Safety evaluation and analgesic studies of defatted methanol extract of *Capparis spinosa* L. (Capparidaceae) fruits and roots bark in albino wistar rats

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Abstract

Capparis spinosa L. is an indigenous plant from Algeria but has widespread distribution in Mediterranean area. It is used by the local populations in traditional medicine for the treatment of various diseases. The purpose of this study is to test toxicity and analgesic effect of defatted methanol extract of fruits and roots bark of this plant in albino Wistar rats. To evaluate the acute toxicity, 500-5000 mg/kg body weight of each extract was administered orally to rats; symptoms of toxicity and mortality were observed for 72 h. The results revealed the absence of toxicity for both extracts. In sub-chronic toxicity, rats were treated, with doses of 100 and 200 mg/kg/day of each extract, they were surveyed for four weeks, no symptoms of toxicity were observed. These results were confirmed by the blood biochemical analyses and the histopathology study of liver and kidney. Peripheral analgesic activity was tested orally at the dose of 100 and 200 mg/kg for each extract against pain induced by acetic acid. The dose of 200 mg/kg of both extracts presented significant analgesic effect, compared to the positive control; the acetylsalicylic acid.

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Introduction

Capparis spinosa L. or *caper plant*, also known as *Kabbar* or *Thailalout* is a long-lived shrubby plant which belongs to the genus of *Capparis* (family Capparidaceae)[1,2] very widespread in the Mediterranean countries.[3,4] In Algeria, it is grown as native shrub especially in Aurès area. The plant is one of the medicinal and aromatic plants with high pharmaceutical and ecological values.[1] It is spontaneous, xerophyte, halophile[1] and provides a required condiment: the caper,[5] the plant is also used as fodder or ornamental plant.[2]

The vegetative parts of *C. spinosa* were reported to have several biological activities such as antioxidant,[6] antifungal,[7] anti-hepatotoxic,[8] anti-inflammatory,[9] antiallergic and antihistaminic,[10] chondroprotective,[11] hypolipidemic[12] and photoprotective[13] activities.

The composition of *C. spinosa* have been widely evaluated, it consists of glycosides, flavonoïdes, alkaloids, terpenes, essential oils, fatty acids, steroids, glucosinolates,[11,14-16] carotenoids and tocopherols.[17] Vitamins such as vitamin C,[16] proteins[18] and biogenic salts like K, Mg, Ca, Na, Zn, Cu, Fe and P.[19,20]

Capparis spinosa have been widely studied[1,6-13] but no work has been done concerning its toxicological profile. The present study aims at assessing the safety of degreased methanol extract of the fruits and roots bark of *C. spinosa* by acute and sub-chronic toxicity studies on liver and kidney functional indices in albino Wistar rats, and estimating the analgesic activity by writhing test.

Materials and Methods

Plant material

The fruits and roots of *Capparis spinosa* L. were collected from Batna region (northeastern Algeria), in October 2012. The plant was identified by Pr. OUDJHIH. Veterinary and Agronomic Sciences Institute, University of Batna 1. The plant parts: roots bark and the fruits, were, separately, air dried and crushed using laboratory mortar (Retsch).

Sample preparation and extraction

Powdered materials were extracted, separately, at a ratio of 1:6 (W/V) by maceration in sequential gradient solvents: petroleum ether, chloroform and methanol with intermittent shaking for 24 hours according to Ene *et al.*[21] The mixtures were then filtered and the methanol extracts were concentrated at 45°C under reduced pressure using rotatory evaporator (Buchi Rotavapor) to obtain a roots bark defatted methanol extract (E_rMeOH) and fruits defatted methanol extract (E_fMeOH).

Animals

Experiments were performed using albino Wistar healthy rats of either sex weighting (140±20) g, obtained from the stock colony of Biotechnology of the Bioactive Molecules and Cellular Physiopathology Laboratory. Animals were allowed to free access to aliment and water *ad libitum,* they were fasted 18 hours before and three hours after the extract administration.

