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**Original Research Article**

# Antinociceptive Properties of *Desmodium adscendens* in Mice

***Mfem Cyprain Charles[1]* Ofutet Emmanuel Oleba[1], Kebe Edet Obeten[2], Ekpe Saviour Obono[1]***
*[1]Department of Physiology, Faculty of Basic Medical Sciences, College of Medical sciences, University of Calabar, P.M.B 1115, Calabar, Cross River State Nigeria*
*[2]Department of Anatomy, Faculty of Basic Medical Sciences, College of Medical sciences, University of Calabar, P.M.B 1115, Calabar, Cross River State Nigeria*

***Corresponding Author:**
Mfem Cyprain Charles
Email: emazoeagape@gmail.com

**Abstract:** This study evaluated the analgesic property of *Desmodium adscendens* (DA) in mice using the Hot plate test and the Formalin test. Eighteen (18) mice of 15-25g body weight were used for this study. The mice were randomly assigned into 3 groups (control High dose (HD) and Low dose (LD) treated groups), n=6. Animals in the control group were given normal rat chow and water while animals in the test groups received different doses (100mg/kg and 400mg/kg) of D.A orally with the aid of an orogastric cannula alongside normal chow and water for 21days. At the end of the feeding period, nociception was evaluated following the hot plate and formalin test. The results showed that aqueous leaves extract of DA significantly ($P<0.001$) reduced the frequency and duration of paw lick but reduced escape latencies in the hot plate test compared with control. Frequency and duration of paw flick and paw attention in the acute phase of the formalin test was significantly increased ($P<0.001$) across the experimental groups compared with control. In the light of the above, low and high doses of DA were observed to reduce pain sensitivity following acute thermal pain stimuli whereas; it increases inflammatory pain sensitivity in mice. This indicates that D.A may be good analgesics of thermal cutaneous pain.

**Keywords:** *Desmodium adscendens*, pain, Hot plate, mice, formalin.

## INTRODUCTION

Before the advent of modern medicine, the use of medicinal plants in the management of several ailments was the mainstay especially in the rural part of our society. This could possibly be due to the fact that many had difficulty in gaining access to modern medicine or the inability to afford the cost of getting one; they use traditional medicine (herbs), as a substitute to the conventional treatment of varies illnesses. Although it has been alleged to be primitive and wrongly challenged with animosity, especially by foreign religions, dating back to the colonial days in Africa and subsequently by the conventional or orthodox medical practitioners, its therapeutic potentials can never be overemphasized owing to their biodiversity and perhaps the rich complement of phytochemicals and secondary metabolites that are embedded in them [1, 2].

*Desmodium adscendens* is a medicinal plant which is widely used in popular medicine in different parts of the world. It belongs to the family "*Fabaceae*" and to the genus *Desmodium*. The plant is commonly called Beggar- lice, Beggar weed, Tick Clover or Tick trefoil [3] and *Mbansang Ekpo* by the people of *Effik* in Cross River- Nigeria. In the Brazilian traditional medicine, the leaves of this plant has been used to treat conditions like leucorrhoea, body aches, pains, ovarian inflammations, excessive urination, gonorrhea, and diarrheas [4] In vivo hepatoprotective effect of *D. adscendens* has also been reported [5, 6].In the African traditional medical practice, aqueous leaf extract of *D. adscendens* has been used in the treatment of diseases associated with smooth muscles and some respiratory diseases like asthma, fever, pain and epilepsy. In view of the multiple therapeutic effects of *D. adscendens*, this study was aimed at evaluating the antinociceptive activities of its aqueous leaf extract in mice.

## METHODOLOGY

### Animal Preparation

Two weeks old mice (19-25 g) were used for this study. The mice were obtained from the department of Pharmacology University of Calabar and were kept under standard conditions in the animal house, department of Physiology university of Calabar. They were housed in plastic cages and given free access to rat chow and water *ad libitum*. All animals were allowed to acclimatize for one week under standard laboratory condition before conducting the experiment.

