Research article

Analgesic Activity of the Methanolic Leaf Extract of Jatropha Curcas (Linn)

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ABSTRACT: This study evaluated the analgesic activity of the methanolic leaf extract of Jatropha curcas (Linn) in vivo using analgesic models viz: Hot plate method in mice, tail flick or immersion method in rat and acetic acid-induced writhing reflex model in mice. In all the models, acetylsalicylic acid (ASA) was used as the reference drug. In the hot plate and tail flick models, the oral administration of J. curcas extract at the doses of 100, 200 and 400 mg/kg and the reference drug ASA (400 mg/kg) showed potent analgesic effect by significantly (P<0.001) increasing the pain reaction time dose dependently in mice and rats. Also the reference drug and the extract of J. curcas significantly (P<0.0001) decreased the number of the abdominal contortions in the acetic acid-induced writing reflex in mice and increased the percentage protection in a dose dependent manner. In conclusion, this study indicates that the methanolic leaf extract of Jatropha curcas has significant analgesic properties and may be acting through both peripheral and central pain mechanisms.

Key words: Analgesic, Jatropha curcas, hot plate, tail flick, writhing reflex, acetylsalicylic acid.

INTRODUCTION

Jatropha curcas (Linn) or physic nut is a perennial poisonous shrub which grows up to 5m high and belongs to the family Euphobiaceae. (Gadekar, 2006). The plant originated from Central America but has spread to other tropical and subtropical countries and mainly grows in Asia and Africa (http://www.jatropha.wur.nl). The leaves are usually green to pale green in colour, the flowers are unisexual but occasionally haemophrodite. The fruits are produced mainly during the rainy season and the seeds are mature if the capsule changes from green to yellow (Deghan and Websetter, 1979). The plant has been employed for both medicinal system in Nigeria, the fruits of J. curcas and the stem bark of Cochlospermum plachonii are combined for the treatment of diabetes mellitus (Igoli et al, 2005). Also it is used traditionally for the treatment of pains in the South Eastern part of Nigeria. The use of the aqueous extract of the seed and the nut as a contraceptive have been reported (Goonasekera, et al, 1995). The leaf extract also have been shown to have a potent cardiovascular action(Fojas et al, 1986). Other uses include; the use of the seeds for making soap, candles, detergents, lubricants and dyes. The bark is used as fish poison and the oil from the seed is used as biodiesel (Achten et al,
2008). The sap from the stem is used to stop bleeding from wound and the plant is also used as living fence to protect garden and fields from animals (Gadekar et al., 2006). The present study was carried out to investigate the analgesic (antinociceptive) activity of the methanolic leaf extract of J. curcas (Linn) with the aim of establishing the pharmacological basis for the use of J. curcas leaves decoction for the treatment of pains in traditional medicine in the South Eastern part of Nigeria using standard in vivo analgesic models.

MATERIALS AND METHODS

Collection and extraction of the plant materials.
Fresh leaves of J.curcas were collected from the premises of Michael Okpara University of Agriculture, Umudike, Abia State and identified by the forestry Department of the University. A voucher specimen was deposited in the University herbarium. Extraction was done by cold maceration method. The leaves were air dried, cut into small pieces, pulverized into a coarse powder of about 1mm in diameter. 600 g of the plant material was extracted in 80% methanol for 48hours with intermittent shaking at 2 hr interval. The extract was filtered using Whatman no 1 filter papers and later concentrated in vacou using rotary evaporator at 40°C and 210 milibar. The percentage yield was determined to be 8.36% and the extract was stored in a refrigerator until ready for use at 15°C.

Animals
Mature albino Wistar rats (120-160g) and mice (25-35g) both (sex and age matched) obtained from the laboratory animal units of the Faculty of Veterinary Medicine, University of Nigeria Nsukka were used for the experiment. The temperature varied between 25-35°C and lighting hour varied between 12-14 hr with relative humidity of about 45-65%. The animals were kept in stainless steel cages and clean drinking water provided ad libitum to them while they were fed with standard commercial pelleted feed (Vital feed®, Nigeria). Ethical conditions governing the conduction of experiments with life animals were strictly observed as stipulated by Ward and Elsea (1997).

