Study on Inflammation and the Nervous system of Ethanol extract of Jatropha Curcas seed

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ABSTRACT
Introduction: Jatropha curcas L. seeds are used in traditional medicine to treat a variety of diseases or conditions. The aim of this study is to evaluate effects on inflammation and the nervous system of ethanal extract of J. curcas seeds. Materials and methods: It was used 64 mice divided in 8 groups; respectively, 4 groups received 400, 600, 800 and 1000 mg/kg of ethanol extract of J. curcas seed; and the rest extract Diclofenac, Dizepam, Caffeine and a control group not received any substance. The effects on inflammation was evaluated by Carrageenan-Induced paw oedema test and by Paw skin temperature. The effects on the nervous system were evaluated using the Irwin test. Results: According to the paw oedema, it was showed an trend on an inflammatory effect of the seeds of J. curcas; this activity was statistically significant in doses of 1000 mg/kg. Also, the skin temperature measurements outcomes reveal a positive dose response manner. Regard to neurological manifestations, Straub tail was founded in doses of 400 mg/kg. Stereotypies were founded in doses of 400, 600, 800 and 1000 mg/kg throughout the evaluation. Conclusion: J. curcas seeds were showed an inflammatory effect. In addition, effects on the nervous system were founded as stereotypical and Straub tail.

Key words: Jatropha curcas, Seeds, Inflammation, Carrageenan, Nervous System.

INTRODUCTION
Jatropha curcas (Linnaeus) is a plant which belongs to family Euphorbiaceae and genus Jatropha. This plant is native of America and is widely distributed, is also located in Africa, Asia and Europe where it grows in arid, tropical and subtropical environments. Its name derives from the Greek words “Jatros” (doctor) and “trophe” (food) which translates its medicinal traditional use.1

Such medicinal use is reported in diverse consumption of different parts of J. curcas, some of them through infusions for various conditions such as fever, burn, rheumatism, allergies, diarrhea, intestinal cramping, healing, dysentery, parasitic infections, etc.2-5

Thus J. curcas seeds have acquired importance for its extra-medical properties6 and its use in native communities for some conditions as arthritis, gout, jaundice, convulsions, constipation, diarrhea, fever and inflammation.3,5,6

In Peru, this plant is located in departments of Lima, Piura, Cajamarca, San Martin, Cusco, Ucayali and Loreto where its traditional practices were also recognised.3,7,8

Preclinical studies have demonstrated the biological activity of the different parts of J. curcas, so, antifungal and acaricide activity of seeds;9,11 antidiarrheal, anti inflammatory and antimicrobial effect of the root, stem, bark and leaf;12-14 abortifacient activity of fruit15 and activity on the blood coagulation of latex.6

These recognized effects of J. curcas might be supported by presence of secondary metabolites as alkaloids, flavonoids, esters, lectins, tannins, saponins, terpenoids, lipases, among others.19-23

In addition, there are validated methods to evaluate effects on the nervous system and inflammatory activity as Irwin test and Carrageenan-induced paw oedema test, which were used to validate pharmacological effects of some medicinal plants.24-28

Considering the increase of interest for the value of this medicinal plant,29 this study focused on assessment of effects on inflammation and the nervous system of ethanol extract of J. curcas seeds in escalating doses.

MATERIALS AND METHODS

Chemical Sample
Diclofenac ampoules 75 mg/3 mL, code A021143, RS: EG-2537; Lambda Carrageenan 25 g, code 80 K 1334; Caffeine tablet 100 mg, code 1060522, RS: N-24400; Dizepam ampoules 5 mg/m 1x2 mL, code 10950583, RS: NG-3084.

Plant material and extraction
It was used Jatropha curcas seeds which was collected in the Department of Loreto (Iquitos) under the criteria of the Cerrate method E.1969.30
1.- First, the vascular plant was collected at 08:00 hrs
2.- Then, it was put immediately into a field press
3.- After that, it was dried

Berta Loja, who is biologist of The Research Centre of Traditional Medicine and Pharmacology, FMH-USMP, authenticated the taxonomy of the plant. For mark the specimens it was crossed the information with the herbaria of the Universidad Nacional Mayor de San Marcos (USM) and the Missouri Botanical Garden (MO). Finally, it was classified gender and specie as Jatropha curcas according to Peruvian31-35 and all continent America, South Africa, and Asia flora references.36-37

The extract was prepared from dried and ground seeds of J. curcas, this was macerated in 70% ethanol for a week, then the mixture was filtered.
and the obtained residue was dried in an oven for a period of 7 days. The dried sample was ground in a mortar to obtain a fine powder which was stored in airtight containers and refrigerated until its experimental use.

