



Toxicological evaluation of *Thymelaea hirsuta* and protective effect against CCl₄-induced hepatic injury in rats

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ABSTRACT

The aerial part of *Thymelaea hirsuta* (TH) is used as decoction in the treatment of different pathologies in folk medicine in Morocco. The present investigation was carried out to evaluate the potential of toxicity of an aqueous extract of TH and its hepatoprotective activity against CCl₄-induced hepatic injury in rats. In the acute study, there were no signs of toxicity observed after oral administration of single dose of the extract (5 g/kg body weight). In the sub-chronic dose study, (given by gavages (4 ml/kg) to Wistar rats daily at: 0.0 g/kg, 0.5g/kg, 1 g/kg or 2 g/kg body weight for 4 weeks) no variation on biochemical parameters; serum glucose, creatinine and urea levels was observed compared to the control group. However, the serum levels of alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) were markedly decreased ($P < 0.05$ and $P < 0.001$ respectively) as compared to the controls. There were no significant differences in red blood cells count ($p < 0.05$), in Hemoglobin and Hematocrit serum levels but a significant increase in white blood cells count ($p < 0.001$) and in platelets ($p < 0.05$) for all doses tested. Histopathological examination of the liver, spleen and kidneys tissues at the end of the study showed normal architecture. The elevated serum enzymatic activities of ALAT and ASAT, due to carbon tetrachloride treatment (1 ml/kg CCl₄ every 72 h during 15 days) were restored towards normalization by the plant extracts. The biochemical observations were supplemented with histopathological examination of liver sections. The results indicate that this plant possesses no toxicity effects but hepatoprotective properties.

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Keywords: *Thymelaea hirsuta*, Wistar rats, Toxicity, Histopathology, hepatoprotective action, Folk medicine.

INTRODUCTION

Thymelaea hirsuta is a plant belonging to Thymeleaceae family commonly known as "Methnane" in Morocco. The aerial part of

this plant is used as decoction in the treatment of diabetes. *T. hirsuta* has been used in folk medicine for its antimelanogenesis (Kawano et al., 2007;

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Villareal et al., 2010), and antioxidant (Djeridane et al., 2005) properties. It was shown that the aqueous extract of *T. hirsuta* possesses both hypoglycemic and antidiabetic effects in normoglycemic and streptozotocin-induced diabetic rats (Bnouham et al., 2007, 2010).

The phytochemical compositions of different extracts of *T. Hirsuta* have been well studied (Djeridane et al., 2005; Douhou et al., 2004). This plant contains polyphenols with substantial part of flavonoids, diterpens that can explain its different pharmacological activities. Two new daphnane diterpenoids, hirseins A and B isolated from the aerial parts of *Thymelaea hirsuta* were found to inhibit melanogenesis in B16 murine melanoma cells (Miyamae et al., 2009).

Herbal medicine is gaining popularity in developing countries. Herbal remedies are often believed to be harmless because they are "natural," and are commonly used for self medication without supervision. This increase in popularity and the scarcity of scientific studies on their safety and effective effect have raised concerns regarding toxicity and adverse effects of these remedies (Saad et al., 2006). These products contain bioactive principles with the potential to cause adverse effects (Bent et al., 2004).

Although its use is widespread, there is a lack of detailed toxicological information in the literature or in related cases of poisoning by *Thymelaea hirsuta* in Morocco (data of Anti-poison and pharmacovigilance Center of Morocco). Therefore, the aim of the present study was first to assess the toxicity of an aqueous extract of *T. hirsuta* on Wistar rats by the evaluation of biochemical and hematological parameters and histological studies and then to explore the potential of its *in vivo* hepatoprotective activity against CCl₄-induced hepatotoxicity. The carbon tetrachloride treatment was used extensively to investigate hepatoprotective activity on various experimental animals. It is biotransformed by Cytochrome P450 to active CCl₃ radical, which further reacts with oxygen to form trichloromethylperoxyl radical

(CCl₃O₂•) that bonds covalently to cellular macromolecules and causes peroxidative degradation of lipids membrane of the adipose tissue. This leads to the formation of lipid peroxides, which in turn yield products like MDA, which causes loss of integrity of cell membranes and damage to hepatic tissue (Thabrew et al., 1987). The hepatoprotective activity *in vivo* was studied on rat liver damage induced by CCl₄, by monitoring biochemical parameters and histological changes.

MATERIALS AND METHODS

Animals

Wistar rats weighing (220±20 g) of either sex were purchased from the animal house of the Department of Biology, Faculty of Sciences, Rabat, Morocco. They were kept in departmental animal house in well cross-ventilated room at 25±2 °C, and relative humidity 44–56%, light and dark cycles of 10 and 14 h, respectively, for 2 weeks before and during the experiments. Animals were provided with standard rodent pellet diet, water (the double distilled water) was allowed *ad libitum*. All the experiments were performed in the morning according to current guidelines for the care of the laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals (Zimmerman et al., 1983), in accordance with the internationally accepted principles for laboratory animal use and care (EEC Directive of 1986; 86/609/EEC).

Plant material

The plant material was collected from the local market, identified and authenticated taxonomically at National Scientific Institute, kingdom of Morocco. A voucher specimen of the collected sample was deposited in the institutional herbarium for future reference.

Chemical constituents

The content of saponins was evaluated by the determination of the foam index of plant extract. It was lower than 0.5 cm. The

extraction of the flavonoids was made in a methanolic solution according to the method described by Upson et al. (2000) with modifications. Some chips of magnesium were added to methanolic solution. Then, 5-6 drops of hydrochloric acid concentrate were added. The appearance of a red coloring indicated the presence of the flavonoids and the orange color indicated the presence of flavones (Siddiqui et al., 1997). The presence of alkaloids in the methanolic extract was tested by the reagent of Dragendorff. The alkaloids appear in visible light in the form of orange spots (Merck et al., 1975). The extract was also analyzed by CCM on silica gel. The research of the sterol substances was made by the reaction of Lieberman-Burchard. Their presence gives a red-brown coloring transferring to brown-purplish. The presence of free quinonoids was confirmed by the method described by Ribéreau-Gayon (1968) and the presence of tannins by the method described by Rirk (1975).

The appearance of fluorescence yellow, green, blue, orange after pulverization with KOH (10%) and visualization under UV with 366 Nm, indicates the presence of coumarins (Ladiguina et al., 1990).

