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PRELIMINARY ASSESSMENT OF THE ANTI-NOCICEPTIVE EFFECT OF *STERCULIA SETIGERA* (MALVACEAE) IN EXPERIMENTAL ANIMALS

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ABSTRACT

Sterculia setigera (Malvaceae) is a deciduous tree commonly found growing in the savannah of tropical Africa. A decoction of stem bark extract is used to treat headaches. This study aims to investigate the anti-nociceptive activity of the ethanol extract of *S. setigera.* The acute study involved the administration of extract at 500, 1000, and 2000 mg/kg to determine the lethal dose (LD_{50}). Anti-nociceptive activity was examined using the mouse writhing and the hot plate test methods. Acute toxicity studies did not show any sign of mortality at 2000 mg/kg. The stem bark of *S. setigera* at 200mg/kg produced a significant (p<0.05) reduction in the number of writhes observed in the writhing test. However, oral administration of the extract (100, 200, 400 mg/kg) did not prolong (p>0.05) the reaction times in the hot plate test. This study shows that *S. setigera* possesses an anti-nociceptive effect.

Keywords: Anti-nociceptive, acute toxicity, hot plate, mouse writhing, *Sterculia setigera.*

INTRODUCTION

Sterculia setigera (Malvaceae) is a deciduous tree commonly found in the Sahelo-Sudan and Guinea savannah zones of Tropical Africa (Atakpama *et al*., 2012). It is a vital tree crop used as a gum and exported for commercial purposes (Igoli *et al.,* 2005). *Sterculia setigera* is distributed in Senegal, Sudan, and Ethiopia. It is found growing on rocky soils and in shallow gritty soils (Atakpama *et al*., 2012). In Nigeria, *Sterculia setigera* is commonly called "kukui" in Hausa, "Boboli" in Fulani, and "Ose-awere" in Yoruba (Zaruwa *et al.,* 2016).

Sterculia setigera can reach 18 meters in height and has an open spread crown with a buttressed base. It has a grey, purple bark that peels off in thin, irregular scales leaving pale patches, while a slash reveals a fibrous, brownish-to-red inner bark and a white exudate (Tor-Anyiin *et al*., 2011). The leaves are simple and alternate in arrangement with palmately arranged nerves. The leaf blade is ovate to orbicular in outline, and the upper surface tends to be stellate and tomentose. The flowering period begins towards the end of the dry season (Ayodele *et al.,* 2000). Fruit is a sessile follicle, 6-10 cm long oblong in shape, grey-green or brown in colour, and many-seeded, the follicle can stay on the tree even when seeds fall off. Isolated compounds from the stem bark include(s) lupeol and a class of procyanidin trimmers (Adelakun *et al*., 2014). The sugar properties of the gum obtained from the species consist of D-galacturonic acid, L-rhamnose, and D-galactose and are quite like those obtained from *Sterculia urens* (Idu *et al.,* 2007).

It was reported that *S. setigera* contains active metabolites such as tannins, flavonoids, saponins, phenolics, and glycosides (Idu *et al.,* 2007). The seeds are eaten in Northern Nigeria (Ayodele *et al.,* 2000). The plant is used for managing several ailments in various local communities. A decoction of stem bark extract is used as part of a herbal regimen to treat skin ailments and infections, fever, diarrhoea, and toothache (Farnsworth *et al.,* 1985). A black "soap" preparation from the powder obtained from a burnt mixture of fruits and seeds is used to manage dermatosis (Atakpama *et al.,* 2012). The Methanol extract of the stem bark is used to treat jaundice, diarrhoea, dysentery, and wounds. The exudate (gum) is used in the treatment of snake bites, leprosy, syphilis, coughs, bronchitis, and rickets and to manage insanity (Zaruwa *et al.,* 2016).

