International Journal of Multidisciplinary Research and Development
Online ISSN: 2349-4182 Print ISSN: 2349-5979
www.allsubjectjournal.com
Volume 3; Issue 2; February 2016; Page No. 220-224

Haematological parameters of Diarrhoea Induced Albino rats Treated with Leaf, bark and root of *V. paradoxa* extracted using four solvents

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Abstract
Blood samples of albino rats treated with solvent extracts of leaf, bark and root of *Vitellaria paradoxa* were studied. The rats were grouped into A and B containing five rats each. Group a rats were given *E. coli* ATCC 25922 and ciprofloxacin while group B rats were given *E. coli* ATCC 25922 and treated with plant extract. The procedure was replicated for each test microorganism, each solvent extract and two dosages of the extract. The blood parameters significantly depended (p <0.05) on solvent of extraction and type of plant parts but not significantly (p >0.05) on type of tested microorganism nor on dosage of treatment with higher levels in rats treated with commercial antibiotics, methanol extracts, bark extracts and 50mg/ml concentrations. Rats treated with bark extracts have higher degree of disease resistance and are less likely to develop anaemia than those treated with other parts of *V. paradoxa*.

Keywords: *Vitellaria paradoxa*, solvent extract, treated, omidun, haematological studies

1. Introduction
Due to a rapid increase in the rate of infections, antibiotic resistances in microorganisms and side effects of synthetic antibiotics [1], uses of medicinal plants in the treatment of microbial infections are gaining fast recognitions over antibiotics. Medicinal herbs have curative properties and have been used for years in daily life to treat diseases all over the world [2]. Hence, plants have turned out to be an important source of new potent antibiotics, new drug leads and new chemical entities [3]. They are considered cheapest and safer alternative sources of antimicrobials [4] to which pathogens are not resistant [5].

The effects of many different plant extracts on pathogenic microorganism have been indicated by numerous studies [6, 7]. The leaves, root, fruits and stem bark of *Vitellaria paradoxa* have been used in the treatment of various infections such as wound infections, skin diseases, diarrhoea, dysentery, helminthes and other gastrointestinal tract infections [8]. Some basic parameters influencing the quality of an extract are [9] plant part used as starting material and solvent used for extraction.

Furthermore, "adulteration, inappropriate formulation, or lack of understanding of plant and drug interactions have led to adverse reactions that are sometimes life threatening or lethal [10]. "Proper double-blind clinical trials are therefore needed to determine the safety and efficacy of each plant before they can be recommended for medical use [11]. Although many consumers believe that herbal medicines are safe because they are "natural", herbal medicines and synthetic drugs may interact, causing toxicity to the patient.

Although many consumers believe that herbal medicines are safe because they are "natural", herbal medicines and synthetic drugs may interact, causing toxicity to the patient. A case of major potassium depletion has been attributed to chronic licorice ingestion [12] and consequently professional herbalists avoid the use of licorice where they recognize that this may be a risk. Few studies are available on the safety of herbs for pregnant women [13], and one study found that use of complementary and alternative medicines are associated with a 30% lower ongoing pregnancy and live birth rate during fertility treatment [14].

However, Collinson and Zewdie-Bosuener [15] and Bauer and Moll [16] variously reported works on this plant which focused essentially on the fruit, kernel, seed and the fat from the seed. Study conducted by Abubakar *et al.* [17] also revealed that methanol stem bark extract *Vitellaria paradoxa* may possess anti diarrhoeal property. Phytochemical screening of the stem bark of *V. paradoxa* revealed the presence of carbohydrates, alkaloids, saponins, tanins and cardiac glycosided [18]. Falana *et al.* [19] also revealed that various solvent extracts of leaf, bark and root of *V. paradoxa* possess varying antimicrobial potentials. There is no information on the effect of these solvent extracts on the consumers. Hence, this work on study of haematological parameters of diarrhoea induced albino rats treated with leaf, bark and root of *V. paradoxa* extracted using for solvents.

2. Materials and Methods
This work was conducted as part of Ph.D research work under the Department of Microbiology, Federal University of Agriculture Abeokuta. The work was approved by the University ethical committee.

