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DOI: 10.3923/ijp.2016.549.555
Research Article

Anti-inflammatory and Anti-plasmodial Activities of Methanol Extract of *Pterocarpus erinaceus* Poir. (Fabaceae) Leaves

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Abstract

**Background and Objective:** *Pterocarpus erinaceus* Poir. (Fabaceae) is used in folk medicine in Burkina Faso to treat inflammatory diseases including malaria, ulcer, rheumatism and infectious disease. The aim of this study was to carry out the evaluation of the safety and efficacy of methanol extract of *Pterocarpus erinaceus* leaves studying anti-inflammatory, analgesic and anti-plasmodial activities.

**Methodology:** The anti-inflammatory and analgesic effects of methanol extract of leaves were studied by carrageenan-induced paw edema test and acetic acid-induced writhing test in mice Naval Medical Research Institute (NMRI). Methanol extract effect on Tumor Necrosis Factor alpha (TNF α) and Nitric Oxide (NO) production was evaluated by lipopolysaccharide (LPS)-stimulated RAW 264.7 cells. The 2,2-diphenyl-1-picryl-hydralyl, Sigma (DPPH) and cupric-reducing antioxidant capacity (CUPRAC) methods were used to evaluate the antioxidant activity of the extract. Test against *Plasmodium berghei* infected mice was used to evaluate the anti-plasmodial activity of methanol. **Results:** At doses of 100, 200 and 400 mg kg⁻¹, oral administration of methanol extract significantly produced anti-inflammatory and analgesic effects in dose-dependent manner. Methanol extract inhibited release of NO and TNF α in LPS-stimulated RAW 264.7 cells. The capacity of extract to scavenge free radical was lower than trolox and rutin. Plasmodium inhibition of extract was expressed at 50 and 100 mg kg⁻¹. **Conclusion:** These results indicate that the leaves of *Pterocarpus erinaceus* have anti-inflammatory and analgesic properties, which support its traditional medicine use.

**Key words:** Anti-inflammatory, analgesic, antioxidant, anti-plasmodial, *Pterocarpus erinaceus*

Received: February 05, 2016  Accepted: March 26, 2016  Published: June 15, 2016


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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.
INTRODUCTION

Inflammation is a defensive system that protects the organism against pathogens (bacteria, fungi, virus) and injury. Several pro-inflammatory endogenous mediators such as cytokines and nitric oxide (NO) are produced during inflammatory process. The ROS are a heterogeneous group of oxygen radicals, which play an important role in physiological processes but uncontrolled ROS production can lead to oxidative stress, tissue and DNA damage. Excessive production of ROS and cytokines can maintain or amplify the inflammatory response leading to chronic inflammation. The ROS induce the release of cytokines (IL-1β, IL-6) and NO production by inducible NO synthase (iNOS). The NO induces biosynthesis of inflammatory prostaglandins (PG, PGE2) via activation of cyclooxygenase 2.

Plasmodium infection causes an inflammatory response involving inflammatory mediators production, such as cytokines (TNF, IL-1β, IL-6) and nitric oxide (NO). The parasite major toxin glycophosphatidylinositol (GPI) of Plasmodium is responsible for TNF and NO production. Punswad et al. reported that increasing serum TNF level were associated with increased mortality in children with severe malaria.

In Africa, malaria patients use local medicinal plants to treat malaria with eliminating clinical symptoms such as fever, pain and general malaise. However, there are few scientific data as well as pre-clinical studies about efficacy and safety of traditional herbal medicines.

*Pterocarpus erinaceus* leaves were used folk medicine in Burkina Faso to treat malaria, parasitosis, fever and anemia. Previous biological investigations on *Pterocarpus erinaceus* revealed in vitro anti-malaria and anti-microbial activities. Previous study had shown anti-inflammatory and analgesic properties of *P. erinaceus* stem bark.

The aim of the present study was to investigate the anti-inflammatory activity of methanol extract from leaves of *P. erinaceus* Poir. (Fabaceae) using *in vivo* and *in vitro* assays, then to carry out analgesic and anti-plasmodial activities in *vivo*.

