

«Evaluation of the anti-icterus effect of crude powdered leaf of *Argemone mexicana* L. (Papaveraceae) against CCl₄-induced liver injury in rats».

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ABSTRACT

Leaves of *Argemone mexicana* L. (Papaveraceae) are used in the folk medicine of Burkina Faso (West Africa) to treat a variety of illness. Aqueous decoction of the drug is indicated in the treatment of malaria fever, abdominal pains, and jaundice. A preliminary study led by the authors showed a good anti-icterus (hepatoprotective) activity of leaves extracts on intoxicated Wistar rats.

The aim of the present investigation was to evaluate the anti-icterus activity of crude leaf powder against CCl₄ induced hepatotoxicity in rats. Liver functions were assessed by the activities of liver marker enzymes, ASAT/GOT, ALAT/GPT, ALP, Total Bilirubin (TBIL) and Direct Bilirubin (DBIL).

A crude powdered leaf suspended in acacia gum solution (2% p/w) was administered orally to the animal at doses of 125, 250 and 500 mg/kg attenuated significantly (p<0,05) the elevation of serum enzymes level and bilirubin (total and direct) if compared to the CCl₄ treated groups. Silymarin (100 mg/kg, p.o.), a known anti-hepatoprotective drug was used as reference.

The results showed a dose-dependent anti-hepatotoxic effect against liver injury induced by CCl₄ in rats. These findings give an opportunity for a future elaboration of galenic formulation as phytomedicament.

Keywords: *argemone mexicana*, crude leaf powder, anti-icterus effect.

INTRODUCTION

In the absence of reliable liver protective drugs in modern medicine, it exists in the field of medicinal plants a great number of preparations recommended for the treatment of liver ailment. The use of medicinal preparations is in vogue since centuries and are quite often claimed to offer significant relief. An application of *Argemone mexicana* L. (Papaveraceae) crude leaf powder in traditional medicine in Burkina Faso is for the treatment of jaundice (icterus), particularly in the area of the "Cascades" region. Therefore, the anti-icterus activity of the powdered drug against CCl₄-induced liver damages was investigated.

Argemone mexicana L. (Papaveraceae), originated from Mexico, is a pan-tropical medicinal plant, which has a long history of use in traditional medicine dating back to the Aztecs (Emmart, 1940). It is a well-known plant used in certain regions of the world to treat several diseases: in India, the leaves decoction are indicated for treatment of bacterial pathology in the Ayurvedic medicine (Indranil *et al.*, 2006). In West Africa, *A. mexicana* is used as uncomplicated malaria remedy in Mali (Willcox *et al.*, 2007) and Burkina Faso (Sourabie *et al.*, 2006, 2009, 2010).

Particularly in the case of Burkina Faso (statistics DGPML, 2006), it is estimated that more than 70% of people still rely on medicinal plants for the treatment of various diseases; and *Argemone mexicana* is specially used in the area of Cascades (south western part of Burkina Faso) for the treatment of malaria, fever and jaundice (icterus). According to the traditional medical practitioners in this area, the plant (*A. mexicana*) posses important pharmacological properties explaining its use as anti-inflammatory, analgesic antipyretic, antimicrobial and antispasmodic.

On the chemical way, many authors reported that interesting secondary metabolites are present in the drug (Bose *et al.*, 1963; Harbone and Williams, 1983; Upreti *et al.*, 1991) such as glycosides, tannins, saponins and alkaloids, especially isoquinolein alkaloids type as sanguinarin, dihydrosanguinarin, berberin, protopin, etc.

Thus, the aim of this work was to evaluate the anti-icterus activity of the crude leaf powder on CCl₄-induced hepatic injury in rats in order to give an explanation about utilization of that plant in the folk medicine of Burkina Faso.

