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Evaluation of the antihepatotoxic effect of *Argemone mexicana* leaf extracts against CCl_4-induced hepatic injury in rats

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ABSTRACT

The leaves of *Argemone mexicana* L. (Papaveraceae) are used in traditional medicine in Burkina Faso to treat a variety of diseases. In the "Cascades" region, many people use the leaves of the plant for the treatment of liver ailment. An aqueous extract and a crude leaf powder suspension were tested for their antihepatotoxic action against CCl_4-induced hepatitis in Wistar rats. The aqueous extract (250 mg/kg, p.o.) and the crude leaf powder suspension (250 mg/kg, p.o.), orally administered, significantly attenuated the elevation of serum enzymes (GOT, GPT, ALKP) and direct bilirubin (Dbil) induced by CCl_4 intoxication in rats. These actions were comparable to that of silymarin used as reference substance. For the acute toxicity test any mortality was seen at doses up to 2500 mg/kg.

KEYWORDS: A*rgemone mexicana*, antihepatotoxic, hepatoprotective, aqueous extract.

INTRODUCTION

Argemone mexicana L. is a pantropical plant originating from Mexico. It has a long history of use in traditional medicine dating back to the Aztecs (Emmart, 1940). *Argemone mexicana* is equally known in India where it is used to treat a wide variety of diseases in Ayurvedic medicine (Indranil et al., 2006). In Africa, the plant is used as antimalarial in Benin, Mali and Sudan (Merlin et al., 2007).

More than 70% of people in Burkina Faso still rely on medicinal plants for the treatment of different illnesses. *A. mexicana* L. (Papaveraceae) is one of the numerous medicinal plant species of Burkina pharmacopoeia which possess divers pharmacological properties and are used as anti-inflammatory, analgesic, antipyretic, antimicrobial, antispasmodic, etc. In the Cascade region, the leaves decoction of *Argemone mexicana* are indicated for the treatment of jaundice.

Interesting secondary metabolites such as glycosides, tannins, saponins, anthracenosides and isoquinoleine alkaloids type sanguinarin, dihydrosaguinarin, berberin,

protopin, etc. have been reported to be present in the plant (Bose et al., 1963; Harbone and Williams, 1983; Upreti et al., 1991). The plant has been previously studied in Mali for antimalarial activity (Sidibé, 2006). The aim of the present study was to evaluate the antihepatotoxic activity of the leaf extracts of *Argemone mexicana* L. in order to give an explanation about its utilization in Burkina traditional medicine.

MATERIALS AND METHODS

Plant material

Leaves of *Argemone mexicana* L. (Papaveraceae) were collected in December 2004 in Banfora (450 km from Ouagadougou, Burkina Faso). A specimen sample was identified by Dr BELEM (DPF/INERA, CNRST) by comparison with the herbarium specimen preserved in the museum of Botany Department. The registration number of the specimen was HNBU 762.

Extracts preparation

Decoction: The aqueous extract was obtained by decoction of 500 g of leaf powder in 2 L of distilled water. After filtration and centrifugation (2500 rd/min for 10 minutes), the aqueous extract was freeze-dried. Furthermore, the extract was tested for the presence of flavonoids, glycosides, tannins, steroids and alkaloids using standard methods. Powder suspension: 250 g of the leaves was reduced in very fine powder (diameter of particles, 100 µm). This powder was suspended in normal saline solution before administration to the animals.

Animals

Male and female Wistar rats (200-230 g) were allotted into five groups of six rats each provided by the CIRDES laboratory in Bobo-Dioulasso at 360 km from Ouagadougou. The animals were acclimatized for a week, prior to experiments. They were fed with standard diet and water *ad libitum* and kept in standard animal facility environment, temperature between 25 and 30 °C, 12 h light/12 h dark light cycle).

Acute toxicity

The test groups orally received plant extracts at different doses: 250, 500, 1000, 2000, 2500 mg/kg. Control group received only the vehicle (2% w/v aqueous gum acacia) at the dose of 5 ml/kg. After administration, all the treated animals were observed for signs of toxicity and mortality during 24 h, 48 h, 72 h and beyond (up to when, precise).

Antihepatotoxic activity

The specialized literature shows a great variety of procedures to study the hepatoprotective activity of vegetable drugs; so we adopted the methods of Chattopadhyay et al. (2003) and Ramachandra et al. (2007), since they were appropriated to our context. For this, the animals were divided into five groups (n = 6 per group); Group I, normal animals; Group II, CCl_4 intoxicated control animals; Group III, silymarin treated animals (reference substance), Group IV, decoction treated animals and Group V, leaf powder suspension treated group.

Liver damage was produced by injecting a daily dose of CCl_4 (0.5 ml/kg i.p.). The aqueous extracts (decoction), the crude leaf powder suspension and silymarin were suspended in 2% w/v aqueous gum acacia and administered by gavage (Anubha and Handa, 1995; Rao, 1997).

Group I received daily a single dose of vehicle (1 ml/kg, p.o.) during one week. Group II was given every day by 1 ml of vehicle (2% aqueous gum acacia) during the time of experience. Groups III, IV and V respectively received silymarin (100 mg/kg p.o.), aqueous extract (250 mg/kg p.o.), and crude leaf powder suspension (250 mg/kg p.o.) once a day during seven days. On the fifth day after the administration (daily) of the respective treatments, all the rats (groups II, III, IV and V) received the CCl_4 (0.5 ml/kg, i.p.).

On the seventh day, the animals were anesthetized with ether and blood samples obtained by cardiac puncture for the estimation of biochemical enzymatic markers. Serum was separated by centrifugation (2500 rpm for 10 min); the biochemical parameters particularly affected in liver disease such as transaminases (SGOT, SGPT), alkaline phosphatase (ALKP) and direct bilirubin (DBil) were analysed according to the reported methods. The mean value ± SEM was calculated for each parameter. Percentage reduction of the hepatotoxin was calculated by considering the enzyme level difference between the hepatotoxin-treated and the control group as 100% level of reduction.

