Asian Journal of Medical Sciences 1(2): 35-38, 2009
ISSN: 2040-8773

Submitted Date: June 26, 2009 Accepted Date: July 15, 2009 Published Date: September 10, 2009

Spathodea Campanulata: an Experimental Evaluation of the Analgesic and Anti-inflammatory Properties of a Traditional Remedy

[1]Emmanuel. E Ilodigwe and [2]Peter .A. Akah
[1]Department of Pharmacology and Clinical Pharmacy, Niger Delta University Amassoma, Bayelsa State, Nigeria
[2]Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, Nigeria

Abstract: The analgesic and anti-inflammatory potentials of the ethanol leaf extract of *Spathodea campanulata*, a Nigerian traditional medicinal plant was studied using cold, thermal and chemical-induced pain models, and carrageenan-induced acute inflammation in rats. The acute toxicity and the phytochemical constituents of the extract were also determined. The results showed that the extract (250-1000 mg/kg) significantly ($P<0.05$) and dose-dependently prolonged the pain reaction times in hot-plate and tail flick pain models, and reduced acetic acid –induced writhing. The extract demonstrated significant anti-inflammatory activity against acute inflammation induced by carrageenan. The estimated LD_{50} of the extract was 4500 mg/kg. Phytochemical analysis revealed the presence of tannins, saponins, anthraquinone glycosides and flavonoids. These findings indicate that the leaf extract of *Spathodea campanulata* has both analgesic and anti- inflammatory properties and could be beneficial in alleviating painful inflammatory conditions.

Key words: Analgesic, anti-inflammatory, carrageenan, native medicine, *Spathodea companulata,* rodents

INTRODUCTION

Nonsteroidal anti-inflammatory drugs are still widely employed as analgesics and to relief inflammatory conditions, while the opiates remain effective in intense unrelenting pains (Musa *et al*,, 2008). The adverse effects of these drugs pose some problems and placed limitations in their use. Hence there is a continuous search for effective and safer alternatives. These drugs have their origin in natural products. Medicinal plants are important sources of new chemical substances with potential therapeutic effects (Eisner, 1990), and literature has documented plants with putative analgesic and antinflammatory activities (Akah and Nwambie, 1994, Elisabetsky *et al.*, 1995, Nunez-Guillen *et al*., 1997, Okoli and Akah, 2000, 2006, Akah and Okoli 2004, Okoli *et al*., 2007 Hassan *et al*., 2008).

In Nigeria, many plant products are used for their analgesic and anti-inflammatory effects and their efficacy is traditionally acclaimed. *Spathodea campanulata* Linn (Bignoniaceae) locally known in Ibo tribe of Nigeria as "Imiewu", is one of such plants. It is a tree but a shrub in the open savannah. Its leaf has been reported to possess antiplasmodial activity (Markinde *et al*., 1987). It has astringent properties and is widely used by traditional medicine practitioners as a relief for painful inflammatory conditions (Dalziel, 1948, Oliver, 1960). The aim of this study was to evaluate the analgesic and acute anti-inflammatory effect of the leaf extract of *S.* campanulata in rats in order to justify or otherwise its use in native medicine.

MATERIALS AND METHODS

Plant material: The fresh leaves of *Spathodea campanulata* were collected from Nawfia, Anambra State, Nigeria and authenticated by Mr. P.O. Ugwuozor of the Department of Botany, University of Nigeria Nsukka and confirmed at forestry Research Institute Ibadan, Nigeria. A voucher specimen is deposited in the Herbarium of the Department of Pharmacognosy, University of Nigeria, Nsukka.

Preparation of extract: The leaves of *Spathodea campanulata* were cleaned, air-dried for 5 days and reduced to a coarse powder. The powder (500 g) was cold macerated with four litres of aqueous ethanol (70%) for seven days. The resulting solution was filtered and the filterate concentrated to dryness in vacuo using rotary evaporator. The resultant solid was partitioned three times with 250 ml of petroleum ether (40-60˚c) to remove any fatty material and evaporated to dryness. The yield was 18% w/w.