MATERIALS AND METHODS

Plant material: The leaves of *P. erinaceus* were collected in March, 2009 at Gourcy (150 km from Ouagadougou, Burkina Faso). The *P. erinaceus* was identified by Pr. A. Thiombiano and a voucher specimen number ON-01 has been deposited at herbarium of ecology laboratory of University of Ouagadougou.

Animals and ethical approval: *In vivo* studies were carried out in accordance with guidelines for care of laboratory animals and ethical guidelines for the investigation of experimental pain in conscious animals. The NMRI mice (25-32 g) were housed in temperate rooms (22 ± 2°C) with a 12/12 dark-light cycle. Mice were allowed free access to standard dry pellet diet and given water *ad libitum*. Animals were fasted for 17 h and weighed before the experiments.

Chemicals: The 2,2-diphenyl-1-picrylhydrazyl (DPPH), neocuproin (2,9-dimethyl1,10-phenanthroline) and copper II chloride, carrayeangan, acetic acid (Sigma) and paracetamol (Sigma) were purchased from Sigma (St. Louis, USA). Trolox (Fluka, France), rutin (Merck, France) and ammonium acetate aqueous buffer (Prolabo, France) were also used in this study.

Biological tests

Acute toxicity: Acute toxicity was carried out to determinate Lethal Dose (LD₅₀). Mice fasted overnight were randomly divided into five groups (six per group). Groups were treated with methanol extract at doses of 500, 1000, 1500, 2000 and 2500 mg kg⁻¹ b.wt. Mice were observed from 2-72 h and two weeks period for morbidity or mortality and changes in behavior were recorded compared to control group treated with water.

Anti-edematous activity: Carrayeangan induced paw edema test was carried out according to the method described by Winter et al. Mice were divided into five groups (n = 6 per group). One hour prior to injection of carrayeangan, control group received distilled water, group II, III and IV were orally treated with MeOH extract (100, 200 and 400 mg kg⁻¹ b.wt.) and group V was treated with acid acetylsalicylic (AAS). The edema volume was recorded at 1, 3 and 5 h after carrayeangan injection using plethysmometer (model Ugo Basile, n° 7141, Italy). The average volume of the right hind paw of each mouse was calculated from three reading at each hour. The inhibitory activity was calculated according to the following formula:

\[
\text{Inhibition percentage} = \frac{(C_1 - C_2)_{\text{control}} - (C_1 - C_2)_{\text{treated}}}{(C_1 - C_2)_{\text{control}}} \times 100
\]
where, \( C_t \) is the paw circumference at time \( t \), \( C_0 \) is the paw circumference before carrageenan injection, \((C_t - C_0)\) is edema or paw size after carrageenan injection to control or treated mice at time \( t \).

**Analgesic activity:** The intraperitoneal injection of 0.6% acetic acid solution (10 mL kg\(^{-1}\) b.wt.) provokes abdominal writhing according to Sawadogo *et al.*\(^{13}\). Five groups (\( n = 6 \) mice per group) were formed. Control group (group I) received per os distilled water, group II, III and IV orally received the MeOH extract (100, 200 and 400 mg kg\(^{-1}\)) and group V treated with paracetamol (100 mg kg\(^{-1}\)) 1 h before acetic acid injection. Five minutes after acetic acid injection, the number of writhing was recorded during 15 min. The analgesic effect was evaluated by a percentage reduction of writhes in treated group compared to the control group.

**TNF \( \alpha \) and nitrite assay:** According to Fotio *et al.*\(^{13}\) extracts were dissolved in a solution of Dulbecco’s Modified Eagle’s Medium (DMEM). Murine cells of the bone marrow were differentiated into macrophages after culture of 1.5 \( \times \) 10\(^6\) cells/well in a 24 well plate. Each well contained DMEM supplemented with 10% fetal calf serum and 20% conditioned RAW 264.7 cells as a source of colony-stimulating factor of granulocytes and macrophages.