Determination of DPPH scavenging activity

DPPH radical scavenging activity of the extract was determined according to the classical method with slight modifications. An aliquot of 0.5 ml of sample solution in methanol was mixed with 2.5 ml of 0.5 mM methanolic solution of DPPH. The mixture was shaken vigorously and incubated for 37 min in the dark at room temperature. The absorbance was measured at 517 nm using UV-vis spectrophotometer. Butyl Hydroxy Toluène (BHT) was used as a positive control. DPPH free radical scavenging ability (%) was calculated by using the formula: $[(A_{517 \text{ nm}} \text{ of control} - A_{517 \text{ nm}} \text{ of sample}) / A_{517 \text{ nm}} \text{ of control}] \times 100$.

Preparation of the aqueous extract of *Thymelaea hirsuta*

T. hirsuta aerial parts were prepared

according to the traditional method used in Morocco (decoction): 50 g powdered of the whole plant mixed with 500 ml of distilled water was boiled for 15 min followed by cooling for 15 min. Thereafter, the aqueous extract was filtered and concentrated in rotary vacuum evaporator at a temperature below 50 °C. The dried extracted material (approximately 10% w/w) was stored at -20 °C until use. The aqueous extracts were prepared daily, just before administration, by dissolving it in distilled water.

Evaluation of acute toxicity

According to the Organization of Economic Co-operation and Development (OECD) guideline for testing of chemicals, TG420 (OECD, 2001a, 2003), rats were randomly divided into 2 groups per sex. The extract at a single dose of 5 mg/kg body weight was given orally to treated group (1st group), whereas an equal volume of water vehicle was given to control group (2nd group). Observations were made and recorded systematically 1, 2, 4 and 6 h after test substance administration. The visual observations included changes in the skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system as well as somatomotor activity and behavioral pattern. The number of survivors was noted after 24 h and these were then maintained for a further 14 days with a once daily observation. On the day 15, all rats were fasted for 16–18 h, then anesthetized with ethyl ether and sacrificed.

Toxicological evaluation of the aqueous extract of *Thymelaea hirsuta*

Rats were randomly divided into 4 groups containing five animals per group and placed in separate cages during the study. The aqueous extract was given by gavages (4 ml/kg) to different groups of rats daily at a dose of 0.0 g/kg, 0.5 g/kg, 1 g/kg or 2 g/kg body weight for 4 weeks. The control group received the vehicle only. The general behavior of rats and signs of toxicity were observed continuously for 1 h after the oral

treatment, and then intermittently during 4 h, and thereafter over a period of 24 h (Twaij and al, 1983). The body weight changes were recorded every 15 days (T= 0, T= 15 and T= 30 days). At the end of the 4 weeks period, blood was collected in two tubes from all rats after the animals were fasted overnight, anesthetized with diethyl ether and sacrificed by decapitation: one with the anticoagulant, ethylene-diamine-tetra-acetate (EDTA), and the other without any additive. The serum was prepared by centrifugation (3000 x g, 10 min, at +4 °C) to measure the biochemical parameters.

Measurement of biochemical and hematological parameters

Serum was analyzed for glucose, creatinine, blood urea nitrogen concentrations, for the activity of liver enzymes: alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT). They were determined enzymatically by standard methods with a biochemical automat (Konelab 20 Thermo). The anticoagulated blood was analyzed immediately for hematological parameters: hemoglobin (Hb) concentration, hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), erythrocytes (RBC), white blood cells (WBC), WBC differential count, and platelets.

Hematological examination was performed using a KX21 automatic Haematology analyzer (Sysmex). For measurement of differential leukocytes, aliquots of whole blood samples were fixed on slide and analysed under microscope.

Histopathological examination of the liver, kidney and spleen tissues

After blood collection, the liver, kidneys and spleen were carefully dissected out. Small slices of these freshly harvested tissues were fixed in buffered formaldehyde solution (10%), dehydrated by serial ethanol solution, diaphanized with ethanol-benzene and enclosed with paraffin. Micrometer sections,

cut by a microtome, were stained with hematoxylin-eosin and examined under a light microscope.

In vivo hepatoprotective activity

Animal treatment and preparation of test

Hepatic injury was induced in rats by oral administration of 1 ml/kg CCl₄ mixed with equal volume of olive oil every 72 h during 15 days.

Rats were divided into three groups (n = 5). Group I (normal control) was treated with vehicle (olive oil 1 ml/kg) daily for 15 days. Group II (CCl₄ control) received 1 ml/kg CCl₄ mixed with equal volume of olive oil every 72 h during 15 days. Group III (test group) received simultaneously the extract (1 g/kg) daily and CCl₄ every 72 h for 15 days. Animals were sacrificed 48 after CCl₄ administration, blood sample of each animal was collected and serum was separated and used for estimation of ASAT and ALAT.

Enzyme assays

The enzymes ASAT, ALAT, were determined enzymatically by standard methods with a biochemical automat (Konelab 20 Thermo).

Statistical analysis

The differences among experimental and control groups were determined using the statistical software Graph Pad Prism Ver. 4.0 for Windows XP. Comparisons among different groups were performed by analysis of variance using ANOVA test. Significant difference between control and experimental groups were assessed by student's *t*-test. All data are expressed as mean ± standard error of measurement (S.E.M.); *P*-values less than 0.05 were considered to be significant.

RESULTS

Chemical constituents

From analysis, we confirmed that the aqueous extract of *T. Hirsuta* collected in Morocco was also rich in flavonoids, alkaloids, sterols, caumaris and tannins, and there was no saponins and free quinonoids (Table 1) like described in other studies

(Djeridane et al., 2005; Dohou et al., 2004; El-Beheiry et al., 2000; Garcia-Granados et al., 1980).

DPPH

DPPH is a free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares et al., 1997). The reduction capability of DPPH radical was determined by the decrease in absorbance induced by plant antioxidants. BHT was used as standard.

The extract has a stronger effect of scavenging free radical (80%) than positive control BHT (60%).

It has been found that antioxidant molecules such as ascorbic acid, tocopherol, flavonoids, and tannins reduce and decolorize DPPH due to their hydrogen donating ability (Soares et al., 1997). *Thymelea hirsuta* extract is rich in phenolics which are involved in several biological activities including antioxidant ones.

Effect of acute and sub-acute oral administration of *Thymelea hirsuta* extract on the body weight and mortality

There were no deaths or any signs of toxicity observed after oral administration of a single dose of the aqueous extract (5 g/kg body weight) in the 14 days of observation.

In the sub-acute toxicity study, the extract at doses of 0.5 g/kg, 1 g/kg, and 2 g/kg caused neither visible signs of toxicity nor mortality. Generally, the reduction in body weight gain and internal organ weights is a simple and sensitive index of toxicity after exposure to substance (Teo et al., 2002). Furthermore, gross examination of internal organs of all rats revealed no detectable abnormalities. Daily oral administration of *T. hirsuta* aqueous extract for 30 days did not produce any obvious symptoms of toxicity or mortality up to the highest dose level used of 2 g/kg.