Animals: Sprague - Dawley rats of both sexes (200-250 g) and Swiss albino mice of both sexes (18-22 g) were employed for this study. The animals were obtained from the National Veterinary Research Institute (NVRI), Vom, Nigeria. They were kept in well-ventilated environment, had free access to rodent pellets (Bendel Feeds Ltd, Benin, Nigeria) and water *ad libitum*. The animals were acclimatized for 3 weeks and were fasted over night with free access to water prior to experiments. All animal experiments were conducted in compliance with NIH

Corresponding Author: Peter A. Akah, Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, Nigeria

Guide for Care and Use of Laboratory Animals (pub. No. 85-23 revised 1985).

Phytochemical test: The phytochemical analysis of *S. campanulata* extract was carried out using standard protocols (Odebiyi and Sofowora 1978).

Acute toxicity study: Sixty mice were divided into six groups of ten per group after six hour fasting period. The mice in group 1 received normal saline (10 ml/kg oral) while the mice in groups 2-5 received oral doses of the extract (500, 1000, 2000, 4000, 5000, mg/kg respectively). The animals were observed for obvious toxic symptoms and mortality in each group within 24 hr was recorded. The median lethal dose of the extract (LD_{50}) was estimated using probit analysis (Miller and Tainter, 1944).

Effect on acetic acid-induced writhing: The method described by Koster *et al*, (1959) and Taber *et al*, (1969) was used. Thirty mice were fasted for 6 hours and divided into six groups of five animals per group. Groups 1-4 received the extract orally (250, 500, 750 and 1000 mg/kg, respectively). The 5th group received distilled water (10 ml/kg, oral) while the 6th group was given aspirin (100 mg/kg, oral) 30 minutes before administration of 1% acetic acid solution (0.1 ml/kg, i.p). The writhes (each of which is characterized by a wave of contraction of abdominal musculature followed by extension of the hind limbs) were counted 5 minutes after acetic acid injection for a period of 30 minutes. A reduction in the number of writhes indicates analgesic property.

Effect on tail flick response: This study was based on the method described by Clark *et al*, (1988). Thirty rats were fasted for 6 hours and divided into six groups of five animals per group. Groups 1-4 received the extract orally (250,500,750 and 1000 mg/kg respectively). Group 5 received distilled water (10 ml/kg) while the 6th group received morphine (10 mg/kg, oral). Each rat was closely restrained in a wire mesh cage and the lower half of its tail dipped in a beaker of cold water (0-1˚C). The time in seconds for tail withdrawal from the water was taken as the reaction time. The reaction time was determined before and at 30 minutes interval after administration of the extract, distilled water or morphine.

Effect on hot plate analgesia: Thirty rats were fasted for 6 hours and divided into six groups of five animals per group. Groups 1-4 received the extract orally (250,500,750 and 1000 mg/kg respectively). Group 5 received distilled water (10 ml/kg) while the 6th group received morphine (10 mg/kg p.o). Each mouse was gently placed on the hot plate maintained at 55 ± 0.5˚C and the time required by the mouse to lick the paw or jump were taken as the response (Turner, 1965). The cut-off time or latency response was 15 seconds to avoid tissue damage.

Anti-inflammatory response: The carrageenan-induced acute inflammatory model was employed (Winter *et al*, 1962). Thirty rats were fasted for 6 hours and divided into 6 groups of 5 rats per group. The extract (250,500,750 and 1000 mg/kg, p. o) was administered to groups 1-4 respectively. The 5th and 6th groups received oral administration of acetylsalicylic acid (100 mg/kg,) and normal saline (10 ml/kg,) respectively. Thirty minutes later, 0.1ml of freshly prepared 1% carrageenan in sterile saline was injected into the sub-planter surface of each rat hind paw (Winter *et al*, 1962). The measurement of the linear circumference of each paw before and at 0.50, 1.0, 2.0, 3.0 and 4.0 hours after induction of inflammation was carried out with the aid of cotton thread. The mean paw circumference and percentage inhibition of oedema was determined (Okpo *et al*., 2001 and Oriowo, 1982).

Statistical analysis: The results are represented as means ± SEM and significance of differences between control group and extract treated groups were determined using Student's t-test and two way analysis of variance (ANOVA). P value < 0.05 was considered significant.

