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Research article

INVESTIGATION ON THE ACUTE AND SUBACUTE TOXICITY OFALHAGI GRAECORUM IN EXPERIMENTAL ANIMALS

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ABSTRACT: Little is known about the acute and subacute toxicity of Alhagi Graecorum (Camel Thorn) in mice. The aim of this study is to investigate the acute toxicity (LD50) and sub acute toxicity of camel thorn in mice. For determination of LD50, mice weighing 25-30 g were divided into nine groups each of 6 animals and received 130, 330, 660, 1300, 2600, 4200, 5000, 5900 and 6900 mg/kg orally of camel thorn water extract respectively. The sign and symptoms of toxicity and the number of died animals in each group were registered and the LD50 was calculated.Another group of Male albino mice weighed 25-30 g were divided into control and different treated group each of 6 mice and placed in the metabolism cages that allowed daily measurement of food and water consumption. The control received normal saline whereas the other groups received 130, 660, 1300 mg/kg (i.p, daily) of camel thorn extract (CTE)respectively, for two weeks.. The animals were weighed daily for any sign of reduction or gain of body weight. The food and water consumption were daily calculated. At the end of this experiment, the mice were killed and blood was collected and used for the determination of glucose and the serum creatinine,urea, aspartate, aminotransferase, (AST) and alanine aminotransferase (ALT) levels.The liver of the animals were removed,fixed in 10%formaline for histopathological investigation. The LD50 was equal to 5400mg/kg.Results of sub acute toxicity studies revealed that no significant weight reduction were observed in treated groups as compared to control, however the food consumption was significantly increased in the mice received 660mg/kg of CTE . Also the water consumption was significantly decreased in the animal receiving 1300mg/kg of CTE.

Key words: Alhagigraecorum, camel thorn, acute toxicity, subacute toxicity

INTRODUCTION

Medicinal plants are used as sources of pharmaceuticals and as ingredients of traditional medicines and are of value in new drug discovery. (Phillipson,J.D.1994). Plants have always been a common source of medicaments, either in the form of traditional preparation or as a pure active principle (NR Farnsworth, NR etal 1985**).**Herbal medicine or phytomedicine is recognized as the most common form ofalternative medicine (Ogbonnia et al.,2011). According to The World Health Organization about 80 % of the world's population relies on traditional medicine for primary health care and more than 30% of the plant species have been used medicinally as alternative medicinal treatment especially in the developing countries(Kroll and Shaw, 2003; Ogbonnia et al., 2008). Herbal remedies are considered safer and less damaging to the human body than synthetic drugs (Alam et al., 2011). Although herbal supplements may be considered to be safe, some medicinal plants or herbs are known to be toxic at high doses and others may have potentially adverse effect after chronic use. Peoples are largely unaware that adverse health effects can be associated with the use of herbal supplements resulting from overdosing, contaminated formulations to the inherent toxicity of the herbs of choice (Hazel et al., 1999).Certain medicinal plants may produce harmful effect and several studies shown that many substances, including natural products, are potentially toxic and therefore should be used with care, respecting their toxicological risks (Veiga-Junior et al., 2005). In most cases, the adverse effects of commonly used plants are not well documented in the literature and their long-term use by humans is usually correlated with low toxicity. However, studies have shown that many foods and traditional plants used for their medicinal properties have mutagenic effects (Kassie et al, 1996, Elgorashi et al., 2003).

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Little is known about the acute and sub-acute toxicity of Alhagi Graecorum (Camel Thorn) in experimental animals. The aim of this study is to investigate the acute toxicity and sub acute toxicity of camel thorn in mice

MATERIALS AND METHODS

Materials

Experimental animals:

Albino mice of either sexes weighing 20-30 g, were maintained in the animal house of Faculty of Medicine –Benghazi University, Benghazi, Libya. The mice and rats were bred in the faculty animal house. All animals were kept in standard cages (48x35x22 cm), at room temperature (20±5°C) with artificial light from 7.00 am to 3.00 pm, and provided with food and tape water ad libitum.