MATERIALS AND METHODS

Plant material

Argemone mexicana leaves were collected in December 2004 in two villages (Beregadougou and Fabledougou), located about ten (10) to twelve (12) kilometers from Banfora (450 km from Ouagadougou, south western). A specimen sample of the drug, brought in Ouagadougou was firstly identified in the laboratory of Pharmacognosy (UFR/SDS, University of Ouagadougou). The identification of the specimen sample was also confirmed and certified by the botanical specimen preserved in the museum of Botany Department. The registration number of the specimen was HBNU 762

Preparation of the Crude leaf powder

The leaves after drying in the shade and under ventilation were crushed in order to obtain crude powder. This crude powder was later tamised in fine powder (diameter of particles; 100µm). During the test moment, this fine powder serving as drug will be suspended in in acacia gum solution (2% p/w) before administration to the rats.

Experimental animals

Male and female Wistar rats (200-230 g) were allotted into six groups of six animals each; they provided by the laboratory of CIRDES (360 km from Ouagadougou. Before the commencement of the experiments, the rats were acclimatized to laboratory conditions for one week. They were fed with standard rat feed and water ad libitum and kept in standard animal facility environment, temperature between 25 and 30°C, 12h light/12h dark (light cycle).

Drug and chemicals

All the drug and chemicals used were of analytical grade. Silymarin was purchased from pharmaceutical officin; carbon tetrachloride was purchased by phytochemical laboratory; SGOT, SGPT, ALP and Direct Bilirubin (DBIL) were procured from Univer Biomedical Lab.

Acute toxicity test

The test groups of rats (six groups of 5 rats each) received orally the fine crude leaf powder suspended in acacia gum solution (2% p/w). Several doses of drug corresponding to each group were used: 250, 500, 1000, 2000 and 2500 mg/kg p.o. Control group received only the vehicle (2% w/v aqueous gum acacia) at the dose of 5 mL/kg.

After administration, all treated rats were kept for observation in order to detect signs of toxicity and mortality during 24h, 48h, 72h and beyond.

Animal treatment (anti-icterus test)

The study of anti-icterus (hepatoprotective) properties due to the medicinal plants is characterized by a great variety of protocols (Fleurentin J., 1990). So for the present work, we adopted the method of Rao and Mishra (1998), Al-Qarawi *et al.*, (2003), which methods were appropriated to our laboratory context. For the experience, the rats were divided into six groups of six (06) animals each (n= 6 per group). Group I served as normal control and then received the vehicle (5mL/kg p.o.) during nine (09) days; Group II served as positive control intoxicated (CCl₄), received equally every day the vehicle (0,5mL/kg, i.p.); Group III served as standard, (silymarin is given at a dose of 100 mg/kg); groups IV, V and VI received drug treatment corresponding respectively to the doses of 125, 250 and 500 mg/kg *per os*.

At the seventh day, the animals of all groups, excepted those of group I received carbon tetrachloride CCl₄ (0,5 mL/kg, i.p.). At the end of experimental period corresponding to the tenth day, the rats were sacrificed after ether anesthesia. Blood samples were collected by direct cardiac puncture and kept in EDTA containing vials for 10 minutes, then it is centrifuged for 5-10 minutes at 2500 rpm.

The serum was collected and used for biochemical estimations like aspartate aminotransferase (AST/GOT), alanine aminotransferase (ALT/GPT), phosphatase alkaline (ALP), total bilirubin (TBIL) and direct bilirubin (DBIL) as per standard procedures, mainly those of Mukherjee *et al.*, (2002); Henry R.J., (1974), Enrique E. and Slivo R., (1926). Dosage of biochemical markers was done by an automate type Konelab 20. This instrument uses only specific reagents.

The results were expressed as percent hepatoprotective (anti-icteric) according to the formula of Al-Qarawi *et al.*, (2003). The percentage reduction of the hepatotoxin (CCl₄) was calculated considering the enzyme level difference between the hepatotoxin-treated and the control group as 100% level of reduction

Statistical Analysis

Results were presented as mean ± SEM for all values; One way ANOVA was used to statistically analyzed the results followed by Student “t” test. The level of significance if kept at P<0,05.

