ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2013; 4(5): 918 - 922

ORIGINAL RESEARCH ARTICLE

Aqueous Extractof*Adenia cissampeloides*Modulates Pain in Mice

Adebiyi O.O[1], Adebiyi O.A*[2], Oyeyipo I.P[1], Obembe O[1] and Oladokun O[1]

[1]*Department of Physiology, College of Health Sciences, Osun state University, Oshogbo, Nigeria*
[2]*Department of Pharmacology and Therapeutics, Osun State University, Oshogbo, Nigeria*

Received 11Apr 2013; Revised 14Sep 2013; Accepted 02 Oct 2013

ABSTRACT

The use of A*denia Cissampeloides* leaves extract (ACE) in the treatment of rheumatic pain among local healers has being reported. However there is no scientific experiment conducted to validate this report. This study was designed to investigate this claim.

Fifty male Swiss albino mice (18-22g) were randomly allotted into ten groups of mice (n=5). Groups 1-3 and 5-8 were treated with graded doses of ACE while groups 4 and 10 received normal saline (10 ml/kg) and Group 9 received 1 mg/kg of diclofenac sodium. All drugs and test compounds were administered via the i.p route.

Tail flick latencies test of mice treated with all test doses showed statistically significant increase in tail flick response compared to control ($P<0.05$). The acetic acid writhing test also showed a statistically significant dose dependent increase in the inhibition of abdominal writhes among test groups ($P=0.000$). The study concluded that ACE exhibitsome analgesic activity to warrant its use in the management of pain among local healers.

Keywords: analgesia, tail flick latency and pain.

INTRODUCTION

Indigenous plants, over the years have been shown to play important roles in the treatment of a variety of diseases.A significant proportion of the indigenous populations in developing countries depend on plants as accessible first line and cost effective therapy in the management of common ailments (Annan *et al*., 2012). This trend makes the scientific evaluation of the efficacy and safety of such herbal remedies extremely important in the present day. The plant Kingdom is a rich treasures house of potential drugs and in recent years, there has been an increasing awareness about the importance of medicinal plants (Yadav and Agarwala, 2011).

Adenia cissampeloides, also known as *Adenia gracilis*(Harms, 1897), *Adenia gummifera* (Harv.) (Harms, 1897), *Adenia guineensis*(W.J.de Wilde, 1971) is a climber thatbelongs to the family Passifloreceae. It is widely distributed in sub-Saharan Africa with its area of growth extending from Senegal in West Africa, to Somalia in the east and as far as Mozambique and South Africa in southern parts of Africa (Neuwinger, 2000). Its use in traditional medicine in Africa appears to be widespread. There are reports of its utilization in managing a wide variety of ailments that include gastro-intestinal complaints, pain, malaria, anaemia, liver disorders, insanity, threatened abortion, depression, veneral diseases, excessive menstruation, gall bladder diseases and a host of parasitic disease (Schmelzer and Gurib-Fakim, 2008; Asase *et al*., 2010). Furthermore, a study in humanshas shown that *Adenia cisampeloides*to possess potent anti-hypertensive activity(Nyarko and Addy in 2006).

There are no scientific studies on the efficacy of *Adenia cissampeloides* in the relief of pain which make the conduct of this study necessary. Therefore, this study was designed tovalidate claims made by local healers that *Adenia cissampeloides* is useful in the management of painful conditions.This study investigated the probable analgesic potentials of *Adenia*

*Corresponding Author: Dr. O.A Adebiyi, Email: Olubunmi.adebiyi@uniosun.edu.ng, Phone No: 2348033595834

cissampeloides in a bid to validate its use in herbal remedies.

Fig 1: ***Adenia Cissampeloides.***

MATERIALS AND METHODS

PLANT COLLECTION AND PREPARATION

The leaves and stem of *Adenia Cissampeloides* were collected from Obafemi Awolowo University Ile-Ife, Nigeria during the raining season of the year 2011. The plant was identifiedand a voucher specimen was deposited at the department of Botany Obafemi Awolowo University, Ile-Ife, Nigeria. The collected plants were air dried under room temperature over a 6 week period. The dried leaves were separated from the stem and crushed. The crushed leaves were subsequently weighed (285g) and soaked in distilled water (w/v 1:10) for 72 hours.

