Efficacy and Safety of Leaf, Bark and Root Extract of *Vitellaria paradoxa* on Diarrhoea Induced Albino Rats

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Abstract Leaf, Root and Bark extracts of *Vitellaria paradoxa* have been used to cure many infectious diseases in different parts of Nigeria. However, there is need for further research to better understand the efficacy and safety of these plant parts. Methanol and omidun extracts of these plant parts studied using albino rats which were randomly assigned into four groups A-D containing five rats each. Group A rats were infected with *E. coli* and later treated with ciprofloxacin, group B rats were infected with *E. coli* only, group C rats were infected with *E. coli* and treated with methanol extract of root, while rats in group D were not infected nor treated. The procedure was replicated for *Salmonella typhi*, omidun extracts and two dosages of the extract (50mg/kg and 100mg/kg) respectively. At the end of the experiment, the rats were anaesthetized using chloroform and liver and kidney samples were collected for histopathological studies. Rats in groups A&D have similar presentation with no histopathological changes. The group that received low concentration (50mg/ml) of leaf and bark extracts showed moderate lymphocytic aggregate and the infiltration disappears in rats that received high concentrations (100mg/ml) of leaf and bark extracts showing similar presentation with rats in group A. Organs of rats treated with low concentration of root extract showed lymphocytic aggregate at low concentration and degeneration at high concentration. Leaf and bark extracts of *V. paradoxa* are not associated with any adverse effects at both concentrations of treatment but there was toxicity of the root extracts at high concentration. Hence, leaf and bark extracts are safe for consumption but the root should be consumed with caution and at low concentration.

Keywords: *Vitellaria paradoxa*, efficacy, safety, methanol, omidun, Concentration


1. Introduction

Man has been using natural products from plant sources for thousands of years either in the pure forms or crude extracts for medical treatment [1]. Medicinal plants would be the best source to obtain a variety of drugs probably due to presence of various complex chemical substance of different composition, which are found as secondary plant metabolites in one or more parts of these plants [2].

It is estimated that in the developing countries, about 80% of the population rely on herbal medicine for their primary health care [3], due to their better cultural acceptability, belief of better compatibility with the human body and fewer side effects. Also, the quality of an extract are basically influenced by plant part used as starting material, solvent used for extraction and extraction procedure [4].

Therefore, plants should be investigated to better understand their properties, safety and efficacy [5]. In Africa, one of such plants which is claimed to have anti-diarrhoea property is *Vitellaria paradoxa* (the shea butter tree). The leaves, root, fruits and stem bark have been used in the treatment of various infections such as wound infections, skin diseases, diarrhoea, dysentery, helminthes and other gastrointestinal tract infections [6]. The roots are used as chewing sticks in Nigeria, most commonly in savannah areas. Roots and root bark are ground to a paste and taken orally to cure jaundice [7]. Mixed with tobacco, the roots are used as a poison by the Jukun of northern Nigeria. Chronic sores in horses are treated with boiled and pounded root bark [8]. Antimicrobial potentials of parts of *V. paradoxa* has also been reported [9].

2. Materials and Methods

2.1. Sources of Plant Collection and Antibiotics

Fresh roots of *Vitellaria paradoxa* was collected from Onipako village, Ilorin Kwara State, Nigeria. The plant was identified by trained plant taxonomists at the Federal University of Agriculture Abeokuta, Nigeria. The collected Plant materials was washed with sterile water and dried under shade to prevent direct effect of sun,
which might affect the chemical constituents in the extract. The antibiotic drug (ciprofloxacin) was purchased from a pharmaceutical shop located in Abeokuta, Ogun State.

2.2. Preparation and Extraction of the Plant

Two hundred (200) gram of the root was cut into small pieces with a surface-sterilized scalpel before milling with milling machine. A quantity (100 g) of the fine powder of roots was weighed and suspended into a 2500ml-capacity conical flask. To this was added 1000 ml of 95% methanol and omidun respectively. Each was allowed to stand for 48hours with constant shaking at regular intervals under room temperature. The percolates were then filtered and the solvents (methanol and omidun) were evaporated using rotary evaporator (Stuart, Barloworld model RE 300) to obtain the methanol and omidun extracts of the root. These were stored in sterile air tight container and stored in the refrigerator at 4°C until needed for analysis.

2.3. Experimental Animals

Sixty healthy male and female albino rats (Rattus norvegicus) weighing between 180g – 220g were used for this experiment. They were purchased from the University of Ibadan, Animal House. Rats were maintained according to the NIH guidelines of care and use of laboratory Animals published by Saha et al. [10]. The rats were acclimatized to standard laboratory conditions (temperature 24 ± 1°C and a 12 hours photoperiod), fed twice daily with standard commercial feeds (Vital Feeds, Nigeria) and distilled water adlibitum for two weeks before the commencement of the experiment. The rats were tested four times over the 2-week period to ensure that they were negative for the test microorganisms [11].

2.4. Experimental Design

The albino rats were randomly assigned into 4 groups ‘A-D’ of 5 rats each. The four groups of Albino rats consisting of 5 rats per group were studied to assess the in vivo antimicrobial effect of the root extract against diarrhoeagenic bacteria. Group A rats were induced with organism and treated with Ciprofloxacin (served as positive control). Group B rats were induced with organism only, two subgroups of C were induced with organism and later treated with 2mls of 50mg/kg and 100mg/kg of different extracts (methanol and omidun) of the root respectively, group D rats received nothing. The treatment was repeated for each of the 3 test organisms. The treated rats were then placed in separate cages over clean white paper and observed for the presence of diarrhoea every one hour for six hours for the onset of diarrhoea, number of diarrhoea episodes and mortality rate (numbers of rats that died during observation period in relation to total number of rats used in each group). Incidence of loose stool that was >2 was considered proof of diarrhoea, while presence of loose stool that was = or <2 was recorded as a protection from diarrhoea [13].