The body weights of control and aqueous extract-treated rats are presented in Figure 1. No significant difference in body weight changes was noted between the control group and any of treated groups at any time period.

Effect of sub-chronic treatment of rats with *Thymelea hirsuta* extract on the biochemical and hematological parameters

The effect of sub-chronic treatment of rats with *Thymelea hirsuta* extract on the biochemical parameters is presented in Figure 2. Serum glucose, creatinine and urea levels of all rats treated with any dose of *T. hirsuta* extract (0.5-2 g/Kg) showed no variation compared to the control group. However, the serum levels of ALAT and ASAT were markedly and significantly decreased ($P < 0.05$ and $P < 0.001$ respectively) as compared to the controls (Figure 2). Treated rats did not show significant difference in red blood cells count ($p < 0.05$), in Hemoglobin and Hematocrit serum levels but presented a significant increase in white blood cells count ($p < 0.05$) and in platelets for all doses tested ($p < 0.001$) (Figure 3, Panels F and M respectively). Red blood cell indices (MCV, MCH, and CCMH) are indicated in Figure 3 in Panels J, K, and L. There were no significant changes in these red blood cell indices between the treated rats and the controls.

Histological changes

Histopathological examination of the liver, spleen and kidneys (Figure 4) at the end of the study showed normal architecture suggesting no morphological disturbances rats treated with all doses of the *T. hirsuta* extract tested as compared to controls.

Effect of *Thymelea hirsuta* extract on ASAT and ALAT levels

The results of the hepatoprotective effect of the extract are summarized in Table 2. In comparison with normal group, there were significant increases both in the levels of ASAT and ALAT in CCl_4 -intoxicated rats, reflecting the liver injury by CCl_4 . The elevated levels of serum ASAT and ALAT were significantly reduced in the animals groups treated with the herbal extract.

The histological observations basically support the results obtained from serum enzyme assays. Histology of the liver sections of normal control animals (Group I) showed

normal hepatic cells with well preserved cytoplasm, prominent nucleus and nucleolus and well brought out central vein (Figure 4-A-1). The liver sections of CCl₄-intoxicated rats (Group II) showed massive fatty changes, necrosis, ballooning degeneration, and broad infiltration of the lymphocytes, histiocytes and Kupffer cells around the central vein and the

loss of cellular boundaries (Figure 5-A). The histological architecture of liver sections of rats treated with extract 1g/kg (Group III) showed a more or less normal lobular pattern with a mild degree of fatty change, without necrosis and lymphocyte infiltration almost comparable to the normal control (Figure 5-B).

Table 1: Chemical constituents of the aqueous extract of *Thymelaea hirsute*.

Component	Result
Saponins	-
Flavonoids	++
Alcaloids	+
Stérols	+
Free quinonoids	-
Caumarins	+
Tanins	+

Table 2: Effect of *Thymelaea hirsuta* extract on ASAT and ALAT serum levels.

Groups	Treatments	ALAT (U/l)	ASAT (U/l)
I	Normal (olive oil)	79.5 ± 10 ^a	86.0 ± 2
II	CCl ₄ control	101.0 ± 15	113.0 ± 12
III	Extract + CCl ₄	63.0 ± 13	87.5 ± 5

Values are mean ± S.D. (n = 5), ^ap < 0.05 compared to normal.

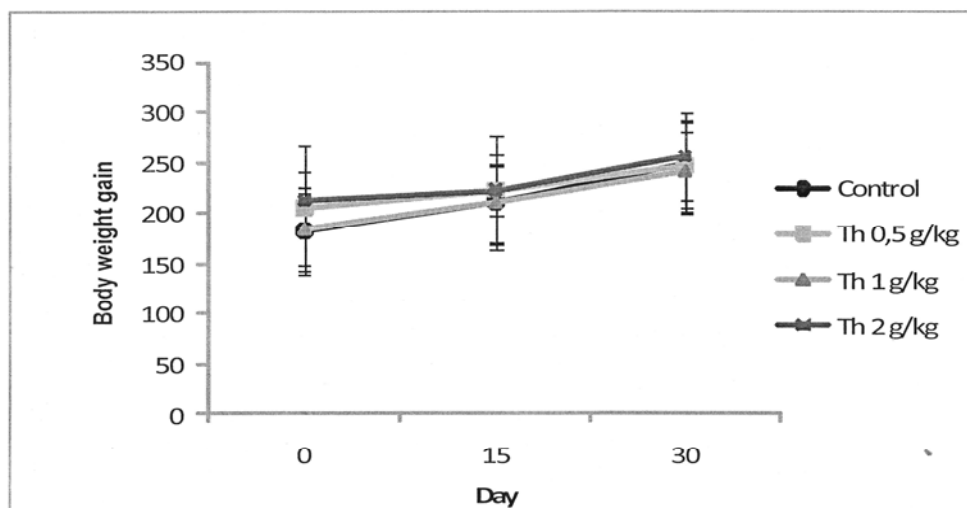


Figure 1: Body weight gain curves of Wistar rats treated orally with aqueous extract of *Thymelaea hirsuta* (Th) (0.5 g/kg, 1 g/kg, 2 g/kg) for 4 weeks. The values are expressed as mean ± S.E.M. (n = 5 rats/group).