Methods

Preparation of the camel thorn extracts (CTE):

An acceptable amount of the camel thorn was collected during April 2006 from Jagbob, a city that lie in the eastern south of Libya, about 35 km away from the Egyptian boundaries. The aerial plant excluding the root was dried in the shade at room temperature then crushed and ground by electrical blender. Using Sox let extraction, the crushed plant was continuously percolated with fresh solvents and the plant material was separated from the extract (Trease & eavns. 1978). Because the constituents being isolated were unknown, the extraction process started with the usual way using a non polar solvent followed by a series of more polar solvents, until several extracts were obtained by increasing solvent polarity. The following solvents were used in sequence (Petroleum ether, Chloroform, Ethyl acetate, Ethanol, and Water).

An Eighty five (85) g of the plant powder was placed in the thimble siphons of Soxhlet apparatus. The flask of the Soxhlet was filled with half liter of the solvent, and heating of solvent stared at a temperature of 60-80^{0}C. By a condensation process, the solvent in the thimble siphons off into the main vessel containing the extract, and the process continued till the extraction was achieved. Extracts were completely evaporated by using rotary evaporator, and kept for use in the pharmacological study. Out of the 85 g of crude plant, 12g water extract (CTE) were obtained.

Toxicity studies

Acute toxicity

The LD_{50} was calculated according to Sperman Karber method as described by WHO guide lines (WHO, 1981).

The acute toxicity of the plant was studied by preparing nine different concentrations of the extract equivalent to the following doses (130, 330, 660, 1300, 2600, 4200, 5000, 5900 and 6900 mg), and administered by the I.P route to nine groups of animals. Fifty-four (54) male albino mice weighing 20 -30 g, divided in nine equal groups were used. The symptoms of toxicity were observed in these different groups, also the number of deaths in each groups of mice recorded.

The principle of the determination of the LD_{50} depends on the determination of the highest dose that dose not kill any animal and referred to as the threshold dose or the maximal tolerated dose LD_0, and determination of the minimal dose that kills the all animals LD_{100}. Several doses had been determined at equal logarithmic dose intervals. The LD_{50} was calculated according to the following formula:

$$M = X_k + \frac{1}{2}d - \frac{d \times r1}{N}$$

Where: M = log, X_k= log dose causing 100% mortality,d = logarithmic interval of doses

r = Sum of the number of the animal dead at each of the individual dose levels

N = Number of the animals on each of the dose levels.

Upon death of animal, its abdomen was opened for biopsy. Samples from liver, lung, and kidney were fixed in 10% formaldehyde, for histopathological examination.

Sub-acute toxicity

Male albino mice weighted 25-30 g were divided into different groups each of 6 mice and placed in the metabolism cages that allowed daily measurement of food and water consumption. The first group (Control) received normal saline. The second, third, and the fourth treated groups were given 130, 660, 1300 mg/kg (i.p, daily) of CTE respectively, for two weeks. All animals were fed by the regular food and water supplied by the animal house of Al Arab Medical University. The animals were weighed daily for any sign of reduction or gain of body weight.

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The food and water consumption were calculated daily for all groups for the period of the experiment (two weeks). At the end of this experiment, the mice were killed and blood collected. Glucose was determined in the sample of blood. Serum was separated by centrifugation at 5000 r.p.m for 15 minutes, divided into aliquots and used for the determination of urea(Talke and Schubert, 1995), creatinine(Jaffe, 1986) , aspartate aminotransferase (AST) and alanine aminotransferase (ALT) Levels(Bergmeyer*et al*., 1986). Samples from liver of all groups were fixed in 10% formaldehyde, for histopathological examination.