2.5. Test Organisms

The test organisms used for this study were clinical isolates (Salmonella typhi) obtained from Sacred Heart Hospital Lantoro, Abeokuta and typed culture (Escherichia coli ATCC 25922), obtained from National institute of medical research, Yaba, Lagos. The organisms were subjected to cultural, morphological and biochemical characterization using protocols described by Cheesbrough [12] for confirmation. Pure cultures of the confirmed isolates were maintained on slants in appropriate media and kept in the refrigerator at 4°C for future use.

2.6. Preparation of Inoculum

The inoculum was prepared from a stock culture of the test microorganisms. Cells of the test organisms were activated further by transferring a loopful of each stored organism onto test tubes containing 10 mls of sterilized peptone water and incubated at 370C for 18-24 hrs. The activated cultures were serially diluted in test tubes to generate concentrations up to 1.0× 10⁹ cfu/ml. A high concentration of the inoculums was prepared in order to increase the probability of establishing the disease condition in the experimental animals.

2.7. In-Vivo Antimicrobial Assay and Pathological Manifestations in the Treated Rats

The rats were randomly assigned into 4 groups A-D of 5 rats each and treated orally as follows; Group A rats were given 0.5ml of 18- hr broth culture of the test organisms to induce diarrhoea and later treated with 2mls of Ciprofloxacin, group B rats were induced with organism only, two subgroups of C were induced with organism and later treated with 2mls of 50mg/kg and 100mg/kg of different extracts (methanol and omidun) of the root respectively, group D rats received nothing. The treatment was repeated for each of the 3 test organisms. The treated rats were then placed in separate cages over clean white paper and observed for the presence of diarrhoea every one hour for six hours for the onset of diarrhoea, number of diarrhoea episodes and mortality rate (numbers of rats that died during observation period in relation to total number of rats used in each group). Incidence of loose stool that was >2 was considered proof of diarrhoea, while presence of loose stool that was = or <2 was recorded as a protection from diarrhoea [13].

2.8. Histology

Following laparotomy, livers and kidneys were quickly excised from each rat and fixed in 10% formalin. Thin sections (3μm) of each organ were histologically processed, stained with Haematoxylin and Eosin (H&E) and observed with light microscope for histopathological changes.

3. Results

Histopathological findings revealed that sections of liver and kidneys of rats that received nothing showed similar presentation with those of rats in the control group (treated with antibiotics) with no significant (p>0.05) histopathological changes as shown in plate 1 (A) and E. However, there was moderate inflammation of liver of rats treated with low concentration of solvents extracts (methanol and omidun) of the root as shown in plate 1(B) while rats treated with high concentration of root extract showed moderate vacuolar degeneration of centrilobular hepatocytes as shown in plate 1 (C). The solvent extracts of the root showed mild to moderate mononuclear cellular infiltrate in the kidney as shown in Plate 2 (F) and at high concentration (100mg/ml), there was inflammation and moderate degeneration of the kidney as shown in plate 2 (G).
Figure 1. Microscopic Presentation of liver of Treated Diarrhoea Induced Albino Rats


Figure 2. Microscopic Presentation of Kidney of Treated Diarrhoea Induced Albino Rats

4. Discussion

The present study was designed to evaluate the effects of methanol and omidun extracts of leaf, bark and root of V. paradoxa using the *in vivo* assay. Livers and kidneys are important internal organs with numerous functions such as elimination of waste products and toxic substances. Dysfunctions of these organs result in leaking of biochemical substances into the blood circulatory system [14].

Histopathological results obtained from this study showed that rats in the infected antibiotic-treated group and rats that received nothing showed similar presentation with no histopathological changes. This agrees with the report that ciprofloxacin was generally considered nontoxic by several authors [15]. However, there was degeneration in the kidneys and livers of rats in the infected non treated group.

Organs of rats treated with low concentration of root extract showed lymphocytic aggregate at low concentration and degeneration at high concentration. Presence of lymphocyte indicates responses of the immune system to strange or toxic substances which enter into the body. Lymphocytes are involved in a variety of immunological function, such as immunoglobulin production and modulation of immune defense [16]. Studies carried out by Ijeh and Agbo [17] indicated the possibility that the use of plant extract in high doses could lead to toxic injury to kidney which may interfere with renal tubular functioning and could induce acute renal failure. A number of herbs are thought to likely cause adverse effects [18]. This inability of root extract to increase or confer protection and prevent damage to organs may be because it cannot be sustained in the body at high enough levels. The histological findings in this work indicated toxicity of root extracts of V. paradoxa especially at high dosage.

Many factors might have caused the root’s toxicity as reported by Lewis [19], he stated that there is a distinction between plants that are poisonous because they naturally produce dangerous phytochemicals and those that may become dangerous for other reasons including the uptake of toxic compounds through response to environmental stress, such as a hard freeze or a drought, contaminated soil or groundwater, by sharply increasing their toxicity.

5. Conclusions

From this study, it can be concluded that methanol and omidun extracts of root *V. paradoxa* showed appreciable antimicrobial effects against all the tested diarrhoea causing microorganisms. The study also revealed that the methanol and omidun extracts conferred similar patterns of protection at both concentrations of treatments from diarrhoea. It was also revealed in histopathology that, leaf and bark extracts were safe at both concentrations of treatments. The root extracts was safe at low concentration but it should be carefully consumed and should be avoided at higher dosage.

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References