Extract preparation

The soaked leaves were subsequently sieved with whatman No 1 filter paper separating the marc from the filtrate. A dark brown filtrate was obtained and evaporated to dryness using rotor evaporator to give a dark brown gelatinous substance with a yield of 5% (14.24g). The extract was stored at 4^{o}C until use.

Animals and experimental groups

Male Swiss albino mice (n=50) weighing 18 to 25 g obtained from the animal house of the college of Health Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria, were purchased for this study. All animals were kept at room temperature (28 ±1°C, 70 to 80% humidity, 12 h light/dark cycle) in the animal holding unit of the medical laboratory of the Osun state university, Oshogbo. They were housed in plastic cages and fed with standard rodent chow and water ad libitum. They were allowed to acclimatize for a period of 7 days before they were subjected to tests. They were randomly divided into nine groups of 5 mice each. Groups 1 to 4 were used for the tail flick test and groups 5-9 were for the acetic acid writhing test. Test compounds and other drugs were administered intraperitoneally.

Antinociceptive Assays:

Acetic Acid Writhing Test.

Mice of either sex with a weight between 18 and 25 g were used. 0.1 ml of a 0.6% solution of acetic acid was injected intraperitoneally to mice (Koster et al. 1959; Taber et al. 1969) Groups of 5 mice were used for controls and treatment groups. The mice were placed individually into glass beakers and five min was allowed to elapse. The mice were then observed for a period of ten minutes and the number of writhes is recorded for each animal. For scoring purposes, a writhe was indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb. The formula for computing percent inhibition is: average writhes in the control group minus writhes in the drug group divided by writhes in the control group times 100%.

Tail Flick LatencyTest.

Male Mice (n=5/ group) weighing 18-22g were selected for this study.Tail flick latency was measured by the Ugo Basile tail flick anagelsy unit 37360 (Italy). The instrument measures the latency of the avoidance response when pain is induced by radiant heat applied to the mouse's tail**.** It basically consists of an I.R. source (50W bulb), whose radiant energy of adjustable intensity is focused by an embodied parabolic mirror on the rodent's tail. An intensity of 5 (out of a possible 100) was selected for this study. The mice were held by the inclined mouse restrainer on the instrument unobstructed upper panel in such a way that its tail, placed over a flush mounted window, received the I.R. energy. The stimulus and the related reaction-time counter were started by function key located on the front panel. When the mouse felt the pain and flickedits tail, a sensor detected it and stopped the reaction-time counter and switched off the bulb. The reaction time of the animal was automatically determined to the nearest 0.1 second. A cut off of 50sec was chosen automatically by the apparatus to prevent thermal injury to the tail of the experimental mouse.

Ethical Considerations

This study was conducted in line with standard practices, procedures, international protocols and accepted principles for laboratory animal use and care as found in the guiding principles for the care and use of animals in research and teaching (Helsinki declaration 2010 update).

Statistical analysis

The test doses for the tail flick latency test were compared with pre-test values by repeated measure ANOVA. The test doses for the acetic acid writhing test were compared with control and standard by one factor analysis of variance (ANOVA).The results were expressed as Mean±S.E.M. analyzed using the Primer biostatistical software. Values of $P < 0.05$ were taken as significant.

RESULTS

There were statistically significant increases in the mean frequency of tail flick latencyamong mice groups treated with graded doses (100mg/kg, 200mg/kg and 400 mg/kg) of ACE compared to their respective pre-test values.Whilethe 100 mg/kg treated group showed significant increase in tail flick latency at all the time intervals tested, the200 mg/kg and 400 mg/kg groups showed significant increases in tail flick latencies only at 60minutes post treatment shown in (**Table 1**)There was a dose dependent percentage prolongation of the tail flick latency by all the test doses, although the different treatment groups appeared to exert their maximal effects at different time intervals for example100 mg/kg showed prolongation of the tail flick latency between 60 and 90 minutes time interval compared to the 200 mg/kg and the 400 mg/kg groups which were observed to slightly prolong the tail flick latency between 30 and 60 minutes time interval shown in (**Fig 2**). The distribution of the mean frequency of abdominal writhes among mice groups treated with graded doses of ACE, normal saline and diclofenac revealed significant reduction in the mean frequency of abdominal writhes among the 200, 400 mg/kg and diclofenac treated groups compared to the control group shown in (**Table 2**).On the other hand the 100 mg/kg group did not show any significant difference in the means of the group and that of the control. There was a dose dependent increase in the percentage inhibition of abdominal writhes of mice treated with graded doses of ACE, normal saline and diclofenacas the dose of ACE was increased from 100 mg/kg through 200 mg/kg to 400 mg/kg. The largest percentage inhibition which was observed in the 400 mg/kg treatment group which showed stronger degree of inhibition of painful abdominal writhes than that produced by diclofenac a known standard used in the treatment of pain.