RESULTS

Acute toxicity of CTE

24 hr observation showed significant increase in the number of dead mice with increase the dose of CTE when compared with control group. As presented in the table 3 and by using of SpermanKarber method (WHO, 1981). As shown in (Table-1) The LD_{50} was nearly equal to 5400 mg/kg. The symptoms of toxicity such as increase the depth and the rate of respiration, lethargy, gathering, hypoactivity (reduction of motor activity), asthenia, and anorexia were seen in the mice up to the dose of 2600 mg/kg immediately 10 to 15 minutes after i.p administration, while at higher doses, in addition to the previous, the symptoms like decrease the rate of respiration, jumping, extension of hind limbs, staggering movement, then convulsion were observed later, and were more pronounced at higher doses (4200 till 6900 mg/kg) and persisted until death.Also as presented in (Table 1), there were no deaths observed after i.p administration of single dose of CTE extract up to 2600 mg/kg. However, the mortality rate increased progressively with increasing dose. The mortality rate was 0% at 130 gm/kg up to a dose of 2600 mg /kg, gradually rose to 100% at 6900 mg/ kg.During examination of the organ of dead animals , the hearts were found in contracted state, there was bleeding in the upper chest of unknown origin, other organs like lung, liver, kidney intestine, and genitalia were intact.The histopathology of the lung, kidney and liver revealed that, normal bronchioles, alveolar ducts and sacs with normal lining epithelium of both control and mice with lethal dose of CTE (6900 mg/ kg) (Fig6. A, B), and also showed normal glomeruli, tubules of the kidney in the mice received the lethal dose of CTE as in the control one. (Fig 6. C, D). The histopathology of the liver in the control mice revealed normal hepatocyte, portal tract and central vein (Fig 6. E), while the liver of mice received lethal dose of CTE demonstrated moderate portal tract chronic inflammatory infiltrations with mild cholestasis (Fig 6, E, and F).

Table 1: Signs & symptom due to acute administration of different doses of CTE in mice

Dose mg/kg	Number	Toxic symptoms	Dead mice	D/T	%of mortality
130	6	None.	0	0/6	0
660	6	Tachypnea, lethargy, hypoactivity.	0	0/6	0
1300	6	Tachypnea, lethargy, hypoactivity.	0	0/6	0
2600	6	Tachypnea, lethargy, hypoactivity.	0	0/6	0
4200	6	Tachypnea, lethargy, hypoactivity, staggering, gasping and death.	1	1\6	17
5000	6	Tachypnea, lethargy, hypoactivity, staggering, gasping and death.	2	2\6	33
5900	6	Tachypnea, lethargy, hypoactivity, staggering, gasping and death.	4	4\6	67
6900	6	Tachypnea, lethargy, hypoactivity, staggering, gasping and death.	6	6\6	100
Total	54		13	13/54	24.1

Sub acute toxicity:

By investigating the sub acute toxicity of CTE and by using the one way ANOVA test followed by LSD, and paired sample test in some studies and as shown in Fig.1, indicated that no significant reduction in body weight of animals given (130, 660, 1300 mg /kg) of CTE as compared to body weight of the animal at the start of the experiment in each group. As shown inFig.2.there was a significant reduction of water intake in the group received 1300 mg/kg of CTE ($p < 0.05$), as compared to the control group. There was a highly significant increase of food consumption in the group received 660 mg/kg of CTE ($p < 0.01$), as compared to the control one as shown in Fig.3.

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With regard to the effect of the CTE on some kidney parameters, and as the data presented in the Fig.4.there was no significant change in blood urea level (P = 0.338) and serum creatinine level (P < 0.182) in different doses (130, 660, 1300 mg /kg) as compared to the control group.The effect of the different doses of CTE on liver transaminases, as shown in the Fig.5.revealed no significant change in ALT (P = 0.182), but there was highly significant increase on AST in the group received 1300 mg/kg of CTE (p < 0.001) as compared to the control group.Histopathological examination of the liver of animals receiving different doses (130, 660, 1300 mg /kg) of CTE showed normal parenchymal architecture with cords of hepatocytes, portal tracts and central veins without noticeable alterations similar to the normal control group (Fig. 7. A, B, C & D).

