PAPER

Pharmacological Effects of a Fraction of The Methanolic Extract of *Prosopis Africana* Fruit (Guill and Perr) Taub

[1]BOSHA, J.A*., [2]ASUZU, I.U .AND [1]ONYEYILI,P.A.

Department of Veterinary Physiology & Pharmacology, University of Agriculture, Makurdi, Benue State. [2]Department of Veterinary Physiology & Pharmacology, University of Nigeria, Nsukka, Enugu State. *Corresponding author: boshajoel@yahoo.com; Phone number: +2348055855125

SUMMARY

The pharmacological effects of some fractions of the Prosopis africana fruits (MEPAF) were investigated. The fractions were obtained by subjecting the MEPAF to accelerated gradient column and Thin-Layer chromatography (TLC). Nine (9) fractions were obtained based on their retention factor (R_f). One of the fractions, fraction G was selected for further studies based on its better local anaesthetic activity using the guinea pig wheal test. The MEPAF did not show any measurable effect on the ocular reflexes and isolated frog rectus abdominis muscles but showed both concentration and time-dependent inhibitions of the intrinsic peristaltic contractions of the rabbit jejunum. It also induced concentration-dependent inhibitions of the contractions induced by acetylcholine (2.5 µg/ml) on isolated rabbit jejunum. These inhibitions are thought to be anti-muscarinic or atropine-like, since the contractions of the jejunal segment is believed to be through muscarinic receptors and could be blocked by atropine and related compounds . Phytochemical spot tests of the fraction G revealed the presence of alkaloids, carbohydrates, saponins, tannins, sterol, terpenes but not flavonoids, polyuronoids and reducing sugar. These findings give credence to the folkloric use of Propsopis africana in the treatment of body pain, toothache and dysentery.

Keywords: Propsopis africana, methanolic extracts, anaesthetics, antiperistaltic, antidiarhoea.

INTRODUCTION

There is a worldwide belief that herbal remedies are safer and less damaging to the human body than synthetic drugs (Williamson *et al*, 1966*;* Murray *et al.,* 2000*;* Schneider; 2004; Valtuena, 2004). They are also cheaper to produce and affordable (Oliver- Bever, 1986; Abdu *et al.,* 2000*),* and are more readily available (Gefu, 2000; Smith 2000; Ghotge and Ramdas 2000). In Nigeria *Prosopis africana* is used to treat body pains, anxiety and toothache (Iwu, 1993; Adikwu, 1994; Okoye, 1999). *Prosopis africana* is a woody tree found commonly in the savannah region of Africa extending from Senegal to Ethiopia. In Nigeria it is found in the middle belt and Enugu state (Keay *et al.,* 1964; Adikwu, 1994). It grows up to 40.60 ft (18-28 m) high with pale drooping foliage and very hard wood. It is known by various local names such as 'Kiriya' (Hausa), 'kohi' (Fulani), 'Sanchilati' (Nupe), 'Gbaaye' (Tiv), 'Ayan' (Yoruba), 'Ubwa' (Ibo), 'Okpehe' (Idoma) (Hutchinson and Dalziel, 1958; Keay *et al.,* 1964; Agishi, 2004)**.** Of many local herbs used in folkloric medicine for treatment of diseases in Nigeria, only very few have been properly identified and documented (Ibrahim, 1984). Out of these, only a very small proportion has been subjected to verification, hence their efficacy still remains in doubt. The present study is aimed at conducting a scientific investigation into the pharmacological activities of the fruit of *Prosopis africana* using both *in vivo* and *in vitro* models. Screening for plant activity based on folkloric use is the best method of approach to new drugs discovery (Sofowora, 2006).

MATERIALS AND METHODS

Sample collection and Identification

The ripe fruits of P. *africana* were collected from the premises of the University of Agriculture, Makurdi, in Makurdi Local Government Area of Benue State, Nigeria, in January, 2007 and were identified by a taxonomist, Mr. Ekuno of the Forestry Department, University of Agriculture,

Makurdi. A sample specimen was deposited in the forestry herbarium with voucher number UAM/FHM 10. They were dried in the sun as described by Pamplona-Rogers, (2004). The dried fruit was subsequently pulverized using pestle and mortar

Preparation of extract

Five hundred (500 g) of the dried and pulverized fruits were extracted with 800 ml of 80% methanol, by cold maceration at room temperature (25°C-28°C), with intermittent shaking for 48hr. Whatman filter paper No1 was used to filter the methanolic extract, which was evaporated to dryness using vacuum rotary evaporator.