### Plant Identification

Leaves of *Desmodium adscendens* were used for this study. The fresh leaves were obtained from *Nsidung* village in Akpabuyo Local Government Area,

Cross River State and identified and confirmed by the Chief Herbarium Department of Botany University of Calabar. The leaves were air-dried, and the dried leaves were grinded into coarse powdered form and were then extracted.

**Extract Preparation**

700g of the powdered leaves of *Desmodium adscendens* were socked in 1000ml of distilled water for 12 hours and stirred at intervals. The mixture was then filtered using a satin mesh material with subsequent filtration using a filter paper to obtain the final filtrate. The final filtrate was evaporated in an aerated oven at a temperature of about 45– $50^0$c to obtain a brown gummy paste of the extract. The extract was be stored in sample bottles and then refrigerated until used.

**Experimental Design**

Eighteen (18) mice were used for this study. They were randomly assigned into three (3) groups, n=6 as follows: Control, Test group 1 (was given Low dose of extract), and Test group 2 (was given High dose of extract)

**Extract Administration**

At the end of the of acclimatization period, animals in test group 1 and 2 were fed with the aqueous leaves extract of *Desmodium adscendens* orally with the help of an orogastric cannula at a dose 100mg/kg and 400mg/kg body weight respectively. While animals in the control group received a quantity of distilled water equivalent to the dose given to the test groups. The animals received the extract once every day for a period of three weeks (21 days).

**Determination of Nociception**

Nociception was evaluated using the hot plate test and the formalin test. Acute thermal pain is modelled by the hot-plate test while continual pain produced by local tissue injury and inflammation was studied using the formalin test [7, 8].

**Hot plate test**

This method of evaluating acute thermal pain is described by Woolfe and MacDonald [9] and used by Nku *et al*,[10] and Ajiwhen and Bisong[7], even though it has undergone modification by several researchers. The hot plate is commercially obtainable and consists of an electrically heated surface. The temperature is regulated for 55° to 56°C. The animals are placed on the hot plate and the timed until either licking or jumping occurrences is recorded by a stop-watch. The latency is recorded before and after 20, 60 and 90 minutes following oral or subcutaneous administration of the standard or the test compound. The paws of mice and rats are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The time until these responses occur is prolonged after administration of centrally acting analgesics, whereas peripheral analgesics of the acetylsalicylic acid or phenyl-acetic acid type do not generally affect these responses.

**Formalin test**

The formalin test is used to determine the potential analgesic effects of compounds for states of relentless pain in which tissue damage occurs. It causes pain by causing local tissue inflammation. The formalin test was carried out in a 30×30×60 cm-sized clear transparent plastic chamber. A mirror placed behind the box allowed for an unobstructed view of the rat's body and the rat's behaviour was recorded on a videotape [10]. The formalin was made of commercially available 37% formaldehyde solution further diluted in isotonic saline. Conscious mice received a subcutaneous injection of formalin solution into the plantar surface of the right hind paw with a 26-gauge needle. The mice were then placed in an individual cage. In our analysis, the pain-related behaviours were quantified by determining the frequency of spontaneous flinching of the injected paw or the cumulative time of licking of the injected paw. Flinching is one of the pain related behaviours in a formalin model and is characterized by spontaneous, rapid, brief shaking or lifting of the paw. Accordingly, each episode of shaking, vibrating or lifting of the paw was counted as one flinch. Flinching and licking were chosen as measures of pain, because they are more spontaneous than other formalin pain related behaviours (e.g. favouring and lifting) and, consequently, are thought to be more reliable for the quantification of the pain-related behaviours. Flinching was counted using the criteria described by Wheeler-Aceto and Cowan [11]: reflexive retraction or shaking of the formalin-injected paw, or flinching of the hindquarters, sometimes including most of the body. A nociceptive score was determined for each 5 minutes by measuring the sum of duration or frequencies of the behaviour.

**Statistical Analysis**

Data obtained from the study were expressed as mean ± SD. Following one-way analysis of variance (ANOVA) and statistical comparisons among the groups was performed with Turkey multiple comparison test using SPSS, version 17.0. $P < 0.05$ was considered significant.