RESULTS

Phytochemical results revealed the presence of tannins, saponins, anthraquinone glycosides, and flavonoids, while the oral LD_{50} of the extract was estimated to be 4500 mg/kg

Effect on acetic acid-induced writhing: The extract evoked significant ($p<0.05$) and dose-dependent reduction in the number of acetic acid-induced writhes in rats. At 1000 mg/kg the effect of the extract was comparable to that of 100 mg/kg of aspirin (Table 1).

Effect on tail flick response: The extract produced a dose-dependent increase in the reaction time to tail flick. The percentage inhibition by the extract at 1000 mg/kg was comparable to that produced by morphine 10 mg/kg (Table 2).

Effect on hot plate analgesia: A significant ($p<0.05$) and dose-dependent elevation of the after treatment reaction time to thermal pain was evident in the extract treated animals. The effect of the extract (1000 mg/kg) was comparable to that produced by 10mg/kg of morphine (Table 3).

Carrageenan-induced oedema: The effect of the extract on carrageenan-induced oedema is shown in Table 4. The extract produced a dose-dependent inhibition of oedema induced by carrageenan. The percentage inhibition produced by the extract (1000 mg/kg) was comparable to that produced by of acetylsalicylic acid at the 3rd hour post-carrageenan injection.

Table 1: Effect of the extract on acetic acid-induced writhing in mice

Treatment	Dose(mg/kg p.o)	No of writhes per 30min	Percentage inhibition
Control	10ml/kg	81.6±3.72	
Extract	250	70.2±2.04*	13.97
	500	62.0±5.10*	24.02
	750	54.4±1.30*	33.30
	1000	48.0±0.63*	41.18
Aspirin	100	52.4±5.24*	35.78

*P<0.05 compared with the control.

Table 2: Effect of the extract on tail flick in rats

Treatment	Dose (mg/kg p.o)	Pre-treatment (sec)	Post- treatment (sec)	% Inhibition
Control	10ml/kg	15.4±1.02	15.6±1.86	1.20
Extract	250	16.8±1.6	25.2±2.54*	33.33
	500	17.0±1.41	34.0±2.83*	50.00
	750	15.7±1.3	37.2±1.90*	57.79
	1000	15.4±0.80	54.6±2.65*	62.98
Morphine	10	15.4±1.02	44.8±2.64*	63.63*

P<0.05 compared with the control.

Table 3: Effects of the extract on hot plate test

Treatment	Dose (mg/kg p.o)	Pre-treatment (sec)	Post- treatment (sec)	% Inhibition
Control	10ml/kg	2.2±0.40	2.2±0.45	-
Extract	250	2.6±0.80	4.2±0.40*	38.09
	500	2.8±0.90	5.4±0.98*	55.56
	750	2.8±1.0	5.4±0.98*	61.11
	1000	3.0±0.87	9.4±049*	68.09
Morphine	10	2.5±0.40	10.0±0.86*	75.00

*P <0.05 compared with the control.

on carrageenan-induced paw oedema. Carrageenan-oedema is a model of acute inflammation used in the study of non-steroidal anti-inflammatory agents (Di Rosa *et al.*, 1971). The effects of the extract was most pronounced, 3 hours after induction of oedema, an action which was similar to that of acetylsalicylic acid, suggesting its usefulness in the management of acute inflammation.

Phytochemcial analysis revealed the presence of tannins, saponins and anthraquinone glycosides and flavonoids. The pharmacological activities of medicinal plants are usually due to their secondary metabolites. Some of the constituents of the extract have been documented to possess analgesic and anti-inflammatory activities (Park *et al*, 2001, Okoli and Akah 2004). The high LD_{50} value of the extract is an indication of its safety (Lorke 1983).

In conclusion, the results of this study provide evidence for the analgesic and anti- inflammatory activity of the ethanol leaf extract of *Spathodea campanulata* thus supporting the validity of its use in painful inflammatory conditions. Bioassay guided fractionation and isolation of the active compound is in progress.