Accelerated gradient chromatography (AGC)

The crude extract of the methanolic extract was subjected to accelerated gradient chromatography (AGC) to separate the components into various fractions. About 10 g of the sample was used for the study using chloroform, ethylacetate and methanol as the solvent system in various combination (Stahl, 1969). All the fractions collected from the separation were spotted on TLC plates coated with silica gel as the stationary phase. The solvent system used for this purpose was chloroform, ethylacetate and methanol in the ratio (1:3:2). From the result of the TLC, similar fractions were recombined.

Animals and treatment

Guinea pigs (*Carvia porcellus)* and rabbits (Oryctolagus cuniculus) were obtained from the laboratory animal facilities of the Faculties of Veterinary Medicine and Pharmaceutical Sciences, University of Nigeria, Nsukka. All the animals were kept in plastic cages and were supplied with clean drinking water and feed was provided *ad libitum*. Feeding was with standard commercial growers mash, (Vital Feeds Jos, Nigeria). The guinea pigs and the rabbit's feeds were also complemented with grasses. Ethical conditions governing the conduct of experiments with life animals were strictly observed (Ward and Elsea, 1977; Zimmennan, 1983; NRC, 1996).

Frogs (*Rana pipiens)* used in this study were obtained from the water tank at Nkrumah Hall, University of Nigeria, Nsukka.

Local anaesthetic and ocular effects of some fractions of the extract

Local anaesthetic effects of fractions code-named B, E and G were carried out in guinea pigs using the wheal test method as described by McLeod, (1970). The effects of the extract fraction on ocular reflexes (corneal, conjunctiva and pupilary) were studied following instillation of the extracts into the eye in rabbits as described by McLeod, (1970).

Reactivity of rabbit's jejunal segments to the G fraction of the extract

Fraction G showed better local anaesthetic activity in the guinea pig and was selected for further studies. A white rabbit, about 1 kg was used for the study. The animal was fasted for 24hr prior to the study. The rabbit was humanely sacrificed and thereafter dissected and the jejunum removed. Segments of the jejunum, about 2-3 cm in length, were cleared of faecal materials with Tyrode's solution. The jejunal preparations were then set up in an organ bath containing Tyrode's solution and aerated with 95% O_2 and 5% CO_2 from oxygen cylinder. Each segment was connected to isotonic myograph transducer and the changes in muscle length recorded on a biography recorder. Varying concentrations (30, 60, 120 and 240 μg/ml) of *Prosopis africana* extract fraction G were added to the organ bath after equilibration time of 30 min. A contact time of 90 sec was allowed for the tissue response. Thereafter, the tissue was washed and the fluid in the bath replaced to allow the tissue to regain its original tone. The procedure was repeated for each concentration of the extract according to Perry, (1970).

The effect of extract fraction G on acetylcholine (Ach) induced contractions of rabbit jejunal segments were also studied using 2.5 μg/ml of Ach and varying concentrations of the extract fraction. The amplitude of contractions were measured

by standard methods and the degree of effect expressed in percentage.

Effect of the extract fractions on Rectus abdominis muscle of the frog

Rectus abdominis muscles of the frog were obtained following humane sacrifice of the animal. The method of Perry (1970) was used. Two to three centimetres (2-3cm) of the muscle was suspended in an organ bath containing Kreb's solution which was also aerated with 95% o_2 and 5% Co_2. A concentration of 2.5 μg/ml Ach was used with different concentrations of the fraction (30, 60, 120 and 240 μg/ml) in the organ bath.

Phytochemical analysis

Phytochemical spot test were carried out as described by Harbone (1991) and Trease and Evans, (1996). The phytochemical study was carried out with the fraction G of the extract. This phytochemical screening was for identification of carbohydrates, tannins, saponins, alkaloids, sterols, terpenes, flavonoids, reducing sugar and polyuronoids.