ANALGESIC SCREENING

Hot plate method in mice.
The experiment was carried out using the method described by Shethy and Anika (1982). Twenty five (25) mature albino mice of both sexes were randomly divided into 5 groups of 5 mice per group. The mice were fasted for 12 h with provision of clean water ad libitum. Each mouse was placed upon the heated metal plate (Hot plate) maintained at the temperature of about 50-55°C within the restraining plastic cylinder. The pain reaction time (PRT) or latency period, which is the time taken for the rats to react to the pain stimulus was determined with a stop watch before drug administration. The cut off time was fixed for 30 second to avoid damage to the tissues of the foot. This served as the control reaction time. The mice were then treated as follows: Group 1 mice received 10 ml/kg of distilled water and served as negative control. Group 2 mice received acetylsalicylic acid 400 mg 1kg (ASA) (positive control) and groups 3, 4 and 5 received 100, 200 and 400 mg/kg of J. curcas extract respectively per os. 30 minutes after drug administration the PRT for each mice was determined again. The response to the heat stimulus varied with the animals and consisted of one and another of the following types of behaviours: kicking its hind foot and jumping about, shaking a foot and licking it or raising one or the other of the hind foot and holding it tightly against the body. Responses involving the fore foot are not considered since they are difficult to distinguish from the normal grooming behaviour of the mice.

Tail flick or Tail immersion method in rats.
The method described by Uma-Davi et al., (1999) was used for this study. 25 mature albino rats of both sexes were randomly grouped into 5 groups of rats per group. The animals were fasted for 12 h with clean water provided ad libitum. Group 1 mice were given normal saline (10 ml/kg). This served as negative control. Group 2 mice were given 400 mg/kg of ASA and served as the positive control. Groups 3, 4 and 5 received 100, 200 and 400 mg/kg of J. curcas extract respectively all by oral intubation. 1 h after drug administration, about 5 cm of the tail of each of the rats was dipped into a water bath containing warm water maintained at the temperature of 50 + 1°C and the period of tolerance to the pain (PRT), i.e the time taken for the rat to flick its tail was recorded for all the rats.

Acetic acid-induced writhing reflex test in mice.
The method of Koster et al (1959) as modified by Dambisya and Lee (1999) was adopted for the study of the analgesic effect of J. curcas in mice. 25 albino mice of both sexes were randomly divided into 5 groups of 5 rats per group and treated as follows: group 1 mice received 10 ml/kg of normal saline (negative control). Group 2 mice received 400 mg/kg of ASA while groups 3, 4 and 5 mice were given 100, 200 and 400 mg/kg of J. curcas extract respectively. 1 h after administration of the drug and extract, 0.7% glacial
acetic acid (10 ml/kg) was given to all the mice intraperitoneally to induce pain characterized by abdominal contortions or writhing. The abdominal contortions consisted of contraction of abdominal muscles and stretching of the hind limbs. The number of writhing observed in each mouse was counted for 30 minutes and recorded. The degree of antinociception (analgesia) was calculated using the following formula according to Dambisya and Lee (1999) and represents the percentage of inhibition of writhing.

\[
\text{Mean control group-mean treated group} \times 100
\]

RESULTS

Hot plate method

The result of the antinociceptive effects of *J. curcas* (Linn) is shown in Table 1. The result showed that there was no significant difference in the pain reaction time (PRT) before drug administration in all the mice. 30 minutes after drug administration, the PRT was significantly (P<0.001) increased by the extract and the reference drug in a dose dependent manner when compared to the normal saline treated group. There was no significant difference between the negative group and the group that received the least dose of the extract (100 mg/kg). In the experiment the reference drug (ASA) was more effective than the extract in reducing the PRT.

Tail flick method

The result of the effect of *J. curcas* on the tail flick method is presented in Table 2. The reference drug ASA and the extract significantly (P<0.0001) increased the latency period from 4.0± 0.71 seen in the negative control group to 9.2± 0.58 sec in group 2 (ASA, 400 mg/kg) and 7.8 ± 0.73 and 8.8 ± 0.58 sec in the 200 and 400 mg/kg treated groups respectively. The effect of the reference drug ASA was also higher than the extract though not significant.

The result of the analgesic in *J. curcas* Table 3, 200 and 400 mg/kg and ASA 400 mg/kg significantly (P<0.0001) reduced the acetic acid-induced writhing when compared to the untreated mice (negative control). There was no significant difference between the reference drug treated group and the 200 and 400 mg/kg extract treated groups. Also, the percentage protection increased with the increase in the dose of the extract from 0% in the normal saline treated mice to 61% at the dose of 400 mg/kg of *J. curcas* and 72% for the reference drug treated group.

**Table 1.** The effect of *J.curcas* extract on Hot plate-induced pains in mice

<table>
<thead>
<tr>
<th>Group/Treatment</th>
<th>Pre-drug ± S.E.M (sec)</th>
<th>Post-drug time ± S.E.M (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal Saline 10 mg/kg</td>
<td>3.2 ± 0.66</td>
</tr>
<tr>
<td>2</td>
<td>Acetylsalicylic acid 400 mg/kg</td>
<td>4.8 ± 1.56</td>
</tr>
<tr>
<td>3</td>
<td><em>J. curcas</em> extract 100 mg/kg</td>
<td>3.0 ± 0.55</td>
</tr>
<tr>
<td>4</td>
<td><em>J. curcas</em> extract 200 mg/kg</td>
<td>2.8 ± 0.58</td>
</tr>
<tr>
<td>5</td>
<td><em>J. curcas</em> extract 400 mg/kg</td>
<td>3.2 ± 0.37</td>
</tr>
</tbody>
</table>

