African Journal of Biotechnology Vol. 3 (5), pp. 284-288, May 2004
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2004 Academic Journals

Full Length Research Paper

Evaluation of the aqueous extract of *Boswellia dalzielii* stem bark for antimicrobial activities and gastrointestinal effects

F.C. NWINYI [1]*, L. BINDA [1], G.A. AJOKU[2], S.O. ANIAGU[1], N.M. ENWEREM[3],A. ORISADIPE[3], D. KUBMARAWA[4], K.S. GAMANIEL[1]

[1]Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD), P.M.B. 21 Garki, Abuja, Nigeria.
[2]Department of Microbiology, Human Virology and Biotechnology, National Institute for Pharmaceutical Research and Development (NIPRD), P.M.B. 21 Garki, Abuja, Nigeria.
[3]Department of Medicinal Plant Research and Traditional Medicine, National Institutefor Pharmaceutical Research and Development (NIPRD), P.M.B. 21 Garki, Abuja, Nigeria.
[4]Department of Chemistry, Federal University of Technology, P.M.B 2076, Yola, Adamawa State, Nigeria.

Accepted 27 April, 2004

The aqueous extract of *Boswelli dalzielii* Hutch (family: Burseraceae) was investigated for therapeutic properties using aspirin-induced ulceration in rats, gastrointestinal motility in mice and castor oil-induced diarrhoea in rats. The median lethal dose (LD_{50}) of the extract was carried out via the oral route in mice. Antimicrobial and preliminary phytochemical screening of the extract was also investigated. The extract did not show toxicity signs or death at doses ≦ 2000 mg/kg p.o. The extract (50-200 mg/kg i.p.) dose dependently reduced acetylsalicylic acid (aspirin) (200 mg/kg p.o.) - induced ulceration in rats. The results obtained compared favourably with cimetidine (100 mg/kg i.p.). The extract (25-100 mg/kg p.o.) dose also dependently reduced intestinal propulsion of charcoal-treated mice. However, the extract (25-100 mg/kg i.p) did not produce significant (P >O.O5) protection against castor oil-induced diarrhoea in rats. No antimicrobial effects were shown by the extract (200 mg/kg) against any of the tested organisms. Tannins were detected in the aqueous extract. The above results show that *B. dalzielii* stem bark probably contains some active ingredients that could be developed for such gastrointestinal problems as have been claimed by traditional medical practitioners.

Key words: *Boswellia dalzielii*, peptic ulcer, gastrointestinal motility, diarrhoea, antimicrobial effects.

INTRODUCTION

Boswellia dalzielii Hutch (family: Burseraceae) is a tree of the Savanna forest recognizable by its papery bark peeling off in a ragged manner. The bark yields a whitish gum resin, which dries readily and is friable. The plant products (such as the gum resin) and different parts of the plant are widely employed in traditional medicine. The gum resin is used along with other medicines as a stomachic and for the treatment of veneral diseases. The bark is boiled in large quantities to make a wash for fever, rheumatism and also taken internally for gastrointestinal troubles. The root decoction boiled along with *Hibiscus sabdariffa* is used for the treatment of syphilis. The root decoction with *Daniellia oliveri* is used on wounds (Dalziel, 1937). In Nigeria (specifically in Adamawa state), the fresh bark is eaten to cause vomiting after a few hours to relieve symptoms of giddiness and palpitations (Burkill, 1985).

There have been reports that a vast majority of the population particularly those living in villages depend largely on herbal medicines (Gupta, 1994). This translates to an increasing need to authenticate these

*Corresponding author. Tel: +234 9 5239089 E-mail: saniagu@yahoo.com.

alleged medicinal plants. Such medicinal plants can be exploited since it has been shown that they are important sources of new chemical substances with potential therapeutic effects (Farnsworth, 1989; Eisner, 1990). The present study was therefore prompted by this need.

MATERIALS AND METHODS

Plant collection and extraction

The stem bark of *B. dalzielii* was collected from kano state, Nigeria between January and April. The plant was identified by Ibrahim Muazzam and the late A.O. Ohaeri, Plant Taxonomists in the Department of Medicinal Plant Research and Traditional medicine, National Institute for Pharmaceutical Research and development (NIPRD), Abuja, Nigeria. The specimen was deposited at NIPRD herbarium with voucher specimen number 4592. The bark was cleaned, air-dried for 7 days and then pounded into a coarse powder using hammer mill. About 200 g of the powder was cold macerated with 1.5 litres of distilled water for 24 h. The product was filtered and freeze-dried. It gave a yield of 25% (w/w).