2. 1 Collection and Preparation of Plant Materials
The plant materials used for this study were leaf, bark and root of *Vitellaria paradoxa* (Shea butter tree) which were collected from Onipako village in Ilorin, Kwara State of Nigeria, confirmed by local farmers and further identified and

authenticated in the Herbarium Laboratory of the Department of Forestry and Wildlife Management, Federal University of Agriculture, Abeokuta. The collected Plant materials were washed with sterile water and dried under shade; they were reduced into small pieces with a surface-sterilized scalpel before milling with an electric blender (Marlex).

A quantity (150 g) of the fine powder of the leaf was each weighed into four 1000 ml-capacity conical flask and 500 ml methanol, sterile distilled water, sterile omidun and non-sterile omidun was added to powder in a conical flask respectively. This procedure was repeated for the root and bark samples to give a total of twelve (12) 1000 ml capacity conical flasks. Each was allowed to stand for 48 hours with constant shaking at regular intervals to facilitate extraction [20].

The percolates were then filtered and the resulting volume on filtration was reduced with a rotary evaporator at 45 ± 10 ºC. Final solvent elimination and drying was done using a water bath at 40 ºC. The extracts were then collected, weighed, packed in sterile air tight containers and labeled. They were kept in the refrigerator at 4 ºC until needed for analysis. Stock solution of each extract was prepared in three test tubes; the test tubes were labeled 1 to 3. A stock concentration of 100mg/ml of the extract was prepared in the first test tube, subsequently; 5ml of distilled water was then introduced into the remaining two test tubes. 5 ml of the stock was withdrawn from the first tube and added to the second test tube which was mixed thoroughly to obtain a concentration 50mg/ml. Another 5 ml was withdrawn from the second tube and then transferred to the third tube which was also thoroughly mixed to give a concentration of 25 mg/ml.

2.2 Experimental Animal

Male and female Albino rats weighing between 180g – 220g were used for this study. 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*. [21]. The rats were acclimatized to standard laboratory conditions (temperature 24 ± 1 ºC and a 12 hours photoperiod), fed twice daily with standard commercial feeds (Vital Feeds, Nigeria) and distilled water adlibitum for one week before the commencement of the experiment.

2.3 Experimental Design

Male and female Albino rats were randomly assigned into 2 groups 'A and B of 5 rats each. Group a received organism and later treated with ciprofloxacin antibiotics while group B received organism and extract. Rats in both groups were observed for six hours for the presence or absence of watery stool then their blood samples were collected and subjected to haematological studies.

2.4 Test Organisms

The test organisms used for this study were clinical isolates (*Shigella dysenteriae* and *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 [22] for confirmation. Pure cultures of the confirmed isolates were maintained on slants in appropriate media and kept in the refrigerator at 4 ^{0}C for future use.

2.5 Hematology

After the *in vivo* experiment, haematological study of blood parameters of the treated rats was carried out. Blood sample (2ml) was collected from their jugular vein with a disposable syringe and needle and immediately transferred into sterile Ethylene Diamine Tetra-acetic Acid (EDTA) embedded vials for haematological study [23] of total erythrocyte (RBC), leukocyte (WBC) counts, Packed Cell Volume (PCV), Hemoglobin (Hb) content. Various hematological indices were calculated from the results obtained. These include Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC).

3. Results

3.1 Effect of Different Extraction Solvent on Haematological Parameters of Blood samples of Diarrhoea Induced Albino Rats

Table 1 shows that level of WBC was high in omidun extracts (5.639), followed by methanol extracts (5.526), sterile omidun extracts (4.841) and sterile water extract (4.641). There was no significant difference (P>0.05) in the levels of WBC of rats treated with omidun and methanol extracts of each of leaf, bark and root of *V. paradoxa*. There was also no significant difference (P>0.05) in the levels of WBC of rats treated with sterile omidun and water extracts of the plant parts but there was significant difference (P<0.05) between these solvent extracts and other solvent extracts (omidun and methanol extracts).

Values of RBC were higher in rats treated with methanol extracts (8.277) and omidun extracts (8.187) followed sterile omidun extracts (7.339) and lower in sterile water extracts (6.819). There was no significant difference (P>0.05) in the level of RBC of rats treated with omidun and methanol extracts but there was significant difference (P<0.05) between these solvent extracts and other solvent extracts (sterile omidun and sterile water extracts).

