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The Central and Peripheral effects of the methanol extract of Fadogia cienkowskii schweinf. var cienkowskii Leaves

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Abstract: *The methanol extract of Fadogia cienkowskii schweinf. var cienkowski leaves was investigated for central and peripherally mediated nervous effects. Oral acute toxicity studies, the effect on phenobarbitone-induced sleeping time, local anaesthetic effects, analgesic activity using acetic acid-induced writhing response, and muscle relaxant effects with hind limb grip reflex and inclined board tests were carried out on the methanol extract following standard models. The extract was tolerated by the oral route up to the highest test dose of 4000 mg/kg; hence, there was no mortality or overt clinical manifestations in mice within 48 h duration of the investigation. The test doses (100, 200 and 400 mg/kg) of the extract significantly ($p<0.05$) potentiated phenobarbitone-induced mean sleeping times in mice from 552.7 ± 2 mins in the control, to 930.0 ± 4, 1009.0 ± 6, and 918.7 ± 5 mins respectively. The extract also exerted a local anaesthetic effect but with reduced potency compared to lignocaine, the reference drug (Lignocaine =100%; 200 mg/kg extract =88.9%). Again, the leaf extract demonstrated a dose dependent analgesic activity in reducing abdominal constrictions evoked in mice in response to acetic acid-induced pain. The results of hind limb grip reflex and inclined board tests revealed that the extract had no effect on muscle relaxation. Thus, the extract exhibited a central nervous-mediated effect when phenobarbitone-induced sleeping time became prolonged, and a peripherally mediated local anaesthetic and analgesic effects. Fadogia cienkowskii schweinf. var cienkowski leaves could be a potential source for isolation of novel analgesic and anaesthetic agents.*

Keywords: *Fadogia; Lignocaine; Phenobarbitone; Analgesic; Anaesthetic*

I. Introduction

The plant kingdom offers prospect as sources of various medicaments and potent chemotherapeutic agents. Natural products from plants play a dominant role in the development of novel drug leads for the treatment and prevention of diseases [1]. It is not an understatement to note that many available drugs are associated with numerous adverse effects. Most anaesthetic agents are known to cause cardiovascular depression, by effects on the myocardium and blood vessels as well as on the nervous system [2]. The prolonged use of analgesic which is a non-selective cyclooxygenase inhibitor blocks prostaglandin and thromboxane production with consequent side effects including gastric ulceration, coagulopathies and sometimes renal failure in patients [3]. Some local anaeaethetics e.g. ropivacaine are also associated with symptoms of mild neurotoxicity [4]. However, a large section of the world´s population relies on traditional remedies to treat plethora of diseases due to their low cost, easy access and reduced side effects [5]. There is urgent need to screen plants for more effective pharmaceuticals with high safety margin, particularly in the light of rapid deforestation and concurrent loss of biodiversity throughout the world.

Fadogia cienkowskii schweinf. var cienkowskii (Rubiaceae), locally called 'ufu-ewureje' is popular among Igede tribe of Benue State within the middle belt of, Nigeria. The leaves were highly acknowledged for their wide therapeutic efficacy in the relief of headache, general body debility, inflammation, diarrhoea and other ailments especially in infants. The plant is a shrub of less than 1 m high usually found in the savanna region and found to be widely dispersed into the drier parts of tropical Africa [6]. The plant is referenced 30H among herbarium species in the South east Dome site of the copper-cobalt belt of the Democratic Republic of Congo and Zambia. This plant could be employed in phytoextraction, a technology of using hyper accumulator plants to extract unwanted trace elements from the soil or mineral wastes with the aim of remediating pollution [7].

However, there was until now, paucity of information on systematic investigation of the medicinal properties of this plant using standard experimental models. The present study therefore sought to evaluate the central and peripheral effects of the methanol extract as an initial step to unravel the pharmacological potentials of this plant.

