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Effects of Methanol Extract of *Jatropha curcas* Leaves on Partial Sciatic Nerve Ligation-induced Neuropathic Pain in Wistar Rats

Mustapha, A.A and *Owoyele, B.V.

Department of Physiology, Faculty of Basic Medical sciences,University of Ilorin, P.M.B. 1515, Ilorin, Nigeria

*Corresponding author:Tel: +234 803 506 5190; Email: deleyele@yahoo.com;owoyele@unilorin.edu.ng

ABSTRACT

The present study was designed to investigate the analgesic potential of methanolic leaf extract of *Jatropha curcas* on neuropathic pain, which tends to be refractory to most medications. Neuropathy was induced via partial sciatic nerve ligation in male albino Wistar rats after which the methanolic extract of *J. curcas* was administered orally at 200 and 400 mg/kg for seven days. Analgesic models viz: hot plate and formalin-induced paw licking test were used to access the behavioural response of the rats to pain perception on the 3rd and 7th day of administration. The results show that the oral administration of *J. curcas* extract at the dose of 400 mg/kg produced potent analgesic effect, significantly (P<0.05) increasing the pain reaction time in the ligated rats when compared with the sham control and non-ligated control. Also the extract of *J. curcas* significantly (P<0.05) decreased the duration of paw licking for both the early and late phases in the formalin paw licking test. In conclusion, this study indicates that the methanolic leaf extract of *Jatropha curcas* has ameliorative potential in the neuropathic pain state.

Keywords: Methanol extract, *Jatropha curcas*, behavioural response,Wistar Rats

INTRODUCTION

Neuropathic pain has been described as "the most terrible of all tortures which a nerve wound may inflict" (Mitchell, 1872). Despite progress in the understanding of this syndrome, the mechanistic details underlying the disease remained elusive. Neuropathic pain is generally characterized by sensory abnormalities such as unpleasant abnormal sensation (dysesthesia), an increased response to painful stimuli (hyperalgesia), and pain in response to a stimulus that does not normally provoke pain (allodynia) (Woolf, 1996). Peripheral neuropathic pain is frequently observed in patients with cancer, AIDS, long standing diabetes, lumbar disc syndrome, herpes infection, traumatic spinal cord injury, multiple sclerosis and stroke (Schmader, 2002; Werhagen *et al*., 2004).

Jatropha curcas is a non-edible oil crop predominately used to produce bio-diesel. It is a species of flowering plant in the genus *Jatropha* in spurge family,Euphorbiaceae, and is native to the American tropics, most likely Mexico and Central America (Janick and Robert, 2008). The plant has been employed for medicinal use in Nigeria, the fruits of *J. curcas* and the stem bark of *Cochlospermumplanchonii* 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 (Omeh and Ezeja, 2010).

Based on the previous analgesic studies, which showed that the plant extracts has a significant anti-nociceptive effect as reported by Omeh and Ezeja, (2010) in acute pain models, the present study was aimed at further investigation into the analgesic activity of the methanolic leaf extract of *J. curcas* on neuropathic pain, a chronic form of pain induced by partial sciatic nerve ligation.

MATERIALS AND METHODOLOGY

Plant materials

The fresh leaves of *Jatropha curcas* were collected from local gardens in Ilorin metropolis. The leaves were air-dried at 45^{o}C for a period ten days, and were subsequently pulverized into a very fine powder using an electric blender (Philips Comfort Blender, Model HR 1727, Holland). The powder (740g) was extracted in 5L of 80% methanol for 48 hours with intermittent shaking at intervals. The extract was filtered using a sieving cloth which had been standardized with Whatman no. 1 filter paper and later concentrated in a water bath at a regulated

temperature of 55^{o}C. The resulting extract weighed 66.8g, giving a percentage yield of 9% and then stored in a refrigerator at a temperature of 15^{o}C until it was ready for use.

Experimental animals

Male Wistar albino rats weighing 180 ± 10 g were employed in the study. Rats were housed in an animal house with free access to water and rat-pellet composed and pelletized by Gbemisola Animals Feeds Company. The rats were kept under standard environmental conditions of temperature (20 - 23^{o}C), and 12 light/ dark cycle.

