#Biological evaluation of anti-inflammatory and analgesic activities of *Argemone mexicana* Linn. (Papaveraceae) aqueous leaf extract#

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RESUME

The present study deals with evaluation of the anti-inflammatory and analgesic properties of a lyophilized leaf extract of *Argemone mexicana* Linn. on laboratory animal. The anti-inflammatory study was done by using carrageenan-induced paw edema method. It was found that lyophilized extract can be effective in acute inflammatory disorders and in that case, it showed significant anti-inflammatory dose-dependent effect ($p<0,001$) at the dose level of 250 mg/kg and 500 mg/kg.

The plant extract was equally tested for its analgesic potential by using the hot plate test method and acetic acid Writhing method. The lyophilized extract was found to exhibit significant ($p<0,01$; $p<0,001$) analgesic activity in tested model. By the hot plate method, the drug extract showed significant ($p<0,001$) increased latency period than the control group at oral dose of 250 and 500 mg/kg.

In acetic acid induced writhing test, the lyophilized extract (250 & 500mg/kg) presented reduced number of writhes at the two dose levels, which were found significant ($p<0,05$; $p<0,001$) if compared to control group. These results support the use of *Argemone mexicana* Linn. for the treatment of pain and inflammation sickness.

Keywords: *argemone mexicana*, lyophilized extract, ant-inflammatory, analgesic

INTRODUCTION

Argemone mexicana L. (Papaveraceae) is a medicinal herbal plant originated from Mexico. It is a pantropical spece which has a long history of use in traditional medicine dating back to the Aztecs (Emmart, 1940). *Argemone mexicana* L. is a very well known plant which has been used in many regions of the world to treat several diseases: in India, the leave decoctions 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 (Sourabié *et al.*, 2006, 2009, 2010, 2012).

Particularly in the case of Burkina Faso (statistics INSD, 2007), 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)* possess important pharmacological properties explaining its use as *anti-inflammatory*, *analgesic*, *antipyretic*, *antimicrobial* and *antispasmodic*.

On the chemical way, many authors have reported that interesting secondary metabolites are shown to be present in this plant (Priya and Rao, 2012; 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.

Inflammation is a biological phenomenon that occurs at the beginning of many pathological situations. It is a process that can be due to the release of histamine, kinins, serotonins, and prostaglandin. The anti-inflammatory agents are those which can normally inhibit the release of these inflammatory mediators (Charde et al., 2010). Inflammation is equally considered according to some authors like Morshed at al. (2011) as a primary

physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli.

It is known nowadays, that a persistent and uncontrolled inflammation can be an etiologic factor for many chronic illnesses (Kumar et al., 2004). Although inflammation is a defense mechanism, the complex events and mediators involved the inflammatory reaction can induce, maintain or aggravate many diseases (Sosa et al., 2002). Moreover, many authors have revealed the link between pain and inflammation in the occurrence of several diseases (Moulisha et al., 2011; Ibironke et al., 2007; Lanhers et al., 1992; Sreelekshmi et al., 2007).

In the domain of struggle against pain and inflammation, numerous plants possessing medical potentialities constitute a good alternative because showing analgesic and anti-inflammatory properties. So, the aim of the present study was to evaluate the anti-inflammatory and analgesic properties of the leaves of *Argemone mexicana* Linn., plant used in the western area of Burkina Faso for several illness treatment as abdominal pain, malaria fever (Graz et al., 2010), jaundice (Sourabié et al., 2012), etc. We have chosen lyophilized decoction because that is the main galenic form used by a great majority of traditional healers in Burkina Faso.

The interest to undertake this investigation is due to the fact that no study regarding the anti-inflammatory and analgesic effect of that plant have been reported by specialized literature concerning the sub west African area. Accordingly, we disclose these pharmacological properties (anti-inflammatory and analgesic effect) of the leaves of *Argemone mexicana* Linn. to further establish the scientific basis of its traditional use.

MATERIALS AND METHODS

Plant material

Argemone mexicana Linn leaves were collected (December 2004) in Banfora, a town located about 450 km far from Ouagadougou. 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 (DPF, INERA/CNRST). The registration number of the specimen was HBNU 762

Biological material

The biological material comprised mainly young male and female white mice (rain NMRI), 4-6 weeks old and weighing 25-30 g; they were used to conduct the *in vivo* experiment. These animals were provided by CIRDES laboratory in Bobo-Dioulasso at 360 km from Ouagadougou. Before pharmacological experimentation, they were kept in the animal house of the Institut (Département Médecine et Pharmacopée Traditionnelles/Pharmacie) and maintained at room temperature between 25 and 30°C, 40-70 % humidity conditions and the natural day-night cycle with an *ad libitum* access to food. The mice had no access to food during the hole day of experiment and the influence of circardian rythms was avoided by starting all experiments at 8.45 a.m.