Animals and study design

64 Mus musculus male albino mice were obtained from National Institute of Health (Instituto Nacional de Salud, INS–Biotério, Chorrillos, Lima–Perú), whose weight average were between 25 and 30 g. Mice were undergone to an acclimatization process in FMH-USMP; they were housed 4 per cage and were maintained at the following conditions: temperature 22°C (± 2), humidity between 30 and 70%, 12 hr light/dark cycles and noise levels less than 70 dB.

Mice were provided with balanced food and water ad libitum and then they were deprived of food 12 hrs before the development of experiment. Animals were divided in 8 groups of 8 mice each group. 4 experimental groups which orally intake ethanol extracts of J. curcas seed in escalating doses of 400, 600, 800 and 1000 mg/kg, 4 control groups which three of them received orally caffeine 32 mg/kg, diazepam 32 mg/kg, diclofenac 8 mg/kg, and a control group who not received any substance. For randomized distribution and allocation of groups, the simple randomization by raffle method was used. Each procedure with all mice during the experiment respected the principles and guidelines for researches in laboratory animals, cited in International Guiding Principles for Biomedical Research Involving Animal (1985).

Irwin Test in mice

The test was conducted as an adaptation of the previously described. The substance was administered to mice in a double-blind manner. Subsequently, the animals were observed during a one hour in time ranges of 15, 30, 45 and 60 min. The end points analyzed were presence or absence of the following: lethality, convulsions, straub tail, sedation, agitation, abnormal gait (rolling or toes), jumps, motor incoordination, piloerection, abdominal cramps, stereotypies (sniffing, chewing or head movements), head shaking, itching, and abnormal breathing.

Carrageenin-induced paw oedema in mice

This was performed as previously indicated. The carrageenin 0.2% was injected subcutaneously into the mice plantar aponoeuris. The volume of the injected paw was measured before and after each injection with a LETICA digital plethysmometer (LE-750). Observations were made on 6 periods: pre-test (before the time of administration), 1, 2, 3, 4 and 5 hrs after administration. The inflammatory activity was calculated using the formula:

\[ \text{Oedema plantar (ml)} = \text{Vt} - \text{Vb} \]

\[ \text{Vt= volume of the foot at measurement point.} \]

\[ \text{Vb= paw volume before carrageenan administration (pre-test).} \]

Measurement of the plantar temperature

The skin temperature was measured using digital thermometer without contact NC 100, ASTME 1965; IEC 60601-1; IEC 60601-1-2 (EMC). The measurements were made periodically on pre-test (before the time of administration), 1, 2, 3, 4 and 5 hrs after administration.

Statistical analysis–

For quantitative variables the following statistics were applied: 1 tail ANOVA, Tukey and Pearson Correlation Coefficient tests. For qualitative variables, Fisher’s statistic was applied. It was considered statistical significance for p<0.05 value and a confidence interval of 95%. It was used like informatics support Microsoft Office Excel 2013 and the Graph Pad Prism Software version 5.01.

RESULTS

Irwin Test

The ethanol extract of the seeds of J. curcas induced neurotoxicity effects during the first hour assessment. The mainly evidenced effects were sedation, stereotypies and Straub tail in groups at doses of 400, 600, 800 and 1000 mg/kg of J. curcas seeds (See Table 1).

Carrageenan induced paw edema

The direct correlation between increased paw edema and administration dose was evidenced. The experimental groups 400, 600, 800 and 1000 mg/kg of J. curcas seeds showed statistical difference versus control groups only during the fifth hour of evaluation. Moreover, the group 1000 mg/kg of J. curcas seeds presented an increasing of plantar edema during all hours of evaluation (See Table 2).

Paw skin temperature

In a dose-response profile, the experimental groups showed increased of the skin temperature in a positive way. Particularly, the group at doses of 1000 mg/kg presented the highest temperature throughout the evaluation (See Table 3).

DISCUSIÓN

The presence of stereotype and Straub tail, would mean a stimulant effect in the the central nervous system level, which may be due to a possible induction of the serotoninergic, dopaminergic, and opioidergic systems. It is recognized the neurotoxicity and fatality affects by the secondary metabolites phorbol esters from the seeds of J. curcas, in contrast, other studies in some varieties of this plant showed poor toxicity. Other secondary metabolites present in the seed of J. curcas are alkaloids, flavonoids, tannins and saponins among others, could be responsible for the effects observed in this study.