Table 4: Effect of the extract on carrageenan induced oedema in rats

Treatment	Dosage (mg/kgp.o)	Paw size(mm)					
		0h	0.5h	1h	2h	3h	4h
Control	10 ml/kg	27.2 ± 0.3	27.4 ± 0.5	29.8 ± 0.84	30.6 ± 0.48	32.2 ± 0.40	30.4 ± 01.35
Extract	250	26.9 ± 0.6	25.2 ± 0.4 (8.30)	25.6 ± 0.49* (14.09)	26.6 ± 0.49* (13.73)	27.4 ± 0.50* (14.91)	27.8 ± 0.84 (8.55)
	500	27.0 ± 0.3	23.2 ± 0.40* (15.33)	24.2 ± 0.80* (18.79)	25.2 ± 0.30* (17.65)	25.6 ± 0.45* (20.50)	25.8 ± 0.40* (15.13)
	1000	27.1 ± 0.5	21.4 ± 0.48* (21.90)	22.2 ± 0.40* (25.50)	22.6 ± 0.42* (28.14)	22.4 ± 0.8* (30.40)	23.2 ± 0.40* (23.68)
Acetylsalicylic acid	100	26.9 ± 0.7	19.6 ± 0.5* (28.47)	20.3 ± 0.30* (31.88)	20.8 ± 0.42* (32.03)	20.9 ± 0.55* (35.09)	21.2 ± 0.61* (30.26)

*P<0.05 compared with the control. Percentage inhibition is shown in parentheses.

DISCUSSION

The results of this study revealed that the ethanol leaf extract of *S. capanulata* possess both peripheral and central analgesic properties. Acetic acid-induced nociception is usually used for the evaluation of mild peripheral analgesic and nonsteroidal anti-inflammatory compounds (Ferrerira and Vane, 1974; Berkenkoff and Weichman, 1998; Hassan *et al*, 2008). On the other hand thermal painful stimuli are selective for the evaluation of centrally, but not peripherally acting analgesic drugs (Chau, 1989). These results suggest that the extract may possess NSAID- like and opiod-like analgesic activities, mediated through both the peripheral and central mechanisms. However, the results of the flick tail and thermal (hot plate) experiments appear to suggest that the extract is more effective in alleviating central pain than peripheral (acetic acid) pain

The carrageenan-induced inflammatory reactions have been shown to be due to the release of inflammatory mediators (Heller *et al*, 1998; Nunez-Guillen *et al*, 1997; Ndebia *et al*., 2007). The extract of *S. campanulata* produced dose-dependent acute anti-inflammatory effects.

REFERENCES

Akah, P.A, and A.I. Nwambie, 1994. Evaluation of Nigerian Traditional Medicine: 1.Plants used for Rheumatic disorders. J. Ethnopharmacol., 42:179-182

Akah, P.A. and C.O. Okoli, 2004. Mechanism of the anti-inflammatory activity of the leaf extract of C*ulcasia* scandens, P. Baeuv., (Araceae). Pharm. Biochem. Bev., 79: 473- 481.

Berkenkoff, J.W. and B.M. Weichmann, 1988. Production of Prostacyclin in mice following intraperitioneal injection of acetic acid, phenylbenzoquinone and zymosan: its role in the writhing response. Prostaglandins, 36: 693-709.

Chau, T., 1989. Pharmacological Methods in the Control of Inflammation. In: Modern Methods in Pharmacology. R. Alan, (Ed.), Liss Inc. New Yrok, 5: 195-212.

Clark, S.J., R.L. Follenfant, and T.W. Smith, 1988. Evaluation of opiod-induced antinociceptive effects in anaesthesized and conscious animals. Br. J. Pharmacol., 95: 275-282.

Dalziel, J.M., 1948. The useful plants of West Tropical Africa. The Crown Agents for Overseas Colonies. London, pp: 397-399.

DiRosa, M., J.P. Giroud and D.A. Willoughby, 1971. Studies of the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. J. Pathol., 104: 15-29.

Eisner, T., 1990. Chemical prospecting. A call for Action. In: Ecology, Economics and Ethics. The Broken Circle. F.H. Borman and S.R. Keller, (Eds.). Yale University Press New Haven, CT.

Elisabetsky, E., F.A. Amador, R.R. Abuquerque., D.S. Nunes, and A.C.T. Carvallo, 1995. Analgesic Activity of *Psychotria colarata*. J. Ethnophamacol., 48: 77-85.