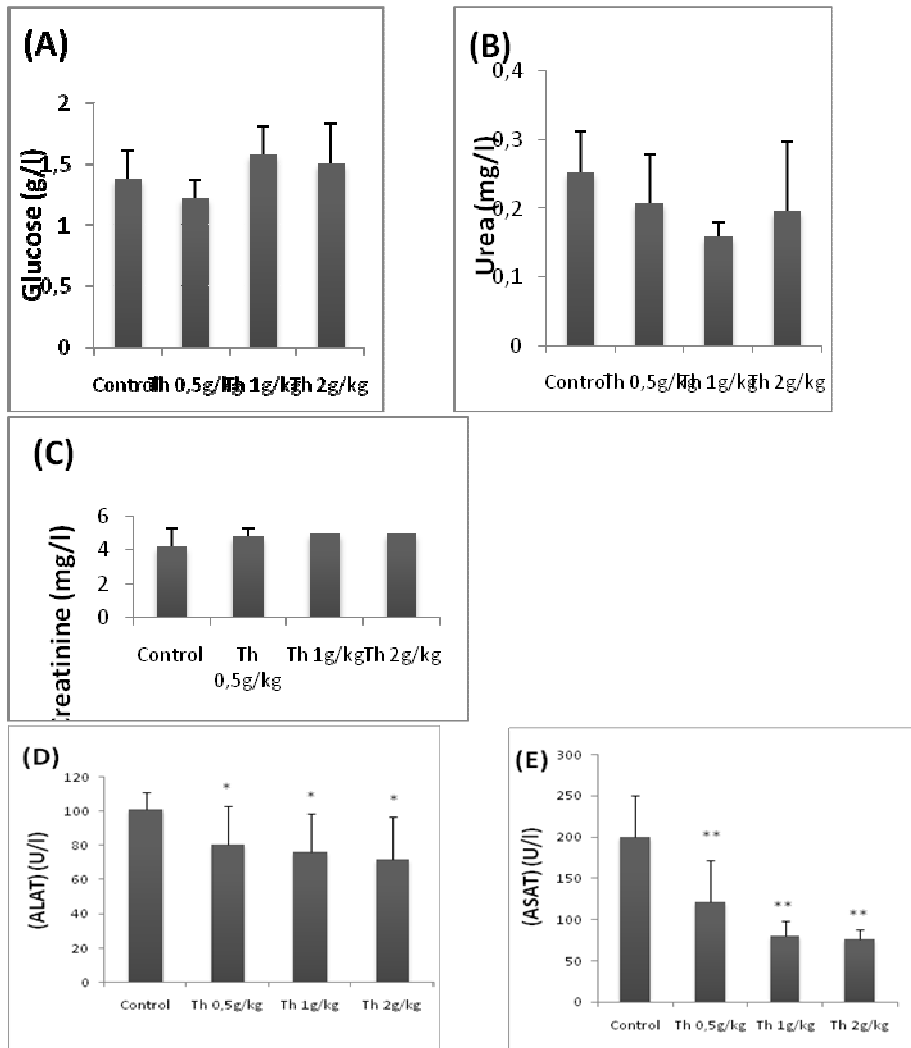
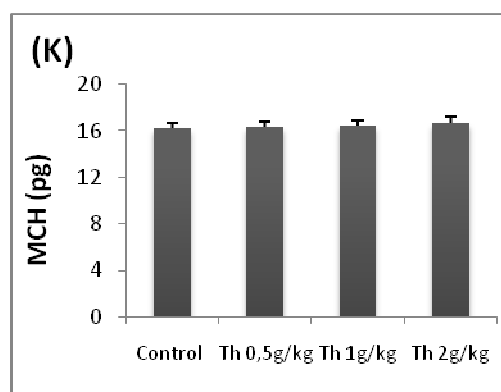
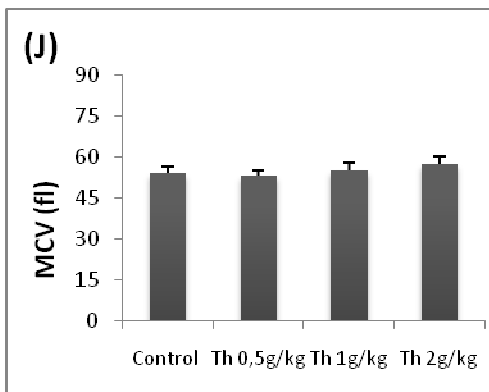
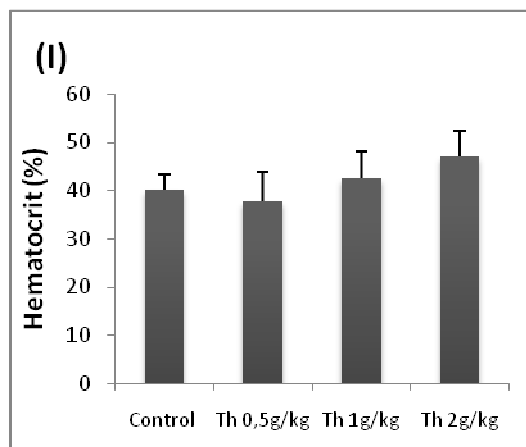
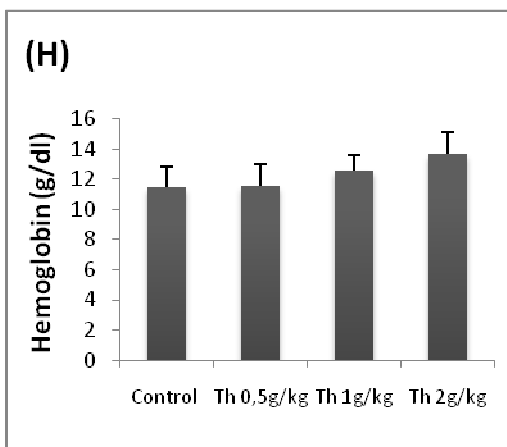
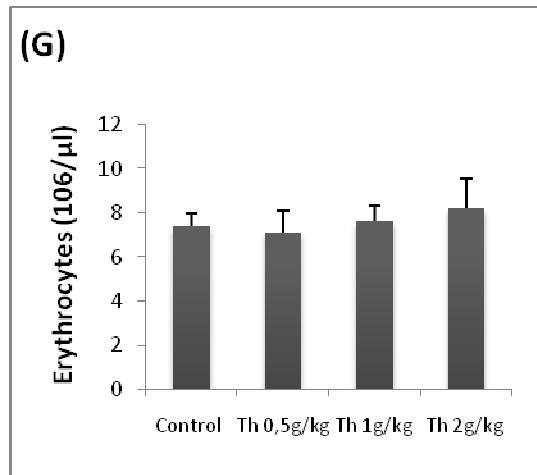
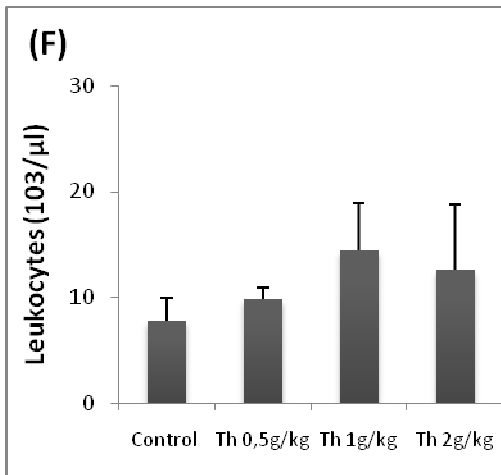


Figure 2: Effect of oral administration of *Thymelaea hirsuta* extract on biochemical parameters. The aqueous extract of the plant was given daily by the oral route to groups of Wistar rats (n =5 per group) at the following doses: 0g/kg, 0.5 g/kg, 1g/kg and 2 g/kg for 30 days. Data are expressed as mean \pm S.E.M.; (*) P <0.05; (**) P <0.01 versus the control group. (ALAT: Alanine aminotransferase; ASAT: Aspartate aminotransferase).