Values of PCV were higher in rats treated with omidun extracts (425.467) and methanol extracts (421.233) followed sterile omidun extracts (382.589) and lower in sterile water extracts (378.365). There was no significant difference (P>0.05) in the level of PCV of rats treated with omidun and methanol extracts but there was significant difference (P<0.05) between these solvent extracts and other solvent extracts (sterile omidun and sterile water extracts).

Generally, there was significant difference (P<0.05) in the levels of LYMPH, MCV, MCHC, MPV and PDW of rats treated with all the solvent extracts (methanol, omidun, sterile omidun and sterile water). However, there was no significant difference (P>0.05) in the levels of MON, GRAN, HGB, MCH, PCT of rats treated with all the solvent extracts (methanol, omidun, sterile omidun and sterile water).

Table 1: Effect of Different Extraction Solvent on Haematological Parameters of Blood samples of Diarrhoea Induced Albino Rats

Parameters	Omidun	Methanol	Sterile Water	Sterile omidun
WBC(µl)	5.639[a]	5.526[a]	4.641[c]	4.841[bc]
LYMPH (%)	4.210[a]	4.257[a]	3.476[b]	3.638[ab]
MON (%)	0.222[a]	0.216[a]	0.159[a]	0.281[a]
GRAN (%)	1.627[a]	1.260[ab]	0.968[b]	1.049[ab]
RBC(µl)	8.187[a]	8.277[a]	6.819[c]	7.339[b]
HGB (g/dl)	141.203[a]	143.656[a]	140.367[a]	138.900[a]
MCV (fl)	58.878[a]	57.783[a]	48.380[c]	53.497[b]
MCH (pg)	22.465[a]	19.010[ab]	17.973[b]	16.058[b]
MCHC (g/dl)	311.217[a]	310.456[a]	280.322[b]	289.367[b]
PCV (%)	425.467[a]	421.233[a]	378.365[b]	382.589[b]
MPV(µl)	6.879[a]	6.847[a]	5.578[c]	6.112[b]
PDW	17.053[a]	15.366[ab]	13.870[b]	14.586[b]
PCT	0.276[a]	0.304[a]	0.295[a]	0.282[a]

Values with different superscript on the same column are significantly different ($p< 0.05$)

3.2 Effect of leaf, bark and root of *V. paradoxa* on Haematological Parameters of Blood samples of Diarrhoea Induced Albino Rats

Table 2 shows the effect of different parts (leaf, root and bark) of *V. paradoxa* on haematological parameters of blood samples of diarrhoea induced albino rats. There was no significant difference ($P> 0.05$) in the level of WBC, HGB and PDW of rats treated with leaf, root and bark of *V. paradoxa*. Level of WBC was higher in rats treated with bark extracts (5.388) and those treated with root extracts (5.289) than those treated with commercial antibiotics (5.191) and leaf extracts (4.808). There was significant difference ($P< 0.05$) in RBC count of rats treated with leaf, root and bark of *V. paradoxa*. An increased RBC count was found in rats treated with bark extracts (8.758), followed by those treated with commercial antibiotics (7.742), those treated with root extracts (7.693) and lower in rats treated with leaf extracts (6.517).

Level of PCV was significantly ($P< 0.05$) high (569.517) in rats treated with bark extracts, followed by those treated with commercial antibiotics (418.512) than in rats treated with root and the leaf extracts (319.924% and 316.282% respectively). However, there was significant difference ($P<0.05$) in the levels of all other parameters (LYMPH, MCV, MCHC, MPV, PDW, MON, GRAN, HGB, MCH and PCT) in rats treated with leaf, root and bark extracts and commercial antibiotics.

Table2: Effect of leaf, bark and root of *V. paradoxa* on Haematological Parameters of Blood samples of Diarrhoea Induced Albino Rats

Parameters	Root	Leaf	Bark
WBC (µl)	5.289[a]	4.808[b]	5.388[a]
LYMPH (%)	4.255[a]	4.024[a]	3.406[b]
MON (%)	0.190[b]	0.141[c]	0.328[a]
GRAN (%)	1.627[a]	1.261[bc]	0.968[c]
RBC (µl)	7.693[b]	6.517[c]	8.758[a]
HGB (g/dl)	140.925[a]	137.325[b]	144.844[a]
MCV (fl)	54.761[a]	50.696[b]	58.447[a]
MCH (pg)	23.847[a]	16.059[c]	16.723[c]
MCHC (g/dl)	333.167[a]	294.429[b]	265.925[b]
PCV (%)	319.942[c]	316.282[c]	569.517[a]
MPV (µl)	6.362[c]	5.958[d]	6.743[b]
PDW	14.961[b]	15.491[b]	15.204[b]
PCT	0.247[b]	0.239[b]	0.382[a]

