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Antinociceptive activities of the methanolic extract of *Prosopis africana* (Guill and Perr) Taub. Fruits *in vivo*

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Abstract
Prosopis africana (Guill and Perr) Taub fruit methanolic extract was tested for its antinociceptive properties. 4.2% w/w dry extract yield was obtained after 48 hours of 80% methanol at room temperature during cold maceration. and occasional shake every two hours. It had a golden brown color, was sticky and odorous, and foamed as it dissolved in distilled water. In mice, the extract did not exhibit any acute hazardous effects in the studied dosage range (250-2000 mg/kg, i.p.). The maximum dose of 2000 mg/kg administered intravenously was well tolerated with just brief indications of drowsiness that persisted for three hours. With a potency equivalent to that of 2% lignocaine hydrochloride, desensitization of the guinea pig skin was demonstrated after intradermal injecting the extract at a 3 mg/ml concentration. The extract and lignocaine hydrochloride had computed slopes of 34.9 and 10.6, respectively. In contrast to previous dosages of the extract (500 and 1000 mg/kg), administration of the extract intraperitoneally (100 mg/kg) 30 minutes before sodium pentobarbitone (35 mg/kg i.p) significantly (p 0.05) decreased the sleeping period in mice. The writhings/contortions caused by acetic acid were considerably (p 0.05) and dose-dependently decreased by the intraperitoneal injection 30 minutes before the 0.7% acetic acid (10 ml/kg) was administered, doses of the extract (250, 500, and 1000 mg/kg) were administered. The extract's inhibitory impact (500 and 1000 mg/kg, i.p.) was equivalent to indomethacin's (10 mg/kg, i.p.) inhibitory effect. The findings of this research have validated the traditional usage of *Prosopis africana* for the relief of headaches, toothaches, and body aches.

Keywords: Antinociceptive, *Analgesic,* Methanolic extract, *Prosopis africana*

Introduction
The amount of time that mice slept was significantly (p 0.05) decreased when the extract (100 mg/kg) was administered intraperitoneally 30 minutes before sodium pentobarbitone (35 mg/kg i.p). However, the extract's other doses (500 and 1000 mg/kg) had no significant impact on the length of time that mice slept. The writhings and contortions caused by acetic acid were considerably (p 0.05) and dose-dependently decreased when the extract was given intraperitoneally at doses of 250, 500, and 1000 mg/kg 30 minutes before 0.7% acetic acid (10 ml/kg). The extract had an inhibitory action that was equivalent to indomethacin (10 mg/kg, i.p.) at 500 and 1000 mg/kg, intraperitoneally. The results of this study support the folklore usage of *Prosopis africana* to cure headaches, toothaches, and bodily aches. Prosopis are a member of the 50-genus Mimosaceae family. Only one species, *Prosopis africana* (Guill & Perr) Taub, formerly known as Prosopis oblonga (Benth), is indigenous to Africa, ranging from Senegal to Ethiopia. There are eight species in the genus Prosopis [9]. It has been used to cure toothache, anxiety, diarrhea, bronchitis, otitis, and bodily problems in Nigeria [13, 6]. In the middle belt of Nigeria, trypanosomosis in cattle is treated using its bark and root decoctions [33, 2, 5]. There are antibacterial properties in its leaf extract [18, 23]. To flavor soup and stew regionally, the seed is used as a fermented condiment [6]. The pods are fed to animals and used as fish poison [22].

The study's main objective was to investigate the antinociceptive qualities of the methanolic extract of fruits using *in vivo* models since different sections of *Prosopis africana* are utilized locally for the treatment of various illness conditions in humans and animals. Due to the lack of scientific techniques and occasionally exaggerated effects of their goods that are based on trial and error, folklore medicine requires this [30]. Most of the time, folk medicine ignores negative symptoms or harmful results [28].

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Materials and Methods
Plant Materials
Prosopis africana (Guill and Perr) Taub was discovered to be the species of ripe fruits in January 2007 on the grounds of the University of Agriculture in Makurdi, Benue State, Nigeria. It was placed in the hebarium as voucher specimen UAM/FHM 10. Using a pestle and mortar, the fruits were coarsely ground after being sun dried and dried in the sun. The mashed fruits were used in 500 g of a 48-hour cold maceration in 80% methanol with occasional shaking every two hours. An *in vitro* vacuum rotary evaporator was used to concentrate the extract.