**RESULTS**

**Comparison of frequency of paw lick in different experimental group during the hot plate test**

The mean ±SEM values for the frequency of paw lick in the control Low dose (LD) and High dose (HD) treated groups were 13.40 ± 0.93, 11.80 ± 2.08 and 7.20 ± 1.07 respectively. The frequency of paw lick was significantly lower ($P<0.05$) in the HD treated group compared with control and LD treated group (Fig. 1).

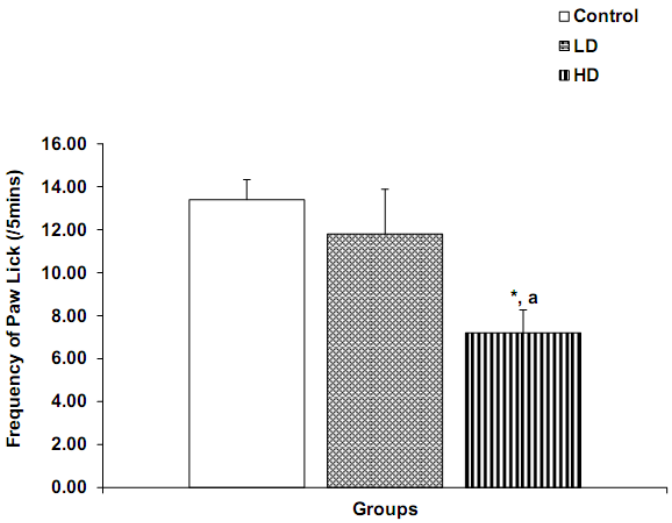


**Fig-1: Comparison of frequency of paw lick in the different experimental groups during the hot plate test**

Values are mean ± SEM. n = 6
*p<0.05 vs Control;
a = p<0.05 vs LD.

**Comparison of duration of paw lick in different experimental groups during hot plate test**

The mean ±SEM values for the duration of paw lick for control, Low dose (LD) and High dose (HD) treated groups were 38.36 ± 1.6, 13.73 ± 1.8, 5.75 ± 0.77 respectively. Duration of paw lick was significantly lower (P < 0.001) in the HD treated group compared with the control. There was also a significant decrease (P<0.01) in the duration of paw lick in the HD treated group compared with LD treated group. The duration of paw lick was also significantly lower (P<0.001) in the LD treated group compared to the control (Fig. 2).

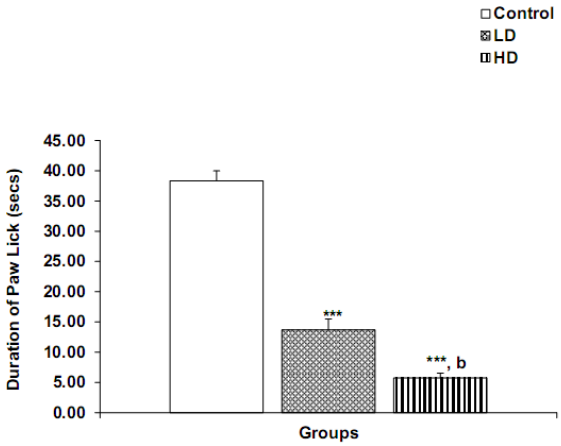


**Fig-2: Comparison of duration of paw lick in the different experimantal groups during the hot plate test**

Values are mean ± SEM. n = 6.
***p<0.001 vs Control;
b = p<0.01 vs LD.

**Comparison of escape latency in different experimental group during hot plate test**

The mean ±SEM values for escape latency in the control, Low dose (LD) and High dose (HD) treated groups were 33.23 ± 1.7, 40.15 ± 0.5, 49.36 ± 2.3 respectively. Escape latency was significantly lower (P<0.001) in the HD treated group compared with control and also significantly lower (P<0.01) compared with low dose treated group. Escape latency was also significantly lower (P<0.05) in LD treated group when compared to control (Fig. 3).

