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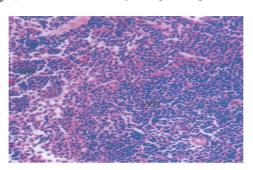
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Fig(11): A section of spleen of male mouse treated with TCA for 6 weeks then treated with a mixture of Capparis spinosa and honey showing white pulp (Wp) and red pulp (RP) with lymphoid depletion and few hemosiderosis (Arrow) (H&E stain (X200)

Conclusion

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

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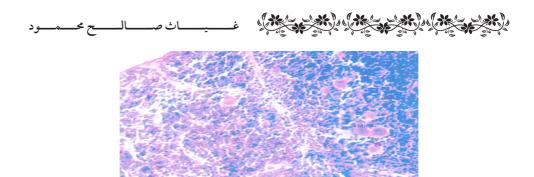
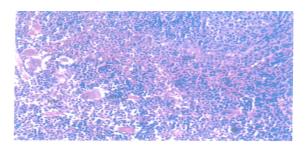


Fig (8): A section of spleen of male mouse treated with TCA for 6 weeks illustrating hyperplasia in white pulp (WP); hemosiderosis hypocellularity and edema in red pulp(RP) Megakaryocyte (MKS) (H&E stain 'X200)



Fif (9): A section of spleen of male mouse treated with TCA for 6 weeks then left for 3 weeks for recovery showing dilatation congestion of blood vessels (BV). Megakarycoytes (MKS). Note necrotic cells with dens nucleir (H&E stain. X200).

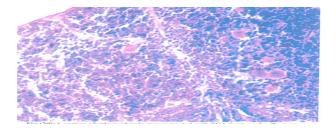
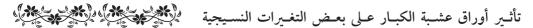
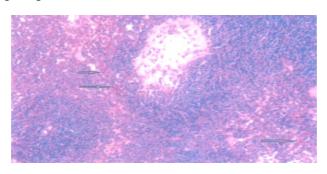
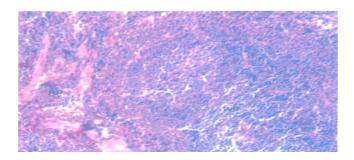


Fig (10): A section of spleen of male mouse treated with TCA for 6 weeks then treated with a mixture of Capparis spinosa and honey (regeneration group) showing red pulp (RP) with few hemosiderosis (Arrow) and white pulp (WP) with less fibrosis and nearly normal architecture (H &E stain 'X200)





Fig(5): A section of spleen of male mouse treated with TCA for 3 weeks showing fibrosis and lymphoid depletion and some necrotic cells in white and red pulp Hemosedrine (Arrows) (H&E stain X200)



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

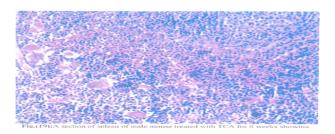
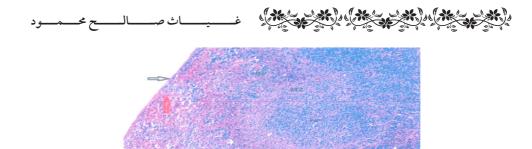


Fig (7): A section of spleen of male mouse treated with TCA for 6 weeks showing hyperplasia in white pulp (WP) hypocellularity and edema in red pulp (RP) Megakaryocyte (MKS) (H&E stain (X200)





Fig(2): A section of spleen of male mouse from control group showing normal architecture of spleen white pulp (WP) Red pulp(RP) capsule (Arrow) marginal zone (MZ) trabeculae (Red Arrow) (H&E stain X200).