Experiments were conducted in accordance with the guideline 420 of the Organization for Economic Cooperation and Development.[22]

Phytochemical screening

Both extracts were tested for the presence of alkaloids, tannins, carbohydrates, reducing sugar, phytosterols and phenolics by a standard phytochemical screening.[23]

Acute toxicity study

The oral medium lethal dose (LD_{50}) was determined according to Hamilton *et al.*[24] For each extract, 36 rats were arranged randomly into six groups (6 per group).

Increasing doses (0, 500, 1000, 1500, 2000 and 5000 mg/Kg) were administered orally in a stepwise procedure (group by group) to rats. The treated rats were kept under observation during three days for any signs of toxicity or mortality.

The LD_{50} value of each extract was calculated arithmetically using the formula:

$$LD_{50} = \text{Least Lethal Dose} - \sum (a \times b) / n$$

Where: a: difference between two successive doses, b: mean mortality, n: number of animals in each group.

Sub-chronic toxicity

In this experience, five groups of six rats (three male and three female) were used, a control group (group 1) received distilled water. The second and the forth group received 100 mg/kg of the E_fMeOH and E_rMeOH respectively. Finally, the third and the fifth group received 200 mg/kg of the E_fMeOH and E_rMeOH respectively.

The treatments were given daily during 28 days, in which rats were supervised for all signs of toxicity or mortality. Their weight was measured weekly.

At the end of experience, blood samples were collected in heparin tubes for biochemical tests (glucose, cholesterol, triglycerides, total bilirubin, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum urea and creatinine) and the animals were sacrificed under ether anaesthesia.

Heart, liver, kidney and lungs of each rat were excised, observed, weighed and the organ/body weight was calculated. A portion of the liver and the kidney was fixed in formaldehyde 10% for histo-pathological studies.

Analgesic activity

In this experiment female rats of 150±10 g body weight were kept at the laboratory animal house under 12h/12h light-dark cycle conditions and were allowed to free access of water and aliments according to the method cited by Siegmund *et al.*[25]

Animal allotment and treatment

The animals were randomly distributed into six groups of six each:

Group 1 served as a neutral control, received distilled water;

Group 2 served as a positive control, received 100 mg /kg acetyl salicylic acid;[26]

Group 3 and group 4 received 100 and 200 mg/kg of E_fMeOH respectively;

Group 5 and group 6 received 100 and 200 mg/kg of E_rMeOH respectively.

Writhing test

One hour after administration of the treatments, 0.1 mL/10 g of acetic acid solution (0.6%) v/v was injected intraperitoneally to each animal. The number of writhing produced in these animals was counted for 20 min, starting 5 min after acid injection. The percentage of the analgesic activity was calculated as follows:

$$\text{Inhibition (\%)} = [(Nc - Nt)/Nc] \times 100$$

Where Nc: mean number of writhing in control group; Nt: mean number of writhing in treated group.

Statistical analyses

Results are presented as mean ±SD of six repetitions. Statistical differences were analysed by the statistical software Graph Pad Prism 5 using one-way analysis of variance (ANOVA) followed by Tukey test.

Results

Phytochemical screening

The results of the phytochemical screening revealed the presence of all tested metabolites except tannins, these findings agreed with other previous studies.[26-28]

Acute toxicity

The present study did not produce any symptom of toxicity (restlessness, hematuria, diarrhea and muscle-coordinated movement) nor mortality during the three days with all the different doses used. No mortality was recorded throughout a week for both sexes. An abdominal muscle contraction in three rats during three hours after gavage with the dose 5000 mg/kg of E_fMeOH was observed. Thus, the LD_{50} is higher than 5000 mg/kg.

Sub-chronic toxicity

In sub-chronic toxicity no signs of toxicity (loss of appetite, abdominal contraction, diarrhea, accelerated breathing and paralysis) were observed during the entire 28 days period of treatment.

At $P \leq 0.05$ no significant differences in body weight gain was observed between the treated and the control (-) group for both extracts and with the different doses as it is indicated in Figure 1.

According to Figure 2, same results were observed for organ body weight. At $P \leq 0.05$ no significant differences was recorded between the treated and the control groups.

