Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 7 (6): 298-304, 2012
ISSN 1816-496X / DOI: 10.3923/jpt.2012.298.304

Antibacterial Activity and Sub-chronic Toxicity Studies of *Vitellaria paradoxa* Stem Bark Extract

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ABSTRACT

This study was designed to assess the antibacterial activity of stem bark extract of *Vitellaria paradoxa* and its effect on haematological and biochemical parameters. The aqueous and ethanol extracts of stem bark of *Vitellaria paradoxa* at a concentration of 50 mg mL^{-1} were tested on clinical isolates of *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Streptococcus pyogenes* using agar well-dilution method. The 12 mg kg^{-1} b.wt. of the ethanol extract was administered for 28 days to a group of rats to assess its effects on the hematological indices, serum levels of creatinine and urea and activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). The LD_{50} of the ethanol extract was determined to be 115 mg kg^{-1} b.wt. in mice. All the bacteria were susceptible except *Pseudomonas aeruginosa* and *Streptococcus pyogenes* that showed resistance to both aqueous and ethanol extracts. Ethanol extract was observed to exhibit more antibacterial activity at equivalent dose compared to aqueous extract. All hematological parameters were not significantly altered except the platelet count which was significantly ($p<0.05$) reduced. Serum levels of creatinine, urea, ALT, AST and ALP were significantly higher ($p<0.05$) in treated rats compared with control.

Key words: *Vitellaria paradoxa*, extracts, antibacterial activity, clinical isolates, hematology, enzymes

INTRODUCTION

The discovery of sulphonamide antibiotics in 1930s and penicillin in 1940s resulted in a dramatic reduction in fatality rates associated with bacterial infections (Butler and Buss, 2006). This breakthrough in the treatment of bacterial infections prompted a concerted effort at searching for more novel antibiotics during the following three decades and this search has produced many of the antibacterial drugs that are of clinical importance today, many of which are of natural product origin (Walsh and Wright, 2005). However, the recent global emergence of bacteria that are totally or almost totally resistant to available antibiotics is a big challenge because treatment options for infected patients are becoming more and more limited. This has necessitated a continuous search for new antibacterial drugs to replace those ones to which bacteria have

developed resistance (Walsh and Amyes, 2004). Many antibacterial agents are available in nature for the treatment of systemic infections. Medicinal plants constitute good source of active agents for this purpose and many plants extracts have been reported to possess various antimicrobial activities (Nawel *et al*., 2005).

Vitellaria paradoxa (Sapotaceae) is a plant of African origin whose leaves, root, fruits and stem bark have been used in the treatment of various infections such as wound infections, skin diseases, diarrhea, dysentery, helminths and other gastrointestinal tract infections (Soladoye *et al*., 1989). This plant flourishes in Savanna zones of Nigeria. It is known as Shea-butter tree in English, 'Kadanya' in Hausa, 'Okwuma' in Igbo and 'Emi' in Yoruba languages. Stem bark of *Vitellaria paradoxa* showed significant antifungal effect against clinical isolates of *Aspergillus niger*, *Aspergillus flavus*, *Epidermophyton floccosum*, *Microsporum audouinii* and *Trichophyton mentagrophytes* (Ahmed *et al*., 2009). The bark is used to suppress cough and also to treat leprosy (Ferry *et al*., 1974). The root of the *Vitellaria paradoxa* when used as chewing stick has been found to be useful in the treatment of oral infection (Ndukwe *et al*., 2005). Phytochemical screening of the stem bark extracts of the plant revealed the presence of carbohydrates, alkaloids, saponins, tannins and cardiac glycosides (El-Mahmood *et al*., 2008). In line with the need to search for more effective and safe antibacterial drugs and to justify the traditional use of *Vitellaria paradoxa* in the treatment of infectious diseases, this study was designed to assess the toxicological effects of ethanol extract and the antibacterial activities of both ethanol and aqueous extracts of the stem bark of *Vitellaria paradoxa*.

MATERIALS AND METHODS

Collection of plant materials: Samples of the stem bark of *Vitellaria paradoxa* were collected in April from the Permanent Site Campus of the University of Ilorin, Nigeria. The stem bark samples were authenticated at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, Nigeria where voucher specimen was deposited. The study was conducted between April, 2011 and July, 2011.