Extract preparation

Decoction: the aqueous extract was obtained by decoction of 500 g leaf powder in 2 L of distilled water during 30 minutes. After this step, filtration and centrifugation (2500 rd/min for 10 min) were performed to freeze-dry the aqueous extract.

Furthermore, the extract was qualitatively tested for the presence of chemical constituents. The phytochemical screening of the extract was performed using the following reagents: alkaloids with general precipitation reagents as Bouchardat, Draggendorf and Valser-Mayer tests; flavonoids with Shibata test (cyaniding test); tannins with ferric chloride solution 1% p/v) saponins with ability to produce significant stable foam and Liebermann-Burchard reagent to highlight triterpenes and steroids.

Acute toxicity

The acute oral toxicity study was performed to evaluate the acute toxic effects and to determine minimum lethal dose (LD_{50}) of the drug extract. The albino mice male and female weighing 25-30 g were used for the experiment. The lyophilized extract was administered orally to the different groups (**n= 6**) of over night fasted mice at the dose of 25, 50, 100, 250, 500 and 1000 mg/kg body weight. After administration of the extract, all the animals were observed continuously for signs of toxicity and mortality during 24 h, 48 h, 72 h and beyond.

- **Anti-inflammatory activity**

Carrageenan induced Paw edema method:

The lyophilized extract on carrageenan induced inflammation in mice paw was investigated by using the method advocated by Winter et al. (1962) with some modifications according to our laboratory conditions. For this, the animals were divided into four groups of six (06) mice each. 0,1 mL of 1% carrageenan in normal saline (0,9% w/v NaCl) was injected to the sub plantar region of right hind paw.

Argemone mexicana L lyophilized extract was administered to the albino mice 30 minutes before carrageenan injection and the basement value was taken at 0 hour. Several groups were treated as below:

***group I (Negative control):** carrageenan (0,1 mL of 1% carrageenan/mouse to the sub plantar region) and saline water 5 mL/kg bw)

***groupe II (positive control):** carrageenan + phénylbutazone (25 mg/kg bw)

***groupe III** (treated group) : carrageenan + lyophilized extract of *Argemone mexicana* (250 mg/kg bw).

***groupe IV** (treated group) : carrageenan + lyophilized extract of *Argemone mexicana* (500 mg/kg bw).

The paw value was measured at 1h, 3h and 5h after carrageenan injection, using Plethysmometer. The left hind paw served as a reference non-inflamed paw for comparison. The average percent increase in paw volume with time was calculated and compared against the control group. Inhibition percentage was calculated according to the following formula (Alam Morshed et al., 2011):

$$\% \text{ Inhibtion of paw edema} = (Vc\text{-}Vt/Vc) \text{ X } 100$$

Where Vc and Vt represent average paw volume of control and treated mice respectively.

Statistical analysis:

Data from the studies were reported as the mean±S.E.M. and analyzed statistically by means of analysis of variance (ANOVA) followed by Student t-test; Values of *p<0,05 are regarded as significant

- **Analgesic activity**

Hot Plate Test Method:

Hot plate method (Morshed et al., 2011) was employed to evaluate the analgesic activity of the extract. Experimental animals (albino mice) were divided into four groups designated as group-I, group-II, group-III and group-IV consisting of six (06) mice in each group. The different groups have been treated as follows:

***group I (Negative control):** solution of 1% (v/v) Tween-80 in distilled water

***group II (positive control):** paracetamol (100 mg/kg p.o.)

***group III (treated group):** lyophilized extract of *Argemone mexicana* (250 mg/kg p.o.)

***group IV (treated group):** lyophilized extract of *Argemone mexicana* (500 mg/kg bw).

The mice were positioned on the hot plate kept at a temperature of 55°C; the reaction time was recorded when animals licked their fore or hind paw, or jumped prior to and 0, 30, 60, 120, 180 and 240 minutes after oral administration of the samples.