II. Materials And Methods

2.1. Selection, plant collection and identification

Selection of the plant was based on oral information from traditional healers on the wide healing effects of Fadogia cienkowskii schweinf. var cienkowskii leaves in diverse diseases especially in children. Fresh leaves of Fadogia were collected in March, 2015 from farm locations in Ochimode village, Oju Local Government Area of Benue State in the middle belt of Nigeria. The leaves were duely identified to be those of Fadogia cienkowskii schweinf. var cienkowskii by a plant taxonomist, Mr. A.O. Ozioko of the Department of Botany, University of Nigeria, Nsukka (UNN).

2.2. Experimental Animals

Inbred albino mice of both sexes weighing 20.7- 38 g, bred in the Laboratory Animal Unit of the Nigerian Veterinary Research Institute (NVRI), Vom, Plateau state, Nigeria were used in the experiments. Male guinea pigs (485-614 g) source locally from Gwagwalada market were also employed in local anaesthetic studies. The mice were kept in the same room with a temperature varying between 28 and 30^0C; lighting period was between 15 and 17 hours daily. The mice were kept in stainless steel wire mesh cages which separated them from their faeces to prevent coprophagy. They were supplied clean drinking water and fed standard feed (Grower mash pellets, Vital feed®, Nigeria). Animals were allowed two weeks to acclimatize before the commencement of the investigations. Ethical rules guiding the use of animals for experimentation were strictly adhered to. The laboratory animals were also used in accordance with laboratory practice regulation and the principle of laboratory animal care as documented by [8].

2.3. Reagents, chemicals and drugs

Methanol was obtained from JHD, Guangdong Guanhua Science Tech. Co. Ltd., China, Picric acid (Lab. Tech. Chemicals, Idia), glacial acetic acid (JHD, China), Indomethacin (SJZ Chem. Pharm. Co. Ltd., China), Lignocaine-adrenaline (Shreechem Pharmaceuticals PVT. Ltd., Mumbai, India), Phenobarbitone sodium (Sterop-Belgium), pancuronium (Rotex Medica Trittau,Germany) were used in the study. The solvents used for extraction were only analar grade reagents.

2.4. Preparation and extraction of the plant material

The plant material was dried under mild sunlight, and then reduced to coarse particles with mortar and pestle before been pulverized into fine particles using a laboratory hammer mill. A measured mass (400 g) of the powdered leaves was exhaustively extracted by cold maceration in 80% methanol with intermittent shaking at 2 h intervals for 48 h. The extract was filtered with Whatman filter paper size-1.0 and the filtrate concentrated in vacuo using a vacuum rotary evaporator. The concentration and percentage yield of the extract were determined. The extract was kept at 4 ^{0}C in a refrigerator for use in further studies.

2.5. Acute oral toxicity studies

Acute toxicity studies were conducted using a modified method described by [9]. In the first phase of the study, (9) matured albino mice of both sexes were marked with 10% picric acid, weighed and randomly separated into 3 groups (A – C) of 3 rats per group. The groups were separately given increased oral doses (250; 500 and 1000) of the extract respectively. The rats were observed for signs suggestive of toxicity and death within 24 h. In the absence of mortality, the second phase of the test commenced with a new set of mice in the same procedure with administration of varyingly higher doses (2000; 3000 and 4000 mg/kg) of the leaf extract to the respective groups. Observation for toxicity also lasted for 24 h. A 4th group that received an equivalent volume (10 ml/kg) of distilled water served as a control in both cases. All treatments were given orally by gastric intubation. The final LD_{50} value was calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose. Animals that survived were further monitored for two weeks for toxic effects. The test was terminated after two weeks and all the animals were humanely sacrificed and postmortem examinations carried out on them.

2.6. Pentobarbitone-induced sleeping time

The method of Perry [10] was used. Four groups consisting of 5 mice per group were randomly selected. Group A which served as the control was injected with phenobarbitone sodium (35 mg/kg) intraperitoneally. Groups B-C received the extract at 100, 200 and 400 mg/kg respectively, 30 min before phenobarbitone administration. The onset of sleep (when the righting reflex was lost) and the time of awakening (when the righting reflex was regained) were recorded. The mean sleeping times of the separate mice groups were calculated and compared.