RESULTS AND DISCUSSION

Results

Phytochemical screening about crude powder leaf of *Argemone mexicana* L. showed in majority the same chemical principles as those revealed in the study of Sourabié (2009) and equally by Léga (2010). These chemical principles identified are summarized in **table 1**. Concerning the acute toxicity test, the suspension of the leaf powder did not show any mortality even at the dose of 2500 mg/kg b.wt (Sourabié *et al.*, 2009).

The effect of crude powdered leaf of *Argemone mexicana* L. on serum transaminases (GOT/AST; GPT/ALT), alkaline phosphatase (ALP), total bilirubin (TBil), and direct bilirubin (DBil) on CCl₄-intoxicated rats are summarized in **table 2**. There was significant ($p < 0,01$) increase in serum GOT (AST), GPT (ALT) and ALP levels in CCl₄-intoxicated group (group II) if compared to the normal control group (group I).

The effect of crude powdered leaf (*Argemone mexicana*) on total bilirubin (TBil) and direct bilirubin (DBil) are equally shown in table 2. For the two parameters, there was significant ($p < 0,05$) elevation of total bilirubin and direct bilirubin in the serum of CCl₄ intoxicated groups (group II) when compared to normal control group (group I). The crude leaf powder (suspension) in the different doses (125, 250 and 500 mg/kg b.wt) reduced the levels of total and direct bilirubin (table 2).

Pharmacological profile of the anti-icterus effect

In order to define how the anti-icterus effect is exhibited, we have determined the “hepatoprotective power” (HP) of the drug from the five (05) investigated biochemical parameters. This has been done in order to know if the anti-icterus effect is *dose-dependent* or not. The hepatoprotective power (H.P.) is calculated according to the following formula:

$$\text{H.P.(\%)} = \text{percent reduction of } [\text{GOT} + \text{GPT} + \text{ALP} + \text{TBIL} + \text{DBIL}] / 5$$

where **H.P.** = hepatoprotective power” of the drug at the dose considered, expressed in mean percentage (%) of reduction.

GOT: glutamic oxalacetic transaminase; **GPT:** glutamic pyruvic transaminase; **ALP:** alkaline phosphatase

TBIL: total bilirubin; **DBIL:** direct bilirubin

[GOT+GPT+ALP+TBIL+DBIL] = the amount of reduction percentage of the 5 investigated biochemical parameters.

The results of Hepatoprotective Power (HP), compared to that of silymarin showed a dose-dependent anti-icterus profile about the crude powdered leaf of *Argemone mexicana* Linn. (**table 2**).

Results presented in table 3 showed effectively presence of anti-icterus effect exerted by the leaf powder. The pharmacological action is present on all the biochemical parameters studied. If compared with silymarin (100 mg/kg), the Hepatoprotective Power of the crude powdered leaf of *Argemone mexicana* Linn. is increased when the dose administered to the rats becomes more and more higher. These results confirm the dose-dependent property that characterizes the crude powdered leaf of *Argemone mexicana* Linn.

Discussion

Protection against CCl₄-induced liver injury has been taken as a test for potential anti-hepatotoxic (hepatoprotective) agent by several investigators (Sha *et al.*, 2009; Sanmugapriya (2006). Furthermore the changes associated with CCl₄-induced liver damage are similar to that of acute viral hepatitis (Suja *et al.*, 2004); so CCl₄-mediated hepatotoxicity was chosen as the experimental model.

Moreover, the ability of a hepatoprotective drug (as the crude powdered leaf of *Argemone mexicana* L.) to reduce the injurious effects or to preserve the normal hepatic physiological mechanism, that have been disturbed by a hepatotoxin (CCl₄; 0,5 mL/kg i.p.), is the index of its protective effect according to Yudav and Dixit (2003).

