Acute toxicity and anti-inflammatory activity of aqueous ethanol extract of root bark of *Ximenia americana* L. (Olacaceae)

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Accepted 14 June, 2011

*Ximenia americana* L. (Olacaceae) is a folk medicinal plant used in Burkina Faso to treat inflammatory, noxious and infectious diseases. The aim of this study was to evaluate anti-inflammatory properties of aqueous ethanol extract of root bark of the plant and evaluate its acute toxicity. Carrageenan was used to induce oedema and leukocyte migration; acetic acid was used to induce vascular permeability in order to evaluate anti-inflammatory properties of the plant extract. The acute toxicity was evaluated using mice. The aqueous ethanol extract of *X. americana* administered intraperitoneally is fairly toxic in mice with a LD₅₀ of 345 mg/kg of body weight. The extract inhibited paw swelling, polymorphonuclear neutrophil and leukocyte migration induced by carrageenan. Moreover, the extract (10, 30 and 100 mg/kg) reduced vascular permeability induced by acetic acid. Aqueous ethanol extract of root bark of *X. americana* (Olacaceae) possesses anti-inflammatory properties by inhibiting oedema, pain, cell migration and increased vascular permeability.

**Key words:** *Ximenia americana*, anti-inflammatory activity, acute toxicity, root bark.

**INTRODUCTION**

*Ximenia americana* L. (Olacaceae) is widely used in folk medicine in West Africa to treat various disorders such as inflammation, pain, fever (Kerharo and Adam, 1974; Tapsoba, 2006; Magassouba et al., 2007), helminthiasis (Maikai et al., 2008), diarrhoea, wounds (koné et al., 2004) and intoxications (Kumar-Roine et al., 2009). Different parts of the plant are used as infusion, maceration or decoction. Published scientific reports of the biological activities of the plant are scanty and include anti toxic (Kumar-Roine et al., 2009), anticancer (Voss et al., 2006) and antimicrobial (Omer and Elnima, 2003; Kubmarawa et al., 2007). In Burkina Faso the root bark of the plant is mostly used in painful and inflammatory diseases. The aim of this work was to evaluate, anti-inflammatory activity, and acute toxicity of aqueous ethanol extract of the root bark of *X. americana*.

**MATERIALS AND METHODS**

**Plant material**

The root bark of *X. americana* L. (Olacaceae) was collected in November 2006 about 15 km from Ouagadougou, the capital of Burkina Faso. The plant was identified by botanists of the Department of Forest production of “Centre National de Recherche Scientifique et Technologique (CNRST)”and attested by the Laboratory of Ecology of the University of Ouagadougou where a voucher has been deposited under the number C01 23/11/2006.
Extraction procedure

Two hundred grams of air dried powdered plant material was extracted by maceration in hydro-alcohol mixture (ethanol-water 80:20 v/v) for 48 h while stirring at 23 to 25°C using an adapted nutrition pump (Ordinal 2, Electrofrance, France). The extract was filtered, and centrifuged at 3500 RPM for 10 min. The filtrate was concentrated to dryness in a rotor evaporator under vacuum and later in oven (45°C). The dried powder was used for pharmacological and toxicological tests.

Pharmacological and toxicological tests

Animals

Male Swiss mice weighing 25 to 35 g were used in the experiments. The animals were provided by “Centre International de Recherche Développement de l’Elevage en zone Subhumide (CIRDES)” of Bobo-Dioulasso, Burkina Faso. The animals were housed in standard environmental conditions (23 ± 1°C, 12 light /dark cycles) with free access to water and standard rodent diet of fish supplemented corn granule. The animals were fasted with access to water at least 14 to 15 h before all experiments.

Acute toxicity study

Animals were randomly allotted in 5 groups of six mice. The aqueous ethanol extract was administered intraperitoneally at doses of 150, 300, 450 and 600 mg/kg of body weight. The control group received only the solvent (normal saline, 10 ml/kg b.w). The animals were observed during the first two hours for toxic signs and then mortality was recorded for each group at 24, 48 and 72 h after dose administration. We used the method of Litchfield and Wilcoxon (1949) for the determination of LD50.