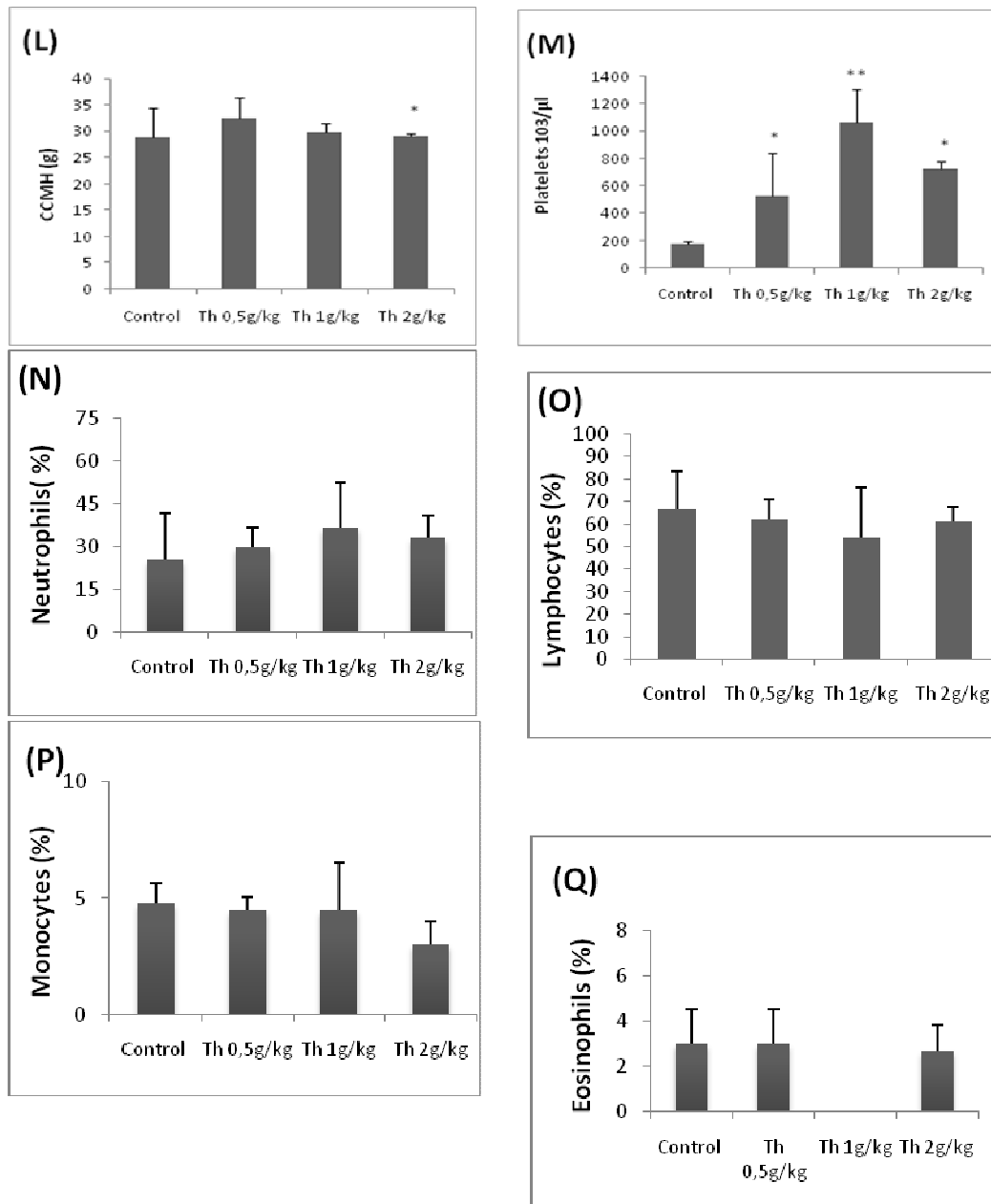


Figure 3: Effect of oral administration of *Thymelaea hirsuta* extract on hematological parameters of rats (MCV; mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: Mean corpuscular hemoglobin concentration).

The aqueous extract of the plant was given daily by the oral route to groups of Wistar rats (n =5 per group) at the following doses: 0g/kg, 0.5 g/kg, 1g/kg and 2g/kg, for 30 days. Data are expressed as mean ± S.E.M.; (*) P <0.05; (**) P <0.01 versus the control group.