After six days of culture, the cell preparation contained 99% of adherent macrophages, which accounted for approximately 10% of total cells initially placed in culture. Macrophages derived from bone marrow bone marrow-derived macrophage (BMDM) were untreated or treated with different concentrations (25, 50 \( \mu \)g mL\(^{-1}\)) extracts for 30 min. The BMDM were stimulated by *Escherichia coli* (serotype 055:B5), LPS (1 \( \mu \)g mL\(^{-1}\)) or LPS/IFN-\( \gamma \) for 24 h. The supernatant was collected for determination of the amount of nitrite and the cytokine Tumor Necrosis Factor alpha (TNF \( \alpha \)). The amount of TNF \( \alpha \) in the cell culture supernatant was measured using the enzyme-linked immunosorbent assay (ELISA) test. Accumulation of nitrite in the culture medium was determined as BMDM indicator of the production of nitric oxide by the Griess reagent (1% sulphanilamide and 0.1% in naphthylenediamid 2.5% phosphoric acid). About 25 \( \mu \)L of the supernatant collected were mixed with 50 \( \mu \)L of the Griess reagent for 5 min. The absorbance was measured at 570 nm and the concentration of nitrite was determined by comparison with a reference to a standard curve of sodium nitrite.

**Antioxidant activity**

**DPPH radical scavenging assay:** Free radical scavenging activity of methanol extract and flavonoids fraction were evaluated by DPPH (2,2-diphenyl-2-picryl-hydralyl, Sigma)\(^{13}\). Trolox and ascorbic acid were used as positive control. The IC\(_{50}\) was considered as the concentration of the substrate that causes 50% loss in DPPH activity and the Anti-Radical Power (ARP = 1/IC\(_{50}\)) was the antioxidant power.

**CUPRAC assay:** Cupric-reducing antioxidant capacity (CUPRAC) assay was used to determine the antioxidant capacity of methanol extract and flavonoids fraction\(^{14}\). Trolox Equivalent Antioxidant Capacity (TEAC) was used to determine the antioxidant capacity of the substrate.

**In vivo anti-plasmodial assay:** *In vivo* anti-malaria activity of methanol extract of *P. erinaceus* leaves was performed in mice based on the 4 day suppressive test\(^{15}\). *Plasmodium berghei* ANKA strain infected blood was obtained by ear, mixed in Phosphate Buffered Saline (PBS). The test mice were infected by i.p., injection with 200 \( \mu \)L (10’ parasitized erythrocytes) on day 0. Infected animals were randomly divided in three groups of six mice. Control group and treated groups received orally respectively distilled water and extract (50 and 100 mg kg\(^{-1}\), b.wt.), 2 h post infection. The groups 50, 100 and 250 mg kg\(^{-1}\) were treated once per day from day 0-3. On day 4 of the test, thin blood smears from the tail of treated mice were performed and fixed in methanol. Blood from animals were strained with Giemsa 10% and microscopically examined.

**Statistical analysis:** Results were expressed as Mean\( \pm \)SEM. GraphPad Prism version 5.0 was used to analyzed study data. Statistical analysis was performed by one-way ANOVA followed by Dunnett’s test. Differences were considered significant compared to control group if \( p<0.05 \).

**RESULTS**

**Acute toxicity:** Oral administration of methanol extract at doses of 500, 1000, 1500, 2000 and 2500 mg kg\(^{-1}\) b.wt. did not provoke any changes in behavior. No mortality of mice NMRI was obtained during two week of observation.

**Anti-edematous activity:** Carrageenan induced-paw edema was used to evaluate the anti-inflammatory effect of the methanol extract. It showed a significant inhibitory effect on paw edema formation from first to 5th h after carrageenan injection. The highest inhibition was observed during the 5th h, which at 200 and 400 mg kg\(^{-1}\) and the percentage of inhibition obtained were, respectively 58.1 and 77.4% (Table 1).
Fig. 1: Effect of methanol extract of *Pterocarpus erinaceus* leaves on nitrites (NO) level in IFN/LPS or LPS-stimulated RAW 264.7 cells. Values are Mean±SEM (n = 8), p<0.05, significant from control (one way ANOVA analysis followed by Dunnett’s test).

Fig. 2: Effect of methanol extract of *Pterocarpus erinaceus* leaves on TNF α level in IFN/LPS or LPS-stimulated RAW 264.7 cells. Values are Mean±S.E.M. (n = 8), p<0.05, significant from control (one way ANOVA analysis followed by Dunnett’s test).