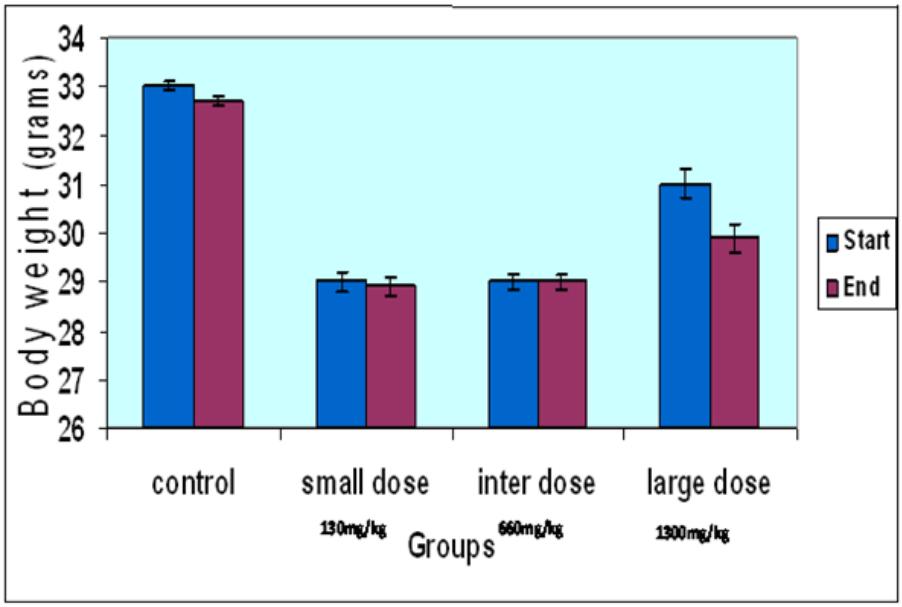

Figure-1: Effect of Different doses of CTE on the change in the bodyweight in miceNo significant Change as compared to the weight at beginning of experiment

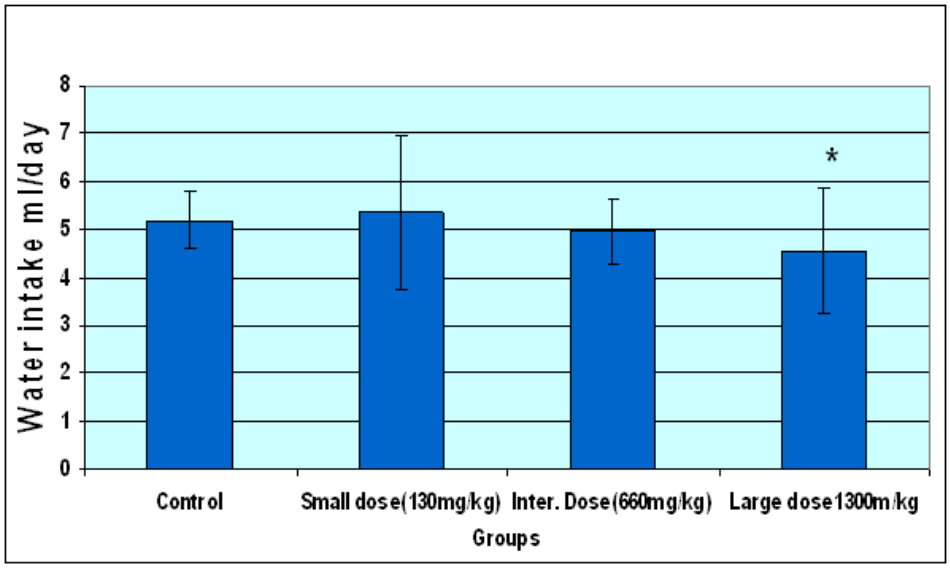

Figure-2: Effect of Different doses of CTE on the change of the water intake in mice* Significantly decreased as compared to the control group (p<0.05).