The inhibition percentage was calculated as:

$$(PAS) = (Tb\text{-}Ta/Tb) \text{ x } 100$$

Where **Tb =** reaction time in seconds before drug administration

Ta = reaction time in seconds after drug administration

****Acetic acid induced method (Writhing):***

For the evaluation of analgesic effects of the extract, the method of Dharmasiri (2003) cited by Morshed et al., (2011) was used with slight modification. Animals were divided into four groups of six mice each and then treated as follows:

***group I (Negative control):** solution 1% (v/v) Tween-80 in distilled water (5 mL/kg bw)

***groupe II (positive control):** solution 1% (v/v) Tween-80 in distilled water + solution of paracétamol (100 mg/kg p.o.)

***groupe III (treated group):** solution 1% (v/v) Tween-80 in distilled water + lyophilized extract (250 mg/kg p.o.*)*

***groupe IV (treated group):** solution 1% (v/v) Tween-80 in distilled water + lyophilized extract (500 mg/kg p.o.*)*

Thirty minutes later, 0,6% acetic acid (10 mL/kg) solution was injected by intra-peritoneal route to all the animals of different groups. The number of abdominal constrictions occurring between 5 to 15 minutes after acetic acid injection was counted. A significant reduction of writhes in the tested animals (mice) compared to those in the control group was considered as an anti-nociceptic response.

RESULTS

Phytochemical screening:

The phytochemical screening revealed the presence of interesting constituents in the aqueous extract (decoction) of the drug. The yield of decoction was 5,13 % and the presence of many chemical constituents has been noted as alkaloids, flavonoids, sugars and glycosides, phenolic compounds as tannins, saponins (Sourabié et al., 2009).

According to many authors as Priya and Rao, (2012), Upreti et al., (1991), Bose et al., (1963), the alkaloïdic components of the plant are isoquinoline type and a great number of them possess bioactive properties as berberine, cheilanthifoline, coptisine, muramine, scoulerine, sanginarine and protopine (Priya and Rao, 2012). The results of phytochemical screening are resumed in Table 1.

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**
Aqueous extract	**5,13**	++++	+	++	+	++

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

Acute toxicity test

The acute toxicity study has shown no toxicity of the drug extract at the dose of 1000 mg/kg p.o. No mortality was recorded in any group of mice after 72h of administering the lyophilized extract to the animals. However, the mice presented muscular weakness with slow movements, which disappeared around the end of observation period (72h).

- **Anti-inflammatory activity**

The effective values determined for each group are shown in table 2. In negative control animal (mice), injection of carrageenan in the sub plantar of mice has produced a local edema that increased progressively to reach a maximal intensity five hours after the injection of the phogistic agent (table 2).

The lyophilized aqueous extract of *Argemone mexicana* leaves exhibited a significant dose dependent reduction when administered both at 250 and 500 mg/kg bw. The maximum inhibition of the edema occurred at 5th hour with the extract administered at dose of 500 mg/kg bw (59,15% percent inhibition).

And the levels of percent inhibition of edema due to the lyophilized extract at 500 mg/kg are statistically interesting (58,82%, at 3th hour; (59,15% at 5th hour) by comparison with the negative control. These results confirm the anti-inflammatory property that has been mentioned by several authors as Priya Lekhya et al., (2012), Sanogo et al. (2007), Sourabié et al. (2012).

Tableau 2: Anti-inflammatory effect and percent inhibition of *Argemone mexicana* L. aqueous lyophilized leaves extract on paw edema induced by carrageenan.

Treatments	**Doses**	**Volume (mL) of paw edema**		
Groups	**(mg/kg)**	**1h**	**3h**	**5h**
Contrôl (Na Cl; 0,09%)	**5 mL/kg**	1,01±0,08	1,36±0,09	1,42±0,07
Phénylbutazone	**25**	0,45±0,06*	0,54±0,08*	0,55±0,06**
(positive control)		**(55,44%)**	**(60,29%)**	**(61,27%)**
Lyophilized decoction	**250**	0,75±0,05*	0,82±0,06*	0,85±0,07**
		(25,74%)	**(39,70%)**	**(40,14%)**
Lyophilized decoction	**500**	0,50±0,07*	0,56±0,03*	0,58±0,06**
		(50,49%)	**(58,82%)**	**(59,15%)**

Values in parenthesis indicate percent inhibition of edema. Values were expressed as mean±SEM. (n = 06 mice per group) *p<0,05 ; **p<0,01 vs control (Student test).