Ferreira, S.H. and J.R. Vane, 1974. New aspects in the mode of action of non-steroidal anti-inflammatory drugs. Ann. Rev. Pharmacol., 14: 57-73.

Hassan, H.S., A.A. Ahmadu, and A.S. Hassan, 2008. Analgesic and anti-inflammatory activities of *Asparagus africanus* root extract. Afri. J. Trad. CAM., 5(1): 27-31.

Heller, A., T. Kock., J. Schemck and V.K. Acker, 1998. Lipid mediators in inflammatory disorders. Drugs, 55: 487-496.

Koster, R., M. Anderson, and E.J. Debeer, 1959. Acetic acid analgesic screening. Fed. Proced., 18: 418-420.

Lorke, D., 1983. A new approach to practical acute toxicity testing. Arch. Toxicol., 53: 275-289.

Markinde, J.M., E.K. Adesogen and O.O.G. Amusan, 1987. The Schizontocidal activity of *Spathoda* campanulata leaf extract on Plasmodium berghei *berghei* in mice. Phytother. Res., 1(2): 65-68.

Miller, L.C. and M.C. Tainter, 1944. Estimation of LD_{50} and its error by means of logarithmic-probit graph paper. Proc. Soc. Exp. Biol. Med., 57: 261-264.

Musa, Y.M., A.K. Haruna, M. Llyas, A.H. Yaro, A.A. Ahmadu and H. Usman, 2008. Phytochemical, analgesic and Anti-inflammatory effects of the ethylacetate extract of the leaves of Pseudocedrall kotschyll. Afr. J. Trad. CAM., 5(1): 92-96.

Ndebia, E.J., R. Kamgang, and B.N. Nkeh-chungag A.nye, 2007. Analgesic and anti-inflammatory properties of aqueous extract from leaves of *Solanum torvum*. (solanaceae). Afr. J. Trad. CAM., 4(2): 240-244.

Nunez-Guillen, M.E., J.A. Silva-Emimi, C. Souccar and A.J. Limdala, 1997. Analgesic and anti inflammatory activities of the aqueous extract of Plantugo major (L). Int. J. Pharmacogn. 6: 465-472.

Odebiyi O.O. and E.A. Sofowora, 1978. Phytochemical screening of Nigerian medicinal plants. Lloydia, 41: 234-235.

Okoli, C.O. and P.A. Akah, 2000. A pilot evaluation of the anti-inflammatory activity of Culcasia scandens, a traditional antirheumatic agent. J. Altern. Compl. Med., 6: 632-637.

Okoli, C.O. and P.A. Akah,.2006. Analgesic activity of leaf extract of Culcasia scandens P. Beauv, Ind. J. Exp. Bio., 44: 422-424.

Okoli, C., P.A. Akah., S.V. Nwafor, U.U. Ihemelandu and C. Amadife, 2007. Antiinflammatory activity of seed extracts of Aframomum melegueta. J. Herbs Spices Med. Plants, 13: 11-21.

Okpo, S.O., F. Fatokun, and O.O. Adeyemi, 2001. Analgesic and anti-inflammatory activity of *Crinum* glaucum aqueous extract. J. Ethnopharmacol., 78: 207-211.

Oliver, 1960. Medicinal plants in Nigeria. Nigerian College of Arts, Science and Technology, Ibadan, Nigeria, pp: 23.

Oriowo, M.A, 1982. Antiinflammatory activity of piperonyl-4- acrylic isobutyl amide an extractive from *Zanthoxylum zanthoxyloids*. Planta. Medica., 44: 54-56

Park, E.H., J.H. Kahng, S.H. Lee, and K.H. Shin, 2001. Anti-inflammatory principles from cactus. Fitoterapia, 72: 288-290.

Taber, R.I., D.D. Greenhouse, J.K. Rendel and S. Irwin, 1969. Agonist and antagonist interactions of opiods on acetic acid induced abdominal stretching in mice. J. Pharmacol. Exp. Therap., 169: 29-37.

Turner, R.A. 1965. Screening Methods in pharmacology. Academic Press; New York, pp: 158.

Winter C.A., E.A. Risley and G.W. Nuss, 1962. Carrageenin induced oedema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol., 111: 533-547.