2.7. Local anaesthetic test

The method described by Anika and Shetty [11] was used with slight modification. The lower back of the guinea pig was neatly shaved a day prior to the conduct of the experiment. Three different concentrations of the extract (10, 20 and 40 mg/ml) and Lignocaine-Adrenaline (20 mg/ml) were used. The concentrations of the extract and lignocaine were injected intradermally to form a wheal, which was outlined with a marking pen on six different sites of the lower back. Five minutes after the injection, the sensitivity of the areas were tested by pricking lightly with a needle six times at the site of injection. Control test was done by pricking the skin as far away as possible from the site of the extract or lignocaine. The response at the site of injection indicated the degree of anaesthesia, which was expressed as the number of negative responses. The test was repeated at 5 min intervals for a period of 30 min after the injection. The total score for each wheal was added up to make the total number of negative responses out of the 36 possible and expressed as percent (%) anaesthesia.

2.8. Analgesic study

The method of Koster et al. [12] on acetic acid writhing response in mice was applied. Twenty-five (25) mice were randomly marked with 10% picric acid, weighed and classed into five groups (A-E). Group A served as the negative control and was given distilled water (10 ml/kg) alone, orally, group B (positive control) received indomethacin (10 mg/kg, p.o.) while groups C, D and E were treated with the extract at 100, 200 and 400 mg/kg, respectively. One hour after, 0.6% acetic acid was injected intraperitoneally at 10 ml/kg to each mouse. Five (5) minutes after acetic acid administration, the number of abdominal constrictions that occur within subsequent 20 min are counted and recorded (i.e. within 15 min).

2.9. Hind limb grip reflex

This test was conducted according to the method adopted adopted by Bolon and St. Omer [13]. Matured albino mice were weighed, marked with 10% picric acid and randomly allocated to 4 groups (A-D). The separate experimental animal groups comprised 5 mice per group. A steel wire (26 cm long by 0.2 cm thick) was supported between two poles. Each mouse was gripped at the base of its tail and suspended above the wire with both paws. The mouse was lowered and when released, pulled up and grasped the wire with all the 4 paws. The maximum time allowed for this synergistic hind limb support was 15 sec. The tests were conducted before intraperitoneal administration of distilled water (10 ml/kg) to negative control (group A) and increasing doses (100, 200 and 400 mg/kg) of the extract to the rest of the test mice in groups B, C and D. The ‘‘fall off time’’ when each mouse fell from the steel wire before and after treatment was noted and subsequently compared with that of other groups.

2.10. Inclined board test

The method of Kitano et al. [14] was adopted. Briefly, a smooth surfaced board was inclined at an angle of 35 ^{0}C to the horizontal and each mouse was placed one after another on the board as a control before treating with the extract. Those mice unable to stay for a minimum of 10 seconds failed the test. Three groups of mice (B, C and D), each containing five mice, were treated with increasing doses (100, 200 and 400 mg/kg, i.p.) of the extract while group A which served as the positive control was injected with pancuronium (0.001 ml, i.p.). Thirty min later, each mouse was put back to the inclined board and those that could no longer stay for 10 seconds failed the test and were recorded as positive for muscle relaxation.

2.11. Statistical analysis

All data generated as experimental results were subjected to one-way analysis of variance (ANOVA) and Duncan multiple range post hoc test. Mean group differences at $p< 0.05$ were considered significant.

III. Results

3.1. Extraction of the plant material

The methanol extract of Fadogia cienkowskii schweinf. var cienkowskii was brownish in colour and odourless. The extraction process gave a yield of 12.4 % (w/w).

3.2. Acute toxicity studies on Fadogia leaf extract in rats

There were no deaths by the oral route even at the highest test dose of 4000 mg per kg body mass within 48 h of the investigation. There were also no changes in faecal consistency or overt clinical manifestations within the period of investigation. At post mortem, there was no observable gross lesion in the liver, gastro-intestinal tract, spleen, heart and kidneys of the experimental rats.

