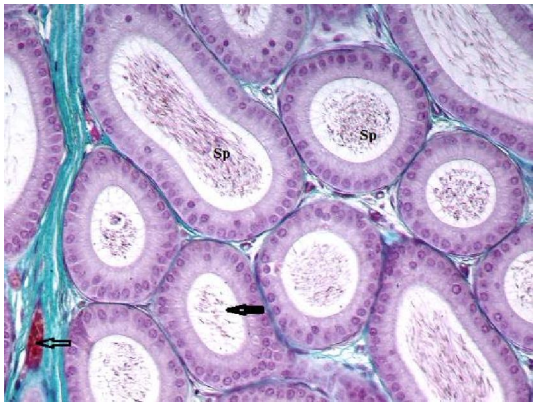


Fig.(14): A section of epididymis of control mouse showing normal epididymal tube contained numerous spermatozoa (Sp) in its lumen. Vascularized connective tissue surrounding epididymal tube (Arrows) (Crossmon's trichrome stain, X400).



Fig(15): A section of epididymis of mouse from *Capparis spinosa* and honey treated group. Epididymal tube occupied with stored spermatozoa(Sp), sections of epididymal tube with decrease in spermatozoa (Thick Arrow), connective tissue (Star), congestion (Arrow) (H&E stain, X400).



Fig(16): A section of epididymis of mouse from *Capparis spinosa* and honey treated group exhibiting normal histological structure. Epididymal tube with dense sperms in its lumen (Sp), sections of epididymal tube with decrease in spermatozoa (Thick Arrow), small hemorrhagic area in connective tissue (Arrow) (Crossmon's trichrome stain, X400).

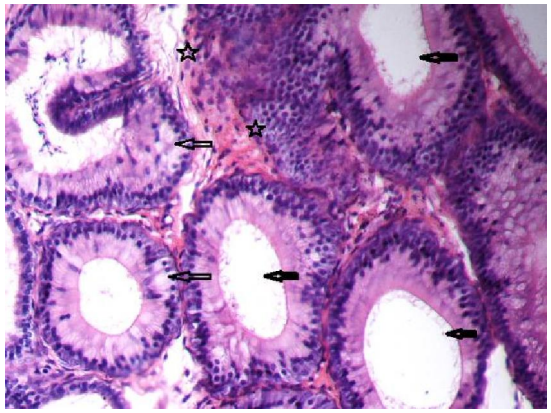


Fig. (17): A section of epididymis of mouse treated with TCA for 6 weeks illustrating necrotic epithelial cells with destructed stereocilia (Arrows). Epididymal tube devoid of sperms (Thick Arrows), mononuclear cells infiltration and edema in connective tissue (Star).Note atrophy of epididymal tube (H&E stain, X200).

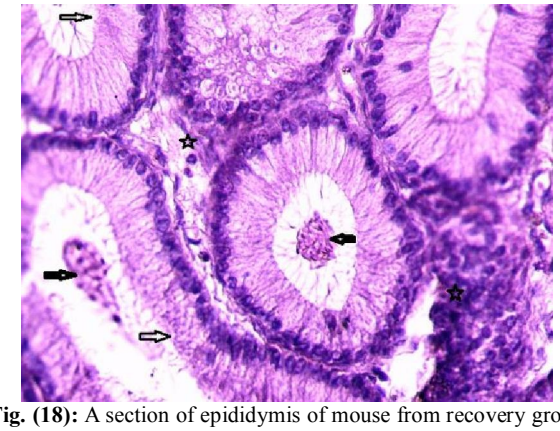


Fig. (18): A section of epididymis of mouse from recovery group illustrating vacuolization ofepithelial cells with destructed stereocilia (Arrows). Note the necrotic debris in the epididymal tube lumen (Thick Arrows), mononuclear cells infiltration and edema in connective tissue (Stars) (H&E stain, X400).

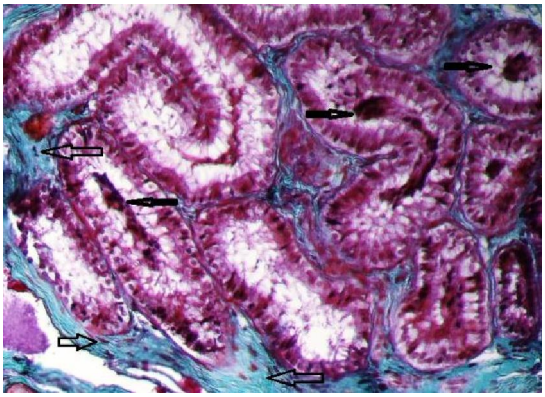


Fig.(19): A section of epididymis of mouse from recovery group showing massive degenerated and necrotic epithelial cells. Necrotic debris in the epididymal tube lumen (Thick Arrows), peritubular connective tissue (Arrows) (Crossmon's trichrome stain, X200)