Experimental animals

Adult Wistar rats (180-250 g) and swiss albino mice (18-22 g) of both sexes were used for the investigations. They were bred and maintained in the Animal Facility Centre, Department of Pharmacology and Toxicology, NIPRD, Abuja, Nigeria, under natural environmental conditions. The animals were fed with standard feed (Pfizer Feeds PLC, Lagos) and water *ad libitum* except when starvation was otherwise needed during the study.

Test organisms

The microorganisms used for the screening include *Bacillus subtilis, Candida albicans, Staphylococcus aureus, Pseudomonas aerugniosa, Proteus mirabilis and Escherichia coli.* The organisms were clinical isolates obtained, standardised and stored by the Department of Microbiology, Human Virology and Biotechnology, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria.

Drugs and chemicals

Cimetidine (Sigma, USA), Acetylsalicylic acid (Sigma, USA), Loperamide (Janssen-Cilag, UK), Castor oil (Bell Sons & Co, England) Nutrient agar (BBL, USA) and Nutrient broth (BBL, USA) were used for the investigation.

Phytochemical tests

The freshly prepared extract was subjected to standard phytochemical analyses for different constituents (Trease and Evans, 1983). The presence of alkaloids, glycosides, tannins, saponins and anthraquinones were tested.

Acute toxicity studies (LD_{50})

The method of Lorke (1983) was modified to evaluate the oral acute toxicity *(*LD_{50}*)* of the extract. The Swiss albino mice used in the study were starved of feed but allowed access to water 24 h prior to the study. The oral administration of widely differing doses of the extract (10, 100, 1000, 2000 mg/kg p.o) to four groups of mice (n = 4) was done to establish the range of doses of the extract that would produce toxic effects. This was done by observing the mice over a 72 h period post treatment for behavioural signs such as excitement, nervousness, dullness, alertness, ataxia or even death. The adopted method estimates LD_{50} by calculating the geometric mean of the dose that caused 100% mortality and the dose which caused no lethality at all.

Studies on acetylsalicylic acid-induced ulceration

The adult Wistar rats used in this study were fasted for 18 h with water given *ad libitum*. The animals were separated into five groups (6 rats per group). The first group received normal saline (30 ml/kg i.p.), the second group received Cimetidine (100 mg/kg i.p), the remaining three groups received various doses of the extract (50-200 mg/kg i.p.). 30 min later, an aqueous suspension of acetylsalicylic (ASA) was administered to each rat at a dose of 200 mg /kg p.o. The rats were then put in restraining cages for 4 h (Aguwa and Mittal, 1987). At the end of the 4 h period, the rats were sacrificed and the stomach removed and opened along the greater curvature. The stomach was rinsed under a stream of water and pinned flat on a cork board. The stomach was coded to avoid observer bias and examined with a hand lens (X10 magnification). The scoring of the severity of ulceration was as described by Asuzu and Onu (1990) and Akah et al. (1998):

< 1 mm (pin point) = 1
1-2 mm = 2
> 2 mm = 3
> 3 mm = 4

The mean ulcer index was determined by dividing the total ulcer indices in a group by the total number of animals in that group. The percentage severity of ulceration was determined by dividing the scores of ulcers of each group by the total number of scores in the control group and the result multiplied by 100 (Main and Piearce, 1977; Akah et al. (1997).

Studies on gastrointestinal motility in mice

The method of Akah et al. (1998) was used to test the effect of the extract on gastrointestinal motility. Adult Swiss albino mice were divided into four groups (of six mice per group). The animals were starved for 24 h prior to the experiment, but were allowed access to water. The first group was given normal saline (20 ml/kg p.o.) while groups two, three and four received the extract at the doses of 25, 50 and 100 mg / kg p.o., respectively. Five minutes after drug administration, 0.5 ml of a 5% charcoal suspension in 10% aqueous solution of tragacanth powder was administered to each mouse orally. The animals were killed 30 min later and the abdomen opened. The small intestines were dissected out and placed on a clean surface. The distance traveled by the charcoal meal from the pylorus was measured. The entire length of the small intestine was also measured. The percentage distance traveled by the charcoal plug along the small intestine (from the pylorus to the caecum) was then estimated for both the extract and the normal saline-treatment groups.