Carrageenan – induced oedema in mice

Anti-inflammatory activity was assessed on the basis of inhibition of paw oedema induced by injection of 0.05 ml of saline solution of carrageenan (1%) (Sigma, France) into the subplantar region of the right hind paw of the mouse according to a modified described procedure (Winter et al., 1962). The mouse was retained in our laboratory. Briefly, six groups of six animals were used. Each group of animals received either aqueous ethanol extract of *X. americana* in exponential increasing concentrations (1, 10 and 100 mg/kg), betamethasone (2 mg/kg) (Sigma, France), indomethacin (5 mg/kg) (Fluka, Italy) or solvent (normal saline) by intraperitoneal route 30 min prior to the injection of carrageenan to all groups. The inflammation was quantified by the difference of measurement of the volume of saline solution displaced by the paw prior to the administration of phlogistic agent (V0) and 0.5, 1, 2, 3, 4, 5 and 6 h after (Vt), using a plethysmometer (model 7150, Ugo Basil). Each measurement was the average of two readings. The percentage of inhibition of paw oedema for treated groups was obtained as follows:

\[
\text{Percentage of inhibition} = \left( \frac{(V_0 - V_t)\text{control} - (V_0 - V_t)\text{treated}}{(V_0 - V_t)\text{control}} \right) \times 100
\]

Leukocyte migration in vivo

Leukocyte migration induced by inflammation was assessed by using the method originally described by Di Rosa (1972) and modified by Griswold et al. (1987). Six groups of six mice were injected with 0.2 ml of carrageenan (1% w/v) in sterilized normal saline into peritoneal cavity. The aqueous ethanol extract (10, 30 and 100 mg/kg b.w); the standard drugs betamethasone (2 mg/kg) and indomethacin (10 mg/kg) were administered orally 1 h before. Because the doses of 10 and 100 mg/kg b.w of plant extract have yielded an effective anti- oedematous effect, we restricted the doses to 10, 30 or 100 mg/kg b.w for the rest of pharmacological evaluations. The control group received distilled water. Four hours later, the peritoneal cavities wash fluids were collected by using 3 ml heparinised phosphate-buffered saline (PBS). Heparin was used to prevent cells adhering to each other. Soft massage was used to homogenize the non-opened peritoneal cavity containing injected fluid and about 1 ml was harvested with needle and syringe. The total and differential leukocyte count was performed on the fluids collected from the peritoneal cavities. Aliquots of the wash fluids were stained with Turk’s solution (0.01% crystal violet in 3% acetic acid), Cell counting was performed using a Neubauer hemacytometer and a light microscope (Leica S11421). Leukocyte cell type identification was brought out on stained smear of wash fluids preparations with May-Grunwald and Giemsa.

Vascular permeability

The *in vivo* vascular test of permeability was carried out according to a modified method originally described by Whittle (1964). Briefly, five groups of six male mice each were fasted for 18 h before the experiment. Three groups received by oral route increasing doses (10, 30 and 100 mg/kg) of the plant extract. A standard reference group received indomethacin (10 mg/kg). The control group received distilled water. Each animal was injected intravenously with 0.2% solution of Evans blue dye 1 h after the administration of drug. Fifteen minutes later, the mice were injected intraperitoneally with 1 ml/100 g of body weight with freshly prepared 0.6% acetic acid in NaCl 0.9% solution. After 30 min, the mice were sacrificed by cervical dislocation and the abdominal cavities were washed twice with 3 ml of PBS. The collected peritoneal fluids were then centrifuged at 4000 rpm for 10 min. The absorbance of the supernatant was measured at 600 nm by using a microplate reader STAT FAX® 3200 (AWARENESS, USA). In order to determine the concentration of the dye in the collected supernatants, a calibration of the spectrophotometer was made. A standard absorbance curve of Evans blue dye concentrations ranging from 1 to 50 µg/ml was built.

Statistical analysis

Statistical analysis of data was performed using Mann-Whitney U-testor Kruskal-Wallis test respectively for the comparison of 2 or several means. Stat view software version 5.0 and PRISM version 3.1 were respectively used for the statistical analysis and graphs representation. Criterion for statistical significance was p < 0.05.

Ethical considerations

The vegetal material was constituted of the root bark of *X. americana*. To save biodiversity, roots were taken from several old trees in an area of high density of the plant. Animals were handled according to ethical guidelines of the Faculty of Health sciences.

RESULTS

Acute toxicity

Graphic representation of cumulative mortality in extract...
treated mice during 24 h allowed determination of the lethal dose (LD₅₀) of the extract by intraperitoneal route (Figure 1). It was 345 mg/kg ranging between 267 and 445 mg/kg b.w. The groups of mice which received more than 150 mg/kg died within 72 h (Table 1). The toxicity was characterized by agitation, abdominal cramps, drowsiness, tendency to gathering, loss of the exploration instinct and the death of the animals.