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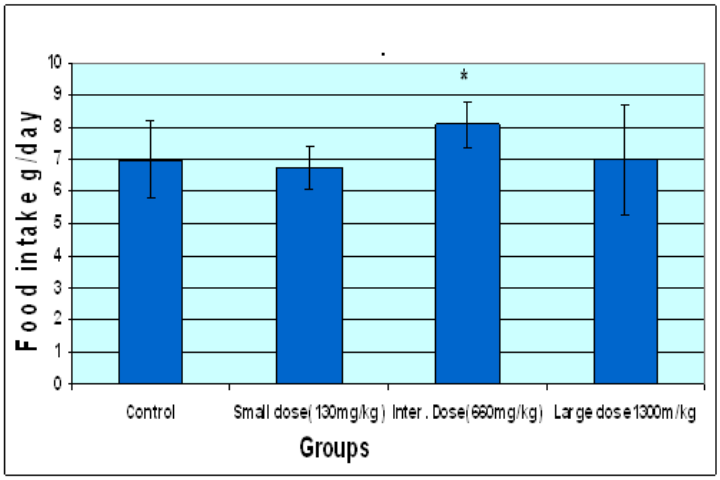

Figure-3: Effect of Different doses of CTE on the change of the food consumption in mice* significantly increased as compared to the control group (p<0.05).

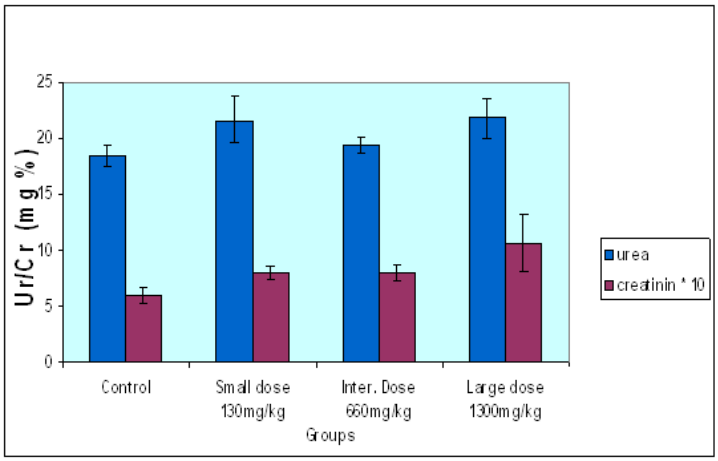

Figure-4: Effect of Different doses of CTE on some renal parametersNo significant change as compared to the control group.

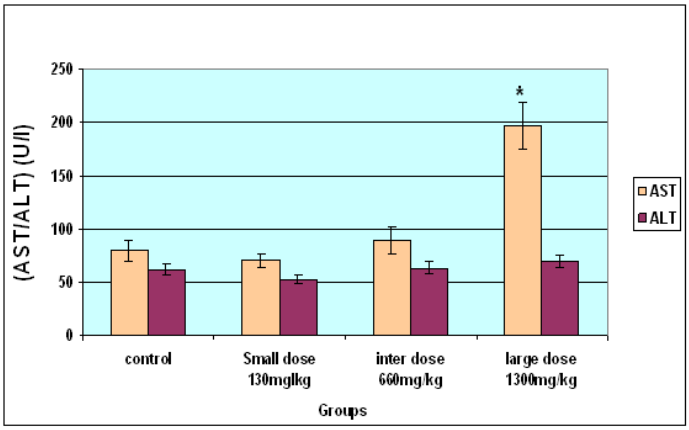

Figure-5: Effect of Different doses of CTE on some liver parameters* Significantly different from other groups (P<0.05) other groups (P<0.05)