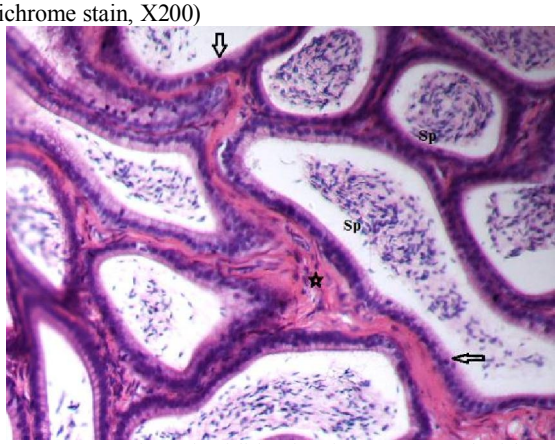


Fig.(20): A section of epididymis of mouse treated with TCA for 6 weeks then treated with a mixture of *Capparis spinosa* and honey (Regeneration group) showing epididymal tube with normal epithelial cells and distinct stereocilia (Arrow). Note density of spermatozoa (Sp), peritubular connective tissue (Star) (H&E stain, X200).

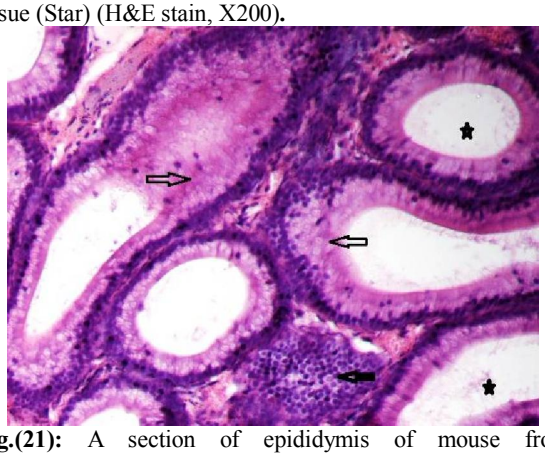


Fig.(21): A section of epididymis of mouse from regeneration group exhibiting epididymal tube with degeneration, necrosis and exfoliated of epithelial cells (Arrows), edema and accumulation of mononuclear cells infiltration in peritubular connective tissue (Thick Arrow), devoid epididymal tubular lumen from the spermatozoa (Stars) (H&E stain, X200)

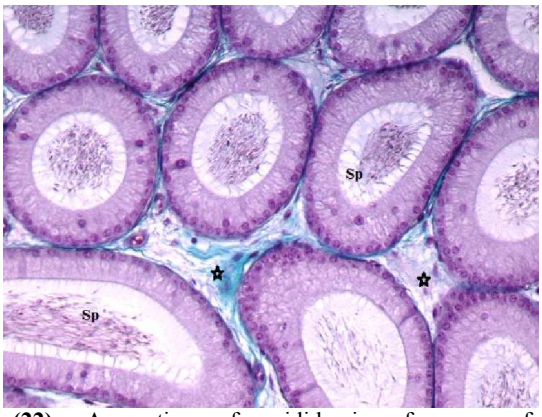


Fig.(22): A section of epididymis of mouse from regeneration group exhibiting decrease of the histopathologicl lesions. Epididymal tube with accumulated sperm in the lumen (Sp), peritubular connective tissue (Stars) (Crossmon's trichrome stain, X400).

4. Discussion

The current study revealed that there were no detectable histopathological alterations in testes and epididymal tissues of mice treated with honey in comparison to the control group. Also, in honey treated rats by 20 mg/ kg body weight/day for 4 weeks, normal and regular seminiferous tubules were clearly appeared. The germinal cells were greatly regulated with normal diameter and mild dilatation of the seminiferous tubules with normal complete spermatogenic series **(Abu-Zinadah *et al*., 2013).**