The ethnomedicinal applications of this plant and the presence of bioactive constituents such as phenolics make it a potential anti-nociceptive agent (Ayodele *et al.,* 2015; Idu *et al.,* 2007). The search for novel anti-nociceptive compounds is continuous and is underscored by the need for much safer and efficacious agents (Islam *et al.,* 2016). The orthodox agents have deleterious effects that can hamper their continued usage thus highlighting the need for much safer agents (Ali *et al,* 2015). Despite its widespread use in ethnomedicine, there is a dearth of pharmacological studies on *S. setigera*. This study aimed to investigate the anti-nociceptive effects of ethanol extract of *Sterculia setigera* bark mice.

METHODS

Plant Collection and Extraction

The stem bark of *Sterculia setigera* was collected in Warri Local Government, Bauchi State, Nigeria in the month of September 2021. The plant was identified and authenticated by Mallam Ibrahim Muazzam Wudil of the Department of Traditional Medicine and Medicinal Plant Research at the National Institute for Pharmaceutical Research and Development (NIPRD) (NIPRID.H.1047). The plant material collected was washed, air-dried to a constant weight, and thereafter pulverized using a mechanical grinder. A Soxhlet apparatus was used to carry out the extraction. The powdered material (300 g) was placed in a Soxhlet apparatus (thimble) and extraction was carried out using 1.5 L of 98 % v/v ethanol concentration for 8 h. After the extraction process, the resulting extract was filtered and concentrated using a rotary evaporator. The dried extract was stored in clean glass containers in the refrigerator at 4ºC.

Animals

All experiments were performed using male Swiss albino mice (7 -9 weeks; 18 - 25g). The animals were procured from the Animal House, Department of Pharmacology, University of Benin, Benin City. The animals were kept in well-ventilated cages in the animal house of the Department of Pharmacology and Toxicology, University of Benin, Benin City. The animals were maintained under standard conditions and were allowed free access to clean drinking water and feed. The handling procedures were approved by the Faculty of Pharmacy, University of Benin Ethical Committee on Experimental Animals (EC/FP/021/11).

Acute Toxicity Study

An acute toxicity study was carried out according to the modified method of Miller and Tainter, 1944). Mice were randomly divided into 4 groups with 5 animals in each group. Mice were treated with *Sterculia setigera,* in groups II, III, and IV at doses of 500, 1000, and 2000 mg/kg respectively. Group I (control group) was given 10 ml/kg,

po of distilled water. The symptoms of toxicity within the period of 24 hours were recorded and the animals that survived after 24 hours of administration were observed for any sign of delayed toxicity for 2 weeks (Miller and Tainter 1944).

Tests for Nociception

Acetic acid-induced mouse writhing assay
The animals (18 – 25 g) were randomly allotted to five groups of five mice each. Group I (control) received distilled water (10 mL/kg) orally, group II received acetylsalicylic acid (100 mg/kg, po) while groups III, IV, and V received orally 100, 200, and 400 mg/kg of the extract. After 1 hour, 0.6%v/v acetic acid (10 mL/kg, ip) was injected into all animals. The number of writhes by each mouse was counted immediately after acetic acid administration at intervals of 5 minutes for a period of 30 minutes (Edosuyi *et al*., 2018).

Hot plate test
This experimental method of Eddy and Leimbach (1953) was used with slight modification to measure the reaction time to thermal nociceptive stimuli. The hot plate apparatus was turned on and allowed to heat up to 55 ± 1ºC. Mice (18 - 25g) were randomly divided into five groups of five mice per group. The pre-drug reaction times of each animal were recorded by placing each mouse on the hot plate and recording the reaction time before drug treatment. After recording the pre-drug reaction times, the animals were treated as stated; Group I received distilled water 10 ml/kg, po, groups II, III, and IV were orally administered 100, 200, and 400 mg/kg of the extract respectively and group V received Pentazocine (10 mg/kg, ip). Thereafter, the animals were individually placed on the hot plate at 0, 30, 60, 90, and 120 minutes. The time interval from placement and shaking/licking of the paw or jumping was recorded as the reaction time.