Data on various blood parameters such as glucose, cholesterol, triglycerides, total bilirubin, SGOT, SGPT, serum urea and creatinine are represented in Table 1.

According to Table 1, glucose, triglycerides, urea and creatinine levels were not significantly affected when compared with control group. In contrary cholesterol level increased in treated animals in a dose dependent manner, whereas the levels of the liver transaminases were decreased especially in treated animals with the dose of 200 mg/kg of E_fMeOH.

No morphologic abnormalities were observed in heart, lungs,

liver and kidney. The naked eye and the microscopic examination of liver and kidney did not show any histo-pathological alterations; in liver, they were characterized by normal hepatic cells; with distinct nuclei and normal eosinophilic cytoplasm with normal sinusoids. The kidney showed a preservation of the cellular renal architecture with normal glomerules, proximal tubules and collecting ducts. The adrenal glands showed normal layer of cells in both cortex and medulla of animals treated (Figures 3 and 4).

Analgesic activity

The oral administration of defatted methanol extract of fruits and root barks of *Capparis spinosa* in rats significantly reduced, in a dose-dependent manner, the number of abdominal constrictions induced by acetic acid as compared to control group. A protective effect of 81.68% and 88.51% were observed in the treated groups by E_rMeOH at doses of 100 mg/kg and 200 mg/kg respectively. However, E_fMeOH showed 80.43% and 67.08% of protective effect, at doses of 200 and 100 respectively. The inhibition produced by the dose 200 mg/kg of E_rMeOH was not significantly ($P \leq 0.001$) different than that observed with acetylsalicylic acid (100 mg/kg) which showed a protective effect of 92.55% (Figure 5).

Discussion

In the literature, there are very few reports on the safety of *Capparis spinosa,* compared to the research on its biological activities, despite it is an ancient herb that has been widely used as condiment and drogue in traditional and alternative medicine.

Our results are in agreement with those found by Karanayil *et al.*,[29] who reported an LD_{50} superior to 5000 mg/kg for *C. spinosa*. However, this dose may cause allergic contact dermatitis with inflamed skin in prolonged use.[30,31]

Liver and kidney were chosen for the histo-pathological study because they play an essential role in the organism, detoxifying the xenobiotics, drugs and toxic molecules. The liver plays a principal role in transforming these molecules leading to the toxicity from these agents.[32-36]

Table 1. Effect of two doses of E_fMeOH and E_rMeOH on blood biochemical parameters of rats.

Parameters	Control (-)	E_fMeOH 100mg/kg	E_fMeOH 200mg/kg	E_rMeOH 100mg/kg	E_rMeOH 200mg/kg
Glucose (g/L)	0.924±0.058	0.900±0.164[a]	0.910±0.044[a]	0.985±0.016[a]	0.985±0.016[a]
Cholesterol (g/L)	0.478±0.072	0.510±0.033[a]	0.635±0.060	0.590±0.066	0.610±0.044
Triglycerides (g/L)	1.178±0.271	1.020±0.285[a]	0.880±0.296[a]	0.955±0.170[a]	1.060±0.285[a]
SGOT (U/L)	132.792±15.171	112.500±1.643	094.000±3.286	116.000±16.432	139.000±8.764[a]
SGPT (U/L)	87.760±9.040	96.000±3.286[a]	59.167±1.941	78.500±2.739[a]	84.000±8.764[a]
Urea (g/L)	0.344±0.031	0.340±0.021[a]	0.390±0.066[a]	0.350±0.033[a]	0.385±0.005[a]
Creatinine (mg/L)	5.000±0.000	5.000±0.000[a]	5.000±0.000[a]	5.000±0.000[a]	5.000±0.000[a]

Values are mean±SD of six animals. [a]test values carrying superscripts different at <0.05 compared to control group.; SGOT, serum glutamate oxaloacetate transaminase; SGPT, serum glutamate pyruvate transaminase.

