Nigerian Journal of Pharmaceutical Research
Vol. 5 No. 1 pp 91-96 (September, 2006)

THE POSSIBLE VALUE OF *Prosopis africana* (LEGUMINACEAE) IN THE TREATMENT OF TUBERCULOSIS

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Abstract

Prosopis africana, Family Leguminoceae, is used in ethnomedicine to treat different ailments including, malaria in Nigeria, male sterility in Sudan and as a cardiotonic agent in Senegal. This research aims at evaluating the anti-tuberculosis activity, analgesic and anti-inflammatory action of the stem bark extract of *P. africana.* Preliminary studies using the agar dilution method show that the stem bark extract of *Prosopis africana* (1000, 1500, 2000 µg/ml) has inhibitory activity against clinical isolates of *Mycobacterium tuberculosis*, the causative organism of tuberculosis (TB). It also significantly inhibited *B. subtilis* and *S. aureus* but not the gram-negative organisms: *E. coli, P. aeruginosa*, *S. typhii* and yeast (*C. albicans*) at all test concentrations (1000, 2000, 3000 µg/ml). Aqueous extracts of *Prosopis* (100 and 200 mg/kg i.p) significantly inhibited acetic acid induced writhing in mice and a dose and time-dependent inhibition of the egg white induced paw oedema ($P<0.05$). The study provides evidence of activity against clinical isolate of mycobacteria, significant anti-inflammatory and analgesic properties, and provides a basis for its possible use as an affordable and effective anti-TB agent from natural product. © 2006: NAPA. All rights reserved.

Keywords: *Prosopis africana*; *anti-tuberculosis*; *anti-inflammatory*; *analgesic*

INTRODUCTION

Prosopis africana is a flowering plant that belongs to the sub-family: mimosaceae family: leguminoceae. *Prosopis africana* has been used for a variety of diseases and conditions in most African countries such as Nigeria, Sudan, Mali, Senegal etc. In Sudan, the dried leaves of *P. africana* are used as an aphrodisiac (Walter, 1977) while in Northern Nigeria, a decoction of the root is used for toothache and the bark, used as a dressing for open wounds (Dalziel, 1937), due to its antiseptic properties. Virtually, all parts of the tree are used for some medicinal purpose.

Previous work carried out shows that it contains alkaloids (Piperidine type), proteins and some inorganic compounds (Khuong, 1982 and 1972). The alkaloids, prosopine (I) and prosopinine (II) were obtained from the leaves, bark or roots of *P. africana* (Datta, *et al.,* 2000). They were found to possess significant activity against *staphylococci* ATCC 6538F and *E. coli*. They were also observed to decrease capillary permeability and possess both local anaesthetic activity and sedative activity on the central nervous system (Bourrinet and Quevauviller, 1968). The selected microbial and pharmacological studies were carried out with a view to establishing its possible anti-tuberculosis activity as well as evaluate its anti-inflammatory and analgesic activities. The

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ISSN 0189-8434 © 2006 NAPA

World Health Organization (WHO) has expressed concern over the emergence of virulent drug-resistant strains of *M. tuberculosis* and is calling for measures to be strengthened and implemented to prevent the global spread of the deadly TB strains. This follows research showing the extent of XDR-TB, a newly identified TB threat which leaves patients (including many people living with HIV) virtually untreatable using currently available anti-TB drugs. MDR-TB (Multidrug Resistant TB) describes strains of *M.. tuberculosis* that are resistant to at least the two main first-line TB drugs - isoniazid and rifampicin. XDR-TB or Extensive Drug Resistant TB (also referred to as Extreme Drug Resistance) is MDR-TB that is also resistant to three or more of the six classes of second-line drugs.

The description of XDR-TB was first used earlier in 2006, following a joint survey by WHO and the US Center for Disease Control and Prevention (CDC). Resistance to anti-TB drugs in populations is a phenomenon that occurs primarily due to poorly managed TB care. The problems include incorrect drug prescribing practices by providers, poor quality drugs or erratic supply of drugs, and also patient non-adherence.

MATERIALS AND METHODS

Antimicrobial screening

Test organisms: *Escherichia coli* (ATCC 11775), *Staphylococcus aureus (*ATCC 12600) *Pseudomonas aeruginosa, Salmonella typhi* (Clinical isolate), *Bacillus subtilis* (ATCC 6051), *Candida albicans* (ATCC 18804).