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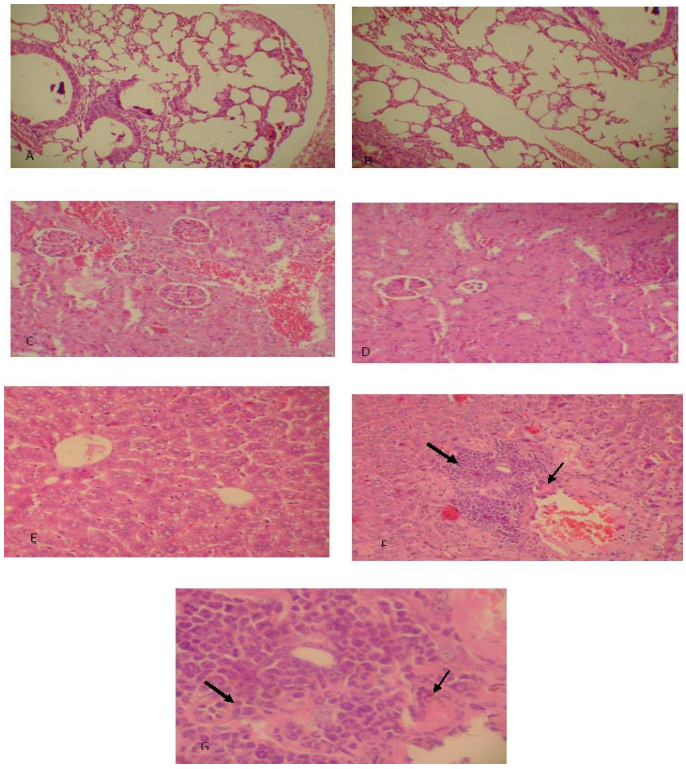

Figure 6: Light microscope of different organs of normal mice (control), and mice treated with lethal dose of CTE. Normal lung parenchyma in both control mice (A) and the mice treated with lethal dose of CTE (B). Normal kidney structure in control mice (C), and the mice treated with lethal dose of CTE (D). Normal liver parenchyma in control mice (E), the liver of mice treated with CTE showed moderate intensity of chronic inflammatory infiltration (thick arrow) and mild cholestasis (thin arrow) (F, G).

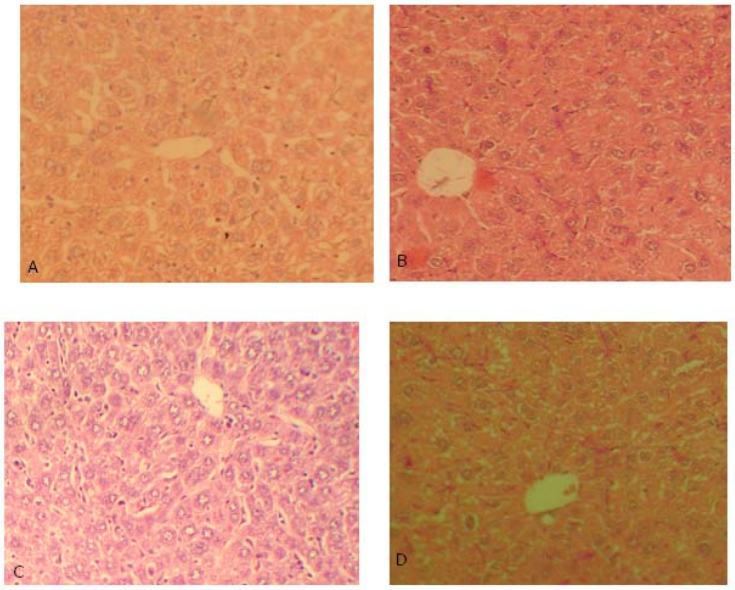

Figure 7 : Light photomicrographs (10 x magnifications) of hematoxylin and eosin–stained sections of liver of normal mice, control (A) and mice received daily injection of different doses of CTE in mice, (130 mg/kg (*B*), 660mg/kg (C) and 1300 mg/kg (D)) All section showed normal liver parenchyma as compared to control.