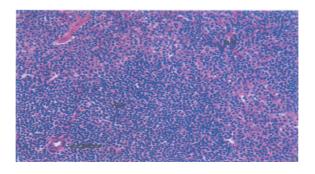
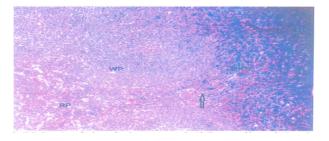


Fig.(3):A section of spleen of male mouse treated with honey showing normal architecture of spleen white pulp (WP) Red pulp (RP) eccentric artery (Arrow) (H&E stain X200)



Fig(4):A section of spleen of male mouse treated with Capper and honey showing normal histological structure of white pulp (WP) and red pulp (RP) Megakaryocytecs (MKS) Trabeculae (Arrow) (H&E stain X200)



for 3 weeks for recovery (recovery group). In addition dilated and congested blood vessels as well as necrotic cell with condensed nuclei were noticed (Fig9) on the other hand. Administration of the mixture of Capparis spinosa and honey (Fig10 and 23) after stoppage of the treatment with TCA; lessened most of the pathological lesions. This may confirm that the treatment of mice with the mixture of Capparis and honey has a better effect in attenuating the adverse effects of toxicity induced bt TCA than the animals left for recovered without treatment. Similarly administration of honey has significantly attenuated the determintal effect of poisonous materials on different organs of the rat; as it provides anti-inflammatory immune -stimulant; antiucler and regerative effect (Fiorani et al. 2006). In addition; Honey possessed some biological properties such as antioxidant (Perez et al 2006) and immunomodulatory effects (Timm et al 2008). Furthermore: It is important for the treatment of acute and chronic free radical mediated toxicity (Abdel- Moneim and Ghafeer (2007) .Also all parts of Capparis spinosa possesed antioxidant effects with certain correlation with their polyphenols and flavonoids contents (Arrar et al 2013). Biological studies revealed important anti-oxidative anti- inflammatory and immunomodulatory properties of Capparis (Tlili et al 2011). Duman et al.(2003) suggested that combination of Capparis with deferasirox may have additive effect on decreasing the oxidative damage and tissue toxicity. Therefore, it is possible to suggest that the effect of Capparis spinosa with honey in attenuating the toxic effect induced by TCA in this study could be partly mediated by their combined counteraction on oxidative stress within the organs via their antioxidant properties.



the spleen sections of mice treated with honey only (Fig3) or with the mixture of leaves powder of Capparis spinosa and honey (Fig4). Our findings werw in agreement with Sini et al (2010) who found that histological examination of the organs did not reveal any abnormalities in rats treated with aqueous leaf extract of Capperis grandiflora by the dose 1000-3000 mg/kg. According to Haque and Haque (2011) no detectable abnormalities were found in the histopathology of the heart liver kidney or lungs in rats treated with the chloroform extract of the roots of Capparis zeylanica Linn at a dose of 300 mg/rat/day for 14 days compared with the control group.

In the current study the spleen sections of mice treated with TCA for 3 weeks (Fig5) and 6 weeks (Fig 6-8) revealed obvious pathological findings disorganization of lymphoid follicles, hyperplasia in white pulp, depletion of lymphocytes in red pulp with edema, and some necrotic cells in white and red pulps. Increasing of megakaryocytes and hemosiderosis as well as fibrosis in the red pulp and some lymphoid follicles were also noticed. Therefor, the cellularity of spleen was affected by TCA administration. However, splenic immunosuppressioon may attributed to the decreased lymphatic cells numbers in the spleen as well as in other immune organs (Monfared et al '2014) .TCA has an ability to induce oxidative – stress responses such as lipid peroxidation and oxidative DNA damage following acute or short-term TCA dosing in mice (Larson and Bull 1992). Moreover a potential mechanism of TCA –induced oxidative stress via macrophage activation was speculated by Hassoun and Ray (2003). Other studies have shown that macrophages can be activated and become a source of reactive oxygen species that may produce damage to surrounding tissues (Karonvsky et al. 1988; Briggs et al. 1986) . Menezes et al (2005) reported that all extensive injuries were repaired with collagen fibers which may lead to the fibrosis observed here in.