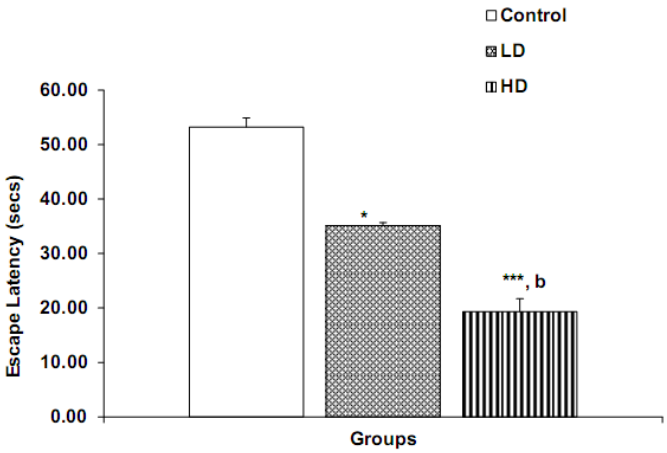


**Fig-3: Comparison of escape latency in the different experimental groups during the hot plate test**

Values are mean ± SEM. n = 6.
*p<0.05, ***p<0.001 vs control;
b = p<0.01 vs LD.

**Comparison of frequency of paw flick in different experimental groups during formalin test**

The mean ±SEM values for the frequency of paw flick in the control, Low dose (LD) and High dose (HD) treated groups in the acute phase were 7.00 ± 0.7, 14.80 ± 1.1, 20.20 ± 0.9 respectively. Frequency of paw flick was significantly higher (P<0.001) in HD treated group compared with control and also significantly higher (P<0.001) when compared with LD treated group. It was also observed that the frequency of paw flick was significantly higher (P<0.001) in the LD treated group when compared with control in the acute phase. The mean values for frequency of paw flick in the control, LD and HD treated groups during the chronic phase were 5.00 ± 1.1, 5.4 ± 1.1, 5.6 ± 1.1 respectively. However, there was no significant difference among the groups during the chronic phase (Fig. 4).

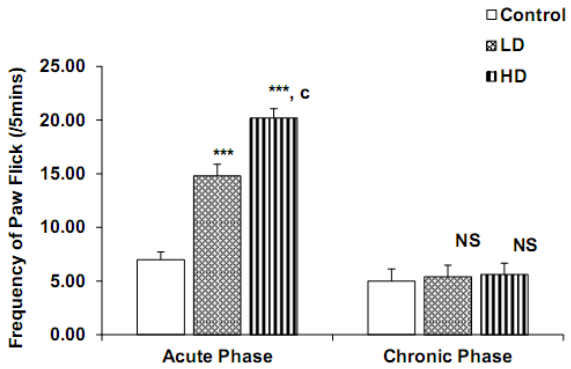


**Fig-4: Comparison of frequency of paw flick in the different experimental groups during the formalin test**

Values are mean ± SEM. n = 6.
***p<0.001 vs Control; c = p<0.001 vs LD;
NS = not significant vs control.

**Comparison of duration of paw flick in different experimental group during the formalin test**

The mean ±SEM values for duration of paw flick in the control, low dose (LD), high dose (HD) treated groups were 35.24 ± 1.7, 43.87 ± 3.0, 56.17 ± 1.9 respectively during the acute phase. Duration of paw flick was significantly higher (P<0.001) in the HD treated group compared with control and LD treated group. The duration of paw flick was significantly higher (P<0.05) in the LD treated group compared to control. The mean values for duration of paw flick in the control, LD and HD treated groups during the chronic phase were 8.9 ± 2.4, 13.03 ± 2.8, 18.77 ± 1.4 respectively. There was a significant increase (P<0.01) in the duration of paw flick in the HD treated group compared to the control (Fig. 5).