Table 1: Table showing the tail flick latencies of mice treated with graded doses of ACE and normal saline on different time scales

Treatment Groups	Pre-test	T= 30 min	T=60 mins	T= 90 mins	T=120 mins
100 mg/kg i.p	6.58±0.21	10.50±0.50*	10.36±0.28*	11.77±0.21*	9.4±0.31*
200 mg/kg i.p	6.78±0.27	10.48±0.50*	11.0±0.84*	8.20±0.47	8.72±0.63
400 mg/kg i.p	6.34±0.07	9.26±0.23	11.40±2.1*	8.32±0.14	9.46±0.32
Normal Saline (10 ml/kg i.p)	5.82±0.39	8.34±0.08	8.16±0.08	8.22±0.43	8.6±0.23

*P = 0.000 (compared to pre-test value for each dose level)

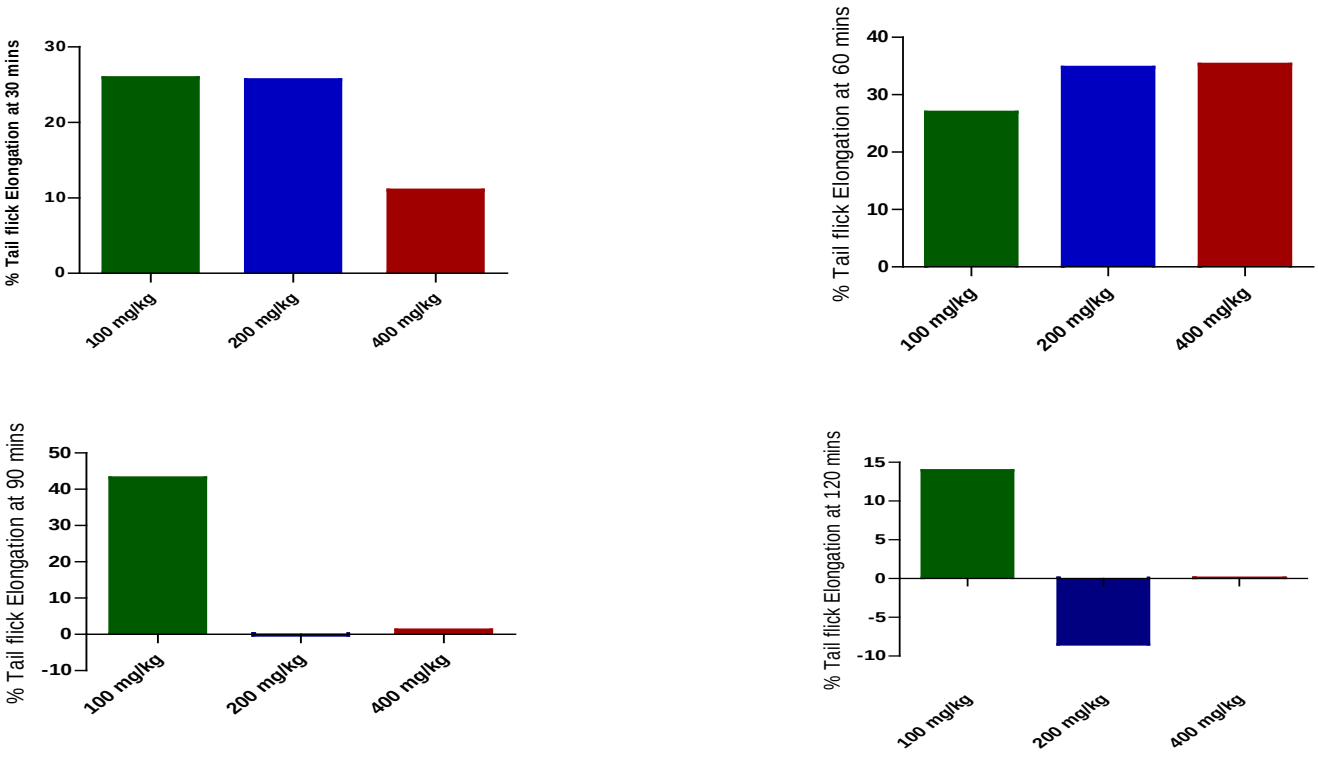

Fig 2: The graph of Percentage elongation of Tail flick latencies of mice treated with graded doses of ACE