Animals: Healthy Swiss mice (20-25 g) and albino Wistar rats (180-200 g) used in the study were obtained from the Animal House of the College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. They were maintained under standard laboratory conditions of humidity (40-60%), temperature (21±1°C) and light (12/12 h light/dark cycle). The animals were acclimatized for one week, fed on rat pellets (Livestock Feeds PLC, Ibadan, Oyo State, Nigeria) and allowed free access to drinking water *ad libitum*. They were fasted overnight before the experiment was carried out. All conditions of animal use were as approved by United States National Institute of Health (NIH) guide for Care and Use of Laboratory Animals and in accordance with the recommendation of IASP (Zimmermann, 1983).

Preparation of the plant extracts: The stem bark of *V. paradoxa* was air-dried under shade to constant weight for 14 days and the dried materials were chopped into small pieces using a pestle and mortar and then pulverized into powdery form using an electric blender. One hundred grams of the powdered plant material was soaked in 500 mL of distilled water or 95% ethanol in separate 1.5 L sterile conical flasks at room temperature. These were subjected to uniform agitation in a shaker water bath for 48 h. The content was then filtered with a piece of muslin cloth and then Whatman filter paper (No. 1). The filtrates were evaporated to dryness and the resultant extracts

were packed in separate clean dry bottles and stored in a refrigerator at 4°C until use. The yields of aqueous and ethanol extracts are 0.6 and 0.8%, respectively.

Collection and identification of bacteria: The clinical isolates of *E. coli, K. pneumonia, P. mirabilis, E. faecalis, S. aureus, P. aeruginosa* and *S. pyogenes* were collected in peptone water from the Microbiology Laboratory of Nigeria Institute of Medical Research, Lagos, Nigeria Preliminary identification of the test organisms were carried out in the Microbiology Laboratory of LAUTECH Teaching Hospital, Osogbo, Nigeria. The organisms were stored on Nutrient agar slants in a refrigerator (2-8°C). Purity of the cultures was checked at regular intervals as described by Acheampong *et al.* (1988).

Determination of antimicrobial activity: Antimicrobial activities of aqueous and ethanolic extracts of *Vitellaria paradoxa* were carried out using the agar well diffusion method (Vanden Berghe and Vlietnck, 1991). The bacterial strains grown on nutrient agar at 37°C for 18 h were suspended in a saline solution (0.9%, w/v) NaCl and adjusted to a turbidity of 0.5 McFarland standard (10^8 CFU mL^{-1}). To obtain the inocula, these suspensions were diluted 100 times in Muller Hinton broth to give 10^6 colony forming units (CFU) mL^{-1}. Standardized inoculum of each test bacterium was spread onto nutrient agar plates so as to achieve even growth and the plates were allowed to dry. Discs were obtained using Whatman filter paper (No. 1). They were prepared by punching and then put in vials-bottles and sterilized in an oven at 150°C for 15 min. Thereafter the discs (9 mm diameter) were aseptically bored into the solid nutrient agar using a sterile cork borer. The test solutions of extracts at concentration of 50 mg mL^{-1} were then introduced into each of the designated discs on each plate while ensuring that no spillage occurred. Sparfloxacin at a concentration of 5 μg mL^{-1} (Solidum Pharmaceuticals, Nigeria) was used as a positive control and distilled water as negative control. The plates were left at room temperature for 1 h, allowed to diffuse into the medium, turned upside-down and thereafter incubated at 37°C for 24 h in an incubator. Clear zones of inhibition were observed. Activity of each extract was tested in triplicate and the diameters of zones of inhibition were measured in millimeter.

Acute toxicity test: The ethanol extract of *V. paradoxa* was tested because of its higher antibacterial activity against all the bacteria considered. Mice were divided into six groups of six animals each. The extract was administered to mice in groups 2-6 in single oral doses of 20, 40, 80, 160, 320 and 640 mg kg^{-1} b. wt., respectively by intra gastric gavage using oral cannula (a feeding needle). The control group (group 1) received an equal volume of distilled water as vehicle. Observations of toxic symptoms were made and recorded within 24 h after administration of the extract. Behavioral parameters and mortality were monitored closely for the initial 2 h and thereafter for 24 h. Lethal dose in fifty per-cent of the total population (LD_{50}) was interpolated using Lorke (1983) method.