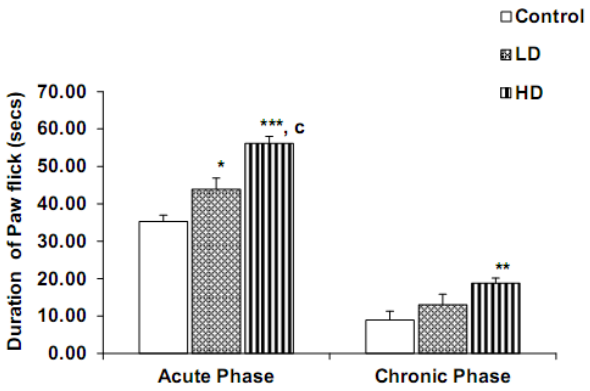


**Fig-5: Comparison of duration of paw lick in the different experimental groups during the formalin test**

Values are mean ± SEM. n = 6.
*p<0.05, **p<0.01, ***p<0.001 vs Control;
c = p<0.001 vs LD.

**Comparison of frequency of paw attention in different experimental groups during the formalin test**

The mean ±SEM value for frequency of paw attention in the control, low dose (LD) and High dose treated groups were 5.2 ± 0.9, 7.6 ± 0.5, 16.00 ± 1.1 respectively during the acute phase. There was a significant increase (P<0.001) in the frequency of paw attention in the HD treated group compared to control and LD treated group in the acute phase of the formalin test. Also, the mean values for frequency of paw attention in the control, LD and HD treated groups during the chronic phase were 3.8 ± 0.9, 4.40 ± 0.4, 6.80 ± 0.58 respectively. There was a significant increase (P<0.01) in the frequency of paw attention in the HD treated group compared to control and LD treated group in the chronic phase of the formalin test (Fig. 6).

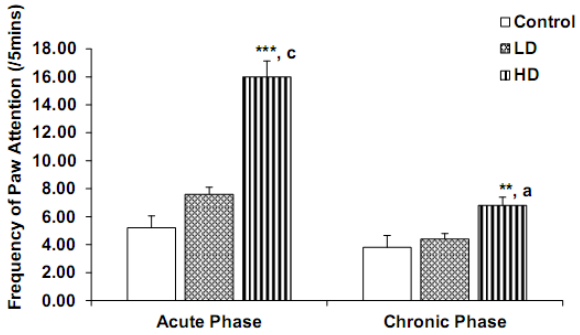


**Fig-6: Comparison of frequency of paw attention in the different experimental groups during the formalin test**

Values are mean ± SEM. n = 6.
**p<0.01, ***p<0.001 vs control;
a = p<0.05, c = p<0.001 vs LD.

**Comparison of duration of paw attention in different experimental groups during the formalin test**

The mean ±SEM value for duration of paw attention in the control, Low dose (LD) and High dose (HD) treated groups were 52.83 ± 3.4, 97.67 ± 2.65, 140.31 ± 4.2 respectively during the acute phase of the formalin test. Duration of paw attention was a significantly higher (P<0.001) in the HD treated group compared to control and LD treated group. There was also significant increase (P<0.001) in the duration of paw attention in the LD treated group compared with the control group. The mean values for duration of paw attention in the control, LD and HD treated groups during the chronic phase were 13.17 ± 1.7, 15.0 ± 2.5, 26.74 ± 1.6 respectively. There was a significant increase (P<0.001) in the duration of paw attention in the HD treated group compared to control and LD treated group.(Fig.7).

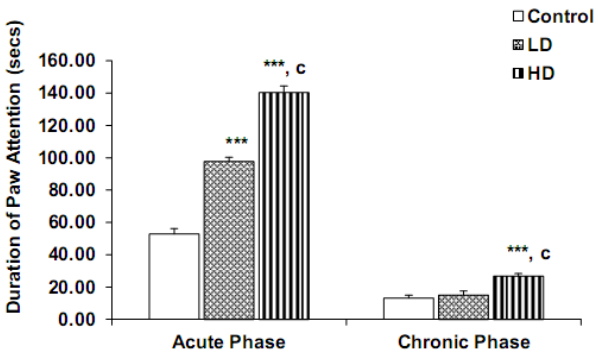


**Fig-7: Comparison of duration of paw attention in the different experimental groups during the formalin test**
Values are mean ± SEM. n = 5.
***p<0.001 vs control;
c = p<0.001 vs LD.