Methodology: Agar dilution method was used for this study. The above organisms were sub-cultured in tryptic soy broth (bacteria) and Sabouraud dextrose broth (yeast) overnight and diluted 1:200 with sterile saline, just before inoculating plates. Plates of nutrient agar and Saboraud dextrose agar containing the test concentrations of the extract were prepared and their dried surfaces, spot-inoculated with 10 µL of each of the standardized inoculums. The inoculated plates were then incubated at 37°C (for bacteria) and 30°C (for yeast) for 24 hrs. Growth of organisms after the incubation is interpreted as lack of activity of the extract at such test concentration. Control for organism viability (OVC) was provided in extract free media.

Preliminary anti-mycobacterial screening

Test organisms**:** *Mycobacterium tuberculosis* (Clinical isolate).

Methodology: The plant extract was screened for anti-mycobacterial activity by the minimum inhibitory concentration method described by Canetti *et al.,* (1969).

Test for analgesic activity in mice

Writhing reflex test

Mice of either sex were divided into three groups of six. One group received vehicle (control) and the other two groups received *Prosopis africana* (100 and 200 mg/kg.) extract at different doses intra-peritoneally, 30 minutes later, 0.1% acetic acid was injected intra-peritoneally. The number of abdominal contractions (Writhing movement) as observed for 15 minutes, starting from 5 minute after the injection of acetic acid, was recorded. The percentage inhibition of writhing movement was then calculated.

Hot plate test

Rats were kept in a glass cylinder (open at both ends) on a hot plate, such that the rats have direct contact with the hot plate maintained at constant temperature of 551°C (Williamson *et al.,* 1996). The time taken for either paw licking or jumping was recorded. Rats were divided into three groups; one group (control) received distilled water, while the other two groups received two dose points of *Prosopis africana* extract (100 and 200 mg/kg. i.p.), 30 minutes before placement on the hot plate.

Test for Anti-inflammatory activity

Acute inflammation was induced by injecting egg-white into the sub-plantar surface of the rat's hind paw. Acute inflammation was measured by increase in the rat's right hind paw linear circumference (Oriowo, 1982). Adult Wister rats, of either sexes (140-170 g) bred in the Department of Pharmacology Animal House; University of Jos, Jos, Nigeria were used for this experiment. The animals were fasted for 12 hours and deprived of water only during the experiment. Oedema was assessed in terms of the difference in zero time linear circumferences at the injected paw and its circumference at 1, 2, and 3 hours intervals after egg-albumin injection (Hess and Milong, 1972; Oriowo, 1982). For routine drug testing, the increase in paw circumference 2 hours after administration of the inflammation-inducing agent, was adopted as a measure of effect (Oriowo, 1982). Animals were divided into three groups of six. One group received distilled water and served as the control while the other groups received the test drug at different concentrations. *Prosopis africana* (100 and 200 mg/kg.) extract was administered intra-peritoneally, 30 minutes before the induction of inflammation. The paw circumference was measured with the aid of vernier calliper at 1, 2, 3and 4 hours. To obtain a measure of oedema, the measurement at zero time was subtracted from the measurement at specific time intervals.

Statistical analysis

Differences between control and treatment groups were by paired student *t*- test (Snedecor and Cochran , 1967).

RESULTS

The intra-peritoneal LD_{50} values of the aqueous extract of *Prosopis africana* was 3.23 g/kg.

Anti-inflammatory activity

The extract of *Prosopis africana* (100 and 200 mg/kg. i p.) showed anti-inflammatory activity against acute inflammation induced by sub-plantar injection of egg-white (Table 2, Table 3). It suppressed, in a dose dependent manner, the increase in the rat paw oedema. The inhibition was significant (P>0.05) 2 hours after the inflammation-inducing agent (Table 2 and 3) was injected in pretreated animals.

Table I: Effect of aqueous extract of *P. africana* on selected micro-organisms

	Extracts Concentrations (g/ml)				Remarks
	1000	2000	3000	OVC	
Organisms					
Ec	+	+	+	+	Inactive
Sa	-	-	-	-	Active
Ps	+	+	+	+	Inactive
S_T	+	+	+	+	Inactive
B_S	-	-	-	+	Active
Ca	+	+	+	+	Inactive
	1000	1500	2000	OVC	
M_T	+	+	-	+	Active

Ec = *Escherichia coli*; Sa = *Staphylococcus aureus*
Ps = *Pseudomonas aeruginosa*; S_T = *Salmonella typhi*
Bs = *Bacillus subtilis*; Ca = *Candida albicans*;
M_T = *Microbacterium tuberculosis;*
- = Inhibition of organism
+ = No inhibition of organism
OVC = Organism viability control