In the present work, the increase of the levels of serum markers GOT, GPT, ALP and Bilirubin (total and direct) are the signs which showed significant hepatic damage in CCl₄ intoxicated rats (group II) as shown in **table 2**. This hepatic injury can be attributed to the structural integrity damage of liver, because these enzymes have a cytoplasmic location and released into circulation after cellular damages, indicating development of hepatotoxicity according to Sallie *et al.*, (1991). Carbon tetrachloride (CCl₄) is the main responsible of that toxicity which causes multiple damages in the liver. Its toxicity is due to the metabolites particularly the *trichloromethyl* (.CCl₃) and the derivative *trichloromethylperoxyde*. These two metabolites are generated by the intermediate of cytochrome P₄₅₀ hepatic oxidase.

Thus, these free radicals can alkylate cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids in the presence of oxygen, to provide lipids peroxides, leading to liver damage (Sarada *et al.*, 2012; Sanmugapriya *et al.*, 2006; Bishayee *et al.* 1995). Hepatocellular necrosis leads to elevation of the biochemical serum enzymes levels, which are released from the liver into blood (Ashok *et al.*, 2002).

So, increased levels of GOT, GPT, ALP and serum bilirubin (TBil and DBil) can be considered as conventional indicators of liver injury according to Achliya *et al.*, (2004).

The results of our study revealed a significant increase of the activities of GOT, GPT, ALP and serum bilirubin levels on exposure to CCl₄, indicating considerable hepatocellular damage. On the other hand, oral administration of crude powdered leaf to the intoxicated rats attenuated the increased levels of the serum enzymes, produced by CCl₄ and caused a subsequent recovery towards normalization like that of silymarin treatment.

This normalization of serum biochemical markers by crude powdered leaf of *A. mexicana* suggests that it is able to condition the hepatocytes so as to protect the membrane integrity against CCl₄-induced leakage of biochemical marker enzymes into the blood plasma. We can affirm with great certitude that the above changes can be considered as an expression of the functional improvement of hepatocytes.

Concerning the mechanism of action of the anti-hepatotoxic effect exhibited by the crude powdered leaf of *Argemone mexicana*, it can be attributed to the phytochemical components highlighted in the powdered suspension extracts. Effectively, according to Rathi *et al.* (2008) protopin, one of the isoquinoleic alkaloids of *A. mexicana* exerts an inhibition action on lipid peroxides formation. And it has been known that lipid peroxidation is one of the important steps of CCl₄ metabolism leading to the degradation of hepatocytes cells membranes. The mechanism of the anti-icterus effect can also be due to some phytoconstituents as sugars and glycosides present in the drug (Table 1). These components according to Chiu *et al.*, (1992) and Ye *et al.*, (2001) derived from polysaccharides that some of them have been well documented in literature about their anti-hepatotoxic property.

The inhibition of lipid peroxidation by protopin creates a good condition for liver protection against CCl₄-intoxication that is known to cause many important damages to the liver. Finally, the mechanism of action exerted by protopin (isoquinoleic alkaloid of *A. mexicana*) is similar to that of silymarin, considered as an inhibitor of cytochrome P₄₅₀ (Sarada K. *et al.*, 2012). The inhibitory role of silymarin was effectively confirmed by Letteron P. *et al.*, (1990) concerning the metabolism of Carbon tetrachloride (CCl₄).

Silymarin inhibits the hepatic oxidase CYP₄₅₀ (Cytochrome P₄₅₀), the main enzyme responsible for the activation of carbon tetrachloride (CCl₄) transformation into its metabolites notably radical *trichloromethyl* which leads to *trichloromethylperoxyde*, free radical. This derivative free radical (*trichloromethylperoxyde*) plays a great role in the lipids peroxidation leading to liver damage.