RESULTS

The results of the accelerated gradient column chromatography analysis showed that the crude extract of *Prosopis africana* fruit in methanol (MEPAF) was separated into several fractions. The TLC result of these fractions showed the presence of nine fractions on the bases of spots with similar retention factor (R_f). These fractions that had same R_f values were grouped as F_A-F_I (Table 1). Fraction I had the highest volume followed by G and H, while the quantity of fraction A was the least. Following concentration, fraction E had the highest extract concentration followed by fraction D, while fraction G was the third. Fraction A was observed to have completely evaporated without extract present (Table I).

TABLE I: Fractions obtained from column chromatography of MEPAF

No of test tubes	Volume (ml)	Group	Final weight (mg)
1-20	3	A	00
20-30	5	B	10
31-51	90	C	230
52-59	73	D	890
60-76	100	E	4890
77-80	37	F	390
81-100	160	G	730
101-120	150	H	140
121-255	800	I	200

The three (3) fractions (B, E and G) tested showed some local anaesthetic activities. Fractions G, B, and E showed 38.9, 30.6 and 27.8% local anaesthetic effects respectively at 0.6mg/ml (Table i). Fraction G had the best anaesthetic effect. The three fractions G, B and E produced better anaesthesia than lignocaine hydrochloride, a standard anaesthetic drug.

TABLE II: Local anaesthetic effects of some fractions of the extract of Prosopis africana

FR	Conc.	Duration of activity (seconds)							T	%AN
	(mg/ml)	0	5	10	15	20	25	30		
B	0.6	0/6	3/6	1/6	1/6	2/6	2/6	2/6	11/36	30.6
	0.2	0/6	0/6	1/6	0/6	0/6	0/6	1/6	2/36	5.6
E	0.6	0/6	3/6	0/6	1/6	3/6	1/6	2/6	10/36	27.8
	0.2	0/6	0/6	0/6	0/6	0/6	0/6	1/6	1/36	2.8
G	0.6	0/6	6/6	4/6	2/6	0/6	1/6	1/6	14/36	38.9
	0.2	0/6	0/6	3/6	0/6	0/6	0/6	0/6	3/36	8.3
LH	0.1	0/6	2/6	0/6	0/6	0/6	1/6	1/6	4/36	11.1
	0.3	0/6	0/6	0/6	0/6	0/6	1/6	1/6	2/36	5.6

FR, fractions of mepaf; LH, lignocaine hydrochloride; T, total; AN, anaesthesia

The instillation of the fraction G of the methanol extract of Prosopis africana in to the eye of the rabbits did not alter the ocular (corneal, conjunctival and pupilary) reflexes studied. Fraction G of the extract induced concentration-dependent inhibitions of peristaltic and Ach-induced contraction of rabbit jejunum. The inhibition of the peristaltic movement of the jejunum was concentration and time-dependent (Fig 1). The inhibition increased with increase in concentration as well as increase in time. At 30 μg/ml the maximal percentage inhibition was 19.1% while values of 29.5, 37.5 and 55.0% were obtained at 60, 120 and 240 μg/ml extract concentrations respectively (Table III).

TABLE III: Effect of G fraction of the methanol extract on the reactivity of the rabbit jejunal segments.

Extract Conc.	% inhibition/ Time (seconds)							
(µg/ml)	0	1	15	30	45	60	75	90
30	0	0	5.4	8.9	8.9	12.5	15.9	19.1
60	0	6.3	6.3	10.4	10.4	20.0	25.1	29.5
120	0	12.5	12.5	25.0	25.0	31.3	35.4	37.5
240	0	15.0	32.5	32.5	47.5	52.5	52.5	55.0

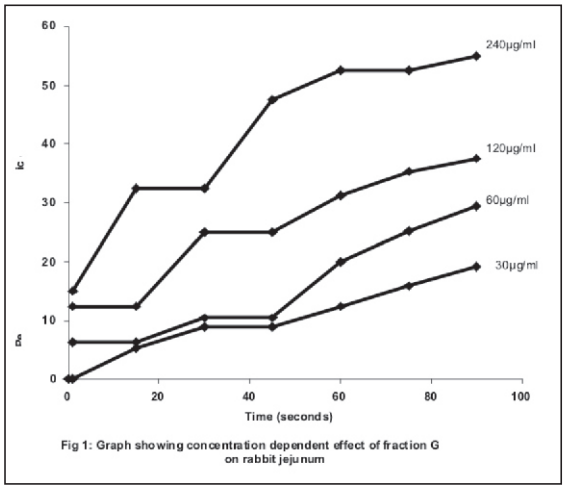

Fig 1: Graph showing concentration dependent effect of fraction G on rabbit jejunum