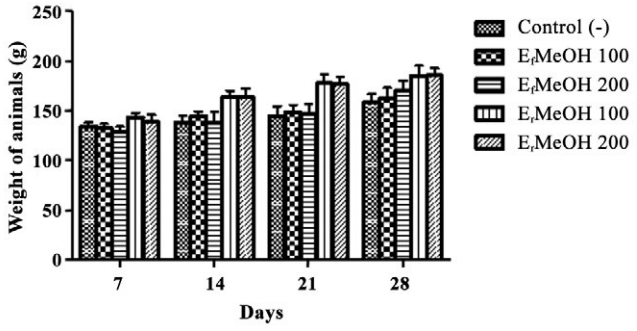

Figure 1. Evolution of the body weight of animals during the experimental period. Values are mean±SD of six animals.

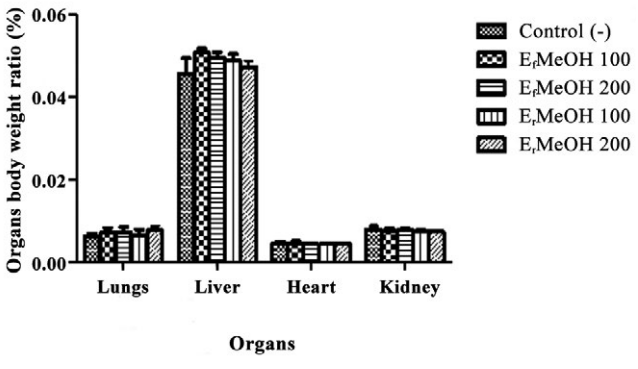

Figure 2. Effect of different doses of E_fMeOH and E_rMeOH on organs body weight ratio of the experiment rats. Values are mean±SD of six animals.

Several research evaluating the safety of the genus *Capparis;* the effect of *C. erythrocarpus* on chronic administration using histopathological studies was published,[32] according to their results, the morphology of the liver, kidney and heart tissues was not affected. Same results were found for *C. sepiaria* ethanolic extract[33] and *C. grandiflora*,[34] which did not show any acute, sub-acute or chronic toxicity. Otherwise, *C. tomentosa* has been reported to be toxic. A depression in central nervous system was observed with aerial parts of *C. moonii* ethanolic extracts.[31]

The biochemical parameters of cholesterol and liver transaminases showed a significant difference ($P<0.05$). However, according to Sharp and La Regina,[35] the ranges of clinical chemistry for rats are large (52-224 IU/l range interval of SGPT for example).

These results were concluded the safety of this plant and justify its traditional use by peoples. Despite the importance of these results, clinical tests are necessary.

Acetic acid writhing test is used to evaluate the compounds for peripheral antinociceptive activities.[37] It is useful to discriminate central and peripheral nociception.[37] The cramps appeared following the injection of the acetic acid are produced by peripheral mediators such as prostaglandins. It is suggested that the extract expressed this antalgic capacity by the inhibition of the synthesis of these mediators.