## DISCUSSION AND CONCLUSION

### Discussion

Natural products of plant origin have been widely reported to exert profound and long lasting effect on human health due to the enormous phytochemical compounds embedded in them [1]. In this study, the effect of aqueous leaves extract of *Desmodium adscendens* on hot plate and formalin induced pain was evaluated in mice. The Hot Plate test is a common sensor motor task that measures thermal nociception in rodents. It measures the nociceptive responses of mice when they are placed on a warmed metal plate with varying temperature, starting from non-noxious levels to a standard, constant temperature. The latency to a nociceptive response is recorded and defined as the time elapsed until the subject licks or flicks its hind paw. In our study; after the mice were subjected to the hot plate test, jumping latencies, frequency and duration of paw licks were significantly lower in the extract treated groups compared with control, which may suggest that *D. adscendens* may possess analgesic properties. This outcomes is in line with findings by Guarin *et al,* [4] where leaves extracts of *D. adscendens* where reported to treat pain and ovarian inflammations. It also corroborates with studies by Addy & Awumey[12] where authors reported an analgesic potentials of leaves of *D. adscendens.* Also the plants extracts of *D. adscendens* was also reported by N'gouemo *et al,*[13]to induced hypothermia and had analgesic effect in mice.

Formalin test is made of two phases, the acute and chronic phases. Here, parameters such as the frequency and duration of hind paw lick, frequency and duration of hind paw attention were used to assess the change in sensitivity of pain sensation. Mice which feel more pain will exhibit more of these behaviours. The frequency and duration of hind paw lick and hind paw attention in the test groups were significantly higher compared to the control, thus, indicating increased in pain sensitivity. This study reveals that there was a significant increase in pain sensitivity during the acute phase much more than in the chronic phase of the formalin test in the test groups compared with the control. This increase may be due to the release of chemical pain excitants such as bradykinin, serotonin, histamine, prostaglandin and proteolytic enzymes which stimulate chemosensitive pain receptors and excite pain by making their membranes more permeable to ions and greatly decrease the threshold for stimulants of pain receptors [14]. Our findings on the formalin test are at variance with studies by Addy & Awumey[12] where *D. adscendens* was reported to reduces reduce local tissue inflammations and anaphylactic reactions, interfere with histamine-induced contractions guinea pigs.

*D. adscendens,* one of the commonly used medicinal plants has been reported to contain several phytochemical compounds including flavonoids and other polyphenols. Prelude studies have indicated that flavonoids and other classes of polyphenols may interfere with the mechanisms of inflammation via its ability to hamper the production of reactive oxygen or nitrogen compounds [15]. There have also been proposed to inhibit the pro-inflammatory activity of enzymes involved in free radical production such as cyclooygenase, lipooxygenase or induce nitric oxide synthesis [15, 16]and to modify intracellular signalling pathways in immune cells or in brain cells after a stroke [17].

Procyanidins, a class of flavonoids, have been shown in preliminary research to have anti-inflammatory mechanisms including modulation of the arachidonic acid pathway, inhibition of gene transcription, protein expression and activity of inflammatory enzymes, as well as secretion of anti-inflammatory mediators [17], a possible mechanism for the analgesic property of *D. adscendens* observed in our study following the hot plate test. In summary, our results shows that aqueous leaves extracts of *D. adscendens* lead to a dose dependent decrease in pain sensitivity following the hot plate test compared with control. However, *D. adscendens* potentiates the action of formalin by increasing pain

sensitivity in a dose dependent manner compared with control following the formalin test.

## CONCLUSION

Low and high doses of *D. adscendens* reduced pain sensitivity following acute thermal pain stimuli whereas there it increases inflammatory pain sensitivity in mice. This indicates that *D. adscendens* may be good analgesics of thermal cutaneous pain.

## CONFLICT OF INTEREST

Authors hereby declare that there is no conflict of interest whatsoever.

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