Effects on hematological and biochemical parameters: Ten albino Wistar rats were used for the study. They were randomly divided into 2 groups of 5 rats each. Group 1 served as the control and received normal saline (5 mL kg^{-1}). Group 2 was treated with 12 mg kg^{-1} of the extract for 28 days. All drugs were administered orally. On the 29th day, rats were anaesthetized in a glass chamber containing cotton wool soaked in diethyl ether and blood samples were collected by cervical decapitation into clean lithium heparin bottles. The blood samples were centrifuged for 5 min and

the supernatant plasma was subsequently used for the assay of biochemical parameters while whole blood was used for hematological assays. An automated hematologic analyzer (Sysmex KX-21, Japan) was used to analyze the hematological parameters of Hemoglobin (Hb), Packed Cell Volume (PCV), Red Blood Cell (RBC), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC), White Blood Cell (WBC), neutrophils, lymphocytes and platelets. AST, AL, ALP, serum urea and creatinine levels were determined as described by Toro and Ackermann (1975).

Statistical analysis: All data were expressed as Mean±SEM and statistically analyzed by Student's t-test at 95% confidence limit.

RESULTS

Oral dosing of 20, 40, 80, 160, 320 and 640 mg kg^{-1} of the ethanol extract resulted in different percentage death of the rats as shown below (Table 4). LD_{50} was calculated as 115 mg kg^{-1} from a curve of log dose vs. %response. Behavioral changes observed include reduced motor activity, increased breathing rate and abdominal writhes.

Both aqueous and ethanol extracts demonstrated antimicrobial activity against five out of the seven bacteria strains but ethanol extract demonstrated higher antibacterial activity especially on *K. pneumoniae*, *E. faecalis* and *S. aureus*. *P. aeruginosa* and *S. pyogenes* were completely resistant to both extracts. These results are presented in Table 1.

Following the treatment of rats with ethanol extract of *V. paradoxa* for 28 days, ALP, ALT, AST, creatinine and urea plasma levels of the treated rats increased significantly ($p<0.05$) compared with the control (Table 2). There was no significant difference between the control and test groups in the haematological indices tested except the platelets which significantly decreased in the test group (Table 3).

Table 1: Antibacterial activity of stem bark extract of *Vitellaria paradoxa*

	Zone of inhibition (mm)		
Organism	Aqueous extract	Ethanol extract	Sparfloxacin
Klebsiella pneumoniae	7.2±0.7[a]	9.0±0.7[a]	34.1±4.1[a]
Proteus mirabilis	6.5±0.8[a]	8.3±0.9[a]	30.6±2.8[a]
Enterococcus faecalis	10.0±0.3[a]	19.5±0.8[a]	32.3±3.2[a]
Escherichia coli	9.1±0.7[a]	10.5±0.7[a]	32.7±2.5[a]
Staphylococcus aureus	11.4±0.9[a]	21.0±0.5[a]	30.2±4.0[a]
Pseudomonas aeruginosa	na	na	26.0±2.9[a]
Streptococcus pyogenes	na	na	24.1±3.7[a]

Value are represents Mean±SEM for three trials, na: Non-measurable zone of inhibition, [a]$p<0.05$ compared with negative control which has no antibacterial activity

Table 2: Effects of stem bark ethanol extract of *Vitellaria paradoxa* on biochemical parameters in rats

Parameter	Control	Test
ALT (IU L^{-1})	79.8±6.90	181.8±41.5[a]
ALP (IU L^{-1})	100.5±11.1	126.0±14.7[a]
AST (IU L^{-1})	452.2±19.3	493.8±98.1[a]
Urea (mmol $L^{-}1$)	7.3±1.00	13.0±0.4[a]
Creatinine (mmol L^{-1})	89.2±11.6	106.0±15.0[a]

Values are Mean±SEM (n = 5), [a]$p<0.05$ compared with control

Table 3: The effects of stem bark ethanol extract of *Vitellaria paradoxa* on haematological parameters in rats

Parameter	Control	Test
RBC ($\times 10^6$ μL^{-1})	43.9±0.3	47.3±0.3
Hemoglobin (g dL^{-1})	12.1±1.1	13.3±0.6
Haematocrit (%)	41.8±2.3	45.5±1.9
MCV (fL)	60.4±0.5	62.3±1.1
MCH (pg)	17.2±0.8	18.2±0.3
MCHC (g dL^{-1})	28.6±1.3	29.3±0.5
WBC ($\times 10^3$ μL^{-1})	10.2±1.6	9.8±2.1
Neutrophil (%)	64.2±1.5	65.7±1.2
Leukocyte (%)	35.2±1.2	33.2±1.5
Platelets ($\times 10^3$ μL^{-1})	709.6±62.2	514.8±48.4*

