Effects of *Dombeya Buettneri* Extracts on Heamapoietic Indices of Wistar Albino Rats.

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Abstract

The effects of Dombeya buettneri extracts on electrolyte and heamapoietic indices of wistar albino rats were studied. The results showed that there was a significant increase in red blood cells, white blood cells and hemoglobin, resulting in hemopoiesis and synthesis of hememoiety for hemogloglobin. The phytochemical composition showed low phytic acid (trace) which suggests antioxidant activity, enhancing immunocompetence. The LD50 obtained was 2.58mg/kg, suggesting that dosage of Dombeya buettneri below this LD50 is safe. This work suggests that Dombeya buettneri may be useful in the treatment of anemia and may boost immune response, and that administration (dosage) below 2.58mg/kg is safe.

Key Word: *Dombeya buettneri,* heamapoietic indices, wistar rats, and electrolytes.

Introduction

Man has been dependent on medicinal plants as curatives or palliatives of main health problems based on cultural instinct of preservation and protection (Vattem et al, 2005).

Dombeya buettneri is a plant found in the southern part of West African sub-region, and is useful for prevention and treatment of gastrointestinal disorders including peptic ulcers (Eastmond *et al.* 2008). Phytochemical analysis of the shrub revealed that it contains polyphenols, alkaloids, tannins, flavanoids, cardiac glycosides, and anthrouinones (Okwori *et al.,* 2000; (Rifat-Uz-Zaman *et al.,* 2004); Hotz and Gibson, 2007).

Gastric ulcer results from imbalance between aggressive factors and the maintenance of mucosa integrity through the endogenous defense mechanism (hyperacidity) (Rifat-Uz-Zaman *et al.*, 2004; Martin, 2005). The ulcer is an open sore in the lining of the stomach (gastric ulcer), or in the upper part of the small intestine or duodenum (duodenal ulcer) (Rifat-Uz-Zaman *et al.,* 2004). Both types are called peptic ulcer. It can also be described as a deep and sharply demarcated break in lining or discontinuation in the epithelium of stomach, duodenum or oesophagus. Stomach ulcer is a small erosin (hole) in the gastrointestinal tract (Manuel *et al*, 20O5); and the signs include burning, cramping, gnawing or aching in the abdomen that comes in waves. Pain is worse before meals and at bed time when the stomach is usually empty due to hyperacidity (Martin, 2005). An ulcer hurts when it penetrates the mucosa into underlying submucosa, which is

rich in nerves and blood vessels (Ologundudu *et al.*, 2000; Martin, 2005, Schubert, 2008).

Gastriculcer results from imbalance between aggressive factors (Hcl, pepsin, ulcerogenic drugs, alcohol, nicotine) and protective factors (secretion of mucus(gastric mucosal blood flow, HCO_3, prostaglandins etc) (Allesandra and Robert, 2005; Mike and Gilani, 2006). Non-steroidal anti-inflammatory drugs (e.g aspirin, ibuprofen, diclofenac, indomethacin, and ethanol) act by inhibiting cyclooxygenase enzyme important for production of prostaglandins that protect, the gastric epithelium (Rifat-Uz-Zaman *et al,* 2004; Schubert, 2008), thereby destroying the gastric mucosa barriers. Aspirin treatment increases formation of malondialdehyde (MDA) which is highly indicative of oxidative damage due to accumulation of toxic free radicals which cause lesion of mucosal cells (Ologundudu et al, 2008).