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Statistical Analysis

The experimental results were expressed as the mean ±S.E.M. and are accompanied by the number of observations. Data were assessed by the method of analysis of variance (ANOVA). If this analysis indicated significant differences among the group means, then each group was compared by rather the LSD, or paired sample test. A P value of 0.05 was considered statistically significant, P value of 0.01 was considered statistically highly significant.

DISCUSSION

During the past decade, traditional systems of medicine have become increasingly important in view of their safety(Krishnarajuet et al,2006). For this reason, research is carried out in order to determine the toxicity of medicinal plants. Current estimates suggest that, in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs (Ngutaetal 2012.) Camel thorn is customarily used in folkmedicine as a remedy for rheumatic pains, bilharziasis, liver disorders, and for various types of gastrointestinal discomfort (Bolus, 1983), but there is no scientific background that supports this use The present study was proposed to investigate the safety and toxicity, of the aqueous extract of Camel thorn, despite the widespread use, few scientific studies have been undertaken to ascertain the safety and efficacy of traditional remedies. The present investigation shows that the CTE is non toxic via the intra-peritoneal route in mice, at least up to a maximum dose of 4.2 g/kg. In this study, mortality and symptoms of pronounced behavior were noted only after intra-peritoneal administration of relatively high doses of Camel thorn aqueous extract in mice (LD_{50} = 5.4 g/kg); these findings, indicate the plants with no acute toxicity at a dose lower than 4 g/kg. In one study, the LD_{50} determined up to 10 g/kg orally (Islam, et al 2000,Mohhammad et al., 2007).The toxic effect of aqueous extract on the vital organs (include: liver, kidney and lung) of dead mice after administration of lethal dose (6.9 g/kg) were studied histopathologically and compared with control. These histopathological study revealed that, the lethal dose did not affect the lung and kidney parenchymal tissues, but it demonstrated moderate portal tract chronic inflammatory infiltrations with mild cholestasis.
In concentrated effort to gain insight into the possible sub-acute effect of Camel thorn aqueous extract at different doses; our study revealed that, the treatment of the mice with 130, 660, 1300 mg/kg of CTE for 2 weeks did not affect body weight of the mice. The changes in body weight have been used as an indicator of adverse effects of drugs and chemicals (Theo et al., 2002). These results suggest that at the intra-peritoneal doses of 130, 660, 1300 mg/kg of, theCTE for 14 days is not toxic, compared to the controls. It is known that the liver plays a central role in xenobiotic metabolism. Several chemicals and xenobiotic are able to induce hepatocellular damage (Omiecinski et al 2011).
Measurement of the level of serum transaminases indicates the degree of liver damage produce by hepato-toxins (Ramaiah et al 2011). The serum level of ALT is more specific to the liver and is thus better parameter for detecting liver (Ozer et al, 2008). Since there were no significant changes in the levels of transaminases (ALT, AST), creatinine and urea at a dose of 130, 660 mg/kg which are good indicators of liver and kidney functions.But there was significant change in the level transaminase (AST) at higher dose (1300 mg/kg).The histological assessment of this organ, revealed that even a higher dose (1300 mg/kg), the hepatocytes had normal parenchymal architecture as compared to the other tested doses(130, 660 mg/kg) and to the control. There is no explanation for these divergent results. However may speculate that the hepatocellular damage was very minor and thus it cannot be detected histologically. An important finding during acute toxicity study which may participate in the persistent convulsion and death of animals, was the severe hypoglycemic effect (decrease in the glucose level in some mice up to 30 mg %) which was produced by the lethal dose of CTE. To confirm the toxic nature of any plant product, one has to consider several factors that can alter its toxicity profile, including the growth stage, and the maturity of the plant, the specific part (s) of the plants (such as leaves, roots, bark, flowers, seeds etc..) used, the storage conditions of the product (freshly collected or stored for long time) the seasonal variation in the relative abundance of phytochemicals. (Jaouad et al., 2004). The present work also showed that 660 mg /kg I.P. was well tolerated dose with no adverse reaction and thus it was used during all our experimental work. From this study we may conclude that the camel thorn plant extract is safe.

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