Considering the presence of stereotype and Straub tail, it is possible that the ethanolic extract of J. curcas seed have a lipophilic chemical constitution, which would make it possible to cross the blood-brain barrier, and therefore, it should alert the potential risk during pregnancy and others. In this study, doses of 400, 600, 800 and 1000 mg/kg of ethanol extract of J. curcas seeds showed throughout early hours of evaluation an inflammatary effect similar to control group. Contrary, a research showed an anti-inflammatory effect of methanol extract of J. curcas root at dose of 100 and 200 mg/kg. Similar results were showed in other study with the alcohol extracts of root, stem and leaf, at doses of 200 mg/kg showed a decrease of edema, and with aqueous extract of J. curcas leaves at doses of 300 mg/kg reduced edema significantly.

The contradictory inflammatory effects presented by the seeds of the J. curcas in front of the others parts of the plant might have explanation in the presence or absence of their secondary metabolites, inclusively in the concentration or others, these variables are need to study in future studies.

In addition, the outcomes of the skin temperature are according to the inflammation cascade produced by carrageenan and in this case it is possible that the ethanolic extract of J. curcas induced elevation of the skin temperature by release autacoids like serotonin or prostaglandins, nonetheless, which should be clarified in future studies.
Alexander et al.: Inflammatory and neurobehavior effect of *Jatropha curcas* seeds

**Table 1: Effects of ethanol extract of *Jatropha curcas* seed on Irwin test in the first hour**

<table>
<thead>
<tr>
<th>Behaviours</th>
<th>Control</th>
<th>Caffeine</th>
<th>Diazepam</th>
<th>400 mg/kg</th>
<th>600 mg/kg</th>
<th>800 mg/kg</th>
<th>1000 mg/kg</th>
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<tbody>
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<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Convulsions</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Straub tail *</td>
<td>YES</td>
<td>NO</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Sedation †</td>
<td>NO</td>
<td>NO</td>
<td>YES</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
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<td>Jumps</td>
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<td>NO</td>
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<td>NO</td>
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<td>NO</td>
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</tr>
<tr>
<td>Stereotypies ‡</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
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<td>YES</td>
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<tr>
<td>Head twitches</td>
<td>NO</td>
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</tr>
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<tr>
<td>Increase of respiration</td>
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<tr>
<td>Decrease of respiration</td>
<td>NO</td>
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<td>NO</td>
</tr>
</tbody>
</table>

*Chi square, p<0.05, 400 mg/kg vs. Diazepam and Control (45 min).
Chi square, p<0.05, Diazepam vs. 400, 600, 800 and 1000 mg/kg (30 and 45min), vs. Control and Caffeine (30 min).
† Chi square, p<0.05, Diazepam vs. Control (15 min), vs. 400 and 600 mg/kg (15 and 30 min), vs. Caffeine (30 min), vs. Control, 800 and 1000 mg/kg (45 min).

**Table 2: Effects of ethanol extract of *Jatropha curcas* seed on Carrageenan-induced paw edema**

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Increase of paw edema</th>
<th>1st h</th>
<th>2nd h</th>
<th>3rd h</th>
<th>4th h</th>
<th>5th h *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>400 mg/kg</td>
<td>0.07 ± 0.05</td>
<td>0.04 ± 0.03</td>
<td>0.06 ± 0.04</td>
<td>0.08 ± 0.06</td>
<td>0.06 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>600 mg/kg</td>
<td>0.06 ± 0.04</td>
<td>0.06 ± 0.05</td>
<td>0.06 ± 0.08</td>
<td>0.06 ± 0.04</td>
<td>0.06 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>800 mg/kg</td>
<td>0.06 ± 0.05</td>
<td>0.03 ± 0.03</td>
<td>0.06 ± 0.05</td>
<td>0.07 ± 0.06</td>
<td>0.04 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>1000 mg/kg</td>
<td>0.08 ± 0.05</td>
<td>0.09 ± 0.04</td>
<td>0.09 ± 0.05</td>
<td>0.11 ± 0.08</td>
<td>0.14 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>Diclofenac</td>
<td>0.04 ± 0.05</td>
<td>0.05 ± 0.05</td>
<td>0.05 ± 0.06</td>
<td>0.05 ± 0.04</td>
<td>0.06 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.07 ± 0.07</td>
<td>0.03 ± 0.06</td>
<td>0.06 ± 0.05</td>
<td>0.06 ± 0.06</td>
<td>0.11 ± 0.04</td>
<td></td>
</tr>
</tbody>
</table>

* ANOVA, p<0.05; Tukey, p<0.05; 1000 mg/kg vs. Control, Diclofenac, 400, 600 and 800 mg/kg.
SD: Standard Deviation.