3.3. Phenobarbitone-induced sleeping time.

The mean sleeping time induced in experimental mice by phenobarbitone alone was 552.7 ± 2 mins, the extract of Fadogia leaves however, caused significant ($p<0.05$) increase in the sleeping time values of 930.0 ± 4 , 1009.0 ± 6 , and 918.7 ± 5 mins. at 100, 200 and 400 mg/kg, respectively compared to control. There was no significant ($p>0.05$) different in the mean sleeping time values of mice treated with increasing doses (100, 200 and 400 mg/kg) of the extract (Table 1).

Table 1

Effect of extract on Phenobarbitone-induced sleeping time in mice

Animal group	Treatment	Dose	Mean sleeping time (mins)
A (Control)	-	-	552.7 ± 2
B	Extract	100 mg/kg	930.0 ± 4*
C	Extract	200 mg/kg	1009.0 ± 6*
D	Extract	400mg/kg	918.7 ± 5.0*

*****significant at $p < 0.05$

3.4. Local anaesthetic effect

The extract exerted appreciable and significant ($p<0.05$) local anaesthetic effect at different test doses relative to lignocaine, the reference drug used. Lignocaine had 100% anaesthetic effect but the crude extract produced 55.6%, 88.9% and 66.7% at 10, 20 and 40 mg/ml respectively. The extract displayed a maximal local anaesthetic effect at the medium test concentration of 20 mg/ml (Table 2).

Table 2

Local anaesthetic effects of F. cienkowskii schweinf leaf MeOH extract in the guinea pig

TMt	Conc.	Mean no. of negative responses at 5 min interval.					Total	%Anaesthesia (mg/ml)		
		5	10	15	20	25	30			
Lignocaine	20	6/6	6/6	6/6	6/6	6/6	6/6	36/36		100
Extract	10	1/6	5/6	4/6	2/6	6/6	2/6	20/36		55.6
Extract	20	6/6	6/6	5/6	6/6	6/6	3/6	32/36	88.9	
Extract	40	4/6	1/6	6/6	6/6	3/6	4/6	24/36		66.7

TMt=Treatment, n: number of animals per treatment = 3.

3.5. Analgesic effect of the extract

The test doses (100,200 and 400 mg/kg) of the crude extract of F. cienkowskii schweinf. var cienkowskii demonstrated a significant ($p<0.05$) analgesic effect in reducing the number of abdominal constrictions evoked in mice as a response to acetic acid-induced pain. Untreated mice in the control (group A) that were given distilled water produced 14.8 ± 4 as the mean number of abdominal constrictions relative to 7.8 ± 2 with indomethacin, a popular analgesic while the extract dose dependently reduced the mean number of the abdominal constrictions to 9.3 ± 3, 7.3 ± 2 and 3.2 ± 0.6 at 100, 200 and 400 mg/kg, respectively (Table3). The analgesic effect of the extract was most significant ($p<0.01$) at 400 mg/kg when abdominal constrictions became drastically reduced from 14.8 ± 4 in the negative control to 3.2 ± 0.6.

Table 3

Analgesic activity of the extract against acetic acid-induced writhing response in mice

Group	Treatment	Dose	Mean no. of abdominal constrictions
A (control)	Distilled water	10 ml/kg	14.8 ± 4
B	Indomethacin	10 mg/kg	7.8 ± 2*
C	Extract	100 mg/kg	9.3 ± 3*
D	Extract	200 mg/kg	7.3 ± 2*
E	Extract	400 mg/kg	3.2 ± 0.6**

n=5 mice per group; *;**significant at $p<0.05$; $p<0.01$ respectively.

3.6. Test for grip reflex

The crude extract of F. cienkowskii leaves did not exert significant ($p>0.05$) effect on hind limb grip reflex in mice, not even at the highest test dose of 400 mg/kg. The display of synergistic hind limb support was intact when treated mice pulled up and grasped the wire with all the four paws.

3.7. Inclined board experiment

Mice treated with pancuronium (0.001 ml) failed the test but all the extract-treated mice were able to stay at leisure on the inclined plane for more than10 seconds.