Treatment with Ach (2.5 mg/ml) in the presence of fraction G without washing resulted in a concentration-dependent percentage inhibition of Ach-induced contractions (Fig. 2). The 30 μg/ml of the Fraction G reduced the Ach induced contractions of the rabbit jejunum by 8.6%, while at 240 μg/ml the extract decreased the rabbit jejunum induced contraction by 44%.

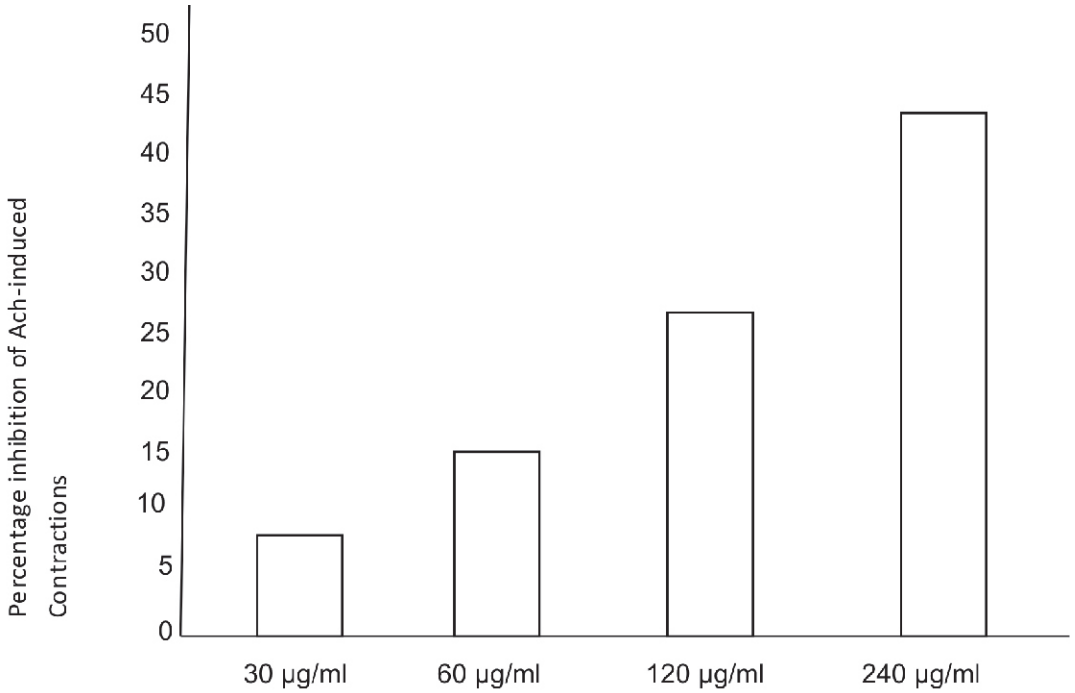

Fig. 2: Effect of fraction G of methanolic extrac of *Proosopis africana* on Ach- induced contraction of rabbit jejunum.

Administration of various concentrations of fraction G methanol extract (30, 60, 120 and 240 μg/ml) did not show any measurable activity on the frog rectus abdominis muscle. Phytochemical analysis of the fraction showed that fraction G contains alkaloids, saponins, tannins, sterols, terpenes and carbohydrates. There was complete absence of flavonoids, polyuronoids and reducing sugar (Table IV)

TABLE IV: Phytochemistry of the G fraction of the methanolic extract of Prosopis africana

Chemical constituent	Test	Concentration
Alkaloids	Dragendorff's	+++
	Wagner's	++
	Mayer's	+
Saponins	Emulsifying	+
Tannins	Ferric chloride	++
Carbohydrates	Molisch's	++
Reducing sugars	Fehling's	_
Flavonoids	Ammonia	_
Polyuronoids	Ethanol	+++
Sterols & Terpenes	Acetic anhydride	+++

- , absent; +, low concentration; ++, moderate concentration; +++, high concentration