Obvious increase in the number of megakaryocytes and hypocellularity were evident in the red pulp in spleen tissue of mice received TCA for 6weeks then left



for 6 successive weeks then treated orally by oral gavage with a mixture of Capparis spinosa and honey at dose level 40 mg/kg body weight one per day for 3 successive weeks and known as regeneration group.

Acute toxicity studies:

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

Histopathological studies:

For the light microscopic examination, the spleen was carefully dissected out and quickly fixed in Bouain's fluid, dehydrated in ascending grades of ethyl acohol, cleared in xylene, impreganted in paraffin wax and sections of 5-7 um thickness were taken. The deparaffinized sections were stained with Harri's haematoxylin and eosin (H&E) and periodic acid Schiff (P&S) according to Bancroft & Gamble (2008).

Histological sections were examined by light microscope with digital camera (Nikon Eclipse E400)

Result and Discussion Histopathological study:

Examination of the spleen sections of control mice showed normal architecture. It was composed of white and red pulps surrounded by a capsule of dense connective tissue. White pulp was consisted of lymphoid nodules with central artery located eccentrically. Lymphoid nodules of white pulp separated from red pulp with well visible marginal zone. Red pulp was composed of splenic cords and sinusoids Megakaryocytes with an irregularly lobulated nucleus were visible among the cell of red pulp (Fig 2) No obvious histopathological changes was detected in





the prescriptions given by traditional healers. The dose was determined according to Paget and Barnes (1964).

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



Fig (1): Capparis spivnsa

Experimental design:

100 healthy adult male mice were divided into 5 groups of 20 mice each and subjected to the following treatments:

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

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

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

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

Group V: Received TCA at dose level 500 mg/kg body weight in drinking water



Materials and methods Experimental animals:

Healthy adult male Swiss albino mice (Mus-musculus) 8 to 10 weeks old and weighing 224 gm were obtained from the Animal Breeding House of faculty of veterinary medicine. Omar El- Mukhtar University. Albayda. Libya. They were housed in the labratory animal room in clean plastic cages under controlled coditions of temperature (20 2) C and photoperiod (14h light: 10 h dark) cycle.

The animals were maintained on standard commercial pellet diet clean drinking water adlibitum. Mice were acclimatized for 1 week prior to the start of experiments.

Materials used:

Fresh plants of Capparis spinosa (fig. ۱) were collected from Algabal Alakhder in Al– Bayda – Libya between march and April ۲۰۱۲. The plant was authenticated by Department of Botany، Faculty of Agriculture، Omar El– Mukhtar university، Al Bayda– Libya. Only the leaves were used. They were cleaned, air–dried and then powdered mechanically.

Honey sample:

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

Preparation of the mixture of Capparis spinosa and honey:

Leaves powder of Capparise spinosa (400mg) were well mixed with 40 gm of Seder honey and used at a dose level of 40 mg/kg body weight (0.1 ml/mouse) (equivalent to dose used by a human weighting 70 kg in traditional medicine). The mixture of Capparis spinosa leaves powder and honey was prepared according to



antidiabetic (Ziyyat 1997) antihepatotoxic (Gadgoli and Mishra 1999) antifungal (Ali-shtayeh et al 1999) diuretic antihypertensive and poultice (Calis et al. 1999) antihyperlipidemic (Eddouks et al.2005) activities and antihelminthic properties (Mustafa 2012)

Trichloroacetic acid (TCA) (CC13COOH) is mainly used in the production of its sodium salt which is used in many industries; as herbicide etching agent and antiseptic (Lin et al. 2005). TCA is a colorless to white crystalline solid with a sharp pungent odor (NIOSH 2003). It is formed from organic material during water chlorination (Coleman et al. 1980 and IPCS 2000) and has been detected in groundwater surface water distribution systems and swimming pool water. TCA was detected in vegetables fruits and grains (Reimann et al. 1996) and can be taked up into foodstuffs from the cooking water (U.S. EPA 2005). Therefore human exposure to TCA can also occur via food consumption. Oral half lethal does (LD50) of 4970 mg/Kg of body weight for TCA have been reported in mice (Woodard et al. 1941).