Laboratory animals
Wistar albino adult mice, guinea pigs, and rabbits were used in the tests and were obtained from the University of Nigeria Nsukka's faculty of veterinary medicine and pharmaceutical sciences' experimental animal facilities. All of the animals had access to clean drinking water and were kept in plastic cages. as well as unlimited amounts of commercial producers mash to eat (Vital feeds Jos Nigeria). Green grasses were added to the rabbits' and guinea pigs' diets. The temperature in the room where the animals were housed fluctuated between 28 and 30 degrees, and there was between 12 and 14 hours of illumination each day.

Acute toxicity test in mice
Both male and female Wistar albino mice, weighing between 16 and 26 g, were divided into five groups, each having six mice. Different intraperitoneal dosages of the extract were administered to Groups I–IV (250 mg/kg, 500 mg/kg, 1000 mg/kg, and 2000 mg/kg, respectively) (i.p). Group V acted as the control group and received distilled water. The mice had unrestricted access to food and water, and they were monitored for 24 hours for toxic effects such as lethargy, dullness, excitability, depression, diarrhea, and death [20].

Local anaesthetic effect in guinea pig and rabbit
We employed both male and female guinea pigs (Cavia porcellus) weighing between 223 and 250 g. The guinea pigs' dorsums were shaved with a razor blade and left to rest for 24 hours. The intradermal administration of lignocaine hydrochloride (1 mg/ml and 0.3 mg/ml), a common local anesthetic, and two different doses of the extract (3 mg/ml and 1 mg/ml) were performed. The gradient was computed for the extract and lignocaine using a graph of log concentration against % anaesthesia [20].
White rabbits were also subjected to ocular reflex tests. While using one eye, the other one was employed for control. Two drops of the extract containing 0.3 mg/ml were injected into each eye using a cotton swab, and corneal, light, and pupillary responses were measured using a pupilometer and torch light

[26]. The extract's concentration was raised to 1.0 mg/ml.

Effect on sodium pentobarbitone sleeping time in mice
Twenty Wistar albino mice, Both sexes were divided into four groups of five mice each at random weighing between 16 and 39 g. Only sodium pentobarbitone 35 mg/kg intraperitoneally was administered to Group I. (i.p). 30 minutes before to the intraperitoneal injection of 35 mg/kg of sodium pentobarbitone, the extract was administered to Groups II–IV at doses of 100 mg/kg, 500 mg/kg, and 1000 mg/kg, respectively (i.p.). The mice were watched as they slept, from the point at which the righting reflex was lost and the mouse was laying on its dorsal recumbency until the point at which the animal returned to its regular posture on the ventral recumbency and resisted turning upside down. For each group, the times for each mouse were recorded, represented as means and standard errors of means (SEM), and then one-way analysis of variance was applied (ANOVA). P 0.05 was used to evaluate significance.

Analgesic effect in mice
Thirty-five Wistar albino mice were split into five groups of seven animals each at random. weighing between 13 and 20 g. Acetic acid 0.7% at 10 ml/kg intraperitoneally was administered exclusively to Group I as a negative control (i.p). Within 30 minutes of receiving 10 ml/kg of 0.7% acetic acid intravenously, Groups II–IV received extract doses of 250 mg/kg, 500 mg/kg, and 1000 mg/kg, respectively. Indomethacin 10 mg/kg i.p. was administered to Group V as a positive control, 30 minutes before 10 ml/kg, 0.7% acetic acid, was administered intravenously [16]. The extract and medication administration times were recorded. For each group, the beginning of the reaction (writings or contortions) and the total number of contortions throughout the course of 30 minutes per mouse were recorded, quantitatively assessed using one way analysis of variance and provided as a mean with standard error of the mean (SEM) (ANOVA). LSD was used to compare the means of the two groups using Microsoft SPSS. The cutoff for significance was 0.05.
All the animals used for the experiments were subjected to minimum pains and distress [21].

Results
The yield of the methanolic extract of *Prosopis africana* fruits (MEPAF) was 4.2% weight to weight. It was golden brown, viscous, and had a pleasant, fragrant smell. In distilled water it dissolved, but not in 5% Tween-20. Even at the maximum dose (2000 mg/kg i.p.) administered over the course of 24 hours, no deaths were reported. The extract had a local anesthetic effect in the guinea pig wheal test but not in the rabbit ocular reflex test. (Fig 1, Tables 1 and 2).