DISCUSSION

The accelerated gradient column chromatography analysis resulted in the separation of the methanol extract into several fractions, about two hundred and fifty five test tubes of ten ml each. The result of the TLC of these fractions allowed for the recombination of these fractions into nine (9) making use of the retention factor (R_f). Local anaesthetic study indicates that the three methanol extract fractions (B, E and G) tested contained some pharmacological principles, which have action on the peripheral nerves observed as local

anaesthesia in guinea pigs. The local anaesthetic effect lasted for over 30 min, which is a significant observation especially at the highest concentration of 0.6 mg/ml of the extract. The duration of local anaesthetic noticed in this study appeared to be similar to that of lignocaine hydrochloride (Lawrence, et al 1997), but the percentage anaesthesia was higher in the fraction. The model has been used in laboratory animals to evaluate the local anaesthetic effect of drugs (Shetty and Anika, 1982). Phytochemical analysis showed the presence of alkaloid in the fraction G of the methanol extract. It is therefore possible that the local anaesthetic effect observed in the study may be attributed to the alkaloid acting alone or in combination with other constituents in the extract, since alkaloids have varying clinical uses (Olaniyi, 2000).

The contractile responses of the normal isolated jejunal segments and those treated with Ach were dose-dependently inhibited by fraction G of the methanol extract of *Prosopis africana* fruit. The contraction of the jejunal segment by Ach is believed to be through the muscarinic receptors which could be blocked by atropine and related compounds (Sanni *et al*, 2005). The G fraction of the *Prosopis africana* may contain compounds, which may be acting as antagonists of muscarinic receptors, since it inhibited Ach-induced jejunal contraction in the present study. The inhibitory effect of the above named extract fraction on Ach-induced gastrointestinal tract (GIT) motility could be exploited in the treatment of non-bacterial diarrhoea especially in the presence of tannins in the extract. Tannic acid, an astringent of the GIT is a constituent of many commercial anti-diarrhoea compounds (Brander and Pugh, 1992).

Finally, the use of the methanol extract of *Prosopis africana* in folk medicine has a positive correlation with scientific data since its intradermal administration has shown significant local anaesthetic activity in guinea pigs. It also produced a significant inhibitory effect on normal and Ach treated isolated jejunal segments. Further studies to isolate and identify the active principle as well as determine the mechanism(s) of action are now in progress.

ACKNOWLEDGEMENT

The first author acknowledges the support of the University of Agriculture Makurdi for granting him study leave to conduct the research.

REFERENCES

ABDU, P. A., JAGUN, A. G; GEFU, J. O; MOHAMMED, A. K; ALAWA C. B. I and OMOKANYA, A. T. (2000): A survey of ethnoveterinary practices of agro-pastoralists in Nigeria. In: *Ethno-veterinary practices, Research and Development Proceedings of International Workshop on Ethnoveterinary Practices*. Jerome O. Gefu, Paul A. Abdu and Clement, B. Alawa. (Eds) pp. 25 – 37.

ADIKWU, M. U (1994): Physico-chemical properties and pharmaceutical application of *Prosopis africana* (Guill & Perr) Taub. gum. *Ph.D Thesis Department of Pharmaceutics University of Nigeria, Nsukka (unpublished)*.

AGISHI, E. C (2004): Etulo, Idoma, Igede, Tiv and Hausa Names of Plants. Agiterb Publishers Makurdi, Nigeria. pp. 25-55

BRANDER, G. C. and PUGH, D. M. (1992): Veterinary Applied Pharmacology and Therapeutics 4nd ed. Bailiere Tindall, London. Pp 281-299.

GEFU, O. J. (2000): Ethnoveterinary Practices, Research and Development. National Animal production Research Institute. Shika Zaria International Workshop: Gefu, J.O, Abdu, P.A, Alawa C.B. (Eds.). p. 5

GHOTGE, N. S. and RAMDAS, S. R (2000): Improving livestock health by integrating healthcare delivery systems. Some experiences in India International Workshop on Ethnoveterinary Practice. Kaduna Nigeria. pp. 129 – 137.

HARBORNE, J. B. (1991): Phytochemical methods. A guide to modern Techniques of Plant analysis, 2nd ed Chapman and Hall London pp. 1–236.

