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Antianaemic Potentials of Aqueous Telfairia Occidental is Leaf Extract in Trypanosoma Brucei Infection in Albino Rats

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ABSTRACT

Anaemia is a hall mark pathological effect of trypanosomiasis. Therefore, the effect of Telfaira occidental is leaf extract on African trypanosomiasis induced in albino rats with Trypanosoma brucei that was examined to see its potentials against anemia. Six groups of three rats were used. Group one was infected and treated with 64mg/kg of the fresh leaf extract. Group 2 was treated with 64mg/kg of the dry leaf extract. Group 3 was infected but untreated. Group 4 was uninfected but administered 64mg/kg of the fresh leaf extract. Group 5 was uninfected but administered 64mg/kg of the dry leaf extract. Group 6 was uninfected and untreated. Administration of the extracts was by oral route. Parsitemia, packed cell volume (PCV) and liver function parameters were monitored. The results showed that T. occidental is leaf extract significantly increased the PCV and tend to stabilize the liver function parameters. Parasite proliferation was reduced and the lifespan of the treated rats was prolonged. The leaf extract, therefore had a positive effect on the treated experimental rats, and posits some hope in the effort towards the control of trypanosomiasis.

Keywords: *Telfairia occidental is, Trypanosoma brucei, anemia, PCV*

1. INTRODUCTION

African trypanosomiasis is a parasitic disease in people and animals. It is caused by protozoa of genus Trypanosoma and transmitted by the tsetse fly and other insects. This disease leads to the damage of the central nervous system and results in sleeping sickness syndrome. Some of the fatal consequences of African trypanosomiasis are anemia and tissue damage [1]. A hall mark pathological effect of trypanosomiasis is anemia potentiated by a variety of substances released by the parasites.

Purified trypanosomes have been found to release several proteinaceous substances not necessarily proteases, which in addition to proteases, might be responsible for haemolysis and the consequent anemia in African trypanosomiasis [2]. Another possible mechanism for the pathogen city of anemia is the impairment of bone marrow function. Studies have shown that bone marrow is severely stressed during infections with T. brucei and T. musculi [3, 4]. Also, over production of nitric oxide by inducible nitric oxide synthase (iNOS) in macrophages is another mechanism proposed for the pathogen city of anemia and tissue damage in African trypanosomiasis [5].

Telfairia occidental is (family cucurbitacea) is a tropical vine grown in West Africa and highly reputed in traditional medicine [6]. It is commonly called ugu in Nigeria. Reports hold that the aqueous crude extract of Telfairia occidental is leaves has haematinic activity [7] yields positive results in the treatment of sudden attack of convulsion, malaria and anemia (Gbile, 1986) and significantly increased red blood cell count, white blood cell count, packed cell volume and hemoglobin concentration in rats [9].

Therefore the work was designed to investigate the Antianaemic potential of the leaves extract of T. occidental is and hepatic indices in trypanosomiasis using albino rats as model.

2. MATERIALS AND METHODS

2.1 Plant Collection and Preparation

Fresh leaves of T. occidental is were purchased from a market in Chobe junction, Jos, Plateau state, Nigeria. Aqueous extracts were prepared from the fresh and dry leaves.

For the extraction of dry ugu leaf, the leaves were shredded, shade-dried and reduced to a powdery form by pounding. 500 g of powdered sample was soaked in distilled water overnight, after which it was filtered. The filtrate was then evaporated in water bath to dryness. The solid extract was then weighed. 100g of the solid dry extract was then dissolved in 300ml of distilled water and used for the study.

For the extraction of fresh leaves, the sample was blended using a homogenizer and then thoroughly drained. The liquid extract was filtered and evaporated in a water bath to dryness. The solid extract was then weighed. 150g of the solid extract was then dissolved in 470ml of distilled water and used for the study.