Values with different superscript on the same column are significantly different ($p< 0.05$)

3.3 Effect of difference in concentration of extracts on Hematological Parameters

Table 3 shows the effect of different in concentration of extracts on haematological parameters of blood samples of diarrhoea induced albino rats. There was a decrease in the level of WBC of rats treated with 100mg/ml concentration (5.043) of extracts than those treated with 50mg/ml concentration (5.281) of extracts although there was no significant difference ($P> 0.05$) in the level of these parameters in rats treated with both concentrations of extracts.

There was a significant difference ($P<0.05$) in the levels of RBC of rats treated with 50mg/ml concentration (7.449) and those treated with 100mg/ml (7.263) concentration of extracts. There was a decrease in the level of PCV of rats treated with 100mg/ml concentration (387.777) from those treated with 50mg/ml concentration (416.050) and there was significant difference ($P<0.05$) in the levels of PCV of rats treated with 50mg/ml concentration and those treated with 100mg/ml concentration of extracts.

There was no significant difference ($P> 0.05$) in the levels of LYMPH, MON, GRAN MPV and PDW of rats treated with the different concentrations of extracts but there was significant difference ($P<0.05$) in the levels of HGB, MCV, MCH, MCHC and PCT of rats treated with the different concentrations of extracts.

Table 3: Effect of difference in concentration of extracts on Heamatological Parameters

Parameters	50mg/ml	100mg/ml
WBC (µl)	5.281[a]	5.043[a]
LYMPH (%)	4.035[a]	3.755[a]
MON (%)	0.212[a]	0.227[a]
GRAN (%)	1.390[a]	1.062[a]
RBC (µl)	7.449[a]	7.263[b]
HGB (g/dl)	140.641[b]	141.761[a]
MCV (fl)	55.484[a]	53.784[b]
MCH (pg)	18.710[b]	19.043[a]
MCHC (g/dl)	285.353[b]	310.328[a]
PCV (%)	416.050[a]	387.777[b]
MPV (µl)	6.367[a]	6.341[a]
PDW	14.844[a]	15.593[a]
PCT	0.301[a]	0.277[b]

Values with different superscript on the same column are significantly different ($p< 0.05$)

3.4 Main effect of different type of organism on Haematological Parameters

Table 4 shows the effect of different diarrhoea causing microorganism on haematological parameters of blood samples of diarrhoea induced albino rats. Although, levels of haematological parameters vary slightly form diarrhoea inducing microorganism to microorganism but there was significant difference ($P< 0.05$) in the levels of WBC, RBC, HGB, MCH, MCHC, PCV, MPV and PCT of rats induced with different diarrhoea causing microorganisms.

However, there was no significant difference ($P>0.05$) in the levels of LYMPH, MON, GRAN, MCV and PDW of rats induced with different diarrhoea causing microorganisms.

Table 4: Main effect of different type of organism on Haematological Parameters

Parameters	*S. typhi*	*Sh. flexneri*	*E. coli* ATCC 25922
WBC (µl)	5.646^{ab}	4.797b^{c}	4.515^{c}
LYMPH (%)	3.915^{ab}	3.800^{ab}	3.639^{b}
MON (%)	0.315^{a}	0.124^{b}	0.293^{ab}
GRAN (%)	1.368^{a}	1.267^{a}	1.328^{a}
RBC (µl)	6.8181^{b}	7.8032^{a}	7.7528^{a}
HGB (g/dl)	136.421^{b}	142.194^{ab}	135.875^{b}
MCV (fl)	55.053^{ab}	55.762^{a}	53.735b^{c}
MCH (pg)	20.627^{ab}	15.374^{b}	17.651^{ab}
MCHC (g/dl)	342.521^{a}	283.375^{b}	332.097^{a}
PCV (%)	468.472^{a}	399.931^{b}	367.915^{b}
MPV (µl)	6.302^{b}	6.239^{b}	6.601^{a}
PDW	16.840^{a}	14.867^{a}	14.665^{a}
PCT	0.328^{a}	0.317^{a}	0.246^{b}