Table 2: Effect of *Prosopis africana* extract on egg-white induced inflammation

	Average inflammation (Increase in Paw circumference) mm+ SEM					
Dose (mg/kg)	1hr	Inh(1hr)	2hr	Inh(2hr)	3hr	Inh (3hr)
Control	3.41±0.64	-	3.46±0.45	-	3.34±0.63	-
-						
100	2.75±0.74	0.66	2.35±0.77	0.11	1.89±0.43	1.45
200	1.44±0.33	1.97	1.25±0.11	2.21	1.15±0.12	2.19

Note: Inh = inhibition, n=6

Table 3: Effect of aqueous extract of *Prosopis africana* on acetic acid- induced writhing

Treatment	Dose (mg/kg, i.p)	Number of writhing	Inhibition (%)
Control	-	26.17±4.12	-
Extract	100	4.17±2.99*	
Extract	200	3.00±2.53*	

Values are mean±S.E.M., * P<0.05 vs. control, student's *t*-test, n=6

Table 4: Anti-nociceptive effect of aqueous extract of *Prosopis africana* on the hot-plate test

Treatment	Dose (mg/kg i.p)	Latency (s)
Control	-	1.79±0.67
Extract	100	1.78±0.55
Extract	200	1.99±0.41

Values are mean±S.E.M., P>0.05, paired student's *t*-test, n=6

DISCUSSION

The results of the preliminary microbial screening showed that the extract is inactive against the gram-negative organisms (*E. coli, P. aeruginosa*, *S. typhi* and yeast (*C. albicans*) at test concentrations used for this study while activity was observed against *B. subtilis* and *S. aureus* (Table I). Inhibition of growth was slight at 1000 and 1500 g/ml of the aqueous extract against *M. tuberculosis* but there was complete inhibition at 2000 g/ml. These antimicrobial results are similar to that carried out by earlier workers such as Bourrinet and Quevauviller (1968). Tuberculosis is a mycobacterial disease, which causes disability and death in many parts of the world (Benenson, 1990). If untreated, about half the patients will die within a two-year period but appropriate chemotherapy nearly always results in a cure. Early signs such as fatigue, fever and weight loss may occur while localizing symptoms of

cough, chest pain, haemoptysis and hoarseness become prominent in advanced stages (Benenson, 1990).

P. africana was observed from our studies to effectively protect male and female wistar rats against pain induced by intra-peritoneal injection of acetic acid (Table 3), but not that induced by heat (Table 4). The aqueous extract was also found to have anti-inflammatory properties (Table 2). These findings may not be unrelated to the presence of flavonoids, which are known to be responsible for the anti-inflammatory properties of most medicinal plants (Manthey, 2000).

Prosopis africana extract (100 and 200 mg/kg) significantly inhibited acetic acid-induced writhing in mice but did not significantly affect the stay time of rat on the hot plate. *Prosopis africana* can then be deduced to be effective against peripherally, but not centrally induced pain (Turner, 1965).

The aqueous extract of *Prosopis africana* (100 and 200 mg/kg) significantly inhibited egg-albumin induced rat paw oedema. The extracts showed a dose and time-dependent inhibition of the egg white induced paw oedema (Table 2). At the time interval of 2 hours *Prosopis africana* extract produced significant inhibition ($P<0.05$) of the egg-albumin induced oedema.

The aqueous extract of *Prosopis africana* has been demonstrated to be of great advantage in the management of disease conditions in man, animals and plants. In Northern Nigeria, medicinal plants are used to complement orthodox medicines. Most of these plants contain pain relieving and/or anti-inflammatory agents. Additional activity of the plant could be of advantage, especially if it is effective against the underlying cause of pain. The results obtained from this study show that the aqueous extract of *Prosopis africana* has anti-microbial activity. It is advisable to treat the underlying cause of pain rather than treating the pain, which is a signal that something is wrong with the body. Traditional healers use this herb alone or in combination with other herbs for the treatment of pain resulting from the aches of Malaria, inflammatory and infectious conditions. The aqueous extract of *Prosopis africana* can be said from the above result to be effective in treating pain and the underlying cause of pain in infectious diseases. The results also show that instead of using separate analgesic and antibiotic to manage infections diseases, *Prosopis africana* can effectively combat the disease states and relieve pain and inflammation. These results support the claim by the traditional healers that the plant has anti-inflammatory and analgesic activity.

Conclusion

The present study reveals that the aqueous extracts of the *Prosopis africana* (100 and 200 mg/kg possesses anti-inflammatory and analgesic activity in experimental animals.

The antimicrobial properties as well as the analgesic and anti-inflammatory activities of *Prosopis africana* make it a potential candidate in herbal medicine for the treatment of tuberculosis and the overall results of this study seem to confirm the traditional uses of *Prosopis africana*. Comparative activity with standard chemotherapeutic agents among other investigations could be carried out against the promising sensitive strains and the active principles against *M. tuberculosis* identified by spectral studies and other analytical methods.