Effects on castor oil-induced diarrhoea

The method of Pulok et al. (1998) was adopted. Rats of either sex were fasted for 18 h. They were separated into five groups (of six

Table 1. Effect of the aqueous extract of *B. dalzielii* (BD) on acetylsalicylic acid (200 mg/kg p.o) induced ulceration in rats.

Treatment	Dose (mg/kg i.p)	Mean ulcer index ± SEM	% Severity of ulcer
Normal Saline	30	0.80 ± 0.15	100. 00
BD	50	0.18 ± 0.02*	22.50
BD	100	0.05 ± 0.01*	6.25
BD	200	0.00*	0.00
Cimetidine	100	0.00*	0.00

*Means significant (P < 0.05) reduction in mean ulcer index (one-way ANOVA + student t-test; n = 6).

rats each). Normal saline (20 ml/kg i.p.) was given to the control group. The second group received loperamide (10 mg/kg i.p.) as the reference standard while graded doses of the extract (25, 50 and 100 mg/kg i.p.) were given to the remaining groups respectively. At 1 h post-treatment, 1 ml of castor oil was given orally to each animal and the rats were observed for defecation. Over 4 h observation period following castor oil administration, the presence of characteristic diarrhoea droppings were noted on transparent surfaces spread beneath every cage.

Antimicrobial studies

Agar dilution method was to determine the antimicrobial activity (Jones et al., 1989). The inoculums for each organism (*B. subtilis, C. albicans, S. aureus, Ps. aeruginosa, Pr. mirabilis and E. coli*) were prepared from broth cultures containing approximately 5 x 10^6 to 9 x 10^7 colony forming units (CFU)/ml. The diluted (1:20) innoculum was applied as a streak with a loop calibrated to deliver 0.002 ml and containing 9 x 10^3 CFU. The plates were incubated for 24 h at 37°C. Extracts were prepared at a concentration of 2 mg/ml. The extracts were dissolved in sterile water which was also used as control. Observations were performed in duplicates.

Statistical analysis

The results were generally expressed as mean ± SEM. The significance of difference between the control and treated groups were determined using one-way analysis of variance (ANOVA) followed by Student t-test. P values < 0.05 were taken to be statistically significant.

RESULTS

Phytochemical tests

The preliminary phytochemical screening of *B. dalzielii* revealed that alkaloids, saponins, anthraquinones and glycosides were absent while tannins were present.

Acute toxicity studies (LD_{50})

At doses ≤ 2000 mg/kg p.o., no obvious behavioural changes or toxicity signs or even death were seen within the observation period of 72 h.

Studies on acetylsalicylic acid-induced ulceration

The aqueous extract of *B. dalzielii* stem bark (50-200 mg/kg i.p.) significantly (P < 0.05) inhibited ulceration induced by acetylsalicylic acid (200 mg/kg p.o.) dose-dependently in rats. The results obtained compared favourably with Cimetidine (100 mg/kg i.p.; Table 1).

Studies on gastrointestinal motility in mice

The aqueous extract of *B. dalzielii* (25, 50 and 100 mg/kg p.o.) inhibited the intestinal propulsion in charcoal-treated mice dose dependently. The inhibitory effects were significant (P < 0.05) at the tested doses (Table 2).

Effects on castor oil-induced diarrhoea

The aqueous extract of *B. dalzielii* stem bark did not produce a significant (P > 0.05) inhibition of diarrhoea induced by castor oil in rats at the tested doses of 25, 50 and 100 mg/g i.p. (Table 3).

Antimicrobial studies

The antimicrobial studies revealed that the aqueous extract of *B. dalzielii* (2 mg/ml) did not inhibit the growth of all the tested organisms. The observed results were like that of the control (not shown).

DISCUSSION

The dose dependent reduction in acetylsalicylic acid-induced ulceration by the aqueous extract of *B. dalzielii* probably suggests the presence of some active ingredients which act through one or more ulcer protecting mechanisms. The results obtained were comparable to that of cimetidine which has been reported to have ulcer healing mechanism involving competitive inhibition of H_2 receptors (Zeitoun et al., 1982; Brunton, 1990).