Table 1: Anti-inflammatory effect of methanol extract of *Pterocarpus erinaceus* leaves on carrageenan induced hind paw edema

<table>
<thead>
<tr>
<th>Extract</th>
<th>Increase in paw volume (ΔV mL)</th>
<th>Edema inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
<td>3 h</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>0.21±0.03</td>
</tr>
<tr>
<td>MeOH extract</td>
<td>100</td>
<td>0.18±0.03*</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.13±0.02***</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>0.11±0.04***</td>
</tr>
<tr>
<td>Acid acetylsalicylic</td>
<td>100</td>
<td>0.14±0.04**</td>
</tr>
</tbody>
</table>

Values are Mean±SEM (n = 8), *p<0.05, **p<0.01, ***p<0.001 significant from control (one way ANOVA analysis followed by Dunnett’s test).

Table 2: Analgesic effect of methanol extract of *Pterocarpus erinaceus* leaves on acetic acid-induced writhing

<table>
<thead>
<tr>
<th>Extract</th>
<th>Doses (mg kg⁻¹ b.wt.)</th>
<th>Number of writhing</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>66.2±2</td>
<td>-</td>
</tr>
<tr>
<td>MeOH extract</td>
<td>100</td>
<td>45.5±1.8***</td>
<td>31.20</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>26.2±2.0***</td>
<td>60.50</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>21.0±3.0***</td>
<td>68.30</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>100</td>
<td>34.83±1.47**</td>
<td>47.36</td>
</tr>
</tbody>
</table>

Values are Mean±SEM (n = 6), **p<0.01, ***p<0.001 significant from control.

**TNF α and NO determination:** The effect of methanol extract on TNF α and NO productions in RAW 264.7 cells simulated by LPS or LPS/IFN was investigated. The TNF α and NO productions were more in the response to LPS/IFN than LPS only in cells. In Fig. 1 and 2, at 25 and 50 µg mL⁻¹, the methanol extract inhibited the level of nitrite (equivalent to the NO level) and the TNF α level in culture supernatants. The highest inhibition (95.14%) of methanol extract was obtained at 50 µg mL⁻¹ on NO production in LPS-stimulated RAW 264.7 cells.

**Antioxidant activity:** The free radical DPPH⁺-scavenging capacity of methanol extract and flavonoids fraction was less.
than trolox (ARP = 4.55) used as reference substance. The antioxidant power using CUPRAC method of methanol extract and flavonoids fraction was less important than rutin (TEAC = 2.9) (Table 3).

**In vivo antiplasmodial assay:** Methanol extract of *P. erinaceus* leaves exhibiting in vivo anti-inflammatory activity was tested for in vivo anti-plasmodial activity using three doses (50, 100 and 250 mg kg\(^{-1}\) b.wt). Results presented by Fig. 3 showed suppressive capacity of extract against *Plasmodium berghei* ANKA strain. The anti-plasmodial effect of extract was significant (p<0.05) and observed in a dose dependent in manner.

**DISCUSSIONS**

The leaves of *Pterocarpus erinaceus* are used to treat inflammatory diseases (malaria, anemia, ulcer, rheumatism and dermatitis) in traditional medicine in Burkina Faso. In the present study, the pharmacological properties of methanol extract were investigated in paw edema induced by carrageenan, writhing induced by acetic acid, antioxidant capacity, on TNFα and NO production in IFN/LPS or LPS-stimulated RAW 264.7 cells and against *Plasmodium berghei* infected mice.

Before pharmacological studies, the acute oral toxicity of methanol extract was initially investigated in NMRI mice. Up to 2,500 mg kg\(^{-1}\) b.wt, no mortality and no changes in behavior in treated mice were observed. This suggests that methanol extract can be classified to be safe and low toxic drug according to Hodge and Sterner\(^{16}\).

Carrageenan-induced paw edema is wildly used to evaluate the anti-inflammatory effect of natural substances or new drugs. Carrageenan injection induce the inflammatory responses, which is biphasic events: Histamine, serotonin, bradykinins are released in the initial phase (1 h) and the second phase (over 1 h) is due the prostaglandins and cytokines (TNF α, IL 1β, IL 6) production\(^{17,18}\). Paw edema was reduced by methanol extract at 1, 3 and 5 h after carrageenan injection. The edema inhibition suggests that the methanol extract acts on the release of different pro-inflammatory mediator. Edema reduction was important at 5 h after carrageenan injection, suggesting that methanol extract inhibited the release of prostaglandins and cytokines.