Prostaglandins may have a dual role in regulating acid secretion in the damaged stomach, an inhibitory effect at the parietal cells and an excitatory effect, probably through enhancing the release of mucosal histamine, a potent cAMP inhibitor, increases HCI and pepsin secretion (Mike and Ganelin 2006). Thus, histamine antagonist can be used for the treatment of gastric ulcer and related disease (Liu, 2004). Nicotine in tobacco increases the volume and concentration of stomach acid, thus increasing the risk of an ulcer (Schubert, 2008). Dietary minerals are the trace elements required by living organisms other than the basic elements of life, carbon, hydrogen, nitrogen and oxygen, which are present in organic molecules for the maintenance of life through metabolism (Alessandra and Robert, 2005). However, despite the beneficial effects of Dombeya buettneri in the management of gastrointestinal disorders, and other hypersecretory conditions, the effects of the phytotoxicants such as hydrocyanic acid, tannins, phytic acid, and oxalates predispose to anemia; alteration in mineral absorption mechanisms; thereby affecting mineral bioavailability and metabolism; and establishing a diagnosis of right dosage could be difficult since herbal therapy may not be monitored.

Tannins are astringent, bitter plant phenols, which inhibit the absorption of minerals such as irons, leading to anemia. (Gilani *et al*, 2005). Tannins are metal ion chelators, and tannin-chelated metal ions are not bioavailable. Tannins interfere with iron absorption in the gastrointestinal lumen, decreasing the bioavailability of iron. However, tannins can also be effective in protecting the kidneys (Cheng *et al,* 2002; Seeram *et al*, 2005). Tannins have been used for immediate relief of sore throat, diarrhea, dysentery, skin ulcer, and are potential antiviral (Chengetal, 2002; Lu *et al*, 2004; Liang, 2008). Oxalates aggravates kidneys disorders, rheumatoid, arthritis (Morozumi *et al.*, 2006); Oku and Ndu, 2006). Phytic acid is a strong chelator of important minerals such as calcium, magnesium, iron and zinc and may contribute to mineral deficiency (Hurrel, 2003). However, phytic acid is protective against osteoporosis (Lopez-Gonzalez, *et al.*, 2008). It is also a chelator of vitamin Niacin, causing pallegra. It is an anti-nutrient (Lopez-Gonzalez, *et al.,* 2008). Phytic acid has anti-oxidant effect, preventing colon cancer by reducing oxidative stress in the lumen of the intestinal tract (Vucenie et al, 2003; Xu *et al.,* 2008). Phytic acid crosses the blood brain barrier (Grasses *et al.*, 2001; Okwu & Ndu, 2006). Hydrogen cyanide ions interfere with containing respiratory enzymes causing death (Ernst, *et al.*, 2004; Mathew, 2004; Oboh and Oladunmoye, 2007).

The aim of the study is to focus on the assessment of the heamapoietic indices of wistar albino rats, mineral profile, and LD_{50}, an indicator of hepatotoxicity.

Materials and Method

Collection and preparation of *Dornbeya buetneri.*

The leaves sample collection and preparation of *Dombeya buettneri* were harvested in a plantation in Makurdi and was identified by M. T. Okoh of the Department of Botany, Federal University of Agriculture, Makurdi, where species voucher was

preserved in their herbarium.

The leaves were sun dried for 2 days and then oven dried at 46°C+ 1°C until it was brittle. About 50 g of the dried material was blended into powder using an electric blender (MSE, London, UK) and used for phytotoxicant assessment. From another 50 g portion of the dried material by modified method described by Obiefuna *et al.*, 1994. The 50 g dried material was pulverized and macerated for 12 h in 200 ml deionized water. This was filtered and the residue discarded, the filtrate was then evaporated to dryness in an aerated oven at 46°C to yield a dark brown extract from which a stock concentration of 1 g 100ml was prepared and used for animal studies. It was stored in capped bottles until required for electrolyte and haematological studies.

Experimental Animals

Twenty (20) wistar albino rats weighing 130-150 g, housed in plastic cages, were kept under standard condition of temperature 28^{0C} ± 1C), relative humidity 650' + 5 % and light (12 hours day/dark cycle) were used for the experiment. They were divided randomly into four groups of 5 rats per group. Animals were fed ad libitum with water and rat chow - (livestock feeds Ltd = Calabar-Nigeria). All experimental animal were approved by Ethical and Animal Welfare Committee of the Medical College, Benue State University, Makurdi, Nigeria.