Table 2. Effects of aqueous extract of *B. dalzielii* (BD) on intestinal propulsion of charcoal-treated mice.

Treatment	Dose (mg/kg p.o)	Mean intestinal length (cm)	Mean distance traveled by by charcoal (cm)	Percentage of intestinal propulsion of charcoal
Normal Saline	20	46.38± 2.9	34.00 ± 8.6	73.31
BD	25	38.25 ± 2.5	20.63 ± 3.3	53. 93*
BD	50	35.93 ± 1.0	17.50 ± 3.1	48.71*
BD	100	39.10 ± 1.4	17.98 ± 3.3	45.98*

*Significant ($P < 0.05$) decrease in intestinal propulsion (One way ANOVA + student t- test; n = 6).

Table 3. Effect of aqueous extract of *B. dalzielii* (BD) stem bark on castor oil-induced diarrhoea in rats.

Treatment	Dose mg/kg i.p.	Number of mice with diarrhoea	% inhibition of diarrhoea
Normal Saline	20	6	0.00
BD	25	5	16.67
BD	50	6	0.00
BD	100	5	16.67
Loperamide	10	0	100.00*

*$P < 0.05$ means statistically different from control (one way ANOVA, student t-test).

The antimotility effect of the extract was demonstrated in the charcoal-treated mice. The results indicated a reduction in peristaltic activity and ultimately reduction in gastrointestinal motility. This finding has implications on the rates of gastric empty as well as secretory activities. Such effects may reduce the development of ulcer. Also, a delay in gastric emptying of stomach will prevent speedy evacuation of the stomach contents which in turn increases the absorption of orally administered anti-ulcer agents and promotes ulcer healing. These effects are considered to be beneficial to ulcer patients (Bertaccini et al., 1981; Akah et al., 1998). Although antisecretory studies were not investigated, the reduction in motility could also mean a decrease in secretions.

According to Bambery et al. (1992) as well as Mohammed and Hunt (1994), antisecretory drugs have been found to be effective in the treatment of peptic ulcer disease especially where excessive acid secretion is implicated. The extract did not significantly inhibit diarrhoea induced by castor oil rats. Dinesh et al. (1999) classified castor oil as a stimulant laxative. Antimotility drugs such as Loperamide block the actions of castor oil and are used to relieve diarrhoea. The present study has shown that the extract inhibits gastrointestinal tract motility, yet did not stop castor oil-induced diarrhoea.

Therefore, the activity of the extract may not be directed against the stimulatory activity of castor oil on the GIT. Also, preliminary phytochemical screening revealed that tannins are present in the extract. According to Robertson et al. (1956), tannin-bearing preparations are used for arresting diarrhoea because of their ability to coagulate and precipitate proteinaceous materials. Therefore, the extract's inability to inhibit castor oil induced diarrhoea irrespective of its antimotility effect and tannin content could be attributed to other factors.

The extract exhibited no antimicrobal activity against all test organisms used in the study. However, in one of our studies (unpublished), oil from the leaves of *B. dalzielii* exhibited significant activity against *S. aureus, B. subtilis and C. albicans* at the concentrations used in this study. This further confirms that care needs to be taken to specify the exact part of a plant used for a particular study since different parts of the same plant have different properties and also show different activities.

In conclusion, the aqueous extract of *B. dalzielii* showed anti-ulcer activity and reduced gastrointestinal motility. According to Dinesh et al. (1999), advantage can be taken of agents that reduce intestinal motility, gastric secretory effect and are anti-spasmodic as adjunctive treatment in non-ulcer dyspepsia, irritable bowel syndrome, and diverticular disease. Antispasmodics are of value for treating abdominal cramps associated with diarrhoea while antimotility drugs relieve diarrhoea. The results obtained therefore conform to the traditional use of *B. dalzielii* extract for gastrointestinal disorders.

ACKNOWLEDGEMENT

This study was supported by a grant (Ref. No. RMRDC/AF/SEC/S.29/I/11) from Raw Materials Research and Development Council (RMRDC), Abuja, Nigeria. The authors are grateful to the Ibrahim Muazzam and the late A.O. Ohaeri for their botanical assistance.