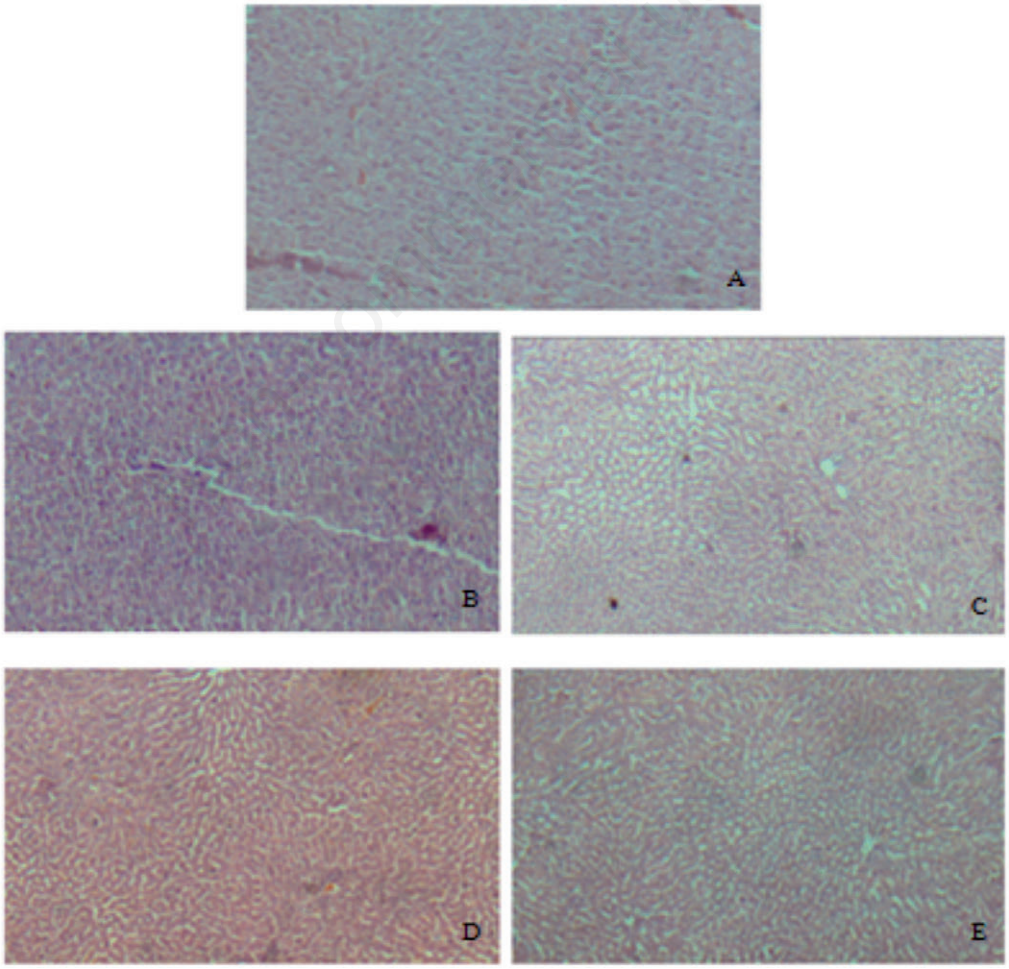

Figure 3. Histopathological sections of liver of rats on sub-chronic toxicity; in order: (A) control group rats; (B, C) rats treated with 100 mg/kg and 200 mg/kg of E_fMeOH; (D, E) rats treated with 100 mg/kg and 200 mg/kg of E_rMeOH.

Results of analgesic activity showed that the extracts tested, have a significant dose-dependent analgesic effects. In fact, a dose of 200 mg/kg has a similar action to that of acetylsalicylic acid (100 mg/kg). Preliminary phytochemical experiments indicated the presence of alkaloids and phenolics, which may be responsible for the analgesic activity. This can be checked only by the realization of better-targeted pharmacological tests to elucidate the exact mechanism of action.

Related studies in Capparidaceae family have demonstrated that different species exhibit an analgesic activity such as: *Capparis zeylanica*[26] and *Capparis ovate*.[38] These results agree with her traditional use especially against the dental pain and rheumatisms (the broadest uses in the area where made harvest).

Conclusions

In conclusion, the results of the present study clearly showed that the E_rMeOH and E_fMeOH under the conditions tested, did not induce acute or sub-chronic toxic effects in albino Wistar rats, and indicate that the extract possess analgesic properties.

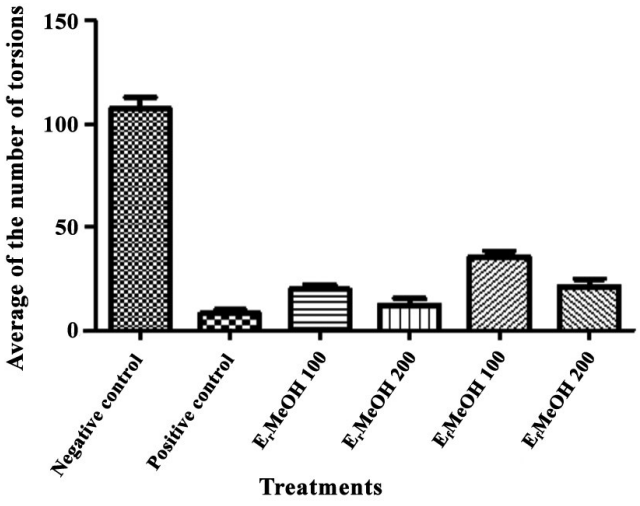

Figure 5. Effects of E_fMeOH and E_rMeOH of *Capparis spinosa* on acetic acid-induced pain in rats. Each bar represents the mean±SD of 6 animals.