### Table 1. Cumulative mortality in mice treated with increase doses of plant extract.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg i.p)</th>
<th>Cumulative mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>150</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>300</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>450</td>
<td>0</td>
</tr>
<tr>
<td>IV</td>
<td>600</td>
<td>0</td>
</tr>
</tbody>
</table>

The number of animal (n) was 6 per group.

### Anti-oedema activity

The sub plantar injection of 1% carrageenan suspension produced a local oedema reaching a steady state in 2 h as observed in control group which received saline solution. The extract of *X. americana* (10 and 100 mg/kg b.w) significantly inhibited carrageenan provoked mice paw swelling (Figure 2, respectively *p*<0.05, **p**<0.01).
Figure 2. Evaluation of paw volume (% oedema) in carrageenan induced oedema in mice. Aqueous ethanol extract of *X. americana* (1 mg/kg, 10 mg/kg, 100 mg/kg), indomethacin (5 mg/kg) and betamethasone (2 mg/kg) treated groups reduced paw volume change in comparison to control group (vehicle treated group). The data represent mean ± sem of 6-12 animals. From 3 to 6 h curves of the plant extract 10, 100 mg/kg and betamethasone are different to control, respectively *p<0.05, **p<0.01, $$$p<0.001.

Table 2. Effect of aqueous ethanol extract of *X. americana* on leukocyte and polymorphonuclear neutrophil influx in carrageenan induced peritonitis.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Leukocytes</th>
<th>Polymorphonuclear neutrophils</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Count (10⁶/ml)</td>
<td>Inhibition (%)</td>
</tr>
<tr>
<td>Control (vehicle)</td>
<td>-</td>
<td>2.67±0.18</td>
<td>-</td>
</tr>
<tr>
<td>Extract</td>
<td>10</td>
<td>1.77 ± 0.28*</td>
<td>33.96</td>
</tr>
<tr>
<td>Extract</td>
<td>30</td>
<td>1.62 ± 0.28*</td>
<td>39.90</td>
</tr>
<tr>
<td>Extract</td>
<td>100</td>
<td>0.90 ± 0.11*</td>
<td>66.34</td>
</tr>
<tr>
<td>Betamethasone</td>
<td>2</td>
<td>0.71 ± 0.10*</td>
<td>73.51</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>1.53 ± 0.13*</td>
<td>42.78</td>
</tr>
</tbody>
</table>

Drugs were administered orally. The values represent mean ± sem; n = 6 animals per group. *p<0.0059 versus control. **p<0.0345 versus 100 mg/kg of plant extract. *p<0.0209 versus 10 or 30 mg/kg of extract.

The intensity of anti-oedema effect of plant extract was proportional to the tested doses. From 3 h of challenge with extract, the swelling of paw were brought from 24.21% in control to 16.27, 9.82 and 5.68%, respectively in groups treated with 1, 10 and 100 mg/kg. That corresponded to 40, 56 and 73%, respectively of oedema inhibition. The decrease of the oedema caused by indomethacin and betamethasone was respectively, 38 and 93% (Figure 2).

Leukocyte migration

As the early stage of the inflammatory process is marked by leukocyte influx to inflamed tissue, we assessed the effect of extract of *X. americana* on peritonitis induced by carrageenan. The extract at 10, 30 and 100 mg/kg resulted in a significant decrease in leukocyte influx to peritoneal cavities (Table 2).

Vascular permeability

As shown in Figure 3, the amount of dye passed across vessel in control group was 0.84±0.05 µg/g b.w as the dye was administered according to body weight. The extract (10, 30 and 100 mg/kg) reduced significantly (p<0.05; versus control group). The amount of dye retrieved in peritoneal fluid was 0.57±0.07 (inhibition 32.85%); 0.51±0.04 (39.34%) and 0.36±0.08 (57.36%)
Figure 3. Effect of the aqueous ethanol extract of X. americana (Olocaceae) on acetic acid induced increase of vascular permeability in mice. The amount of increase in vascular permeability was correlated to amount of dye measured in peritoneal cavity fluid. Values are expressed as mean ± sem (n= 6-12). Significance relative to control values: * p< 0.05; *** p< 0.001.