References

Bars D., Gozariu, L.M, and Cadden W.S. (2001): Animal Models of Nociception. *Pharmacol. Rev.* 53:597-652.

Benenson, A.S. (1990): Control of Communicable Diseases in Man, Fifteenth edition. American Public Health Association, Washington D.C.

Bourrinet, P., and Quevauviller, A. (1968): Prosopinine, an alkaloid from Prosopis Africana. Effect on the central and autonomic nervous systems.*Comptes Rendus des Seances de la societe de Biologie et de Ses Filiales*, 162(5-6) 1138-40.

Bourrinet, P; Quevauviller, A. (1968): Prosopis africana alkaloids. Prosopine and prosopinine , *Annales Pharmacuetiques Francaises*, 26 (12) 787-96.

Canetti, G; Fox, A.; Khomenko, D.A; Mitchison, N.R; Smelev,N.A. (1969): *Bull. Wld. Heal. Org.* 21-48

Dalziel, J. M, (1936): Useful Plants of West Africa. The Crown Agents for Overseas Colomes, London p, 401

Dalziel, J.M. (1937): The useful plants of West Tropical Africa. London Crown Agents, London, Great Britain. An Appendix to Dalziel, J.M. and Hutchinson, J. 1927-1936. The flora of tropical West Africa. London Crown Agents, London. Great Britain.

Datta, A; Ravi, K; Jalluri, S. (2000): Book of Abstracts, 219th ACS National meeting, San Francisco, CA ORGN-762. Publisher: American Chemical Society, Washington, D.C

Hess, S.M. and Milong, R.C. (1972): Inflammation Mechanism and Control. LEPOW, I.H. and WARD, P.A. ed, academic Press New York p.1-2.

Irvine, F.R. (1961). Woody plants of Ghana with special references to their uses. London, OxfordUniversity Press. Pp.868.

Kerharo, J. and Adam, J.C. (1974): La Pharmacopee senegalaise traditionnalle*: Plantes medicinales et toxiques.* Ed. Vigot & Freres. Paris,1011 pges. (ISBN2-7114-0646-6).

Khuong Huu, Q.; Monseur, X.; Gasic, M.; Wovkulich,P.; Wenkert, E. (1982): A carbon-13 NMR spectral analysis of Prosopis Africana alkaloids. *J. Chem. Soc. Pakist.* 4 (4), 267-9.

Khuong Huu, Q., Ratle, G., Monseur, X., and Goutarel, R. (1972): *Piperidine alkaloids.II. Structures of prosopine and prosopinine, alkaloids from Prosopis africana.,* Bulletin des societies Chimiques Belges 81(7-8), 425-41.

Lorke, D. (1983): A new approach to practical acute toxicity testing. *Arch Toxicol* ;54(4):275-87.

Manthey, J.A. (2000): *Biological properties of flavonoids pertaining to inflammation. Microcirculat.* ; 7(6 Pt 2) :S29-34.

Meek, C.K. (1931): *Tribal Studies in Northern Nigeria* R. Eth could be DOD of Ubangian or DOH Dong under Bantoid.

Ngnokam, D.; Ayafor, J.F.; Connolly, J.D.; Nuzillard, J.M . (2003): *Nauclefolinine*: *A new alkaloid from the roots of Nauclea latifolia* . Bulletin of the Chemical Society of Ethiopia pp. 173-176(4)

Oliver, B. (1960): Medicinal plants in Nigeria. Ibadan College of Arts and Sciences and Technology.

Oriowo, M.A. (1982): Anti-inflammatory activity of piperonyl-4-acrylic isobutyl amide, an extractive from *Zanthoxylum zanthoxyloides. Planta Med*; 44(1):54-6.

Rang H.P and Dale M.M. (1991): Pharmacology. Churchhill Livingstone, 2nd edition, Pg 175.

Snedecor, G.W and Cochran, WG. (1967): Statistical Methods. 6th edition. Ames, Iowa: The Iowa State University Press, p. 423

Turner, R.A. (1965): Screening methods in pharmacology. Academic Press, New York :106.

Walter, H. L; Memory P.F, (1977): Medical Botany: Plants affecting Man's Health. Published by John Wiley & Sons. U.S.A Http://www.who.int/tb/xdr/faqs/en/thomasg@who.int

Williamson, E. M; Okpako, D. T; Evans, F. J. (1996): Pharmacological methods in Phytochemistry Research. Volume I, pp 4-5.Robyns and Ghesquiere, 1933

Winter, E.A. , Risley, E.A. and Nuss, G.V. (1962): *Proc. Soc. Expt. Biol. Med*. 111: 544-547.