HUTCHINSON, J. and DALZIEL, J.

M.(1958): *Flora of West Tropical Africa.* Vol. I Part II, R. W. J. Keay (Ed.). Crown Agents, London. pp. 485 – 503.

IBRAHIM, M. A (1984): Screening of some African herbs for activity against Nippostrongylus braxiliensis in rats. M.Sc. Thesis Amadu Bello University, Zaria.

IWU, M. M., (1993): Handbook of African Medicinal Plants. CRC Press Boca Raton, p.48.

KEAY, R. W.J, ONOCHIE, C.F.A and STANFIELD, D.P. (1964): Nigerian Trees Vol II Fed Dept of Forestry Research, Ibadan. National Press Ltd Apapa . pp107-115

LAWRENCE, D.R; BENNETT, P.N and BROWN, M.J. (1997): Clinical pharmacology. International edition. Singapore. Pp579-588.MCLEOD, L. J (1970). Pharmacological experiments on intact preparation.Churchill Livingstone, Edinburgh. pp 34-36

MURRAY, R. K., GRANNER, D. K., MAYES, P. A. and RODWELL, V.W. (2000): Harpers' Biochemistry 25th ed. New York: McGraw-Hill. p. 780.

NATIONAL RESEARCH COUNCIL (1996): 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 p. 46

OKOYE I. E. (1999): Pilot Scale-up Application of gum from *Prosopis africana* family Mimosaceae (Guill& Perr) Taub as a binder in Tablet Technology. *PhD. Thesis Dept. of Pharmaceutical Technology, University of Nigeria, Nsukka.* pp 50-55 (Unpublished).

OLANIYI, A. A. (2000): Principles of drug quality assuarance and pharmaceutical analysis. Mosuro publishers. pp. 341-431.

OLIVER-BEVER P. (1986): *Medicinal Plant in Tropical West Africa*., Cambridge University Press, London. p.1.

PAMPLONA-ROGERS G.D.(2004): *Encyclopedia of Medicinal Plants*. Editorial sefeliez Spain, pp.15-443.

PERRY, W.L.M and STAFF Deparment of Pharmacology University of Edinburg (1970): *Pharmacological Experiments on Isolated Tissues*. Churchill Livingstone pp. 38, 58.

SANNI, S. ONYEYILI, P. A. and SHAMAKI, U. B. (2005): Responsiveness of duodenal smooth muscle to histamine, and carbchol following Escherichia coli infection in chickens. Veterinarski Arhiv, 75: 423-429.

SCHNEIDER E (2004): *Encyclopedia of Medicinal Plants.* Pamplona-Rogers(Ed) Editorial Safeliz Spain p. 19.

SHETTY, S. N. and ANIKA, S. M. (1982): *Laboratory Manual of Pharmacology and Toxicology*. Fourth Dimension Publishers Enugu. pp. 8, 43 -47, 56 – 59, 70 – 82.

SMITH, O. B. (2000): International Workshop on Ethnoveterinary Practices, Kaduna, Nigeria. pp. 19 – 22.

SOFOWORA, A. (2006): Medicinal Plants and Traditional Medicine in Africa 3rd Ed. Spectrum Books, Ibadan. pp. 1 – 150.

STAHL E. (1969): *Thin Layer Chromatography*. A Laboratory Handbook. 2nd ed. Springer-Verlag, Berlin. pp.52 – 85.

TREASE, E. C and EVANS, W. C. (1996): *Pharmacognosy*. 14th ed. W B Saunders Coy, USA. pp. 365 - 650.

VALTUENA, J. A. (2004): *Encyclopedia of Medicinal Plants* Pamplona-Rogers (Ed.) Editorial Safeliz, Spain. p. 17.

WARD, J. W. and ELSEA J. R. (1977): Animal Care and Use in Drug Fate and Metabolism. Methods Techniques. Vol I. Marcel Dekker, Newyork. pp. 372 -390.

WILLIAMSON, E.M., OKPAKO, and EVANS. E. J. (1966): *Selection, Preparation and Pharmacological evaluation of Plant Materia*. John Wiley, Newyork.p 1.

ZIMMERMANN, M., (1983): Ethical guidelines for investigations of Experimental pain in Conscious Animals. *Pain* 16 (2) 109 -110.