Experimental Design

Treatment Regimen

All rats received daily treatments with their test solutions for a period of 30 days. All treatments were conducted between the hours of 9.00h and 10.OOh. Rats in group 1 (control), received a placebo of 5,O rnl distilled water via gastric intubation. The rats in groups 2, 3 and 4 were treated with 200 mg extract/kg, 150 mg extract/kg, 50 mg extract/kg respectively in total vehicle of 5.O ml.

Collection of Urinary Samples and Assay for Urinary Electrolytes.

Daily urine samples were collected from the rats and the volume obtained from each rat was determined by use of measuring cylinder. Urinary sodium and potassium ions were also determined using flame photometry (Vogel, 1962).

Determination of Hydrocyanic Acid, Phytic Oxalic and Cyalic Acid Contents.

The hydrocyanic acid content determination was done using titration method described by Association of official analytical chemists in 1995. *Dombeya buethneri* leaves were blended and soaked in water for four hours, then subjected to extraction. The extract was steam distilled into 2.5 % NaOH (w/v) and titrated against 0.02N AgNO3 solution with 1 mL of 0.5% (w/v) Dithnizone in 95 % ethanol as indicator to an endpoint of red-purple permanent turbidity.

Phytic acid was determined by the method described by Mc Cance and Widdowson (1935). Phytic acid was extracted with O.5N hydrochloric acid (Hcl) and precipitated as ferric 6400/6405 phytate. The absorbance was obtained using 6400/6405 spectrophotometer (Jenway, Essex, England) at 620nml. Total soluble oxalates was determined using the method of Trease and Evans (2002).

Determination of Ld_{50}

Prior to animal studies, LD50 was determined using adult male Albino wistor rats weighing (100-200 g) were randomly assigned 7 groups of 5 rats each. They were injected intraperitonially with a solution of the extract in a dose range of 1i0g/kg body weight using a constant volume of 0.4ml, rats were returned to their cages and allowed free access to food and water. The percentage mortality was plotted against dose of the extract on a special probability-log graph paper from which the LD_{50} value was determined.

Mineral Element Estimation

The mineral element content was estimated by wet oxidatron of sample (Welch and Graham, 2004). About 1 g of the sample was digested with HNO_3 and perchloric acid. The digest was diluted with deionized water and read at wavelength of 422.6nm, 213.9nm, 248.8nm and 440nm specific wave length for calcium, iron and phosphorous respectively.

Preparation of Samples-Serum and Whole Blood Samples.

Hours after exposure should be used after the final exposure, the animals were euthanized by inhalation of overdose chloroform. Blood was collected by cardiac puncture into EDTA sterilized sample bottles (1.5mg/mI). A fraction of 'the blood was collected into plain sample bottles and allowed to clot, then spun in centrifuge at 1,000 g for 1 hour and serum was obtained, and was used for the analysis of sodium and potassium assays while whole blood was used for haematological parameters.

Determination of Erythrocyte and Leucocytes Lencocyte

Red blood cell count was done on blood samples diluted with Hayem's fluid in RBC diluting pipette. The method described by Lewis and Ward (1975) was used. A 1:200 blood sample dilution was charged into the neubear chamber (Hawksley) and viewed with a light microscope. The cells were counted using X40 magnification with observation of the margin rule. The values obtained were multiplied by the appropriate correcting factor to obtain the count in cells/mm3.

White blood cell count was done in a manner similar to red blood cells count. Blood samples were diluted (1:20) with Turk's fluid and charged into the chamber and counted. The number of cells/mm3 was obtained by multiplying by 50 (Dacie and Lewis, 1984).

The improved neubar-heamocytometer was used in counting erythrocyles (RBCs), then the total levcocytes (WBCs) were counted in an improved heamocytometer using track solution as a dilution fluid (glacial acetic acid/mi, 1% aqueous gentian violet/mi, distilled water up to 200m1).

Determination of Heamoglobin Concentration

Heamoslobin concentration was determined by the method described by Dacie & Lewis, 1984. 0.02m1 of the samples were placed in Sahlis tube (0-14g/dl) holding 0.1N HCL in it is 10 unit mark and allowed to stand for 5 minutes. The brown precipitate, acid hematin developed to match the unfading standard colour. The volume of the solution in the graduated sahli's tube was then converted to hemoglobin concentration in g/dl.