Statistical Analysis
Data were expressed as mean ± S.E.M. Statistical analysis was done using one-way analysis of variance (ANOVA) followed by Dunnet *post hoc* test (GraphPad Prism® 6, San Diego, USA). The results obtained were compared with the control group, values of $p<0.05$ were statistically significant.

RESULTS

Acute Toxicity Study
No adverse effect or mortality was observed in the mice and rats 24 h after the administration of each dose of the extract up to 2000 mg/kg. (Table 1).

Table 1: Effect of Acute Administration of Graded doses of *S. setigera* in Mice

Treatments	Convulsion	Sedation	Diarrhoea	Piloerection	Tremors	Urination	Mortality
Control	0/5	0/5	0/5	0/5	0/5	0/5	0/5
SS 500 mg/kg	0/5	0/5	0/5	0/5	0/5	0/5	0/5
SS 1000 mg/kg	0/5	0/5	0/5	0/5	0/5	0/5	0/5
SS 2000 mg/kg	0/5	0/5	0/5	0/5	0/5	0/5	0/5

SS= *Sterculia setigera.* Control (distilled water, 10 mL/kg po)

Acetic acid-induced Mouse Writhing Assay
As shown in Fig 1, intraperitoneal injection of 0.6 % v/v acetic acid increased the number of writhes in a time-dependent fashion, with a peak effect at 30 minutes (122.8 ± 8.6). There were no significant reductions in the number of writhes

observed in mice treated with 100 and 400 mg/kg of the extract. However, at 200 mg/kg of the extract, there was an early onset significant reduction in the number of writhes ($p<0.05$) which was sustained and peaked at 30 minutes (122.8 ± 8.6 vs 8.2 ± 5.3, $p<0.01$). Acetylsalicylic acid, a cyclooxygenase inhibitor, reduced the number of writhes at all grade doses and elicited peak reduction at 30 minutes (122.8 ± 8.6 vs 55.5 ± 4.6, $p<0.001$).

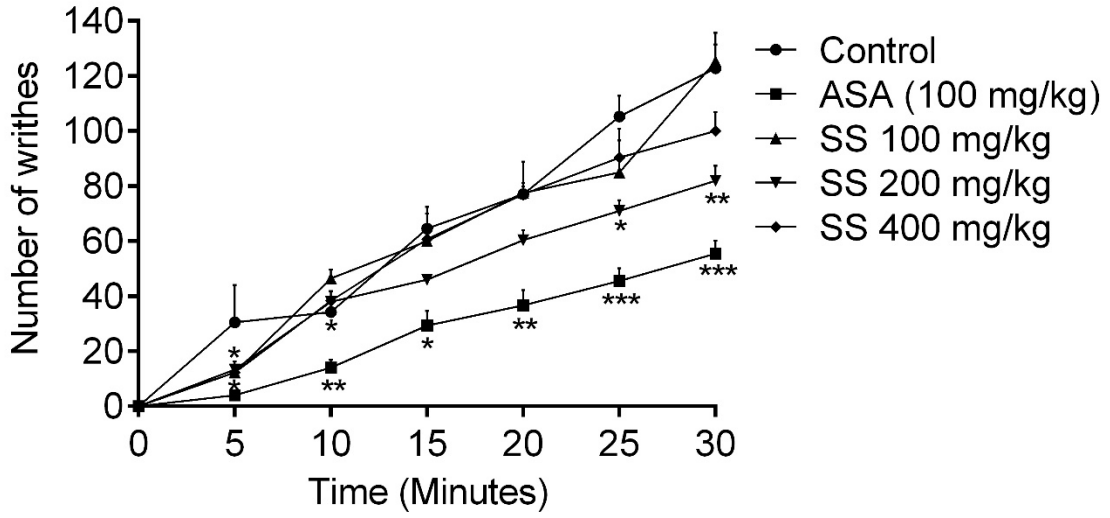

Fig 1: Effect of stem bark extract of *sterculia sertigera* (SS) on acetic acid-induced mouse writhing assay. **p*< 0.05, ***p*<0.01, ****p*<0.001 vs control (distilled water, 10 mL/kg).