2.2 Photochemical Analyses

The extracts obtained were screened for photochemical constituents as follow:

a) Test for alkaloids (using Dragendorff reagent test). To 2.0ml of the extract, few drops of Dragendorff reagent were added. The mixture was then observed for orange coloration.

b) Test for saponins: About 0.5g of the plant extract was shaken with distilled water in a test tube. Frothing which persisted on warming was taken as preliminary evidence for the presence of saponins.

c) Test for phlobatanins: Deposition of red precipitate when an aqueous extract of the plant

leaf was boiled with 1% of aqueous hydrochloric acid was taken for the presence of phlobatanins.

d) Test for flavonoids (using the lead acetate test): To 2ml of extract, 10% lead acetate solution was added and observed for either cream or light yellow coloration which confirmed the presence of flavonoids.

e) Test for tannins: 1.0ml of the extract was diluted with 4.0ml distilled water (in a ratio of 1:4) and then a few drops of ferric chloride solution was added and observed for blue or green precipitate or coloration. This showed the presence of tannins.

f) Test for cardiac glycosides (using Salkowski test): About 0.5g of the extract was dissolved in 2ml chloroform. Sulphuric acid was carefully added along the side of the test tube to form a lower layer. A reddish-brown colour at the interface indicated the presence of cardiac glycosides.

g) Test for terpenes and steroids (using Liebermann- Burchard test): 2.0ml of the extract and 1.0ml of acetic anhydride were mixed. Concentrated acetic acid was carefully added along the side of the test tube. The mixture was then observed for a reddish-brown colour at the interface. This indicated the presence of terpenes and steroids.

2.3 Animal Model and Grouping

Wistar strain female albino rats (150 -170g) purchased from the animal house of the University of Jos Nigeria were used for the study. The rats were housed in wire mesh cages under standard conditions. They were fed with pelletized chick mash purchased from Grand cereals and oil mills limited Jos and clean drinking water ad libitum. They were allowed to stabilize for one month before being used for the experiment.

The experimental animals were then organized in six groups of 3 rats each. Group 1 were infected and treated with 64mg/kg body weight of the fresh leaf extract. Group 2 were treated with 64mg/kg body weight of the dry leaf extract. Group 3 were infected but untreated. Group 4 were uninfected but administered 64mg/kg body weight of the fresh leaf extract. Group 5 were uninfected but administered and received 64mg/kg body weight of the dry leaves extract. Group 6 were uninfected and untreated and served as the control. Administration was by oral route. In the course of the studies parasitemia, PCV and liver function parameters were investigated.

2.4 Trypanosomes and Inoculation of Rats

Bloodstream form of Trypanosoma brucei were obtained from the Nigerian Institute of Trypanosomiasis Research, Vom. Experimental rats were infected with T. b. brucei. Highly infested blood as observed under light microscope was obtained from the tail of an infected rat directly into normal saline, pH 7.0 without anticoagulant. With about 2 trypanosomes per field, 0.3ml of suspension was passaged into the experimental albino rats by intraperitoneal injection.

2.5 Administration of the Extract

Infected/treated rats and uninfected/treated rats were administered 0.2ml solution of the fresh and dry extract containing 64mg/kg body weight orally on the first day of sighting parasite in the blood of infected rats. Administration of the extract continued on daily basis within the experimental period.

2.6 Parasitemia Count

Evaluation of parasitemia was carried out at 24hr interval to monitor infection progress. This was done by counting the number of parasites under the light microscope at X100 magnification from thin blood smear freshly obtained from the tip of the tail of infected rats.

2.7 Collection of Blood Sample

The rats were at the time of sacrifice anaesthetized by placing them in a closed jar containing cotton wool soaked with chloroform. The neck of the anaesthetized rats were cut and Blood was collected from the jugular veins of the animals into sample bottles with and without anticoagulant for packed cell volume and liver function test respectively. The blood with anticoagulant was kept at 4^0C until required for packed cell volume determination, usually not more than 48hrs. Clear serum was obtained from the other blood sample by centrifugation after clotting at 3,000g for 15min and kept frozen until required.

2.8 Packed Cell Volume Analysis

A heparinised capillary tube was filled with two-third of blood gotten from the tail of the animals. One end of the capillary tube was sealed with crista seal. The capillary tube was then spinned in a microhaematocrit centrifuge for 5mins at 12000rpm. The capillary tube was then placed on a microhaematocrit reader and the percentage packed red cell estimated.