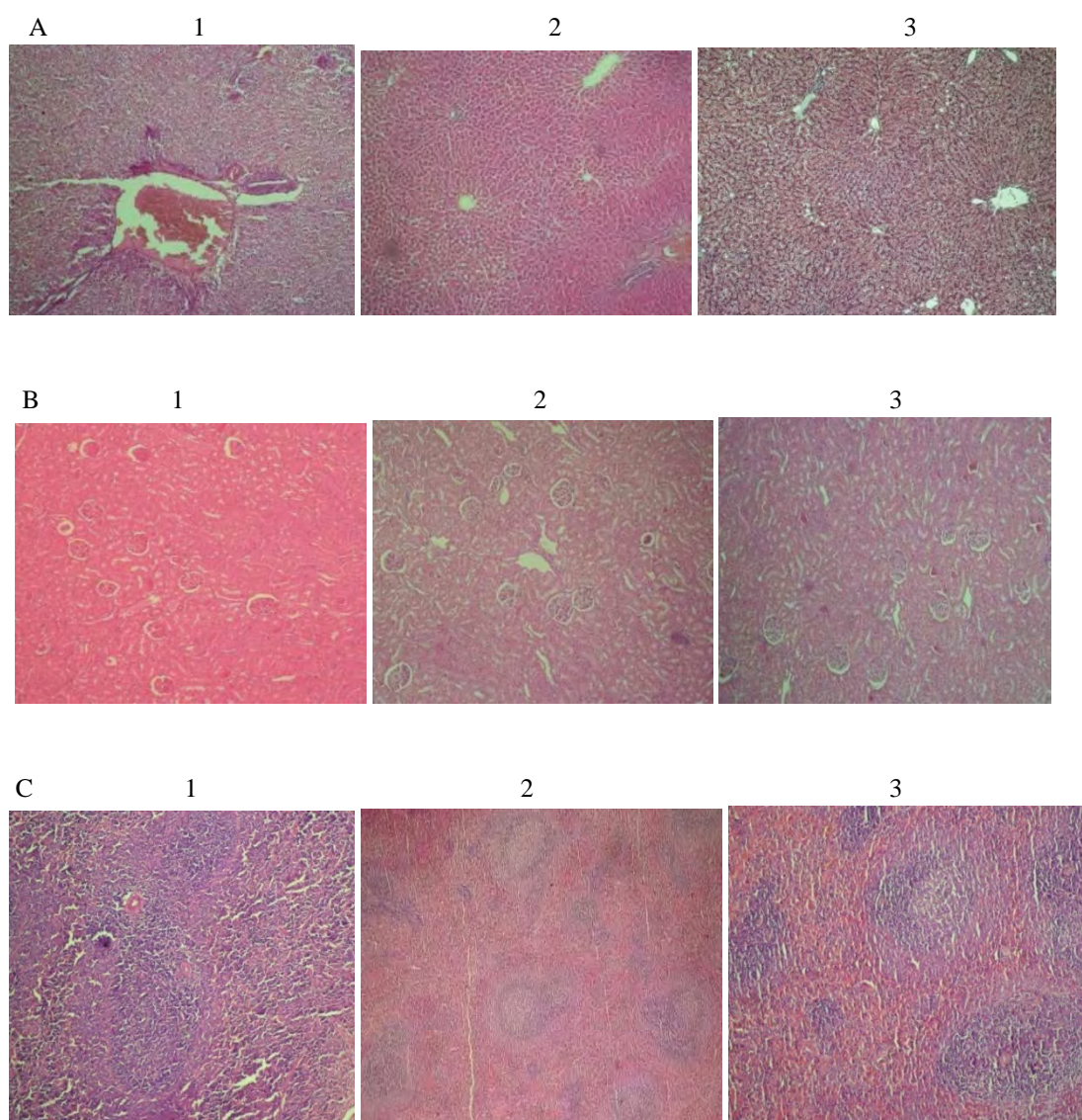


Figure 4: Histological observation of liver, kidney and spleen tissues after sub-chronic treatment with the *Thymelaea hirsuta* extract.

(A) **Liver** sections of normal control rats (1), rats treated with 1 g/kg (2) and 2 g/kg (3) of extract for 30 days showing normal hepatic cells with well-preserved cytoplasm; well brought out central vein; prominent nucleus and nucleolus.

(B) **Kidney** section of normal control rats (1), rats treated with 1 g/kg (2) and 2 g/kg (3) of extract showing a normal architecture of the tissue.

(C) **Spleen** section of normal control rats (1), rats treated with 1 g/kg (2) and 2 g/kg (3) of extract showing a normal architecture of the tissue.

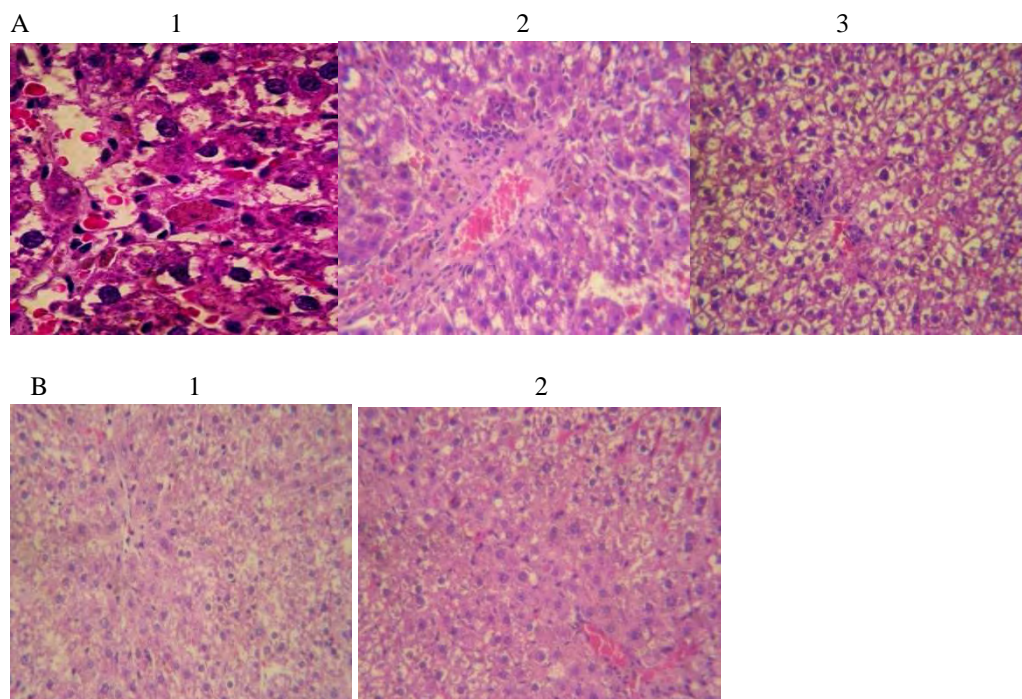


Figure 5. Histopathological changes in CCl₄ intoxicated and *Thymelaea hirsuta* treated rats.

(A) Group II: **Liver section of CCl₄ treated rats.** (1) Cholestase with biliary intracytoplasmic inclusions (HE x100). (2) And (3) Massive fatty changes, necrosis, ballooning degeneration, and broad infiltration of the lymphocytes around the central vein (HE x40).

(B) Group III: **Liver section of CCl₄ and extract treated rats.** (1) And (2) normal lobular pattern with a mild degree of fatty change, without necrosis and lymphocyte infiltration almost comparable to the normal control (HE x40).

DISCUSSION

To determine the safety of drugs and plant products for human use, toxicological evaluation is carried out in various experimental animals to predict toxicity and to provide guidelines for selecting a 'safe' dose in humans. The concordance of toxicity in animals with humans is with hematological, gastrointestinal and cardiovascular adverse effects (Olson et al., 2000).

Furthermore, it is quite difficult to ascertain certain adverse effects in animals such as headache, pain and visual disturbances. In addition, inter species differences in the pharmacokinetic parameters make it difficult to translate some adverse effects from animals to humans. Toxicity tests

are essentially performed on either mice or rats because of their availability, low cost and the wealth of toxicology data in the literature already available for these species (Rollo, 2004).

The evaluation of adverse effects of sub-chronic and chronic dosing in experimental animals may be more relevant in determining the overall toxicity of the plant preparation. Acute toxicity studies with arrange of doses have to be conducted first to select proper dose(s). The present investigation shows that an aqueous extract of the aerial parts of *T. hirsuta* at the dose of 5 g/kg given by oral route doesn't have any effect of toxicity.