Hot Plate Test

As illustrated in Table 2, the extract at all doses did not significantly increase the reaction times of mice in thermal-induced nociception. Pentazocine, an opioid agonist, significantly increased the reaction times to thermal stimuli at 30 (3.7±0.4 vs 5.8±0.6 s) and 60 (4.6±0.2 vs 8.7±1.8 s) minutes (*p*< 0.05).

Table 2: Effect of Stem Bark Extract of *sterculia setigera* (SS) on Thermal-induced Nociception

	Reaction Times (secs)				
Groups	0 min	30 min	60 min	90 min	120 min
Control	4.1±0.4	3.7±0.4	3.1±0.6	4.1±0.3	4.5±0.1
Pentazocine (10 mg/kg)	3.3±0.2	3.4±0.5	5.8±0.6**	6.5±1.4	8.7±1.8*
SS 100 mg/kg	4.6±0.6	3.7±0.2	3.9±0.1	4.6±0.5	1.6±0.1
SS 200 mg/kg	2.9±0.2	2.8±0.4	3.4±0.5	4.2±0.6	3.2±0.6
SS 400 mg/kg	4.1±0.7	4.5±0.9	4.1±0.5	4.7±0.2	3.6±0.6

SS= *Sterculia setigera.* Control (distilled water, 10 mL/kg po). **p*<0.05, ***p*<0.01 vs control.

DISCUSSION

Medicinal plants can be applied for acute or chronic use. Due to inadequate standardization, these medicinal plants could be taken in large quantities for acute or chronic usage. Hence it becomes necessary to evaluate the margin of safety and toxicity. An acute toxicity test is thus necessary to validate the immediate safety of the plant extract at high doses (Lorke 1983; Bello *et al.,* 2016; Edosuyi *et al.,* 2024). In this present study, oral administration of the extract up to 2000 mg/kg did not cause any signs of toxicity, changes in behavior, or mortality within 24 hours.

Anti-nociceptive agents can mitigate nociceptive stimuli via peripheral or central mechanisms (Cavalcante-silva *et al.,* 2014; Edosuyi and Ekanem 2023). Peripherally, these agents block the generation of local impulses at chemo receptors while they act centrally by raising the threshold of pain and reducing the patient's anxiety (Zendehdel *et al*., 2015; Rosenblum *et al.,* 2008; Yin *et al.,* 2016).

Centrally mediated nociceptive stimuli can be induced via thermal stimuli using the hot plate test (Heidari *et al*., 2009). The hot plate test is used in experimental research to assess the effectiveness of compounds by observing the reaction to thermal-induced nociception (Kayani *et al.,* 2016). It is a behavioral model of nociception where behaviors such as jumping and hind paw-licking are elicited following a noxious thermal stimulus. There was no significant increase in the reaction time of the extract when compared suggesting that the extract did not exert any effect against centrally mediated nociceptive stimuli (Schröder *et al.,* 2010).

Acetic acid-induced writhing test is a classical plan model widely used to access novel analgesic agents (Koster *et al*., 1959). It assesses the action of peripherally acting anti-nociceptive agents (Gawade 2012). The intraperitoneal injection of acetic acid causes the release of endogenous substances such as serotonin, histamine, prostaglandins, bradykinins, and substance P and the activation of mitogen-activated protein (MAP) kinases and microglia in the spinal cord (Ren and Dubner 2002). When prostaglandin is released, the nerve endings respond to it through the prostaglandin E_2 receptor by picking up and transmitting the pain and injury messages to the brain and causing visceral writhing (abdominal stretches) stimuli in mice (Rub and Dubner 2002; Calvante-silva *et al.,* 2014). The study revealed that the extract exerted an early-onset action, significantly reducing the number of writhes at 200 mg/kg indicating that the extract possesses an anti-nociceptive effect probably mediated via peripherally.