Table 1: Ocular reflex Tests with MEPAF in Rabbit

Extract	Response	Time in minutes	Result	
			Test	Control
MEPAF (crude) 0.3mg/ml	Corneal reflex	0	Normal	Normal
		15	"	"
		30	"	"
		45	"	"
		60	"	"
MEPAF (crude) 0.3mg/ml	Conjunctival blood vessels	0	Normal	Normal
		15	"	,,
		30	"	,,
		45	"	,,

		60	”		,,	
			Before	**After**	**Before**	**After**
MEPAF 0.3mg/ml	Light reflex (pupil size in mm)	0	7.0	(3.75)	7.0	(3.75)
		15	7.0	(3.75)	7.0	(3.75)
		30	7.0	(3.75)	7.0	(3.75)
		45	7.0	(3.75)	7.0	(3.75)
		60	7.0	(3.75)	7.0	(3.75)

At 100 mg/kg body weight, the extract significantly ($p<0.05$) decreased the Na-Pentobarbitone induced sleeping time in mice but did not significantly alter the sleeping time at higher doses (Fig 2 Table 2). The acetic acid-induced writhing reflex in mice was significantly (P 0.05) reduced by all dosages of the extract. The inhibition at higher doses (500-1000 mg/kg body weight) was comparable to indomethacin at 10 mg/kg body weight (Fig 3 Table 3).

Table 2: Effect of MEPAF on acetic acid induced contortions/writhings in mice

Group	No of mice	Dose of MEPAF (mg/kg, i.p)	Dose of acetic acid (0.7% 10 ml/kg, i.p)	No of contortions in 30 min/gp	Mean ± SE (min)
I	7	Nil	10	396	56.6 ± 2.2
II	7	250	10	257	36.7 ± 4.4a
III	7	500	10	174	24.9 ± 2.7b
IV	7	1000	10	148	21.1 ± 0.7b
V	7	10	10	157	22.4 ± 3.96

a = significant decrease at $p< 0.05$ when compared with the control group, but significant ($p<0.001$) increase with other treated groups
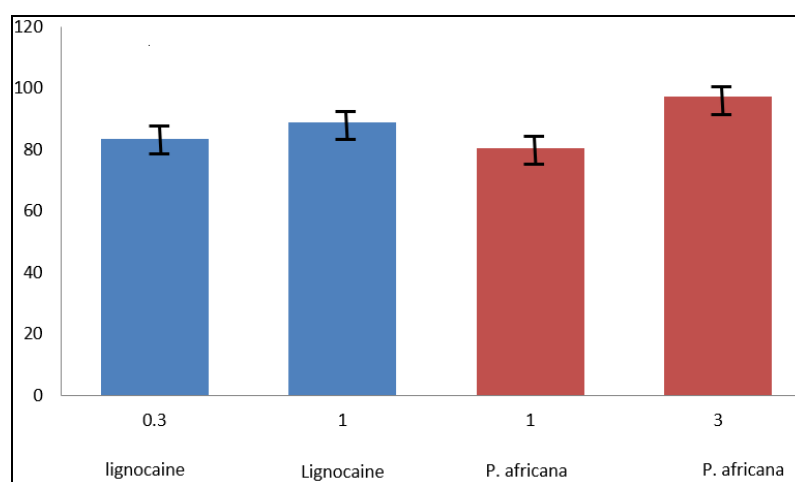b = significant decrease at $p<0.05$ when compared with control and group II