For chronic and sub-chronic studies; the doses selected for chronic and sub-chronic toxicity studies should be at and above the suggested human dose. Daily oral administration of *T. hirsuta* aqueous extract for 30 days did not produce any obvious symptoms of toxicity or mortality up to the highest dose level of 2 g/kg. There was also no change in animal behavior, and the body weight gains were not significantly different in the treated rats as compared to the controls. Since, changes in body weight have been used as an indicator of adverse effects of drugs and chemicals (Teo et al., 2002), the present results suggest that at the oral doses administered of the *T. hirsuta*-extract is non-toxic in rats.

Because kidney and liver toxicity has been reported following the use of phytotherapeutic products (Saad et al., 2006), biochemical parameters evaluation is important. In the present study, creatinine and urea determinations were critical as markers of kidney activity (Newman et al., 1999). There were no significant differences of glucose, creatinine and urea levels in the groups treated with the herbal extract. This was also being confirmed by the absence of histopathological changes in the kidney tissues. At the dose tested *T. hirsuta* extract doesn't acts as hypoglycemic product as reported in others studies (Bnouham et al., 2007) under these experimental conditions.

Among the parameters evaluated, ASAT and ALAT are considered markers for liver function (Hilaly et al., 2004). In the present study, there were significant decreases in ASAT and ALAT values. ALAT is located primarily in the cytosol of hepatocytes, and this enzyme is considered a more sensitive marker of hepatocellular damage than ASAT. ASAT is an enzyme found in the cytoplasm and mitochondria in different tissues, chiefly in the heart and skeletal muscles, liver, kidneys, pancreas, and erythrocytes (Chaves et al., 1998). The decrease of these two enzymes values suggested a hepatoprotective action of the aqueous extract of *T. hirsuta*. So

we tested the effect of daily oral administration of the *T. hirsuta* -extract in CCl₄-intoxicated rats. In fact, the elevated levels of serum ASAT and ALAT in CCl₄-intoxicated rats were significantly reduced in the animals groups treated with herbal extract. Thus it suggests that the *T. hirsuta* -extract have a hepatoprotective effect against liver injury confirmed by histopathological examination of liver tissue. The present study showed for the first time, that aqueous extract from *T. hirsuta* possess hepatoprotective activity as evidenced by the significant inhibition in the elevated levels of serum enzyme activities induced by CCl₄. CCl₄ is bio-transformed by the cytochrome P450 system in the endoplasmic reticulum to produce trichloromethyl free radical ($\bullet\text{CCl}_3$). Trichloromethyl free radical then combined with cellular lipids and proteins in the presence of oxygen to form a trichloromethyl peroxy radical, which may attack lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical. Thus, trichloromethylperoxy free radical leads to elicit lipid peroxidation, the destruction of Ca²⁺ homeostasis, and finally, results in cell death (Reckengel et al., 1989). These result in changes of structures of the endoplasmic reticulum and other membrane, loss of enzyme metabolic enzyme activation, reduction of protein synthesis and loss of glucose-6-phosphatase activation, leading to liver damage (Azri et al., 1992; Wong et al., 2011). In this view, the reduction in levels of ASAT and ALAT by the extracts is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl₄. This effect is agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes (Thabrew et al., 1987; Wong et al., 2011).

Since the preliminary phytochemical analysis of the extracts have shown the presence of flavonoids and phenolic compounds, which have been known for its

antioxidant and hepatoprotective activities (Djeridane et al., 2005), thus it can be concluded that possible mechanism of hepatoprotective activity of *T. hirsuta* may be due to its antioxidant activity, which may be due to the presence of flavonoids and phenolic compounds in the extracts.

Data have shown that hepatic damage induced by CCl₄ is mediated by depleting oxygen radical scavengers in the hepatocytes; free radical metabolites, generated from bioactivation, which interact with unsaturated lipid membrane to cause lipid peroxidation and other cellular macromolecules leading to cell damage (Wong et al., 2011). In this study, the CCl₄ produced significant reductions in the activity of catalase, SOD, peroxidase and GSH and *T. hirsuta*-extract significantly increased CCl₄-diminished hepatic SOD, catalase, peroxidase and GSH activities (data not shown).

The preservation and induction of the activity of in vivo antioxidant enzymes suggest that the aqueous extract of *T. hirsuta* promotes the scavenging of reactive free radicals thus protecting the histostructural integrity of the liver cells.

Analysis of blood parameters is relevant to risk evaluation as the changes in the hematological system have a higher predictive value for human toxicity, when the data are translated from animal studies (Olson et al., 2000). In the present study, with the exception of a significant increase in white blood cells count and in platelets for all doses tested, there were no significant alterations in the others hematological parameters of rats. These results can suggest an immune-stimulatory action of *T. hirsuta* on the production of immune cells.

Conclusion

The studies carried out suggest that at all the oral doses tested, *Thymelaea hirsuta* can be considered safe as it did not cause either any lethality or adverse changes in the general behavior, histological or hematological and biochemical parameters in

the acute and in sub-chronic toxicity studies in rats. Further studies in non-rodents must be performed to prove its safety. The results of this study strongly indicate that *T. hirsuta* has a potent hepatoprotective action against carbon tetrachloride induced hepatic damage in rats related to glutathione mediated detoxification as well free radical scavenging activity. Further studies are in progress for better understanding of the mechanism of action and to evaluate the efficacy of *T. hirsuta* on liver organelle that are possibly damaged during experimental hepatitis.

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REFERENCES

- Azri S, Mata HP, Reid LL, Gandolfi AJ, Brendel K. 1992. Further Examination of selective toxicity of CCl₄ rat liver slices. *Toxicol. Appl. Pharm.*, **112**: 81–86.
- Bent S, Ko R. 2004. Commonly used herbal medicines in the United States: A view. *The Am. J. Med.*, **116**: 478 – 485.
- Bnouham M, Merhfouf FZ, Legssyer A, Mekhfi H, Maâllem S, Ziyyat A. 2007. Antihyperglycemic activity of *Arbutus unedo*, *Ammoides pusilla*, and *Thymelaea hirsuta*. *Pharmazie*, **62**(8): 630-632.
- Bnouham M, Merhfouf FZ, Ziyyat A, Aziz M, Legssyer A, Mekhfi H. 2010. Antidiabetic effect of some medicinal plants of Oriental Morocco in neonatal non-insulin-dependent diabetes mellitus rats. *Hum Exp Toxicol.*, **29**(10): 865-871.
- Chaves CB, Silva RA. 1998. Contribuicao do laboratorio de patologia clinica à pratica da antibioticoterapia. In *Farmacologia*, Silva (ed). Guanabara Koogan: Rio de Janeiro; 949– 958.
- Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. 2005. Antioxidant activity of some Algerian medicinal plants extracts containing