The significant analgesic activity demonstrated by the extract may be due to the presence of a significant number of flavonoids and tannins (Idu *et al.,* 2007). Flavonoids, tannins, saponins, alkaloids, and other polyphenols have been linked with various degrees of anti-nociceptive and anti-inflammatory activities (Hanrahan *et al*., 2011). However, further investigations on its mechanism of action, isolation, and characterization of its active principles are needed for proper elucidation of the plant extract and its possible mechanisms of action.

CONCLUSION

The ethanol extract of *S. setigera* bark has been shown to possess anti-nociceptive properties which appeared to be peripherally mediated.

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Conflict of interest

The authors have declared that no conflicts of interest exist.

References

Adelakun KM, Mustapha MK, Ogundiwin DI and Ihidero AA. (2014). Nutritional and anti-nutrient composition of karaya gum tree (*Sterculia setigera* seed): a potential fish feed ingredient. *Journal of fisheries*. 2(3), 151–156.

Ali, OK., Ahmed, A.JS., and Malwood, AG. (2015). Effects of Tramadol on Histopathological and Biochemical Parameters in Male Rabbits. *American Journal of Biology and Life Sciences*, *3*(3), 85–90. http://www.openscienceonline.com/journal/ajbls

Atakpama, W., Batawila, K., Dourma, M., Pereki, H., Wala, K., Dimobe, K., Akpagana, K. and Gbeassor, M. (2012). Ethnobotanical Knowledge of *Sterculia setigera* Del. in the Sudanian Zone of Togo (West Africa). *ISRN Botany*. 2012(4)1–8.

Ayodele JT, Alao OA and Olagbemiro TO. (2000). The chemical composition *of Sterculia setigera. Nigerian Journal of Animal Science*, 4(5), 310–319.

Bello, I., Bakkouri, A., Tabana, Y., Al-Hindi, B., Al-Mansoub, M., Mahmud, R. and Asmawi, M. (2016). Acute and Sub-Acute Toxicity Evaluation of the Methanolic Extract of *Alstonia scholaris* Stem Bark. *Medical Sciences*. 4(1), 4-14.

Cavalcante-silva, L.H.A., Alice, M., Falcão, P., Carolina, A., Vieira, S., Denisson, M., Viana, M., Araújo-júnior, J.X. De, Celestino, J., Sousa, F., Maria, T., Barbosa-filho, J.M., Noël, F., Miranda, G.E.C. De, Viviana, B., Santos, D.O. and Alexandre-moreira, M.S. (2014). Assessment of Mechanisms Involved in Antinociception Produced by the Alkaloid Caulerpine. *Molecules*. 7361(8), 14699–14709.

Eddy, N.B. and Leimbach, D. (1953). Synthetic analgesics. II. Dithienylbutenyl-and dithienylbutylamines. *Journal of Pharmacology and Experimental.* 107(8), 385–393.

Edosuyi, O. and Ekanem, S. (2023). The Antinociceptive Property of *Brachystegia eurycoma* (Fabaceae) Partly Involves Stimulation of Opioid Pathways and Mitigation of the Action of Reactive Radicals. *Journal of Science and Practice of Pharmacy*. 9(1), 459-466.

Edosuyi, O., Igbe, I. and Iniaghe, L.O. (2018). Antinociceptive and antioxidant activities of *Hunteria umbellata* stem bark: Possible role of the serotonergic, opioidergic and dopaminergic pathways. *Journal of Complementary and Integrative Medicine*, 15(1), 1-10.

Edosuyi, O., Omo-Erhabor Aladuna J, Osaghae I., Odore S. and Igbe I. (2024). Acute and Sub-Acute Oral Safety Profile of Fumarate in Normotensive Wistas Rats. *Journal of Pharmaceutical and Allied Sciences*. 20(2), 3991–4000.

Farnsworth NR, Akerele O, Birgel AS, Soejarto DD and Guo Z. (1985). Medicinal plants in therapy. *Bulletin of World Health Organization*. 63(6), 965–981.

Gawade, S.P. (2012). Acetic acid-induced painful endogenous infliction in writhing test on mice. *Journal of Pharmacology and Pharmacotherapeutics*. 3(4), 348-358.