Fig 1: Percentage Anesthesia of Lignocaine and P. Africana at different concentrations
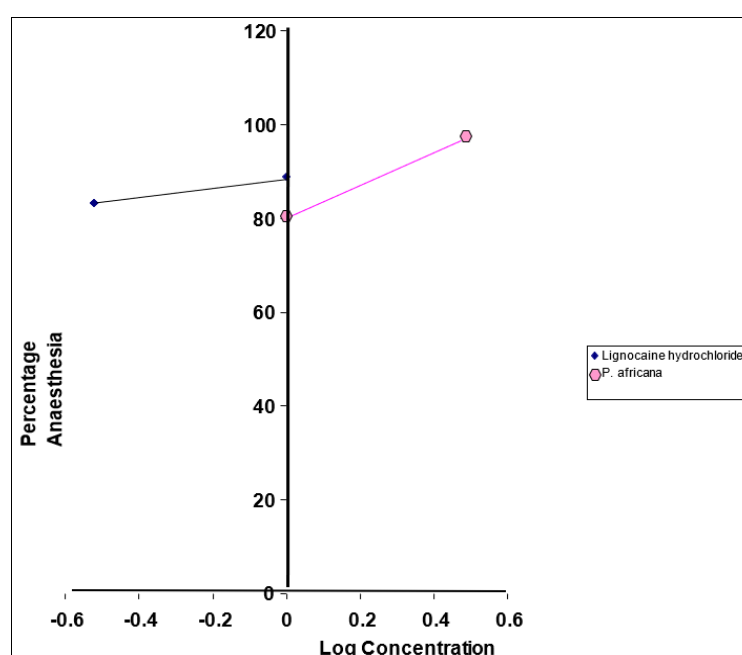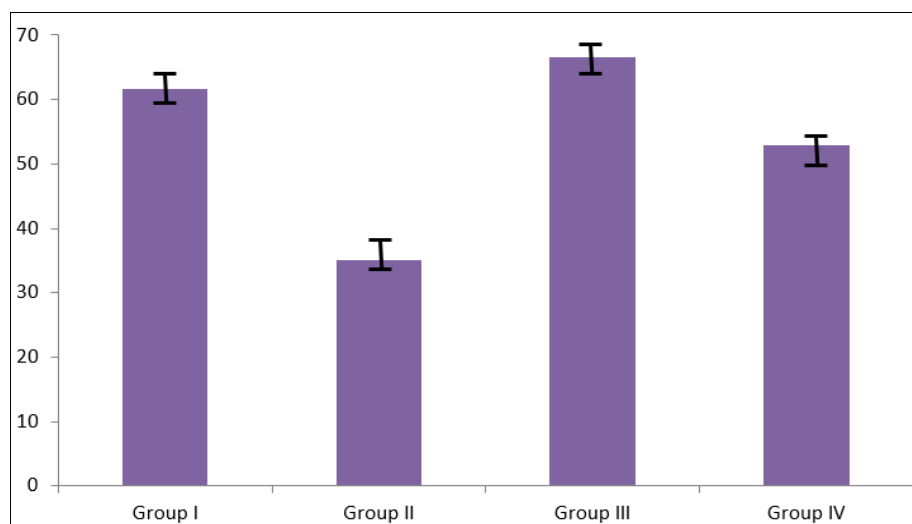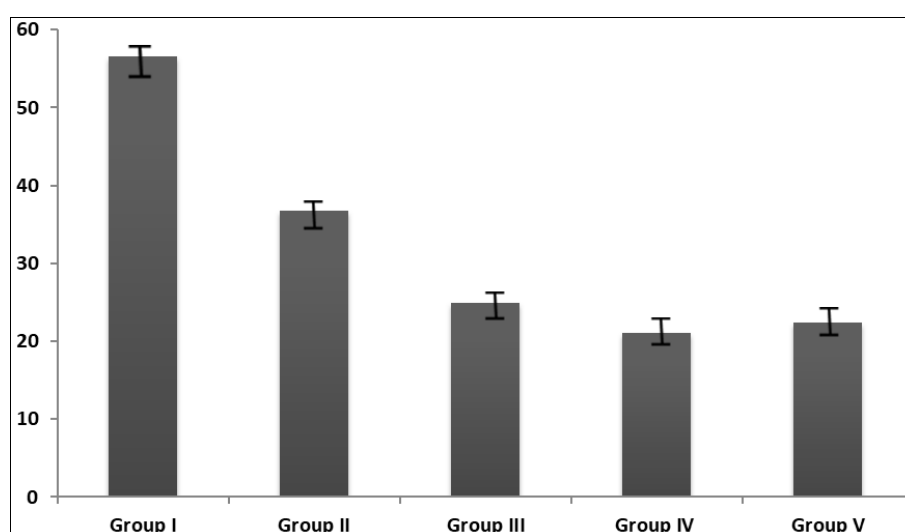

Fig 2: Graph showing the slopes of P. Africana, and lignocaine hydrochloride

Key: Group I control distilled water: Group II 100 mg/kg, Group III 500mg/kg, Group IV 1000 mg/kg

Fig 3: Effect on sodium pentobarbitone sleeping time in mice

Key: Group I Control, distilled water, Group II 100 mg/kg, Group III 500 mg/kg, Group IV 1000 mg/kg, Group V indomethacine 10mg/ml