Table 2: The table of the mean frequencies of abdominal writhes of mice treated with graded doses of ACE, normal saline and Diclofenac

Treatment Groups	MEAN FREQUENCY OF WRITHES± SEM
100 mg/kg i.p	27±1.00
200 mg/kg i.p	24±1.38*
400 mg/kg i.p	4.2±0.58**
Sodium Diclofenac (1mg/kg i.p)	15±0.4*
Normal saline (10 ml/kg i.p)	29±3.98

*P<0.05, **P<0.001(in comparison to control)

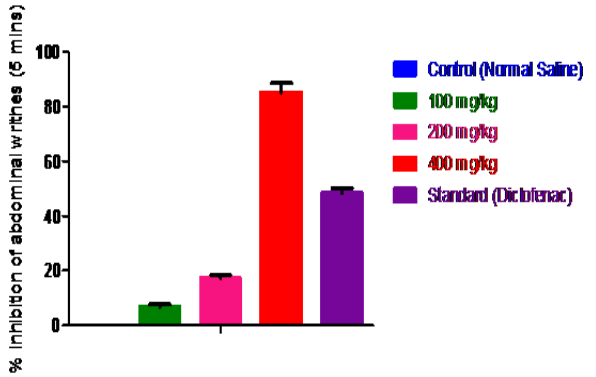

Fig 3: The graph of Percentage inhibition of abdominal writhes of mice treated with graded doses of ACE, normal saline and diclofenac

DISCUSSION

The apparent significant increase and prolongation in tail flick latencies in mice groups treated with graded doses of ACE in this study compared to the control might suggest that ACE possess some pain modulating activity. Thisobservation is supported by theunderstanding from studies that radiant heat testis a very effective screening method for the estimation of the efficacy and potency of potential centrally acting analgesic drugs (D'Amour and smith, 1941; Vogel*et al,* 1998). Tail flick latency test is a modification of the radiant heat method of assessing the analgesic potentials of new test compounds which discriminates between analgesic and non-analgesic substances (Harris *et al.,* 1988). Studies which utilized tail flick latencies as a method of screening for analgesic potentials of test compounds often describe significant increases in tail flick latencies in treated versus untreated controls as being an indication of ability of such test compounds to protect against pain (Vogel *et al*, 1998). It is also true that test compounds that show no significant increases in tail flick latencies compared to control might not possess pain modulating activity.It is therefore possible to attribute the reduced sensitivity of treated mice to the painful stimulus to possible protection from pain. More centrally acting analgesic compounds have been noted to be particularly sensitive to tail flick latency testing (Hajhashemi *et al*, 2010). The acetic acid writhing test result further lends support to the ability of ACE in providing pain relief. Furthermore, it appears that ACE exerts this pain modulating activity via two different mechanisms. This mechanism might include a central action and a peripheral action. This is because pain induced by injection of irritants such as phenylquinone or acetic acid into the peritoneal cavity of mice is often utilized in evaluating test compounds that have peripheral analgesic activity (Vogel *et al.,* 1998). The injection of an irritant like acetic acid causes the release of prostaglandins such as PGE_2 and $PGF_{2\alpha}$ with increased levels found in the peritoneal fluid (Neto *et al*, 2005). Prostaglandin $F_{2\alpha}$ causes severe pains in the peritoneal cavity which was observed as abdominal writhes in the experimental animal. The ability of a test compound in relieving this pain suggests that such compoundmay possess peripheral analgesic effect. In this study, the significant reduction of abdominal writhes amonggroups treated with ACE (200 mg/kg and 400 mg/kg) in contrast to the control group point to the potentials of ACE in modulating pain.Most of the standard peripheral analgesics areknown to possess anti-inflammatory properties and in some cases also antipyretic activity besides analgesia, for many of them the mode of action has been elucidated as an inhibition of cyclooxygenase an important enzyme in the prostaglandin synthesis pathway (Vogel *et al.,* 1998). However it cannot be conclusively said that ACE exerts its pain modulation by interfering with the cyclooxygenase enzyme system although this is suggested. Further studies are needed to investigate this possibility.