IV. Discussion

The extract did not cause death or visible clinical manifestation within 48 h of the investigation even at the highest oral test dose (4000 mg/kg), an indication that the leaf extract had a wide safety margin in the experimental mice. The various test doses (100, 200 and 400 mg/kg) of the extract significantly ($p<0.05$) potentiated phenobarbitone-induced sleeping time values in mice from 552.7 ± 2 mins in the control, to 930.0 ± 4, 1009.0 ± 6, and 918.7 ± 5 mins, respectively (Table 1). Components of the extract could mediate a central nervous effect to induce uncounsciousness and sleep or had the ability to synergise with phenobarbitone. An ideal general anaesthetic should induce unconsciousness, analgesia, amnesia and skeletal muscle relaxation [15]. However, the effect of the extract at prolonging phenobarbitone-induced sleeping time in mice in this study was not observed to be dose dependent.

The extract exerted a profound local anaesthetic effect at all the test concentrations (10, 20 and 40 mg/ml) relative to lignocaine (20 mg/ml), a reference anaesthetic agent. Lignocaine displayed 100% anaesthetic effect but the crude extract produced 55.6%, 88.9% and 66.7% at 10, 20 and 40 mg/ml respectively (Table 2). It is rational that the extract exhibited a reduced local anaesthetic potency compared to lignocaine due to the fact that, the extract is crude and a mixture of impurities while lignocaine is a pure compound. Local anaesthetics stabilize peripheral nerve cell membranes and cause a reversible loss of sensation in a localized area of the body [16]. The extract demonstrated its maximal local anaesthetic effect at the medium test concentration of 20 mg/ml. The leaf extract induced a remarkable dose dependent analgesic activity with reduction in acetic acid-induced abdominal constrictions in mice. Distilled water-treated mice had 14.8 ± 4 as mean number of abdominal constrictions but indomethacin treatment was effective at reducing these constrictions to 7.8 ± 2. The crude extract however, exerted a better efficacy in reducing the average constrictions to 9.3 ± 3, 7.3 ± 2 and 3.2 ± 0.6 at 100, 200 and 400 mg/kg, respectively (Table 3). The extract exhibited a maximal analgesic potency at 400 mg/kg when the activity became significant ($p<0.05$) and greater compared to the effect of indomethacin (10 mg/kg), a popular analgesic. Indomethacin is a non-steroidal anti-inflammatory agent as well as a non-selective cyclooxygenase inhibitor that blocks prostaglandin biosynthesis [2]. Prostaglandins are known to sensitize nociceptors to pain stimuli. Stimulation of nociceptors leads to release of substance P in the neurogenic (early) phase within 5 minutes but late phase (15-30 minutes) of pain is due to release of histamine, serotonin, bradykinin and prostaglandins [17]. The number of abdominal constrictions that occur in a mouse five minutes after acetic acid administration is an indication of response to the severity of pain stimulus [12]. Mouse writhing assay is a test useful for evaluating mild analgesic property that suggests peripherally mediated analgesic effect [18]. The extract could have acted peripherally to relieve acetic acid-induced pain in the experimental mice.

The results of the hind limb grip reflex and inclined board tests revealed that the leaf extract had no effect on muscle relaxation. This was demonstrated by the presence of intact synergistic hind limb support that enabled extract-treated mice to pull up on release to grasp the test wire with all the four paws. In inclined board experiment, mice treated with pancuronium (0.001 ml) failed the test due to muscular paralysis but all the extract-treated mice stayed at leisure on the inclined plane for more than10 seconds. Pancuronium is a long acting (90-180 min), competitive, non-depolarizing, neuromuscular relaxant similar to tubocurarine, metocurine, doxacurium, pipecuronium, and gallamine [19].

V. Conclusion

The extract exhibited a central nervous-mediated effect in potentiating phenobarbitone-induced sleeping time and a peripherally mediated local anaesthetic and analgesic effects. The results of the study provide a basis for exploiting components of the extract of F. cienkowskii schweinf. var cienkowskii leaves for anaesthetic and analgesic effects as adjuncts to complement surgical procedures.

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