Fig 4: Effect on acetic acid induced writhings in mice

Discussion

When compared to lignocaine hydrochloride, a local anesthetic drug, the methanolic extract of *Prosopis africana* fruits (MEPAF) demonstrated effective desensitization of the guinea pig skin at a dosage of 3 mg/ml (Fig. 1). MEPAF's computed slope was 34.9, compared to lignocaine hydrochloride's 10.6 (Fig 2). Lignocaine hydrochloride's duration of action was likewise comparable. This matters in clinical practice [5]. This demonstrates that MEPAF, while being in a primitive form, has considerable potential to be a therapeutically beneficial local anesthetic. Receptor sites inside the sodium channel pore and block ion transport. This inhibits a significant rise in the excitable membrane's permeability to sodium ion. The direct interaction with voltage-gated sodium channels is what causes this effect [10, 8, 11]. Numerous professionals have said that local anesthetics function by blocking sodium channels [27, 10, 31, 8, 24, 11]. Voltage-gated sodium channels, which are vital for electrogenesis and the transmission of nerve impulses, are targets for essential therapeutically helpful analgesics like lidocaine [31, 24, 11].

Sodium channels with specific properties occur as isomers [31, 24, 11, 34] for a variety of circumstances. With sodium channel blockers, a number of pain conditions can be managed. There are tetrodotoxin-sensitive (TTX-S) and tetrodotoxin-resistant (TTX-R) sodium channels, and tetrodotoxin, a puffer fish poison, works similarly to local anesthetics [11, 3]. The action of these mediators is modulated by the administration of prostaglandin E2 (PGE2), serotonin, and carrageenan, among other acute inflammatory mediators.

Fish are stunned with the aid of local anesthetics [22]. Tropical Africa uses the stem bark and fruits (pod) of *Prosopis africana* to stun fish [22, 14]. Because there are saponins present, this substance has a more harmful effect on fish and other cold-blooded creatures [36]. Therefore, MEPAF may be regarded as harmless or having minimal acute toxicity in mammals, meaning that humans who consume fish that has been stunned by it do not run the risk of becoming ill. The acute toxicity test in mice, which revealed no mortality even at the maximum dose (2000 mg/kg, i.p.), supports MEPAF's safety. The mice only displayed brief dullness that persisted for three hours. This result is consistent with some of the preceding results [1, 36, 17, 5, 6].

The mice only displayed temporary dullness that persisted for three hours. This result is consistent with some of the preceding results [1, 36, 17, 5, 6].

The length of mice's sleep-induced by sodium pentobarbitone was influenced by the intraperitoneal injection of MEPAF (Fig 3, Table 1). When compared to the group of mice treated with sodium pentobarbitone alone, the mice given 100 mg/kg of MEPAF had significantly less time to sleep. Additionally, it was noted that there was no discernible difference in sleep duration between the mouse groups given 500 mg and 1000 mg/kg of sodium pentobarbitone and the control group given 35 mg/kg. This behavior demonstrates that, at 100 mg/kg, MEPAF significantly reduced the amount of time spent asleep rather than potentiating the sleep brought on by sodium pentobarbitone.

When compared to the negative control, it was shown that all of the MEPAF dosages of 250, 500, and 1000 mg/kg significantly suppressed or reduced the writhings or contortions caused by acetic acid (Table 2, Fig 4). The number of writhings was significantly reduced from an average of 57 in the negative control to 37 when MEPAF at 250 mg/kg was given 30 minutes before acetic acid. This represents a 35% inhibition. Giving 500 mg/kg and 1000 mg/kg to the group resulted in substantial reductions in 56 and 63%, respectively (table 4). Indomethacin (10 mg/kg) caused a 61% inhibition, which is equivalent to MEPAF at 500 and 1000 mg/kg. MEPAF generally suppressed the acetic acid-induced writhings in a dose-dependent manner similar to that of indomethacin at higher dosages of 500 and 1000 mg/kg. Endogenous mediators such as prostaglandins, bradykinins, serotonin, histamine, nitric acid, and glutamate are involved in the mediation of acetic acid-induced pain [19, 4]. This indicates that MEPAF may exert its effects via preventing the production of prostaglandins or other endogenous mediators. Most non-steroidal anti-inflammatory medicines (NSAID), which are cyclooxygenase (cox) inhibitors, may work in this way [15, 8, 19, 4, 12].

Experiments using the extract on the eyes had no positive effects. The autonomic nervous system is often affected by local anesthetics in a qualitative, "all or none," fashion [26].