Values with different superscript on the same column are significantly different ($p< 0.05$)

4. Discussion

There is general agreement among clinical pathologists and toxicologists that concurrent control data for a parameter in question are best for comparison and for determining a potential test article related finding [24]. Comparison of the effect of different treatment (commercial antibiotics, leaf, bark and root of *V. paradoxa*), different solvent extracts (methanol, omidun, sterile omidun and water), different treatment concentration (50mg/kg and 100mg/ml concentrations) and different diarrhoea inducing bacteria tested (*S. typhi, Sh. Flexneri* and *E. coli* ATCC 25922) on blood parameters of the experimental rats showed significant (p <0.05) reduction in the values of red blood cells (RBC), white blood cells (WBC) and packed cell volume (PCV) of the treated rats from the commercial antibiotics but with no significant difference (p >0.05) among all the plant parts.

Although, these major blood parameters were found higher in rats treated with bark extracts, followed by those treated with root extracts and leaf extracts respectively. The parameters were also found higher in rats treated with methanol extracts and omidun extracts more than other solvent extracts. This is an indication that rats treated with methanol and omidun extracts as well as those treated with barks extracts were capable of generating antibodies in the process of phagocytosis and have high degree of resistance to diseases [25] and enhance adaptability to local environmental and disease prevalent conditions [26], than those treated with root and leaf extracts. Since the major functions of white blood cell and its differentials are to fight infections, defend the body by phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response. Thus, lower WBC counts in rats treated with leaf and root extracts is an indication that the rats were exposed to high risk of infection by the tested microorganisms than the rats treated with bark extracts.

Red blood cells (erythrocytes) serve as carrier of haemoglobin. It is this haemoglobin that reacts with oxygen carried in the blood to form oxyhaemoglobin during respiration [27]. According to Isaac *et al.* [26] red blood cell is involved in the transport of oxygen and carbon dioxide in the body. Thus, a reduced red blood cell count in rats given leaf extracts than in those treated with root and bark extracts implies a reduction in the level of oxygen that would be carried to the tissues of the treated rats as well as the level of carbondioxide returned to the lungs, which is an indication of anaemia [26]. While the increased RBC count in rats treated with bark extracts implies increase in the level of oxygen that would be carried to the tissues of the treated rats as well as the level of carbon dioxide returned to the lungs.

Packed Cell Volume (PCV) measures the percentage (%) of red blood cells in blood [28]. According to Isaac *et al.* [26] Packed Cell Volume is involved in the transport of oxygen and absorbed nutrients. Increased Packed Cell Volume of rats treated with bark extracts means better oxygen transportation and thus results in an increased primary and secondary polycythemia. While a decrease in PCV of rats treated with leaf extracts means anaemia in the rats. Previous reports stated that Packed Cell Volume, haemoglobin and mean corpuscular haemoglobin are major indices for evaluating circulatory erythrocytes, and are significant in the diagnosis of anaemia and also serve as useful indices of the bone marrow capacity to produce red blood cells as in mammals [27].

There is no significant difference (p>0.05) in the effect produced by the different diarrhoea inducing microorganisms. This may mean that the solvent extracts were effective on all the diarrhoea inducing microorganisms tested. However, all values of these major parameters were dose dependent as the values were lower with increased dose of treatment (100mg/ml concentration). This may be an indication that the extracts were nontoxic to the rats treated with low concentration (50mg/ml concentration) of the extracts and may be toxic to the rats with increased concentration of extracts. As reported by Isaac *et al.* [26] animals with good blood composition are likely to show good performance. According to Khan and Zafar [29], haemtological studies are useful in the diagnosis of many diseases as well as investigation of the extent of damage to blood. Khan and Zafar [29] also stated that hematological parameters are good indicators of the physiological status of animals. Other literature states that blood act as a pathological reflector of the status of exposed animals to toxicant and other conditions [30].

5. Conclusions

Findings in this study indicates that rats treated with methanol and omidun extracts are likely to show good performance with good immunological response and can withstand the disease condition better than rats treated with other solvent extracts. It also reveals that rats bark extract and low concentrations (50mg/ml) of treatment were non-toxic to the rats than rats treated with other plant parts and increased concentration of extracts.

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