2.9 Determination of Liver Function Parameters

a) **Total Protein Test:** Three test tubes were labeled: blank, standard and sample. 0.02ml of distilled water was then pipetted into the test tube labeled blank; 0.02ml of protein (standard) into the tube labeled: standard; and 0.02ml of serum into the test tube labeled sample. One ml of biurret reagent was added to the content of each tube. The test tubes were then mixed and incubated for 30mins at 20-25^{0}C. The absorbance of the sample and standard were then measured spectrophotometrically at a wavelength of 546nm against the blank. The concentrations were then calculated.

b) **Albumin Test:** Albumin reacts quantitatively with bromocresol green at pH 4.15 to form a green colored complex. The colour formed is proportional to the amount of albumin present. It is within 28-42g/L for both males and females.

c) **Bilirubin Test:**

i) **For Total Bilirubin (TB):** Two test tubes were labeled as blank and sample. 0.2ml of sulphanilic acid was pipetted into each; 0.05ml of sodium nitrite added to the content of the tube labeled sample; 1.00ml of caffeine and 0.2ml of serum were also added to each tube. The content of the test tubes were then mixed and allowed to stand for 10mins at 20-25^{0}C. 1.0ml of sulphanilic acid was then added to the two test tubes. The test tubes were mixed again and allowed to stand for 25-30mins at 20-25^{0}C. The absorbance of the sample was read against the sample blank.

ii) **For Conjugated Bilirubin (CB):** Two test tubes were labeled as blank and sample. 0.2ml of sulphanilic acid was pipetted into each tube; 0.05ml of sodium nitrite added to the sample; 2.00ml of NaCl (9g/l) added to each tube and 0.2ml of serum were added to each. The content of the test tubes were mixed and allowed to stand for 5mins at 20-25^{0}C. The absorbance of the sample against the sample blank was then read.

d) **Alanine Amino Transferase (Alt) Test:** Two test tubes were labeled as reagent blank and sample. 0.1ml of serum was pipetted into the sample test tube; 0.5ml of phosphate buffer into the two test tubes and 0.1ml of distilled water into the reagent blank test tube. The test tubes were then mixed and incubated for 30mins at 30^{0}C. 0.5ml of 2, 4-dinitrophenyl hydrazine was then pipetted into the two test tubes. The test tubes were mixed and allowed to stand for 20mins at 20-25^{0}C. 5.0ml of NaOH was then pipetted into the two test tubes. The test tubes were mixed and the absorbance of the sample against the reagent blank was read after 5 mins.

e) Measurement against sample blank: Two test tubes were labeled sample blank and sample. 0.1ml of serum was pipetted into the sample test tube and 0.5ml of phosphate buffer into the two test tubes. The test tubes were mixed and incubated for 30 mins at 37^{0}C. 0.5ml of 2, 4-dinitrophenyl hydrazine was pipetted into the two test tubes and 0.1ml of serum into the two test tubes. The test tubes were then mixed and allowed to stand for 20mins at 20-25^{0}C. 5.0ml of NaOH was pipetted into the two test tubes. The test tubes were then mixed and absorbance of the sample against the reagent blank read after 5 mins. The range is within 3-15U/l for both males and females.

f) **Aspartate Amino Transferase (AST) Test:** Measure against reagent blank: Two test tubes were labeled reagent blank and sample. 0.1ml of serum was then pipetted into the sample test tube; 0.5ml of phosphate buffer into the two test tubes and 0.1ml of distilled water into the reagent blank test tube. The test tubes were mixed and incubated for 30mins at 37^{0}C. 0.5ml of 2, 4-dinitrophenyl hydrazine was pipetted into the test tubes. The test tubes were mixed and allowed to stand for 20mins at 20-25^{0}C. 5.0ml of NaOH was then pipetted into the two test tubes. The test tubes were mixed and the absorbance of the sample against the reagent blank was read after 5 mins.

g) **Measurement against sample blank:** Two test tubes were labeled sample blank and sample. 0.1ml of serum was pipetted into the sample test tube and 0.5ml of phosphate buffer into the two test tubes. The test tubes were mixed and incubated for 30mins at 37^{0}C. 0.5ml of 2, 4-dinitrophenyl hydrazine was pipetted into the two test tubes and 0.1ml of serum into serum blank test tube. The test tubes were mixed and allowed to stand for 20mins at 20-25^{0}C. 5.0ml of NaOH was pipetted into the two test tubes. The test tubes were mixed and the absorbance of the sample against the sample blank was read after 5 mins. The range is within 5-18U/l for both males and females.

h) **Alkaline Phosphatase (ALP) Test:** Into three test tubes separately labeled standard, control and sample 0.5ml of alkaline phosphatase substrate was dispensed and equilibrated to 37^{0}C for 3mins. Then 0.05ml (50µl) of each standard, control and sample was added to their respective test tube. The content of each test tube was mixed gently and deionised water was used as blank. The tubes with content were then incubated for exactly 10mins at 37^{0}C after which 2.5ml alkaline phosphatase colour developer was added to each and mixed well. The wavelength of the spectrophotometer was set at 590nm and zeroed with reagent blank before the absorbance was read and recorded.