The ocular blood vessels and eye reflex were unaltered in MEPAF. Its concentrations were increased to 1.0 mg/ml, however this had no impact on the ocular reflexes.

Conclusion
Conclusion: Given its high degree of safety in mice, even in its crude form, the methanolic extract of *Prosopis africana* fruits [MEPAF] employed in this study indicates that it has tremendous potential to be a therapeutically effective local anaesthetic. Therefore, MEPAF can be characterized as safe or having minimal acute toxicity in animals. MEPAF exhibited powerful analgesic action in mice that was dose-dependent and equivalent to indomethacin at 10 ml/Kg, reduced Na-pentobarbitone-induced sleeping duration in mice at 100 mg/kg, and had good local anaesthetic effect in the guinea pig. The acute toxicity test in mice, which revealed no mortality even at the maximum dose (2000 mg/kg, i.p.), supports MEPAF's safety. This behavior demonstrates that, at 100 mg/kg, MEPAF dramatically reduced the amount of time spent asleep rather than potentiating the sleep brought on by sodium pentobarbitone.

Prosopis africana has a pharmacological foundation for its traditional uses, which include the stunning of fish, the treatment of body pain, and the management of various inflammatory disorders.

Acknowledgement

Ethical statement
The authors attest that the journal's aesthetic standards, as listed on the author guidelines page, have been followed and that the necessary ethical review committee permission has been obtained. According to the authors, they adhere to US guidelines for the protection of animals used in research.

References
1. Adikwu MU. Physico-chemical properties and pharmaceutical application of *Prosopis africana* (Guill & Perr) Taub. Gum. Ph.D. Thesis Department of Pharmaceutics University of Nigeria, Nsukka (unpublished); c1994.
2. Atawodi SE, Ameh DA, Ibrahim S, Andrew JN, Nzelibe HC, Onyika EO, *et al*., Indegenous Knowledge system

for treatment of Trypanosomosis in Kaduna State of Nigeria. Journal of Ethnopharmacology. 2002;79(2):279-282.

3. Black JA, Lius Tanaka M, Cummins TR, Waxman SG. Changes in the expression of tetrodotoxin-sensitive sodium channels within dorsal root ganglia neurons in inflammatory pain, International Journal of Gastroenterology (Digestion). 2008;77(2):20-28. black@yale.edu.

4. Bose A, Mondal S, Gupta JK, Gosh T, Dash GK, Si S, *et al*. Analgesic, anti-inflamatory and antipyretic activities of the ethanolic extract and its fractions. Cleome Rutidosperma Fitoterapia. 2007;78(7-8):515-520.

5. Bosha J, Asuzu I. Pharmacological activities of *Prosopis africana* Fruits. Lambert Academic Publishing. 2011;1-12:40-45.

6. Bosha JA, Asuzu IU, Onyeyili PA. Pharmacological effects of a Fraction of The Methanolic Extract of *Prosopis africana* Fruit (Guill and Perr) Taub. Nigerian Veterinary Journal. 2010;31(3):235-241.

7. Lal K, Bhojak N. Adsorbents for removal of hazardous organic substance from waste water and natural water samples: A review. Int. J Adv. Chem. Res. 2021;3(2):17-19. DOI: 10.33545/26646781.2021.v3.i2a.37

8. Budsberg CS. Pain management. In: Textbook of Veterinary Internal Medicine. Diseases of the dog and cat 6th ed. Ettinger, J. S., and E. C. Feldman, (Eds.), Elsevier Saunders. 2005;1:22-24.

9. Burkil HM. The useful plants of West Africa. 3rd ed. Royal botanic gardens Kew. 1994;4:751-753.

10. Catterall W, Mackie K. Local anaesthetics. In: Goodman and Gilman's The pharmacological basis of therapeutics. 10th ed. Hardman, J. G., and L. E. Limbird, McGraw-Hill; c2001. p. 367-380.

11. Cummins TR, Sheet PL, Waxman SG. The roles of sodium channels in nociceptoin: Implications for Mechanisms of pain. Pain. 2007;131(3):243-257.

12. Doucet MY, Bertone AL, Hendrickson D, Huges F, MacAllister C, McClure S, *et al*. Comparison of efficacy and safety of paste formulations of Firocoxib and Phenylbutazone in horses with naturally occurring osteoarthritis. JAVMA. 2008;232(1):91-97.