Determination of Packed Cell Volume

The anticoagulated blood was filled to2/3 of the capillary tube and was spun at 200O g, for 5minutes using the microheamatocrit centrifuge. The PCV was read using microheamartocrit reader as a

Table 1: *Phytotoxicant Composition of Dombeya Boettneri*

ANALYTE	CONCENTRATION (MG/100G)
Hydrocyanic acid	1.45 ± 0.16
Phytic acid	0.25 ± 0.57
Oxalic acid	
Total oxalate	15.17 ± 0.07
Soluble Oxalates	12.17 ± 0.07

Table II presents the concentrations of mineral elements in mg/L. Calcium was present at levels of 183.2 ± 0.05mg/I, Iron 59.00 ± 0.05 mg/dh, zinc 0.61±0.O7 mg/dL and phosphorous 36.7±0.O5mg /d/L. The levels of Calcium and Iron were high.

Table 2: Selected Mineral Element Composition of *Dombeya buettneri*

ANALYSIS	CONCENTRATION (mg/L)
Calcium	183.20±0.05
Iron	59.00±0.05
Zinc	0.61±0.07
Phosphorous (%)	36.70±0.05
Mean ± SD of 3 determinants	

Table III summarizes the effect of aqueous extract of Dombeya buettneri on heamatological parameters in wistar rats. There were significant (P<0.05) increase in RBC, WBC and Hb Values in the test groups. Compared to the controls. The values obtained were 6.90±0.08, 14.30±0.60 and 12.40±0.30 (gIdI) for RBC, WBC and Hb respectively in the test group versus 4.40± 1.10, 8.70±0.30, 11.70±0.40 (g/dL) for the control groups. There was also a decrease in MCV in the test groups compared to the controls, but MCH and MCHC values showed no significant changes in the two groups.

TABLE 3: Effects of aqueous extract of *Dombeya buettneri* on haematological parameters.

Parameters	Control	Treatment group (N)
Red blood cell	4.40±0.10	6.90+0.08*
White blood cell	8.70±0.30	14.30±0.0 6*
Hemoglobin	11.70±0.4	12.40±0.30*
Pack cell volume	41.00±0.50	42.80±0.30
Mean cell volume	92.40±0.40	27.20± 1.10
Mean cell hemoglobin	25.60±0. 10	24.90±.060
Mean cell hemoglobin concentration	28.10±0.6	28.30±0.50

Table IV presents the results of the effects of aqueous extracts of Dornbeya buettneri on urine output and urinary sodium and potassium in wistar rates. There is no significant change in urinary sodium but urinary potassium ions decreased significantly (P<0.05) in the test groups compared to the controls.

Table 4: Effects of aqueous extract of *Dombeya buettneri* on Urine Output and Urinary Sodium and Potassium in male Wistar rats.

Parameters	Control	Treatment group (N)
Urine Outp ut (mI/24 hrs)	3.70±0. 10	3.30±0. 10
Urine Na (mmol/L)	725.60±28.50	706.60±38.80
Urine K^+ (mmol/L)	229.60±8.60)	177.50±3.60*

N = 8 values expressed as mean ± SEM * P<0.05

Table V presents the results of the effects of aqueous extracts of Dombeya buettneri on serum sodium and potassium ion levels in wistar rats. The results showed that there were no significant changes in serum electrolutes.

TABLE 5: Effects of aqueous extract of *Dombeya buettneri* on Serum Sodium and Potassium ion levels in Wistar rats.