Animal grouping

Rats were grouped as follow:

Group A: Sham control; Group B: Non-Ligated (10 ml/kg normal saline); Group C: Ligated (10 ml/kg normal saline); Group D: Ligated (400 mg/kg extract); Group E: Ligated (200 mg/kg extract); Group F: Ligated (40 mg/kg imipramine); Group G: Non-Ligated (200 mg/kg extract); Group H: Non-Ligated (400 mg/kg extract). Measurements of behavioural responses to nociceptive stimuli were carried out in the morning.

Induction of neuropathy by partial sciatic nerve ligation:

Partial sciatic nerve ligation is a novel model of neuropathic pain developed by Seltzer *et al*. (1988), characterized by allodynia similar to von Frey hair stimulation and hyperalgesia to both thermal and mechno-noxious stimuli within hours of ligation; the symptoms last for over 7 months.

In brief, rats were deeply anaesthesized with ketamine [60 mg/kg intraperitoneally (i.p)]. The skin of the lateral surface of the left thigh was incised and a cut made to expose the sciatic nerve. Thereafter, the sciatic nerve was ligated using a suture (nylon) thread and immediately after the incised skin was sutured back. Some rats were subjected to surgical procedure to expose sciatic nerve without any ligation, known as the sham control.

Behavioral paradigms for assessment of pain

Hot Plate Test: Hot plate test is one of the thermal pain models used to evaluate anti-nociceptive effects. Thermal hyperalgesia was assessed by placing animals on a hot plate metal surface maintained at a temperature of 50±1^{0}C, on the third and seventh day after partial sciatic nerve ligation. The latency of jumping response to avoid thermal pain was taken as an index of pain threshold. A cut off time of

30 seconds was maintained to avoid tissue damage.

Formalin-Induced Paw Licking Test: The formalin-induced paw licking test was carried out in accordance with the method described by Hunskaar and Hole (1997). The rats were subcutaneously injected with 0.1ml of 4% formalin into the plantar surface of right hind paw. The rats were immediately placed in a transparent glass compartment, where they were observed. The licking time -an indication of pain in the injected paw, was determined by measuring the amount of time spent in licking the injected paw in two phases (early and late phases). The first 5 min post injection was considered as the early phase, after which the animal was allowed to rest for 15 min and a late phase of 10min is then recorded making 30 min in all.

Statistical Analysis

All the results were expressed as mean and standard error of means (S.E.M). The data of behavioral results were statistically analyzed by one-way anova analysis of variance followed by Duncan's post hoc test by using SPSS (Statistical Package for Social Sciences) as the application software (version 16, SPSS Inc, Chicago). Means were considered significant at *p*-value $\leq$ 0.05 was considered to be statistically significant.

RESULTS

Hot plate test

The result for the hot plate test is shown in Table 1. This shows that the extract caused a significant increase in pain reaction latency of the ligated group at high dose (400 mg/kg) of the extract when compared with the sham control and the normal control groups for both days. There were no significant differences in the latencies of the extract administered groups and the ligated control. Also the results showed that continuous administration of the extract at low doses (200mg/kg) till day 7, significantly (P<0.05) increased the pain reaction time for the ligated group when compared with the sham control and ligated normal saline group.

Table 1: Effects of administration of *Jatropha curcas* extract on hot plate latency in Wistar rats.

GROUPS*	Reaction time (s)*	
	Day 3	Day 7
Sham control	1.83±0.65[a]	1.42±0.15[a]
Non-Ligated control (10 ml/kg normal saline)	1.71±0.25[a]	0.97±0.45[a]
Ligated control (10 ml/kg normal saline)	3.45±0.61[a,b]	3.06±0.90[a,c]
Ligated (400 mg/kg extract)	5.92±1.46b	4.53±1.60[b,c]
Ligated (200 mg/kg extract)	3.76±1.08[a,b]	6.4±2.08[c]
Ligated (40 mg/kg imipramine)	3.54±0.44[a,b]	3.57±0.35[a,c]
Non-Ligated (200 mg/kg extract)	1.52±0.76[a]	3.19±0.78[a,c]
Non-Ligated (400 mg/kg extract)	2.97±1.00[a,b]	2.84±0.80[a]

*Each value is the mean ± S.E.M.[a , b, c]Means in columns with different subscript alphabets are significantly different from each other, P < 0.05, n = 5 rats.

Formalin-induced paw licking test

The results in table 2 show that high dose (400 mg/kg) of extract produced significant inhibition of paw licking in the early and late phases of the test compared with the three controls. The 200 mg/kg was less active than the 400 mg/kg.