DISCUSSION

We demonstrated the anti-inflammatory activity of aqueous ethanol extract of the root bark of X. americana (Olocaceae) and evaluated the acute toxicity of the extract by intraperitoneal route in mice. The anti-inflammatory activity of the plant extract was evaluated on manifestations accompanying the inflammatory reaction like oedema, increased vascular permeability and finally on leukocyte migration into inflamed tissue. The aqueous ethanol extract of the plant at 10 and 100 mg/kg b.w reduced carrageenan induced paw oedema of mouse comparatively to control group from 3 to 6 h (*p<0.05, ##p<0.01). Contrary to plant extract, betamethasone had precocious effect on oedema since 30 min after carrageenan challenge (Figure 2). That could prevent the release of histamine, serotonin and kinines in the first hour, then prostaglandins and lysozymes during second to third hour as described previously (Di Rosa, 1972).

To understand more the effect of the extract of the plant, we assessed its action on amplification phase of inflammation: Vascular and cell migration events. In peritonitis model induced by intra peritoneal injection of carrageenan; an influx of leukocytes is observed from 4 h of challenge (Di Rosa, 1972; Griswold et al., 1987). The aqueous extract of X. americana significantly inhibited cell migration to inflamed tissue (Table 2). The importance of the effect was correlated to the increase of the dose of the plant extract.

In the inflammatory process, leukocyte extravasation is the result of chemical mediators (prostaglandins, bradykinin, histamine...) which increase capillary permeability, the expression of adhesion molecules (selectins, VCAMs) by endothelial cells and cytokines release (IL1, IL6, TNFa,...) (Rang et al., 2003). Because the first cells to reach injured tissue are the polymorphonuclear neutrophils, we performed a differential count of leukocytes in the wash fluid from the peritoneal cavities. The characterization of cell type in the infiltrates showed an important presence of polymorphonuclear neutrophil in control group (2.33 ± 0.18 10⁶ cells/ml about 87% of leukocytes) (Table 2). The extract at tested doses significantly (p<0.0056) prevented polymorphonuclear neutrophil infiltration to peritoneal cavity induced by carrageenan. The inhibitory effect of extract at 100 mg/kg (72%) showed difference with that of the standard reference drugs, namely: indometacin 10 mg/kg (68%) and betamethasone 2 mg/kg (78%) (Table 2). The mechanism of inhibition of polymorphonuclear neutrophil migration during inflammation is well documented (Rang et al., 2003; Gupta et al., 2005;
Zhang et al., 2008). As polymorphonuclear neutrophils are sensitive to cytokines action, betamethasone could inhibit their migration by modulating cytokine release by mast cells and macrophages by its immunosuppressive properties. Likewise non-steroidal anti-inflammatory drugs, e.g. indomethacin, at high dose were reported to inhibit the action of cytokines (Rang et al., 2003).

Vascular permeability change participates in pathophysiology of inflammation with leakage of vascular contents to interstitial tissue. That was assessed by the amount of Evans blue dye which extravasated to peritoneal fluid in acetic acid induced peritonitis in the mice. The extract of X. americana reduced significantly vascular permeability (Figure 3). The extract contains chemical components which could interfere with the metabolism or the targets of released vascular active mediators as aforementioned.

Anti-inflammatory effect of the aqueous ethanol extract of the plant could be assigned to chemical compounds like flavonoids, tannins, saponins, triterpens and sterols which have been characterized in X. americana (Olocaecae) by our laboratory (data not shown) and others (James et al., 2007; Soro et al., 2009). Moreover, one should be aware of the potency of the plant extract. The range of active doses reported here was in order of about ten milligrams. The LD₅₀ (lethal dose) of the extract was 345 mg/kg b.w by intraperitoneal route in mouse. This toxicity of extract of the roots of X. americana has been reported by others (Voss et al., 2006; Soro et al., 2009). The LD₅₀ was 160 mg/kg b.w and all animals which received 300 mg/kg died in 72 h. This suggests that the plant extract should be used with caution in particular in systemic administration.

In conclusion the aqueous ethanol extract of root bark of X. americana (Olocaecae) results in an anti-inflammatory activity which could justify traditional use of that part of the plant. But systemic important dose should be avoided due to the toxicity risk. Further bioassay-led fractionation and isolation of active principles are to be undertaken.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Maîmouna Belem of the Department of Forest production of “Centre National de Recherche Scientifique et technologique (CNRST)” for her help in identifying the plant. Dr My Lac is acknowledged for his critical reading of the manuscript.

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