*P<0.001 when compared to negative control

**Table 2.** The effect of *J.curcas* extract on Tail-flick response in rats

<table>
<thead>
<tr>
<th>Group/Treatment</th>
<th>Mean Pain Reaction time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal Saline 10 mg/kg (p.o)</td>
</tr>
<tr>
<td>2</td>
<td>Acetylsalicylic acid 400 mg/kg</td>
</tr>
<tr>
<td>3</td>
<td><em>J. curcas</em> extract 100 mg/kg</td>
</tr>
<tr>
<td>4</td>
<td><em>J. curcas</em> extract 200 mg/kg</td>
</tr>
<tr>
<td>5</td>
<td><em>J. curcas</em> extract 400 mg/kg</td>
</tr>
</tbody>
</table>

*P<0.001 when compared to negative control

**Table 3.** The effect of *J.curcas* extract on Acetic acid-induced writhing in mice

<table>
<thead>
<tr>
<th>Group/Treatment</th>
<th>Pre-drug ± S.E.M (sec)</th>
<th>Post-drug time ± S.E.M</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal Saline 10 mg/kg</td>
<td>83.8 ± 3.02</td>
</tr>
<tr>
<td>2</td>
<td>Acetylsalicylic acid 400 mg/kg</td>
<td>23.7 ± 2.20*</td>
</tr>
<tr>
<td>3</td>
<td><em>J. curcas</em> extract 100 mg/kg</td>
<td>76.4 ± 1.50</td>
</tr>
<tr>
<td>4</td>
<td><em>J. curcas</em> extract 200 mg/kg</td>
<td>34.0 ± 3.65*</td>
</tr>
<tr>
<td>5</td>
<td><em>J. curcas</em> extract 400 mg/kg</td>
<td>32.6 ± 2.84*</td>
</tr>
</tbody>
</table>

*P<0.001 when compared to negative control

DISCUSSION

Animal tests of analgesic drugs commonly measure nociception and involve testing the reaction of an animal to painful stimuli (Rang et al, 2003). It is possible to assume that certain noxious stimuli (thermal, mechanical of chemical) are painful and that reflex movements or behaviours resulting from such
stimuli are indicative of a pain threshold (Shethy
and Anika, 1982), hence the use of the three
antinociceptive
models viz: Hot plate, Tail flick and Acetic acid – induced writing reflex models for the study of the
analgesic activities of J. curcas. In the hot plate and tail
tail flick models, increase in the pain reaction time (latency
period) indicates the level of analgesia induced by the
drug or extract (Ramadran and Bansinath, 1986). J. curcas
extract produced a dose dependent and signiﬁcant (P<0.001) increase in pain threshold in the
rats and mice in these models that was comparable to
the reference drug ASA. In these models, increase in
stress tolerance capacity of the animals indicates the
possible involvement of a higher centre (Vogel and
Vogel, 1997). It is therefore thought that the analgesic
effect of J. curcas seen in this study may involve
central activity.

Several chemicals e.g phenylquinone and acetic acid
could induce writhing response in laboratory animals
(Berkentopf and Weichman 1986). Intraperitoneal
injection of acetic acid in this experiment produced
abdominal contorsions by activating the chemosensitive
nociceptors in the animals (Onasanwo and Elegbe,
2006) the percentage reduction in the number of
abdominal contorsions indicates the level of analgesia
in the acetic acid induced writhing reflex model
(Machioro et al, 2005). The methanolic leaf extract of
J. curcas exhibited high analgesic activity by
significantly (P<0.0001) reducing the number of
abdominal contortions in acetic acid induced writhing
reflex in mice. Also at the doses of 200 and 400 mg/kg
the extract produced analgesic effect that was
comparable to the reference drug ASA by increasing
the percent protection by 59% and 61% respectively.

The constriction response induced by acetic acid is
a sensitive procedure to establish peripherally acting
analgesics and the response is thought to involve local
peritoneal cells and mediated by prostaglandin pathway
(Ronaldo et al, 2000). This suggests that the analgesic
activity of J. curcas may also involve the peripheral
pain mechanism or may be through inhibition of
prostaglandin activities or synthesis.

In all the models used the extract at the dose of 100
mg/kg did not produce signiﬁcant analgesic effect
which suggests that 100 mg/kg may be a sub analgesic
dose of J. curcas. In conclusion, the methanolic leaf
extract of J. curcas demonstrated significant analgesic
activity and may be acting through both peripheral and
central pain mechanism or through suppression of
prostaglandin activities. However, more work is
required in the isolation and characterization of the
active ingredient and the determination of the exact
mechanism of action.

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