Values are Mean±SEM (n = 5), *p<0.05 compared with the control

Table 4: Acute toxicity of ethanol extract of *Vitellaria paradoxa* stem bark

Dose (mg kg^{-1})	No. of animals	No. of death	Death (%)
20	10	0	0
40	10	0	0
80	10	4	40
160	10	6	60
320	10	10	100
640	10	10	100

DISCUSSION

Ethanol extract of *V. paradoxa* exhibited inhibitory effect on the growth of *E. coli, K. pneumonia, P. mirabilis, E. faecalis* and *S. aureus* but not on *P. aeruginosa* and *S. pyogenes*. The organisms that are susceptible to the extract are known to cause many life-threatening diseases such as meningitis, endocarditis urinary tract infections and gastrointestinal tract infections among others (Adebayo and Ishola, 2009). So the extract could be a good alternative in the treatment of these infectious diseases. *S. aureus* exhibited the highest sensitivity followed by *E. faecalis*. This is an indication that Gram-positive bacteria are more susceptible to the antimicrobial effect of *V. paradoxa* than Gram-negative bacteria. This is consistent with previous studies on other medicinal plants (Ceylan and Fung, 2004; Lopez *et al*., 2005). The significant differences in the outer layers of Gram-negative and Gram-positive bacteria may be responsible for this observation. The resistance of Gram-negative bacteria towards antimicrobial agents is related to the hydrophilic surface of their outer membrane which is rich in lipopolysaccharide molecules, presenting a barrier to the penetration of numerous antibiotic molecules (Shan *et al*., 2007). It is also associated with the enzymes in the periplasmic space, which are capable of breaking down the molecules introduced from outside (Russel, 1991). Gram-positive bacteria lack this outer coating and cell wall. Therefore the cell wall of Gram-positive organisms is more prone to destruction by antibacterial agents. However it should be noted that the extract also exerted some moderate activity on Gram-negative bacteria used for the study except in *P. aeruginosa* in which it was completely devoid of activity. The sensitivity of *K. pneumoniae*, *P. mirabilis* and *E. coli* to the extract agrees with the work of El-Mahmood *et al*. (2008). Ethanol extract of the plant has higher antibacterial effect than water extract. This is probably because ethanol extracted more of the phytochemical constituents of the plant that are responsible for its antibacterial activities.

Phytochemical studies of stem bark of *V. paradoxa* revealed the presence of alkaloids, tannins, saponins, steroids and carbohydrates (Ndukwe *et al.*, 2007). These bioactive compounds are likely responsible for the antimicrobial activity of the plant against the organisms (Ndukwe *et al.*, 2005). There was significant increase in the plasma levels of ALT, AST and ALP of rats treated with ethanol extract of the plant for 28 days. Increase in the levels of these enzymes is an indicator of tissue damage and altered membrane permeability (Kolawole *et al.*, 2011). Damage to liver, kidney, bone and small intestine may increase the level of ALP in blood (Adedapo *et al.*, 2007). Increase in creatinine and urea levels is an indication of injury to the kidney (Aliyu *et al.*, 2007).

The platelets count in the treated rats was significantly lower ($p<0.05$) than that of the control. Platelets are involved in the regulation of clotting when there is injury to the blood vessel walls. When platelet count is low, clot retraction is deficient and there is poor contraction of ruptured vessels (Ganong, 2002) which results in increasing bleeding tendency after a minor injury.

CONCLUSION

This work has demonstrated that *Vitellaria paradoxa* stem bark extract possesses broad spectrum antibacterial activity against some clinical isolate of pathogenic bacteria. The finding justifies the use of the plant in traditional treatment of wounds, leprosy, oral infection and other infectious diseases and therefore has promising potential to provide lead molecules for the development of novel antibacterial drugs. The study also showed that prolonged administration of the plant extract at high doses may adversely affect the functions of some vital organs. Further study of the plant is necessary to isolate the active principles and determine the mechanism of action.

REFERENCES

Acheampong, Y.B., M.A. El-Mahmood and P.F. Olurinola, 1988. The antibacterial properties of the liquid antiseptic TCP. Indian J. Pharm. Sci., 50: 183-186.

Adebayo, E.A. and R.O. Ishola, 2009. Phytochemical and antimicrobial screening of the crude extracts from the root, stem bark and leaves of *Bridelia ferruginea*. Afr. J. Biotechnol., 8: 650-653.

Adedapo, A.A., M.O. Abatan and O.O. Olorunsogo, 2007. Effects of some plants of spurge family on the haematological and biochemical parameters of rats. Vet. Archiv., 77: 29-38.