- **Analgesic activity**
  - ✓ **Method of the hot-plate**

The results of Hot-plate test about the lyophilized aqueous extract of *A. mexicana* are presented in Table 3.

Lyophilized extract at 250 and 500 mg/kg showed here also a dose dependent increase in the latency time when compared to control group (group I).

The two different doses of the plant extract exerted a parallel crescent inhibition of the pain comparatively to the standard.

On the kinetic way, the times 120 min and 240 min were characterized by a higher percent inhibition exerted by the two different doses (250 and 500 mg/kg bw). These inhibition were 45,97% and 6,37% for the dose of 250 mg/kg bw; 76,75% and 34,50% for the extract at 500 mg/kg bw. These results were found to be significant (p<0,001) statistically but remained lower when compared to the standard group in the same interval of time (table 3).

The results of table 3 showed an important decrease of the analgesic effect of the extract at the time 240 min (4th hour) about 6,37 % (250 mg/kg) and 34,50 % (500 mg/kg bw) against 52,4 % for the reference drug (Paracetamol).

Table 3: effect of *Argemone mexicana* lyophilized extract on mice pain induced by hot plate

Treatments	**TREATED GROUPS**			
	Control	**Paracetamol**	**lyoph*250**	**lyoph*500**
0 Min	12.42±1.23	9.50±0.65	12.04±1.52	8.08±1.41
30 Min	10.80±0.89	12.26±0.44	13.83±1.64	10.90±1.25
		(29.10%)	**(14.90%)**	**(35.00%)**
60 Min	9.27±0.66	13.77±0.47**	15.43±1.26*	12.93±0.95**
		(45.04%)	**(28.35%)**	**(55.24%)**
120 Min	8.18±0.60	15.35±0.50***	16.62±0.77***	13.40±1.02***
		(61.83%)	**(38.25%)**	**(65.40%)**
180 Min	6.70±0.55	13.38±0.55	17.55±0.59***	14.26±0.97**
		(80.10%)	**(45.97%)**	**(76.75%)**
240 Min	6.05±0.54	14.47±0.29***	11.28±0.87*	10.86±0.67***
		(52.47%)	**(6.37%)**	**(34.50%)**

Values were expressed as mean ± SEM (n = 6 mice per group). *p < 0,05 ; **p < 0,01 ; ***p< 0,001 vs contrôl.

Lyoph*250: lyophilized extract (250 mg/kg); **Lyoph*500:** lyophilized extract (500 mg/kg)

- ✓ **Acetic acid induced writhing in mice**

Lyophilized extract (250 and 500 mg/kg p.o.bw) and paracetamol (standard drug), when administered orally to the mice, induced a great decrease in the number of writhes if compared to the control as shown in **table 3.**

The drug extract at both concentrations exhibited significant analgesic effect. The percent inhibitions of writhes were 32.95% for the extract at 250 mg/kg and 37.90% at 500 mg/kg. These inhibitions are lower than that of reference drug (58% percent inhibition of writhes).

Table 4: effect of lyophilized extract (250 and 500 mg/kg p.o.) on acetic acid-induced writhing in mice.

Groups	Nb of writhings (5-15 Min)
Negative control (NaCl)	44,52±3,52
Standard (paracetamol)	18,87±0,45*** **(58%)**
Lyoph*250	29,93±0,50** **(32,95%)**
Lyoph*500	34,22±1,51** **(37,90%)**

Values were expressed as mean ± SEM. n = 6 animals per group. *p<0.05, **p<0.01,***p<0.001 vs. Negative control

Lyoph*250: lyophilized extract at 250 mg/kg p.o.

Lyoph*500: lyophilized extract at 500 mg/kg p.o.

DISCUSSION

anti-inflammatory activity

Carrageenan-induced edema involves the synthesis or release of mediators at the injured site. Among these mediators we can distinguish prostaglandins, histamine, bradykinins, leucotriene and serotonin which also cause pain and fever (Asongalem et al.,.2004). Inhibition of these mediators from reaching the injured site or from bringing out their pharmacological effects normally ameliorates the inflammation.

In the case of the present work, it has been shown that the lyophilized leaf extract of *Argemone mexicana* possessed a significant anti-inflammatory effect on paw edema induced by carrageenan. And the maximum activity (76.75 % paw inhibition) showed by the extract at 500 mg/kg bw during the 3th hour was found to be highly significant (p<0.001) if compared to negative control.