Mild histopatological lesions were observed in testes and epididymis of mice treated with themixture of leaves powder of *Capparis spinosa* and honey. In the same respect Al-Sammarae *et al*. (2009) concluded that *Capparis spinosa* extract given intraperitonially by the dose 500, 700mg/kg for 35 days had neither genotoxic nor clastogenic effects in mouse germinal cells (*in vivo*). Moreover, **Sini *et al*. (2010)** recorded that the histopathological examination of the organs did not reveal any abnormalities in rats treated with aqueous leaf extract of *Capperis grandiflora* at dose 1000-3000 mg/kg. Regarding dilation of blood vessels in the interstitial tissue of testes of mice treated with aqueous extract of *Capparis spinosa* in the present work. This effect may produced by stimulation of regional blood flow or initial vasodilation **(Stanic and Samarzija, 1993)**. However, **Eddouks *et al*. (2002)** reported that *Capapris spinosa* fruits are used in the traditional health care in the treatment of cardiovascular disorders. On the other hand, severe pathological changes were observed in the seminiferous tubules of rats treated with the whole plant ethanolic extract of *Capparis aphylla*at dose level 50, 100, 200 mg/kg body weight through intraperitoneal route for 55 days. Seminiferous epithelium underwent extensive vacuolation,

disorganized and distorted but the interstitium was not affected. In the higher dose group the entire seminiferous tubules were collapsed, completely arrest spermatogenic cycle, degenerating germinal elements or detached germ cells were filled in the lumen **(Revathi *et al*., 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).** Moreover, there are no enough documents in the literature about the probable toxic effectsofmedicinal plants **(Monfared, 2013). 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. Furthermore, herbal remedies may be contaminated with excessive amount of banned pesticides, microbial contaminants, heavy metals, chemical toxins adulteration with synthetic drugs **(Bogusz *et al*., 2002; Chan, 2003 & Idodo-Umeh and Ogbeibu, 2010).** However, 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).** This may explain some alterations in the testes and epididymis of mice treated with mixture of *Capparis spinosa* and honey and aqueous extract of leavespowder of *Capparis spinosa* in the present work.

In the present work, sever lesions were detected in the testes and epididymis of mice treated with TCA for 3 and 6 weeks. It is likely that these structural alterations would affect the epididymal epithelium, and biochemically making it non conductive for sperm maturation and survival **(Chinoyand Bhattacharya, 1996). Creasy (2001)** observed that the epithelial cells lining the cauda epididymidis, of mice administered TCA at dose 500mg/kg, showed vacuolization which might be associated with significant alterations in sperm and fertility parameters**.** However, the toxicity of trichloroethylene in rodents is mediated primarily through its metabolism to chloralhydrate, trichloroacetate (TCA) and dichloroacetate **(Lash *et al*., 2000).** A study showing that the enzyme responsible for conversion of trichloroethylene to toxic metabolites, is present in the epididymal epithelium. This toxicity has a high probability of affecting spermatozoa that develop and mature within the epididymal environment **(Forkert *et al*., 2002).** It had been suggested that TCA is the major metabolite of trichloroethylene **(Elcome *et al*., 1985; Fisher *et al*., 1991)** and may be involved in epididymal damage in trichloroethylene-exposed mice **(Odum *et al*., 1992).** An inhalation study reported reductions in sperm count and motility as well as, serum testosterone in rats exposed to trichloroethylene at dose 2030 mg /kg 4 hours day for 12 - 24 weeks. Also reduced fertility was reported when the males mated with untreated females **(DEFRA, 2004)**. The percentage of abnormal sperm was significantly greater in mice exposed to TCA 2000 ppm 4 hours day for 5 days **(European Union, 2004).** Moreover, **Kan *et al.* (2007)** demonstrated that exposure of mice to trichloroethylene by inhalation 1000 ppm for 6 h/day for 5 days/week for 1 to 4 weeks imparted severe damage to the epididymis manifested as degeneration and sloughing of epididymal epithelial cells and resulted in abnormal acrosome and tail formation in epididymal sperm as early as 1 week after trichloroethylene exposure, which became more pronounced after 4 weeks. These results suggested that exposure to trichloroethylene by inhalation might lead to infertility in animals. **Bull *et al.* (1990)** demonstrated that TCA appears to increase lipid peroxidation, suggesting that the production of free radicals may responsible for its effects. Additionally, Reactive oxygen species (ROS) cause defective sperm function as a result of lipid peroxidation of the poly unsaturated fatty acids in the head and mid-piece which consequently alter sperm morphology and lead to decreased motility and ineffective spermatozoon-oocyte fusion **(Baumber *et al.,* 2000 and Dandekar *et al.,* 2002)**.