**Table 3: Effects of ethanol extract of *Jatropha curcas* seed on paw skin temperature**

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Basal</th>
<th>1st h</th>
<th>2nd h</th>
<th>3rd h</th>
<th>4th h</th>
<th>5th h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>400 mg/kg</td>
<td>26.89 ± 3.59</td>
<td>29.36 ± 1.10</td>
<td>29.75 ± 2.23</td>
<td>28.39 ± 1.85</td>
<td>29.50 ± 1.34</td>
<td>30.08 ± 1.65</td>
</tr>
<tr>
<td>600 mg/kg</td>
<td>29.09 ± 2.88</td>
<td>29.83 ± 1.96</td>
<td>29.84 ± 1.60</td>
<td>30.68 ± 2.05</td>
<td>30.61 ± 2.45</td>
<td>30.56 ± 2.00</td>
</tr>
<tr>
<td>800 mg/kg</td>
<td>28.96 ± 1.35</td>
<td>29.66 ± 1.84</td>
<td>29.49 ± 2.38</td>
<td>30.75 ± 3.09</td>
<td>31.73 ± 0.71</td>
<td>31.25 ± 1.83</td>
</tr>
<tr>
<td>1000 mg/kg</td>
<td>28.00 ± 2.27</td>
<td>30.75 ± 2.21</td>
<td>30.70 ± 1.57</td>
<td>31.23 ± 1.08</td>
<td>30.61 ± 1.39</td>
<td>31.33 ± 1.40</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>28.88 ± 1.17</td>
<td>30.36 ± 1.44</td>
<td>29.99 ± 1.79</td>
<td>30.36 ± 0.98</td>
<td>29.18 ± 2.29</td>
<td>30.04 ± 1.92</td>
</tr>
<tr>
<td>Control</td>
<td>28.96 ± 2.98</td>
<td>29.83 ± 1.96</td>
<td>30.03 ± 1.75</td>
<td>30.94 ± 1.69</td>
<td>30.06 ± 2.51</td>
<td>29.70 ± 2.16</td>
</tr>
</tbody>
</table>

* ANOVA, p>0.05; Pearson correlation p<0.05, r=0.04269
SD: Standard Deviation.
CONCLUSION

The ethanolic extract of the seed of *J. curcas* presented inflammatory activity and central nervous system activity like stereotype and Straub tail profile.

AUTHORS’ CONTRIBUTIONS

Author contributions to the study and manuscript preparation are as follows: ZHA, ZHR, GLS, and SGA conceived and designed the study. ZHA, ZHR, GLS, ZFE, GBJ, CSA and PMC performed the experiment. ZHA, ZHR, GLS, ZFE and PMC acquired of data. ZHA, ZHR, and GLS developed statistical analysis. All authors analysed and contributed to data interpretation. SGA drafted the manuscript. All authors revised and approved the final manuscript.

ACKNOWLEDGMENTS

This research was supported by the authors and FMH-USMP. We would like to express our appreciation and gratefulness to Frank Lizaraso Caparo M.D. (Dean of the FMH-USMP), Benjamin Castañeda Castañeda M.D. (Past former director and researcher of the Institute of research of the FMH-USMP), Yordan Martinez Espichan, Gabriela Gonzales Pariona and Jonathan Troncoso Zuñiga (medical students of FMH-USMP) for their collaborations.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

ABBREVIATION USED

None.

REFERENCES


Figure 1: Correlation between dose of *J. curcas* and paw skin temperature.
Alexander et al.: Inflammatory and neurobehavior effect of Jatropha curcas seeds


PICTORIAL ABSTRACT

SUMMARY

- The ethanol extract of J. curcas seeds were showed effects on inflammation and the nervous system.

- The ethanol extract of J. curcas seeds showed an inflammatory effect, effects on the nervous system were found as stereotypes and Straub tail through Irwin test.
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