Statistical analysis

For the determination of significant intergroup differences, each parameter was analysed separately and one-way analysis of variance was carried out. Dunnett's test was used for individual comparisons.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

A qualitative preliminary screening led to the identification of interesting constituents in the aqueous extract (decoction) (Table 1).

The presence of alkaloids, major compounds has been also noted in the flowers and the stem of *A. mexicana* L. (Papaveraceae). Upreti et al. (1991) have founded other isoquinoleic alkaloids like berberin, protopin, sanginarin, argemonin, etc. The yield extract of alkaloids was respectively 0.97% for the flowers and 0.63% for the stem.

Acute toxicity test

Crude leaf powder suspension and the decoction of *Argemone mexicana L.* did not show any mortality, even at the maximal dose (2500 mg/kg, p.o.). However, from 2000 to 2500 mg/kg, animals presented weakness in the muscles with slow movements which disappeared during the observation period (72 h).

CCl_4-induced hepatotoxicity

Carbon tetrachloride (CCl_4, 0.5 ml/kg, i.p.) induced increase in biochemical markers SGOT, SGPT, alkaline phosphatase (ALKP), direct bilirubin (Dbil) levels. The treatment with the decoction and the crude leaf powder suspension (250 mg/kg, p.o.) has significantly brought down the high levels of SGOT, SGPT, ALKP and Dbil during seven days comparatively to silymarin (100 mg/kg, p.o.), used as a reference antihepatotoxic agent. The results are reported in Table 2. The extracts (aqueous decoction and crude leaf powder suspension) did not show any acute toxicity by oral route up 2500 mg/kg. This can be considered that the drug extracts of *Argemone mexicana* L. are weakly toxic if compared to the toxicity level of Litchfiel et al. (1949). The low toxicity reported in the present study is also confirmed by Sidibé et al. (2006) whose findings showed no evidence of adverse effects at dose up to 3,2g/kg p.o. equivalent to 35 g/kg of plant powder.

Table1: Phytochemical components of *Argemone mexicana* L. leaf suspension.

Extract	Yield (%, w/w)	Chemical components				
		Ak	Flav	Cg	St	Pc
Decoction	5.13	+++	+	++	+	++

Ak= alkaloids, Flav = flavonoids, Cg = sugars and glycosides, St= steroids, Pc = phenolic compounds (tannins); +++ = abundant; ++ = present ; + = slightly present

Table 2: Effect of decoction and the crude leaf powder suspension of *A. mexicana* L. (Papaveraceae) on CCl_4-induced hepatotoxicity in rats.

Treatment	Dose (mg/kg, p.o.)	SGOT (UI/L)	SGPT (UI/L)	ALKP (UI/L)	DBil (mg/dl)
Control (vehicle)	-	63,95±2,51	97,18±5,02	130,50±4,34	1,52±0,20
CCl_4 (vehicle)	-	145,50±6,46	212,72±3,73	219,42±16,83	2,46±0,22
Silymarin+CCl_4	100	79,92±2,37 (**87.77%**)	118,97±2,01 **(81.31%)**	148,71±0,02 (**79.97%**)	1,75±2,53 **(75.53%)**
Decoction+CCl_4	250	83,99±3,61 **(75.42%)**	123,95±15,05 **(76.83%)**	158,86±11,78 **(68.50%)**	1,97±0,59 **(52.12%)**
Crude leaf powder suspension +CCl_4	250	87,16±4,25 **(71.53%)**	132,39±15,08 **(69.52%)**	132,39±15,08 **(63.43%)**	2,04±0,46 (**47.87%**)

Values are expressed as mean ± S.E. n = 6; P<0.01 vs CCl_4; one way analysis and Dunnett's test; percentage reduction (%) between bracket; SGOT = serum glutamyl oxaloacetate transaminase; SGPT= serum glutamyl pyruvate transaminase; ALKP = alkaline phosphatase; Dbil = direct bilirubin.

The increased levels of serum enzymes (SGPT, SGOT, ALKP) comparatively to the control group (CCl_4–treated ,Group II) was indicative of pathological conditions of the liver. The treated animals (groups IV and V) showed a significant decrease in SGOT, SGPT and ALKP enzymes level when compared to the hepatotoxic group (group II). The percentage reduction of hepatotoxicity in test groups (IV and V) with the two extracts is similar (profile) to that silymarin used as antihepatotoxic reference substance. That constitutes a proof of the therapeutic efficacy of *Argemone mexicana* L. as hepatoprotective plant used for jaundice treatment in traditional media.

Both the decoction and the crude leaf powder suspension presented the same profil for liver protection and these findings seem interesting in terms of drug formulation. Our results present similar profile with those achieved by Thiombiano et al. (1987) and Ouattara (1999) concerning the antihepato-toxic activity of two other medicinal plants (*Nauclea latifolia* Sm, Rubiaceae and *Cochlospermum tinctorium*, Cochlosperma-ceae). The leaf decoction of the two plants is much recommended for the treatment of jaundice in the Cacades region in Burkina Faso.

Conclusion

The present study has demonstrated the pharmacological potential of leaves of *Argemone mexicana* L. (Papaveraceae) in the treatment of hepatic disorders and justifies the use of the leaves decoction of the plant by local population of Cascades region to treat liver ailments. These promising results encourage further investigations such as the mechanism by which the plant extract exert its hepatoprotective action on CCl_4-intoxicated animals and also other pharmacological indications of the plant (anti-inflammatory, antispasmodic, antipyretic, analgesic).

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