Concerning the interstitial tissue; it was evident that, marked atrophy of seminiferous tubules associated with edema in the intertubular tissue and decrease Leydig cells occurred in mice treated with TCA. These findings could be confirmed by the speculation of **Ozguner *et al.* (2005)** that the presence of interstitial edema might decrease the size of seminiferous tubules or led to their atrophy.Moreover**,** increased oxidative stress is very harmful for Leydig cells (**Aitken *et al.,* 1989; Aitken *et al.,* 1993 and Sultan *et al.,* 2010)** especially that these cells locate close to blood vessels which expose them to a high risk of exogenous toxicants (**Boekelheide, 1993). Guney *et al.* (2007)** added that the testicular tissues are very sensitive to reactive oxygen species effects. However, the change in the number and structure of Leydig cells could affect the testosterone level **(Ross *et al.,* 2003)**. In addition, the change in Leydig cells character was an evidence of non functioning cells as the fate of these phenomena could lead to fatty degeneration **(Wang *et al.,* 2003).** Furthermore, **Behnaz (2014)** regard the decline in serum testosterone level to the absence of Leydig cells in testes.

Finding in the current study revealed that mice treated with TCA exhibited disorganization and decrease of germ cells in some seminiferous tubules. Sloughing of germ cells with accumulation of necrotic debris in the tubular lumina were also observed. As well as, some of the seminiferous tubules were

completely devoid of mature sperms and absence of spermiogenesis in the majority of the seminiferous tubules were noticed. **De Rooij and Russell (2000)** reported that the spermatogonia are highly sensitive to toxicants because of their mitotic activity. Moreover, the defects of spermatogonia will affect the development of the following stages of spermatogenesis **(Elshennawy and Abo Elwafa, 2011)**. **Monsees *et al.* (2002)** suggested that the decreased testosterone level reflected on Sertoli cells function leading to loss of germ cells, loss of contact between them, decrease the thickness of the seminiferous epithelium and finally, destruction of testicular tissue. Generally, Sertoli cells form the sites of attachment of germ cells and provide physical support to them **(Richburg, 2000 and Sawada and Esaki, 2003).** Moreover, **Xie *et al.* (2014)** speculated that the pathological changes of seminiferous epithelium may cause the disruption of Sertoli and germ cells, which results in impaired spermatogenesis and may also lead to germ cell loss**.**

The appearance of hemorrhage in peritubular connective tissue surrounding the epididymal tube in mice treated with TCA in our study could clarify the increased oxidative stress due to the increase in the oxygen tension. The oxygen-induced damage mediated by lipid peroxidation may also damage membrane integrity with increased cell membrane permeability, thus leading to structural damage of DNA and cell death **(Aitken *et al.*, 1989; Aitken *et al.*, 1993 and Sharma and Agarwal, 1996).** Alteration of DNA especially in the reproductive cells even if it not affect the current generation directly, but it may appear on the next generationsleading to increased cell mutation **(Paulraj and Behari, 2006).**

In contrast to the above mentioned results, mice received mixture of *Capparis spinosa* and honey for 3 weeks after stoppage of administration of TCA in regeneration group lessened most of the histopathological lesions which may give an evidence of repair and regeneration of testicular and epididymal tissues. These ameliorating changes may supported and explained by that reported previously where natural honey-bee products such as pure honey, royal jelly and pollen grains had been found to have antioxidant and antimutagenic factors. Several studies have shown that these products are rich in flavonoids, phenolic acids, some enzymes, ascorbic acid, carotenoid -like substances, organic acids, amino acids and proteins **(Vela *et al*., 2007; Estevinho *et al*., 2008; Alvarez-Suarez *et al*., 2010).** Many of these compounds have been shown to be cytoprotective by scavenging reactive oxygen species and reducing lipid peroxidation **(Beretta *et al*.,2007; Alvarez Suarez *et al*., 2010).** Moreover, *Capparis spinosa* extracts may improve animal fertility and it can cross testis barriers **(Al-Sammarae *et al*., 2009)**. The accumulation of mononuclear cells infiltration in interstitial tissue in regeneration groupgave an evidence in support of repair and regeneration of new cells, while clearing off the old cell debris.The disappearance of massive changes in the testes and epithelial cells lining the epididymis of regeneration group may be an indicative of the unaltered level of testosterone. Furthermore, the reappearance of spermatozoa in the lumen of the epididymis following supplementation with mixture of *Capparis spinosa* and honey after TCA intoxication may indicate the amelioration in the spermatogenic activity. Also, histological improvement in seminiferous and epididymal tube might be due to ability of mixture of *Capparis spinosa* and honey to stimulate and enhance the proliferation, maturation and differentiation of spermatozoa. It is also likely that the antioxidants present in the leaves of the plant, acting in concert with the antioxidant system present in the epididymis further preserved and enhanced the process of spermatogenesis.