Table 2: Effects of administration of *Jatropha curcas* extract on formalin induced paw licking in Wistar rats

GROUPS*	DAY 3		DAY 7	
	EARLY PHASE	LATE PHASE	EARLY PHASE	LATE PHASE
Sham control	56.1±7.0[a,e]	32.5±13.6[c]	54.9±6.3[c]	34.8±6.5[b,c]
Non-Ligated (10ml/kg Normal saline)	49.9±2.2[e]	46.4±7.0[c]	37.9±6.5[b,c]	43.6±7.7[c]
Ligated (10ml/kg Normal saline)	56.3±3.5[e]	36.2±7.0[c]	48.2±2.2[b,c]	31±4.1[b,c]
Ligated (400mg/kg Extract)	31.9±8.3[a,b,c]	1.7±1.3[a]	33.8±7.0[b]	3.1±3.1[a]
Ligated (200mg/kg Extract)	36.8±7.9[b,c,e]	7.7±3.5[a]	50.3±7.3[b,c]	16.9±2.8[a,b]
Ligated (40mg/kg Imipramine)	11.2±5.3[a]	0±0[a]	4.6±7a	0.2±0.2[a]
Non-Ligated (200mg/kg Extract)	36.7±10.5[b,c,e]	29.7±9.2[b,c]	42.9±2.4[b,c]	28.5±9[b,c]
Non-Ligated (400mg/kg Extract)	22.2±6.1[a,b,c]	11.4±2.8[a,b]	14±5a	20.9±7.3[b]

* Each value is the mean ± S.E.M., [a , b, c, d, e]

Means in columns with different subscripts alphabet are significantly different from each other, P < 0.05, n = 5 rats

DISCUSSION

This study was designed to investigate the analgesic potentials of the extract of *Jatropha curcas* on peripheral nerve injury neuropathy. The chronic constriction nerve injury used in this study is one of the established model for the neuropathic pain in rodents (Terada et al, 2011, Karimi et al, 2010).In the hot plate model, increase in the pain reaction time (latency period) indicates the level of analgesia induced by the drug or extract (Ramadran and Bansinath, 1986). The hot plate test result showed that the partial sciatic nerve ligation increased the pain reaction time of the ligated control when compared with the non-ligated control, this might be due to delay in the conduction of thermal stimuli (acute pain) from the peripheral nociceptors to the higher centers for pain perception. Also from the hot plate results, the extract at lower dose (200mg/kg) showed more analgesic properties by increasing the pain reaction time, when administered continuously till day 7.

The reduction in the duration of paw licking indicates the level of analgesia in the formalin induced paw licking model (Seltzer *et al*., 1988). The partial sciatic nerve ligation leads to a significant increase in the duration of paw licking (hyperalgesia) during the early (neurogenic pain) phase of formalin-induced paw licking test. For the ligated group, high dose of the extract was more effective in suppressing the hyperalgesia than the low dose during the early phase. But, on the 7^{th} day the extract was no longer effective in alleviating the hyperalgesia, probably due to increased hyperalgesia as the day progresses in the ligated rats. While for the non-ligated group, higher dose of the extract was more effective than the low dose in reducing the duration of paw licking for both days.

For the late phase (inflammatory pain) of formalin-induced paw licking test, partial sciatic nerve ligation didn't lead to significant inflammatory pain (hyperalgesia) when the ligated controls were compared with the non-ligated controls. Also the extract reduced the duration of late phase paw licking dose dependently for both days. Imipramine administration seems to be more effective than the plant extract but this may be due to the use of an extract which still needed to be characterized further for the isolation of its principal ingredients. Such ingredients when isolated may demonstrate better antinociceptive effect than imipramine.

The analgesic activity of the extract in the present study on neuropathy might be due to alkaloid and flavonoid contentsof the extract as reported by Uche and Aprioku

(2008). While alkaloids and flavonoids are known for their anti-nociceptive abilities (Okwu and Josiah, 2006).

CONCLUSION

Thus, aqueous extract of *J. curca* has the ability to moderately thermal and inflammatory pain in chronic sciatic nerve constriction model of neuropathic pain. The effect is pronounced in the inflammatory pain especially after administration of the higher dose. The extract could serve as a complementary analgesic therapy in managing pain due to peripheral nerve injuries (neuropathic pain) and thus enhancing the economic values of *Jatropha curcas* to the pharmaceutical industries.

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