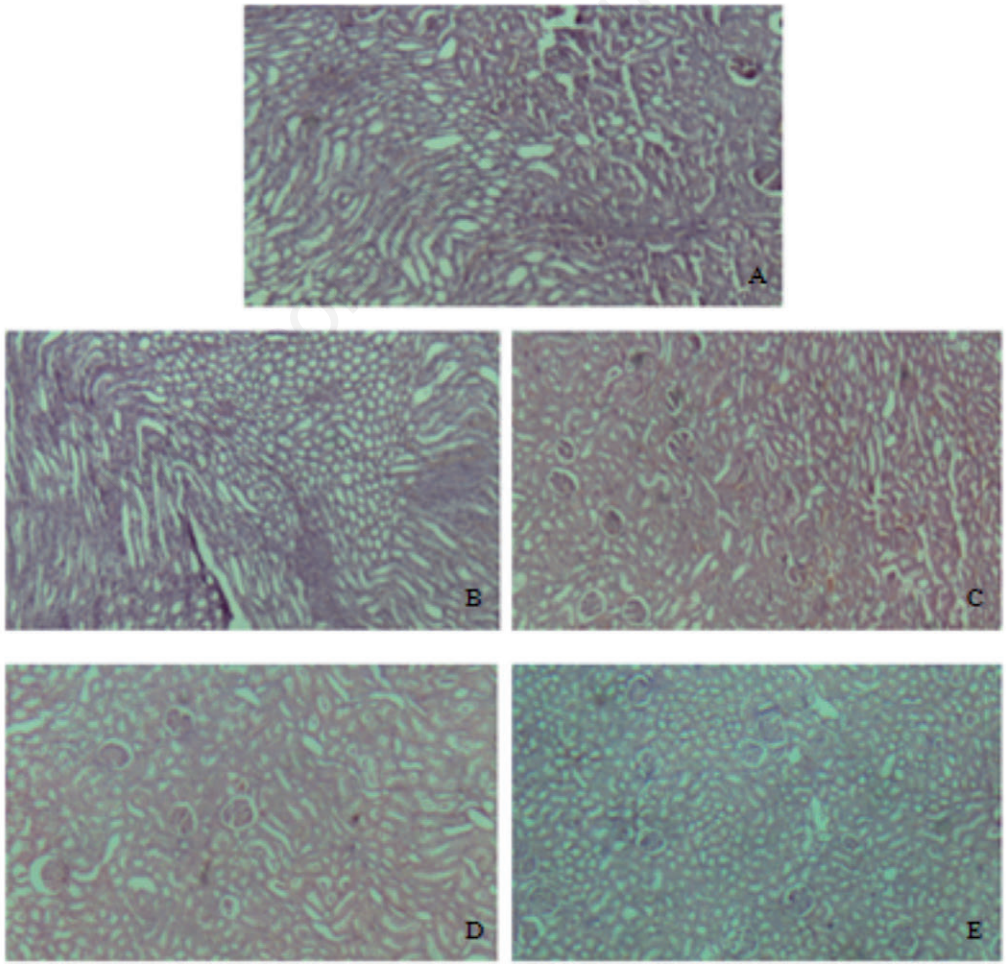

Figure 4. Histopathological sections of kidney of rats on sub-chronic toxicity; in order: (A) control group rats; (B, C) rats treated with 100 mg/kg and 200 mg/kg of E_fMeOH; (D, E) rats treated with 100 mg/kg and 200 mg/kg of E_rMeOH.

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