The present work is of a great interest by the results obtained; in fact, the anti-icterus effect revealed by the leaf powder is similar to those shown by leaves extracts (Sourabié *et al.*, 2012). And chemically, the phytoconstituents revealed in the crude powder leaf are practically the same discovered in the lyophilized extracts; this means that it is possible to administrate directly the powdered form of the drug to the patients. Another interest provided by these results is that the traditional medical practitioner can propose the powdered form of the drug for the treatment of their patients. By this way, the decoction step is avoided and this constitutes an advantage on therapeutic way.

Finally for the researchers in the domain of medicinal plants, these findings provide a good opportunity to apply the results of fundamental research into applied one. In the case of the crude powdered leaf of *A. mexicana* Linn., it is possible to make a galenic formulation of the drug powder in order to elaborate a Traditional Ameliorated Medicament (TAM) called also Phytomedicament. The presentation in capsule form of this phytomedicine can follow the same procedure of formulation as FACA capsules, a phytomedicine produced by the Research Institut of Health Service (IRSS) and which is used in the treatment of acute crisis of sickly cell disease (drepanocytosis).

CONCLUSION

The results of the present work showed the anti-icterus (anti-hepatotoxic) effect of crude powdered leaf of *Argemone mexicana* L. (Papaverceae) in dose-dependent manner similar to those of lyophilized extracts of the same drug. These results are very interesting since they constitute a good example for applied research by the elaboration of phytomedicine available for the treatment of icterus disease. Finally, our study gave a scientific basis to the traditional use of different parts of *Argemone mexicana* Linn. in Burkina folk medicine.

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Table 1: Phytochemical compounds of *Argemone mexicana* L. leaf powder suspension; (In study of Sourabié *et al.* (2009)).

PHYTOCONSTITUENTS						
Drug yield (%)	AK	Flav	Cg	St	Pc	
Leaf powder	5,13	++++	+	++	+	++

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

Tableau 2: effects of *Argemone mexicana* crude leaf powder on various biochemical parameters in rats with carbon tetrachloride induced hepatotoxicity.

BIOCHEMICAL PARAMETERS						
Grps	Doses (mg/kg)	GOT (UI/L)	GPT (UI/L)	ALP (UI/L)	TBil (mg/dL)	
DBil (mg/dL)						
I	--	65,6±2,68	98,7±2,56	131,2±3,02	1,57±0,06	1,50±0,06
II	--	148,19±2,99**	119,37±1,06	219,80±2,29*	2,58±0,07*	2,46±0,07
III	100	80,10±0,88	119,37±1,06	149,15±1,30	1,84±0,05	1,76±0,05
		(83,42%)	(81,79%)	(79,74%)	(73,26%)	(72,91%)
IV	125	96,37±1,11	145,10±1,05	145,26±1,33	1,90±0,09	1,79±0,09
		(62,74%)	(81,79%)	(84,13%)	(67,32%)	(69,79%)
V	250	87,60±1,01	131,91±0,96	142,06±1,21	1,81±0,08	1,73±0,09
		(73,36%)	(70,73%)	(87,74%)	(76,23%)	(76,04%)
VI	500	78,84±0,90	118,72±0,86	137,20±1,08	1,58±0,09	1,69±0,08
		(83,96%)	(82,37%)	(93,22%)	(82,17%)	(80,20%)

Values are mean ± S.E.; n = 6, values within parentheses represent percent hepatoprotection; *p<0,05; ** p<0,01 . Compared with normal control vs liver injured rats.

Table 3: Dose-dependent anti-icterus profile of crude powdered leaf of *A. mexicana* L. (Papaveraceae) on CCl₄-intoxicated rats.

	Doses/crude powdered leaf (mg/kg)			
	Silymarin*	125 mg/kg	250 mg/kg	500 mg/kg
H.P.*(%)	78,22%	73,15%	76,81%	84,38%

Silymarin*: 100 mg/kg p.o.

HP*: Hepatoprotective Power