REFERENCES

Aguwa CN, Mittal GC (1987). Study of anti-ulcer activity of an aqueous extract of leaves of *Pvrenaeailiha mandili* (family: Leacinaceae) using various models of experimental gastric ulcers in rats. Eur. J. Pharmacol. 74: 215-219.

Akah PA, Gamaniel KS, Wambebe CN, Shittu A, Kapu SD, Kunle OO (1997). Studies on the gastrointestinal properties of *Ficus exasperata*. Fitoterapia 68: 17-20, 1997.

Akah PA, Orisakwe OE, Gamaniel KS, Shittu A (1998). Evaluation of Nigerian Traditional medicines II: Effects of some Nigerian folk remedies on peptic ulcer. J. Ethnopharmacol. 62: 123-127.

Asuzu IU, Onu OU (1990). Anti-ulcer activity of the ethanolic extract of *Combretum dolichopetalum* root. Int. J. Crude Drug Res. 28: 27-32.

Bambery P, Cawell CM, Frame MH (1992). A meta-analysis comparing the efficialy of omeprazole with H_2-receptor antogonist for acute treatment of duodenal ulcer in Asian patients. J. Gastroenterol. Hepatol. 7: 577-585.

Bertaccini G, Castiglione R, Scapignato C (1981). Effect of substances and its natural analogues on gastric emptying in the conscious rat. Brit. J. Pharmacol. 72: 221-223, 1981.

Brunton LL (1990). Drugs affecting gastrointestinal function. Pp. 1264-1310. In: The Pharmacological Basis of Therapentics (Ed: Gilman GA, Rall TW, Nies AS, Tylor, P), Pergamon press, New York.

Burkill HM (1985). Useful plants of west Tropical Africa. Second ed., vol. 1. Royal Botanic Gardens Kew, pp. 300-301.

Dalziel JM (1973). The useful plants of west Tropical Africa. The Crown Agents, London, pp. 314-315.

Dinesh KM, Martin J, Furniss L, Hargreaves AM, Whitley LJ (1999). British National Formulary (BNF) Vol. 37. William Clowes, British. P, 31-53.

Eisner T (1990). Chemical prospecting. A call for action. In: Borman FH, Kellert, SR, eds Ecology, Economic and Ethics, The Broken circle. Yale University Press, New Haven, CT.

Farnsworth NR (1989). Screening Plants for new medicines, pp. 83-97. In: Wilson EO (ed), Biodiversity, part II. National Academy Press, Washington.

Gupta SS (1994). Prospect and perspectives of Natural Plants Products in medicine. Indian J. Pharmacol. 26: 1-12.

Jones RN, Gavan TL, Thornsberry C, Fuchs PC, Gerlach EH, Knapp JS, Murray P, Washington JA (1989). Standardization of disk diffusion and agar dilution susceptibility tests for *Neissieria gonorrhorea*: Interpretative criteria and quality control guidelines for Ceftriaxone. J. Clin. Microbiol. 27: 2758-2766.

Lorke D (1983). A new approach to practical acute toxicity testing. Arch. Toxicol. 54: 275-287.

Main IHM, Piearce JM (1977). Histamine out from the rat isolated gastric mucosa during acid secretion stimulated by pentagastrin, methacholine and dibutylyl cyclic AMP. Brit. J. Pharmacol. 61: 461.

Mohammed AH, Hunt RH (1994). The rationale of acid suppression in the treatment of acid related disease. Alimentary Pharmacology and Therapentics 8: 3-10.

Pulok KM, Kakali S, Murugesan T, Mandal SC, Pal M, Saha BP (1998). Screening of anti-diarrhoeal profile of some plant extracts of a specific region of west Bengal, Indian J. Ethnopharmacol. 60: 85-89.

Robertson P, Herer WY (1956). Pharmacognosy 2nd ed. J.B. Lippincott company, Philadelphia, pp. 118-129.

Trease GE, Evans MC (1983). Textbook of Pharmacognosy 12th ed. Bailiere, Tindal, London, pp. 343-383.

Zeitoun R, Soliman MRL, Abou Zeit Har MS, El-Zayaddi AEB, Abel Galil AA (1982). Preventive effect of some drugs against experimental induced duodenal ulcer in the rat. Bull. Alexandria Fac. Med. 17: 685-697.