Parameters	Control	Treatment group (N)
Serum Na+ (mmol/L))	143.00±50	142.80 ± 0.40
Serum K+ (mmol/L)	6.80±0.20	6.70±0.20
Urine K^+ (mmol/L)	229.60±8.60)	177.50±3.60*

N = 8 values expressed as mean ± SEM

Discussion

Most photochemicals antagonize nutrient absorption but have the therapeutic values. These anti-nutrient properties could be exhibited by either chelating minerals element thereby inhibiting some biochemical pathways in the body. This highlights the possibility of herb-food or herb-berb interactions. Phytochemicals occur naturally in plants and may affect health in cellular remembrances and metabolic activities within the cells (Ding *et al.,* 2009).

The results of the phytotoxicant composition showed that amount of Oxalic acid concentration of *D. buettneri* is higher than the other phytotoxic components. This could be an indication that *D. buettneri* has high carbohydrate content since Oxalic acid arises from incomplete oxidation of carbohydrates. Oxalic acid is an anti-nutrient which combine with calcium, iron and magnesium forming crystals which can inadvertently affect the gut and kidney (Coe *et al.*, 2005) leading to condition of nephrolithiasis, and gall bladder stones. Thus if supplements a calcium-rich food are taken with *D. buettneri*. It can lead to precipitation and crystallization of calcium oxalates thereby inhibiting calcium absorption as well as formation of crystalas (Stones) (Coe *et al.*, 2005).

Phytic acid was found to be trace *D. buettneri*, and Iron content was high. This means the content is insignificant hence Iron can be easily absorbed on consumption of *D. buettneri*. Though phytic acid chelates calcium, magnesium, iron and zinc (Hurrell, 2003) and also chelates niacin but in *D. buettneri*, the phytic acid content is very trace. Moreover, it has been established (Dewanto, *et al.*, 2002) that oxalic acid produces ascorbic acid during metabolism. This could account for the reduced phytric acid effect on chelation of iron and other minerals. Phytic acid has been known to have antioxidant effect, reducing oxidative stress in the lumen of the intestinal tract (Vucenik *et al.*, 2003). Phosphorous level was noted be low in *D. buettneri*. This may result from low phytic acid which is the principal storage form of phosphorous (Hurrelt, 2003,).

In the study of the effect of *D. buettneri* on heamatotogical parameters of wistar rats, the significant increase in RBC, WBC and Hemoglobin concentrations following treatment with aqueous extract of *D. buettneri* extracts explains that the extract stimulates heamopoiesis, by a mechanism yet unknown to be elucidated. Both red blood cell count and hemoblobin concentrations of the groups treated with *D. buettneri* were higher than the controls. This could be because the extract contains high content of Iron which could enhance the synthesis of heme moiety of hemoglobin. This suggests that this plant may be useful in management of anaemia.

The PCV values of the treatment groups though was not significantly different from those of controls. PCV is a measure of the erythrocyte balance. The result suggests reduction in erythrocyte balance. This is confirmed by the results of mean cell volume, which was lower in treatment groups compared to controls. The reduction in MCV is due to the non-significant increase in PCV in the test groups compared with control. The total WBC was also higher in the treatment groups compared with controls. This implies that the extract can boost the immune system. Suggestive of a beneficial effect of plant in increasing white blood cells which are vital in defence mechanisms of the body.

Many drugs and crude extracts are known to alter the regulation of fluid and electrolyte concentration in the extracellular fluid. *Dombeya buetttneri* did not significantly alter the urinary Na but there was a decrease significant decrease (P<O.05) in K after administration of the extract.

The decrease in urinary K may be due to the presence of cardiac glycosides in the extract as cardic glycosides have been reported to lower K excretion in dogs (Dacie, *et al.*, 1961). However, this effect is not followed by a change in serum K secretion.

The LD_{50} reported for *D. buettneri* was 2.58mg/kg. This indicates that dosage above this will be deleterious to health.

Conclusion

In conclusion, the results from this study have shown that aqueous extract of *Dombeya bretneri* to albino wistar rats resulted to a significant increase in red blood cells, white blood cells and heamoglobin. This *Dobeya buettneri* may be useful in the treatment of anaemia, and may boost immunity *D. buettneri*, is a rich source of calcium and iron. This work also established below LD_{50} Dombaya brethneri is safe.

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