Numerous studies pointed to the elevation of a variety of detoxication and antioxidant enzymes and biomarkers as a result of treatment with phytochemicals isolated from medicinal plants **(Faizi *et al*., 1994 and Kumar and Pari 2003).** Antioxidants play a major role in preventing the formation of free radicals, which are responsible for many oxidative processes leading to cell damage **(Sakr and Badawy, 2013).** Many studies showed that all parts of *Capparis spinosa* possess antioxidant effects with certain correlation with their polyphenols and flavonoids contents **(Arrar *et al*., 2013).** These flavonoids display a remarkable role in various pharmacological activities including anti-allergic, anti-inflammatory and antioxidant effects **(Middleton and Kandaswami 1992; Harborne and Williams 2000; Berg and Daniel 1988).** Many plant-derived antioxidants have been experimentally proved and used as effective protection against free radical induced tissue damage and oxidative stress **(Lampe, 2003).** Orally administered *Capparis spinosa* seed extracts at dose 50, 100, or 200 mg/kg daily for 8 weeks provided significant protection against D-galactose induces increased production of free radicals and damage of DNA bands in mice **(Turgut *et al*., 2014).** Generally, it was reported that oxidative stress has been postulated as one of the mechanisms leading to testicular damage **(El-Dakdoky and Helal, 2007).** Antioxidant foods that are rich in flavonoids are protective agents against these ailments **(Perez *et al*., 2006).** Furthermore, administration of antioxidants enhanced the testicular functions, epididymal sperm motility and fertilizing ability in rats **Suzuki and Sofikitis (1999)**.

Also, administration of honeyat dose 20 mg/ kg body weight/day for 4 weeks reduced the testicular

lesions characterized by moderate to severe degenerative changes of seminiferous tubules and incomplete arrest of spermatogenesis induced by octylphenol in rats. This effect of honey could be partly mediated by its counteraction on oxidative stress within rat reproductive organs via its antioxidant properties. Moreover, it is also possible that honey could ameliorate the toxic effect of octylphenol on testicular function partly by improving testicular blood flow and spermatogenesis via the oestrogenic activity of its phenolic compounds **(Abu-Zinadah *et al*., 2013).**This observation is in line with the previous publications of many authors where a higher sperm count was observed in many studies following the oral administration of Malaysian honey for 28 days in rats **(Mahaneem *et al.*, 2007).** A significantly higher epididymal sperm count was also found in adult rats following the daily treatment of5% Palestinian honey for 20 days **(Abdul-Ghani *et al.*, 2008).** In addition, honey has the potential to stimulate inflammatory cytokine production from monocytes, thus increasing the tissue protection from various scavenging oxidants **(Harman *et al*., 2005)**. The above finding may support and explain the improvement in testicular tissue and epididymis of mice intoxicated with TCA and treated with mixture of leavespowderof *Capparis spinosa* and honey observed in the present work.

However the reduction in histopathological alterations in testis and epididymis tissues of mice intoxicated with TCA and treated with the mixture of leavespowderof *Capparis spinosa* and honey may reverse the potential effects of this plant for recovery; since many sections of testis and epididymis showed normal histological structure with multiple sperms in the tubular lumina, although the mixture of *Capparis spinosa* and honey did not lead to complete recovery. On the other hand, No ameliorating changes were noticed neither in the testes nor in the epididymal of mice received TCA for 6 weeks then left for 3 weeks for recovery (recovery group). This may confirm that the treatment of mice with the mixture of *Capparis spinosa* and honey has a better effect in attenuating the adverse effects of toxicity induced by TCA than theanimals left for recovered without treatment.

Conclusion

It can be concluded that TCA produce testicular and epididymal injury. The results suggest that histopathological changes are dependent upon the duration of exposure to TCA. The mixture of leaves powder of *Capparis spinosa* and honey (40mg/kg) bears the potentiality to ameliorate TCA induced alterations in testes and epididymis. These observations represent novel findings of mixture of leavespowderof *Capparis spinosa* and honey in testes and epididymis of mice intoxicated with TCA.

Further investigations are needed to elucidate protective role or side effects on other organs and systemto suggest the using of this medicinal plant in therapy. Also, further studies are needed to determine the effects of *Capparis spinosa* on an animal fetuses, on pregnant animals, and their reproductive capacity to complete the safety profile of this herb.

Acknowledgements

This study was financially supported by Libyan authority for research science and technology. Authors wish to thank Dr. Huda for her help in authenticated the plant. Also, the authors appreciate all who help us to complete the present work.

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6/29/2015