13. Iwu MM. Handbook of African Medicinal Plants. CRC Press Boca Raton. c1993.p.48.

14. Jonathan GC, Robert AB, Stephen GW, Noel LO. Naturally Occuring Fish Poisons from Plants. Journal of Chemical Education. 2004;81(10):1457-1461.

15. Khan E. Pain management. In: Khan (Ed.). Mercks Veterinary Manual 9th ed; c2005. p. 1690-1705.

16. Koster R, Anderson M, Debeer EJ. Acetic Acid for Analgesic Screening. Federation Proceedings; c1959. p. 18-124.

17. Lorke D. A new approach to practical acute toxicity testing. Archives of Toxicology. 1983;54:275-287.

18. Makinde AA. Ethnoveterinary Medicine Research and Development. The Vom experience and future directions In: Ethnoveterinary Practice Research and Development. Gefu, J. O., P.A. Abdu, and C. B. Alawa. (Eds.); c2000. p. 138-153.

19. Marchioro M, Blank MA, Mourao RVH, Antoniolli AA. Antinociceptive activity of the aqueous extract of Erythrina velutina leaves. Fitoterapia. 2005;76(7-8):637-642.

20. Mcleod LJ. Pharmacological experiments on intact preparations. Churchill Livingstone, Edinburg; c1970. p. 34-36.

21. National Research Council. Institute of laboratory Animal Resources, commission on life sciences. National Research Council Guide for the care and use of laboratory animals Washington, D.C. National Academy Press. c1996. p. 46.

22. Neuwinger HD. Plants used for poison fishing in Tropical Africa. Toxicon. 2004;44(4):417 -430.

23. Ojo OO, Nadro MS, Tella IO. Protection of rats by extracts of some common Nigerian Trees against acetaminophen induced hepatotoxicity. African Journal of Biotechnology. 2006;5(9):755-760.

24. Rogers M, Tang L, Madge DJ, Stevens EB. The role of sodium channels in neuropathic pain. Semin. Cell & Developmental Biology. 2006;17(5):571-81

25. Saganuwan AS. Tropical plants of antihypertensive, antiasthmatic and antidiabetic value. Journal of Herbs, Spices and Medicinal Plants. 2009;15(1):24-44

26. Shetty SN, Anika SM, Asuzu IU, Anaga AO. Laboratory Manual of Pharmacology and Toxicology. 2nd ed. Fourth Dimension Publishing Co. Ltd. Enugu, Nigeria; c2010. p. 41-52.

27. Sindrup HS, Jensen TS. Efficacy of pharmacological treatments of neuropathic pain; an update and effect related to Mechanism of drug action. Pain. 1999;83(3):389-400.

28. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. 3rd Ed. Spectrum Books. Ibadan. c2006. p. 1-150.

29. Trease EC, Evans WC. Pharmacognosy. 16th ed. Saunders Elsevier, Edinburg. c2009. p. 365-650.

30. Wambebe C. Biological Standardiztion of Herbal Remedies. In: Proceedings of an International Wrokshop on Standardization and Utilization of Herbal Medicines: Challenges of 21st Century. Moody, J.O., E. A. Ajaiyeoba, J. O. Adeboye, and O. O. Ogundipe. (Eds.); c1999. p. 5-18

31. Wood JN, Boorman JP, Okuse K, Baker MD. Voltage-gated sodium channels and pain pathways. Journal of Neurobiol. 2004;61(1):55-71. or j.wood.@ucl.ac.uk.

32. World Health Organization (WHO) Bulletin. Plants and Health for all: First symposium of the WHO on Plants and Health, Kobe, Japan; c1991.

33. Zachariya TS, Kapu SD, Nwinyi FC, Barki T, Gamaniel KS. Medicinal Plants used in the treatment of Animal Trypanosoomiasis by Fulani Herdmen in Abuja Municipal Area Council Nigeria: In: Ethnoveterinary Practice Research and Development International Workshop; c2000. p. 120-128.

34. Zhao P, Barr TP, Hou Q, Dib-Hajj SD, Black JA, Albrecht PJ, *et al*. Voltage-gated sodium channel expression in rat and human epidermal keratinocytes: evidence for a role in pain. Pain. 2008;139(1):90-105.

35. Zimmermann M. Ethical guidelines for investigations of Experimental pain in Conscious Animals. Pain. 1983;16(2):109-110.

36. Bruneton J. Pharmacognosy, phytochemistry, medicinal plants. 2nd ed. Lavoisier publishing, New York; c1994. p. 201-880.