Writhing induced by acetic acid injection on animal is a method used to investigate the peripheral analgesic effect of natural drugs. Acetic acid injection releases endogenous mediators including histamine, serotonin, bradykinin, substance P and prostaglandins (PGE\(_{2wa}\), PGF\(_{2al}\)), which are responsible of abdominal writhing\(^{19,20}\). Oral administration of methanol extract significantly reduced abdominal writhing in dose-dependent manner. This result could be suggesting that the analgesic effect was related to inhibition of endogenous mediators, such as substance P and prostaglandins (PGE\(_{2wa}\), PGF\(_{2al}\)).

In vitro anti-inflammatory activity was evaluated on TNFα and NO production in IFN/LPS or LPS-stimulated RAW 264.7 cells. Pro-inflammatory cytokines as TNFα and Nitric Oxide (NO) play an important role during inflammatory diseases, in addition, TNFα and NO stimulate the production of prostaglandins (PGE\(_{2wa}\), PGF\(_{2al}\)), which are involved in inflammation and pain\(^{21,22}\). Methanol extract inhibited the release of TNFα and NO in RAW 264.7 cells. According to results (Fig. 1 and 2), the inhibition of methanol extract of *P. erinaceus* on NO production was superior when compared to TNFα-production. The release of NO and TNFα are due to NF-κB activation\(^{23}\). It suggests that blocking of NO and TNFα production by extract could be linked to the lack of NF-κB activation. In addition, the anti-inflammatory effect of substance could be the fact that it decreases the COX 2 expression and the nuclear translocation of NF-κB\(^{24}\).

Large amount of Reactive Oxygen Species (ROS) generated by activated macrophages and granulocytes, contribute to expand the inflammatory response. During inflammation ROS induce the release of pro-inflammatory cytokines (TNFα, IL 1β, IL 6), which in turn increase ROS\(^{25}\).
The ROS inhibition contributes to the therapeutic anti-inflammatory effect of natural substance. Two methods (DPPH and CUPRAC) were used to evaluate the capacity of methanol extract of *Pterocarpus erinaceus* to inhibit ROS. According to the results obtained through both methods, methanol extract exhibited antioxidant activity but moderated than trolox and rutin used as reference compounds. It could be this antioxidant effect, which contributes to anti-inflammatory and analgesic effect of extract reducing ROS production and pro-inflammatory cytokines release.

Several studies reported the anti-malaria properties of medicinal plants leading to isolate anti-malaria molecules including quinine, artemisinin actually used against malaria. Methanol extract of *P. erinaceus* leaves exhibited anti-plasmodial effect against *Plasmodium berghei* in mice NMRI. Karou et al. demonstrating that the *in vitro* anti-plasmodial activity of ethanol extract of leaves of *P. erinaceus* was performed against fresh clinical isolated *Plasmodium falciparum* with IC₅₀ = 14.63 µg mL⁻¹. According to the classification about anti-plasmodial activity *in vivo*, methanol extract of leaves of *P. erinaceus* exhibited very good activity.

The pathogenesis of malaria is associated with an inflammatory response as a defensive reaction of body leading pro-inflammatory cytokines production. Kassa et al. reported that during Plasmodium infection, hemozoin formed induces the pro-inflammatory cytokines productions. So the anti-inflammatory activity of methanol extract contributes to antiplasmodial activity *in vivo*.

**CONCLUSION**

The present study demonstrated the anti-inflammatory properties, analgesic effect and the capacity to inhibit free radicals of *Pterocarpus erinaceus* Poir. (Fabaceae) leaves. The anti-inflammatory and analgesic effects of extract support the traditional use of this plant in malaria and associated symptoms treatment. These results constitute some scientific data to promote the use of *P. erinaceus* in traditional medicine in Burkina Faso.

**ACKNOWLEDGMENTS**

This research was supported by traditional medicine department of IRSS/CNRST-Burkina Faso and department of Pathology and immunology, CMU, University of Geneva. The authors want to thank Pr Thiomblan Adjoura, at Botany section of University of Ouagadougou, to identify the plant.

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