Ahmed, R. N., A. Sani and O. O. Igunnugbemi, 2009. Antifungal profiles of extracts of *Vitellaria paradoxa* (Shea-Butter) bark. Ethnobotanical Leaflets, 13: 679-688.

Aliyu, R., A.H. Adebayo, D. Gatsing and I.H. Garba, 2007. The effects of ethanolic leaf extract of *Commiphora africana* (Burseraceae) on rat liver and kidney functions. J. Pharmacol. Toxicol., 2: 373-379.

Butler, M.S. and A.D. Buss, 2006. Natural products-the future scaffolds for novel antibiotics? Biochem. Pharmacol., 71: 919-929.

Ceylan, E. and D.Y.C. Fung, 2004. Antimicrobial activity of spices. J. Rapid Methods Automat. Microbiol., 12: 1-55.

El-Mahmood, A.M., J.H. Doughari and N. Ladan, 2008. Antimicrobial screening of stem bark extracts of *Vitellaria paradixa* against some enteric pathogenic microorganisms. Afr. J. Pharm., 2: 89-94.

Ferry, M.P., M. Gessain and R. Gessain, 1974. Vegetative Propagation of Shea, Kola and Pentadesma. Cocoa Res. Inst. Ghana Ann. Rep., 1987/88: 98-100.

Ganong, W.F., 2002. Review of Medical Physiology. 20th Edn., Lange Medical Books/McGraw-Hill, Medical Publishing Division, London, pp: 414-417.

Kolawole, S.O., O.T. Kolawole and M.A. Akanji, 2011. Effects of aqueous extract of *Khaya senegalensis* Stem Bark on biochemical and hematological parameters in rats. J. Pharmacol. Toxicol., 6: 602-607.

Lopez, P., C. Sanchez, R. Batlle and C. Nerin, 2005. Solid- and vapor-phase antimicrobial activities of six essential oils: Susceptibility of selected foodborne bacterial and fungi strains. J. Agric. Food Chem., 53: 6939-6946.

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

Nawel, H., R.B. Ammar, W. Afef, K. Soumaya and M. Amor *et al.*, 2005. Antibacterial and antimutagenic activity of extracts and essential oil from (Tunisia) *Pistacia lentiscus*. Toxicol. Envir. Chem., 87: 567-573.

Ndukwe, I.G., J.O. Amupitan, Y. Isah and K.S. Adegoke, 2007. Phytochemical and antimicrobial screening of the crude extracts from the root, stem bark and leaves of *Vitellaria paradoxa*. Afr. J. Biotechnol., 6: 1905-1909.

Ndukwe, K.C., I.N. Okeke, A. Lamikanra, S.K. Adesina and O. Aboderin, 2005. Antibacterial activity of aqueous extracts of selected chewing sticks. J. Contemp. Dent. Prac., 6: 86-94.

Russel, A.D., 1991. Mechanisms of bacterial-resistance to non-antibiotics- food-additives and food and pharmaceutical preservatives. J. Applied Bacteriol., 71: 191-201.

Shan, B., Y.Z. Cai, J.D. Brooks and H. Corke, 2007. The *in vitro* antibacterial activity of dietary spice and medicinal herb extracts. Int. J. Food Microbiol., 117: 112-119.

Soladoye, M.O., S.S. Orhiere and B.M. Ibimode, 1989. Ethnobotanical study of two indigenous multipurpose plants in the Guinea savanna of Kwara state-*Vitellaria paradoxa* and *Parkia biglobosa*. Proceedings of the 2nd Biennial Conference of the Ecological Society of Nigeria, August 14, 1989, Forestry Research Institute, Ibadan, Pages: 13.

Toro, G. and P.G. Ackermann, 1975. The Practical Clinical Chemistry. 1st Edn., Little Brown and Co., Boston, USA.

Vanden Berghe, D.A. and A.J. Vlietinck, 1991. Screening Methods for Antibacterial and Antiviral Agents from Higher Plants. In: Methods in Plant Biochemistry, Dey, P.M. and J.B. Harborne (Eds.). Academic Press, London, UK., pp: 47-69.

Walsh, C.T. and G. Wright, 2005. Introduction: Antibiotic resistance. Chem. Rev., 105: 391-393.

Walsh, F.M. and S.G. Amyes, 2004. Microbiology and drug resistance mechanisms of fully resistant pathogens. Curr. Opin. Microbiol., 7: 439-444.

Zimmermann, M., 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain, 16: 109-110.