- phenolic compounds. *Food Chem.*, **97**: 654–660.
- Dohou N, Yamni K, Gmira N, Idrissi Hassani LM. 2004. Étude de polyphénols des feuilles d'une endémique ibéro marocaine, *Thymelaea lythroides*. *Acta Bot. Malacitana*, **29**: 233-239.
- El-Beheiry M. 2000. Evaluation of the organic composition of *Thymelaea hirsuta* populations in Egypt. *Bull. Fac. Sci., Assiut Univ.*, **29**(1): 375-383.
- Garcia-Granados A, Saenz de Buruaga A. 1980. Thymeleacea photochemistry. I. Diterpenes, triterpenes and sterols of *Thymelea hirsuta* L. leaves. (Espagnol). *Anales Quim. Ser. C Quim. Org. Bioquim.*, **76**(1): 94-95.
- Hilaly J, Ellsraïli ZH, Lyoussi B. 2004. Acute and chronic toxicological studies of Ajugaiva in experimental animals. *J. Ethnopharm.*, **91**: 43–50.
- Kawano M, Matsuyama K, Miyamae Y, Shinmoto H, Kchouk ME, Morio T, Shigemori H, Isoda H. 2007. Antimelanogenesis effect of Tunisian herb *Thymelaea hirsuta* extract on B16 murine melanoma cells. *Exp Dermatol*, **16**(12): 977-984.
- Ladiguina EY, Safronich LN, Otriacheva VE, Balandina IA, Grinkevich NI, Sorokina AA, Glizin VI, Molodojnikova LM, Mitin YS, Samilina IA, Ermakova VA. 1983. Khimicheskii analiz lekarstvenii rastenii, Moskva vischaya chkola: 172 p.
- Merck E. 1975. Révélateurs pour la chromatographie en couches minces et sur papier. Merck Darmstadt.
- Miyamae Y, Villareal MO, Abdrabbah MB, Isoda H, Shigemori H. 2009. Hirseins A and B, daphnane diterpenoids from *Thymelaea hirsuta* that inhibit melanogenesis in B16 melanoma cells. *J. Nat. Prod.*, **72**(5): 938-941.
- Newman DJ, Price CP. 1999. Renal function and nitrogen metabolites. In *Tietz Text Book of Clinical Chemistry*, Burtis CA, Ashwood ER (Eds). W.B. Saunders Company: Philadelphia; 1204–1270.
- Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, Lilly P, Sanders J, Sipes G, Bracken W, Dorato M, Van Deun K, Smith P, Berger B, Heller A. 2000. Concordance of toxicity of pharmaceuticals in humans and in animals. *Regulatory Toxicol. Pharm.*, **32**: 56–67.
- Organization of Economic Co-operation and Development (OECD) 2001a. *Guideline for Testing of Chemicals, TG420 (OECD, The OECD Guideline for Testing of Chemical: 407 repeated Dose Oral Toxicity Rodent: 28-day or 14-day Study*. OECD: Paris, France.
- Reckengel RO, Glende EA, Dolak JA, Waller RL. 1989. Mechanism of carbontetrachloride toxicity. *Pharm. Ther.*, **43**: 139–154.
- Ribéreau-Gayon J, Peynaud E. 1968. *Les Composés Phénoliques des Végétaux. Traité d'œnologie*. Édition Dunod : Paris ; 254 p.
- Rizk AM, Hammouda FM, Ismail SI. 1975. Phytochemical investigation of *Thymelaea hirsuta*. III. Coumarins. *Acta Chim. Acad. Sci. Hung.*, **85**(1): 107-115.
- Rollo FD. 2004. *Nuclear Medicine Physics, Instrumentation and Agents* (2nd edn). The C.V. Mosby Company: Missouri, U.S.A; 572-627.
- Hilaly J, Ellsraïli ZH, Lyoussi B. 2004. Acute and chronic toxicological studies of Ajugaiva in experimental animals. *J. Ethnopharm.*, **91**: 43–50.
- Saad B, Azaizeh H, Abu-Hijleh G, Said S. 2006. Safety of traditional Arab herbal medicine. *Evidence-based Complementary and Alternative Medicine*, **3**: 433–439
- Siddiqui A A, Ali M. 1997. *Practical Pharmaceutical Chemistry* (First edn). CBS Publishers and Distributors: New Delhi; 126-131.
- Soares JR, Dins TCP, Cunha A P, Ameida LM. 1997. Antioxidant activity of some extracts of *Thymus zygis*. *Free Radical Research*, **26**: 469–478.

- Teo S, Stirling D, Thomas S, Hoberman A, Kiorpes A, Khetani V. 2002. A 90-day oral gavage toxicity study of d-methylphenidate and d,l-methylphenidate in Sprague–Dawley-rats. *Toxicology*, **179**: 183–196.
- Thabrew MI, Joice PDTM, Rajatissa WA. 1987. Comparative study of efficacy of *Paetta indica* and *Osbeckia octandra* in the treatment of liver dysfunction. *Planta Medica*, **53**: 239–241.
- Twaij HA, Kery A, Al-Khazraji NK. 1983. Some pharmacological, toxicological and phytochemical investigations on *Centaurea phyllocephala*. *J. Ethnopharm.*, **9**(2-3): 299-314.
- Upson M, Grayer RJ, Greenham JR, Williams CA, Al-Ghamdi F, Chen F-H. 2000. Leaf flavonoids as systematic characters in genera *lavandula* and *Sabaudia*. *Biochemical Systematics and Ecology*, **28**: 991-1007.
- Villareal MO, Han J, Yamada P, Shigemori H, Isoda H. 2010. Hirseins inhibit melanogenesis by regulating the gene expressions of Mitf and melanogenesis enzymes. *Exp. Dermatol.*, **19**(5): 450-7.
- Wong LL, Fan ST, Man K, Sit WH, Jiang PP, Jor IW, Lee CY, Ling WL, Tam KT, Wan JM. 2011. Identification of liver proteins and their roles associated with carbon tetrachloride-induced hepatotoxicity. *Hum. Exp. Toxicol.*, **30**(9): 1369-1381.
- Zimmerman M. 1983. Ethical Guidelines for Investigations of Experimental Pain in Conscious Animals. *Pain*, **16**: 109-110.