Hanrahan, J.R., Chebib, M. and Johnston, G.A.R. (2011). Flavonoid modulation of GABAA receptors. *British Journal of Pharmacology*. 163(2), 234–245.

Heidari, M.R., Foroumadi, A., Noroozi, H., Samzadeh-Kermani, A. and Azimzadeh, B.S. (2009). Study of the anti-inflammatory and analgesic effects of novel rigid benzofuran-3, 4-dihydroxy chalcone by formalin, hot-plate and, carrageenan tests in mice. *Pakistan Journal of Pharmaceutical Sciences*. 22(4), 395–401.

Idu, M., Uzoekwe, S. and Onyibe, H.I., (2007). Nutritional Evaluation of *Sterculia setigera* Seeds and Pod. *Pakistan Journal of Biological Sciences*. 11(1), 139–141.

Igoli JO, Ogarji. OG, Tor.-Anyim.TA, and Igoli.NP. (2005). Traditional medicine practice amongst the Igede people of Nigeria. Part II. *The African Journal of Complementary and Alternative Medicine*. 2(2), 134–152.

Islam, S., Shajib, M. S., and Ahmed, T. (2016). Antinociceptive effect of methanol extract of Celosia

cristata Linn. in mice. *BMC Complementary and Alternative Medicine*, *16*(1), 1–14.

Kayani, W.K., Dilshad, E., Ahmed, T., Ismail, H. and Mirza, B., (2016). Evaluation of *Ajuga bracteosa* for antioxidant, anti-inflammatory, analgesic, antidepressant and anticoagulant activities. *BMC Complementary and Alternative Medicine*. 16(1), 10-18.

Koster R, Anderson, D.B.E., (1959). Acetic acid for analgesic screening. *Fed Proc*. 18, 412–430.

Lorke, D. (1983). A New Approach to Practical Acute Toxicity Testing, *Archives of Toxicology.*54(4), 275-287.

Miller, L. and Tainter, M. (1944). Estimation of the ED50 and its error by means of logarithmic-probit graph paper. *Proceedings of the society for experimental biology and medicine.*, 57(2), 261–264.

Ren, K. and Dubner, R. (2002). Descending modulation in persistent pain. *Pain*, 100(2), 1–6.

Rosenblum, A., Marsch, L.A., Joseph, H. and Portenoy, R.K. (2008). Opioids and the treatment of chronic pain: controversies, status, and future directions. *Experimental and clinical psychopharmacology*. 16(5), 405–416.

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Schröder, W., Vry, J. De, Tzschentke, T.M., Jahnel, U. and Christoph, T. (2010). Differential contribution of opioid and noradrenergic mechanisms of tapentadol in rat models of nociceptive and neuropathic pain. *European Journal of Pain*. 14(8), 814–821.

Tor-Anyiin TA, Akpuaka MU and Oluma HOA. (2011). Phytochemical and antimicrobial studies on stem bark of *Sterculia setigera*, Del. *Afr. J, Biotechnol*. 10(53), 11011–11015.

Yin, Z.-Y., Li, L., Chu, S.-S., Sun, Q., Ma, Z.-L. and Gu, X.-P. (2016). Antinociceptive effects of dehydrocorydaline in mouse models of inflammatory pain involve the opioid receptor and inflammatory cytokines. *Scientific Reports*. 6(1), 14-21.

Zaruwa, M.Z., Ibok, Nne.I., Ibok, I.U., Onyenonachi, E.C., Danchal, C., Ahmed, A.G., Ahmed, M.U. and Sudi, I.Y. (2016). Effects of *Sterculia setigera* Del. Stem Bark Extract on Hematological and Biochemical Parameters of Wistar Rats. *Biochemistry Insights*. 9, BCI.S36143.

Zendehdel, M., Torabi, Z. and Hassanpour, S. (2015). Antinociceptive mechanisms of *Bunium persicum* essential oil in the mouse writhing test: Role of opioidergic and histaminergic systems. *Veterinarni Medicina*. 60(2), 63–70