In addition, ACE appears to be excellent analgesic candidate because of its greater than 70 percent inhibitory activity against acetic acid induced abdominal writhes in the treated mice groups. Test compounds that inhibit writhing by values greater than 70 % of control values are considered excellent candidates for analgesia whereascompounds with less than 70% inhibition are considered to have minimal activity (Vogel *et al.,* 1998). Although this potential was observed at highest dose used in this study, it suggests that potential analgesic benefit particularly the peripheral analgesic actions would be better felt at higher doses. The pain modulating activity of ACE however appears to be short-lived, its onset

was observed at 30 minutes and peaked between 60-90 minutes and virtually all analgesic action appeared to have disappeared within two hours of its administration.

CONCLUSION

From this study, it is apparent that ACE possesses some analgesic activity andthis activity appeared to be transient.Its use in folklore medicine in the treatment of rheumatic pain is therefore supported by these findings.

REFERENCES

1. Annan K.., Sarpong K.., Asare C., Dickson R., Amponsah KI., Gyan B., Gbedema, SY. In vitro anti-plasmodial activity of three herbal remedies for malaria in Ghana Adenia cissampeloides (Planch)Harns, Terminalia ivorensis A Chev and Elaesis guineensis Jacq. Phcog. Res. 2012; 4: 225-9.
2. Asase A., Akwetey GA., Achel DG. Ethnopharmacological use of herbal remedies for the treatment of malaria in the Dangme West district of Ghana. J. Ethnopharmacol. 2010; 129:367-76.
3. D'Amour FE, Smith DL (1941) A method for determining loss of pain sensation. J Pharmacol Exp Ther 72:74–79.
4. De Wilde, W.J.J.O., 1975. Passifloraceae. In: Polhill, R.M. (Editor). Flora of Tropical East Africa. Crown Agents for Oversea Governments and Administrations, London, United Kingdom. 71 pp.
5. Hajhashemi V., Ghannadi A., Hajiloo M. Analgesic and Anti-inflammatory effects of Rosa damascene hydroalcholic extracts and its essential oil in animal models. Iran J. Pharm. Res., 2010; 9(2): 163-168.
6. Harris DP, Burton R., Sinclair G. A simple microcomputer interface for tail-flick determination. J Pharmacol Meth 1988; 20:103–108.
7. Koster R., Anderson M., De Beer EJ. Acetic acid for analgesic screening. Fed Proc., 1959; 18:412.
8. Neto AG., Costa JMLC., Belati CC., Vinholis AHC., Possebom LS., Cunha WR., Carvahlo JCT., Da silva Filho AA., Blastos JK., Silva MLA. Analgesic and Anti-inflammatory activity of a crude root extract of Pfaffia glomerate (spreng) Pedersen. J. Ethnopharmaco. 2005; 96: 87-91.
9. Neuwinger, H.D., 2000. African traditional medicine: a dictionary of plant use and applications. Medpharm Scientific, Stuttgart, Germany. 589 pp.
10. Nyarko, AA. and Addy ME. Effect of aqueous extract of Adenia cissampeloides on blood pressure and serum analytes of hypertensive patients. Phytother Res 2006; 4: 25-8.
11. Schmelzer GH and Gurib-Fakim A Plant Resources of Tropical Africa 11(1). Medicinal plants PROTA Foundation, Backhuys Publishers, Leiden, Netherlands/CTA, Wageningen. (2008) 34-37.
12. Taber RI., Greenhouse DD., Rendell JK., Irwin S. Agonist and antagonist interactions of opioids on acetic acid-induced abdominal stretching in mice. J Pharm Exp Ther 1969; 169:29–38.
13. Vogel WH Scholkens BA, Sandow J and Vogel WF Pharmacological assays. (1998)Vol 2692-696,716-717.
14. Yadav RNS., Agarwala M. Phytochemical analysis of some medicinal plants. Journal of Phytology 2011; 3(12): 10-14.