Many authors such as Silva et al. (2005), Morshed et al.(2011), Sanogo et al., (2006) are agreed that the development of edema by carrageenan is commonly correlated with early exudative stage of inflammation. Carrageenan edema is also known to be a multimediated phenomenon that liberates a great number of mediators. Thus, the first phase (1h) implicates the release of serotonin and histamine while the second phase (over 1h) is mediated by prostaglandins, cyclo-oxygenase products and the continuity between the two phases is provided by kinins (Perianayagam et al., 2006).

The results achieved in the present study constitute an indication that *Argemone mexicana* Linn. can be effective in acute inflammatory disorders and in that it showed significant result (p<0.001) with both of the 250 mg/kg and 500 mg/kg dose level.

Mechanism of the anti-inflammatory activity

According to Charde et al., (2010) anti-inflammatory activity can be due to inhibition of release of histamine, serotonin, kinins, prostaglandins, etc. in the first hours following injection of carrageenan. But the chemical compounds revealed during the preliminary qualitative phytochemical screening may be also responsible for the observed anti-inflammatory activity. Effectively, phenolic compound as tannins revealed in the aqueous extract are chemical principles known to be potent cyclooxygenase-1 inhibitors and with anti-phlogistic activity (Wagner H., 1989). Flavonoids equally present (slightly) in the aqueous extract (**Table 1**) have been reported as antioxidants and scavengers of free radicals can also exert anti-inflammatory effects (Geronikaki et al., 2006; Van Acker et al., 2000; Van den Berg et al., 1999).

Mechanism of analgesic activity

The hot plate test used to investigate the analgesic activity of *Argemone mexicana* Linn. lyophilized extract has revealed that the drug extract presented a longer latency time than the negative control group in a dose related manner. This method is considered to be selective for the drugs acting centrally; it measures the complex response to a non-inflammatory, acute nociceptive input according to Moulisha et al., (2011).

For Sabina et al. (2009), hot plate test is one of the models normally used for studying central nociceptive activity. This assertion has been confirmed by the findings of Ibironke et al. (2007) on the anti-inflammatory and analgesic properties of *Chenopodium ambrosioides* leaf extract in rats, which concluded that any agent causing a prolongation of the hot plate latency time must acting centrally. Therefore, the lyophilized extract of *Argemone mexicana* Linn., administered at 250 and 500 mg/kg p.o. may have a central analgesic activity.

The study of analgesic activity by acetic acid induced writhing method is normally used to evaluate the peripheral analgesic effect of drugs and chemical (Morshed et al., 2001). The positive responses given by the extract at two doses (250 and 500 mg/kg p.o.) may be an illustration of a peripheral analgesic effect exhibited by the extract when compared to the negative control (**table 4**).

The mechanism of analgesic activity exhibited by the lyophilized extract can be due to the inhibitory effect exerted by some phytochemicals found in the plant extract (tannins, flavonoids and isoquinoline alkaloids, etc.) and capable to block prostaglandin pathway. It has been mentioned in the specialized literature that prostaglandins, particularly prostaglandin PGE2 plays a great role in the advent of pain and inflammation.

Indeed, the studies of Sweeney et al., (2000) and those of Mackray et al., (2008) have showed that *chelerythrine*, *sanguinarin* and *berberin*, three isoquinoleic alkaloids from *Argemone mexicana* leaves have been found to inhibit cyclooxygenase 1 and 2 (COX-1 and COX-2). On biochemical pathway, it is known that prostaglandins release is mediated by cyclooxygenases. So, the inhibition of these enzymes (COX-1and COX-2) by the active principles contained in the plant extract might reduce the production of prostaglandins and consequently the removal of pain. In this work we have demonstrated that lyophilized extract of *Argemone mexicana* Linn. exhibited both types of pain inhibition. According to Sabina et al., (2009), the analgesic effect of the plants in both models suggests that they have been acting through central and peripheral mechanism.

CONCLUSION

The results obtained in the present study showed anti-inflammatory and analgesic activities of *Argemone mexicana* Linn. by reducing significantly the formation of edema induced by carrageenan. The positive reaction with the acetic acid induced writhing model and hot plate test method is an indication that the leaves of *Argemone mexicana* Linn. has anti-inflammatory and analgesic activities. This present work has provided some justification for the traditional medical use of that plant in Burkina Faso, particularly in the Cascades region where it has been collected.

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