The spleen is the largest secondary lymphoid organ; is considered the draining site for compounds that are administered intravenously; and is therefore, considered an important organ to evaluate for treatment – related lesions. Due to the presence of B and T lymphocytes; the immunotoxic effects of xenobiotics or thier metabolites on these cell population may reflected in the spleen. Therefore, it is one of the recommended organs to evaluate for enhanced histopathology of the immune system (Elmore, ۲۰۰٦). The present work aimed to study the possible protective role of Capparis spinosa leaves and their efficacy as used in traditional medicine in Libya on histopathological alterations of the spleen induced in an animal model intoxicated with tichloroacetic acid.



Abstract

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

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

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

Introduction

Capparis spinosa L. Family Capparidaceae is one of the most common armotic plants growing in wild in the dry regions around the west or centeral Asia and the Mediterranean basin capparis spinosa is well know with its common name "Capers" in different countries (Azaizeh et al . 2003 and Tlili et al . 2011). It has been known for centuries in traditional phytomedicine (Benzidane et al . 2013). In Libya and many other countries Capparis spinosa was found to be used traditionally for treatment of a variety of diseases and cancer (Kulisic – Bilusic et al . 2012). Capparis spinosa considered as a very important source of medicine for



تأثير أوراق عشبة الكبار على بعض التغيرات النسيجية المرتبطة بتعرض الفئران لحامض الخليك ثلاثي الكلور

غياث صالح محمود رئيس قسم التحليلات المرضية كلية السلام الجامعة، بغداد - العراق

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

اشتملت التجربة على مائة من ذكور الفئران بوزن تراوح ما بين ٢٠ -٢٦ جم، وتم تقسيمها الى خمسة مجموعات مجموعة ضابطة والمجموعة الثانية عوملت بعسل السدر عن طريق الفم بجرعة ٤٠ مل / كجم من وزن الجسم لمدة ثلاثة اسابيع . والمجموعة الثالثة عوملت بمخلوط اوراق الكبار والعسل عن طريق الفم بجرعة ٤٠ مل / كجم لمدة ثلاثة اسابيع . والمجموعة الرابعة تم معاملتها بحمض الخليك ثلاثي الكلور في ماء الشرب بجرعة ٥٠٠ مجم / كجم وتم تجميع العينات من بعض الحيوانات بعد ثلاثة اسابيع وبعد ستة اسابيع وتركت باقى الحيوانات بدون علاج للتعافي والمجموعة الخامسة عوملت بحمض الخليك ثلاثي الكلور لمدة ستة اسابيع ثم عوملت بمخلوط الكبار والعسل بجرعة ٤٠ مل / كجم لمدة ثلاثة اسابيع.

وعلى مستوى الفحص النسيجي اظهر طحال الحيوانات المعاملة بحمض الخليك ثلاثي الكلور حدوث تغيرات نسيجية مرضية واضحة، اشتملت على عدم انتظام العقيدات اللمفاوية وفرط تنسج اللب الابيض وهزال لمفاوي في اللب الاحمر مع وذمة تحت المحفظة . علاوة على وجود نخر في بعض خلايا اللب الابيض واللب الاحمر وزيادة الخلايا النواء وصبغ الهيموسدرين وتليف اللب الاحمر وبعض العقيدات اللمفاوية . وادى اعطاء الفئر ان مخلوط الكبار والعسل بعد ايقاف معاملتها بحمض الخليك ثلاثي الكلور إلى تخفيض معظم التغيرات النسيجية المرضية المحدثة نتيجة التسمم بحمض الخليك ثلاثي الكلور الى تخفيض معظم التغيرات النسيجية المرضية المحدثة نتيجة التسمم بحمض الخليك ثلاثي الكلور.