3. RESULTS

The photochemical analyses carried out revealed that the aqueous extract of T. occidental is contains alkaloids, flavoids, saponins and tannins while steroids, terpenoids, cardiarc glycosides and phlobatanins were not detected (Table 1).

Figure 1 presents the effect of the extracts on the parasitemia within the experimental period. The extracts suppressed the level of parasites and so prolonged the life

span of the treated experimental animals when compared with the diseased animals that were not treated.

Table 1: Photochemical component of Telfairia occidental is crude extracts

Photochemical	Detection
Alkaloids	+
Flavonoids	+
Saponins	+
Steroids	-
Tannins	+
Phlobatanins	-
Terpenoids	-
Cardiac glycosides	-

Key: + = Present, - = Absent.

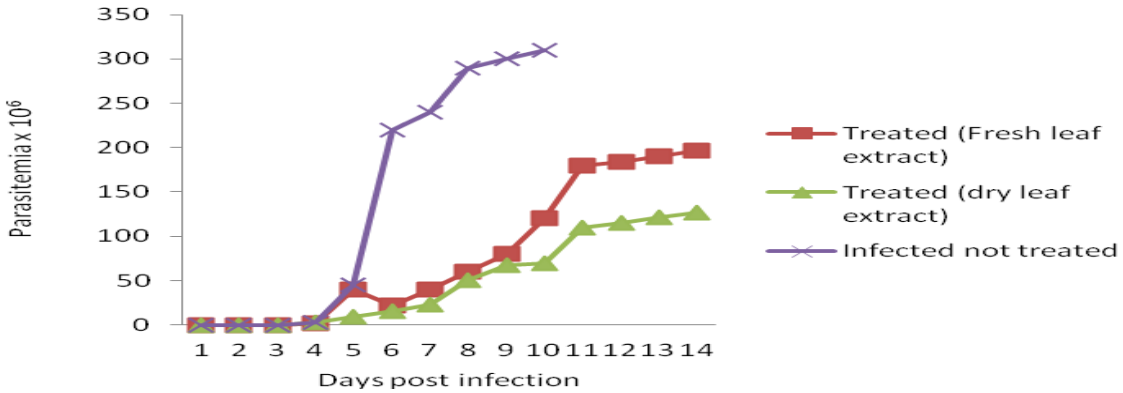

Fig 1: Effects of treatment with T. occidental is leaf extracts on parasitemia

The PCV, as shown in Figure 2, reveals that the extracts resulted in significant increases when contrasted with the negative control group. Table 2 presents data from liver function tests showing the levels of TP, ALT, ASP and ALP. TP levels reduced to similar levels in both diseased animals and those treated compared to the normal animals. ALT significantly increased in the treated animals compared to the normal animals; however, the treatment with extracts from fresh leaves tend to lower the ALT level. The effect of the disease and treatment on AST gave similar trends as seen in ALT. On the other hand, the disease lowered ALP level while the treated groups did not give a significant difference compared with the normal rats.

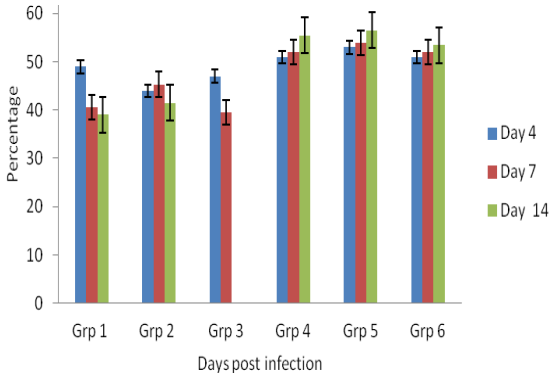

Fig 2: Packed cell volume (%) experimental rats

Table 2: Liver function tests of the experimental rats

Group	TP	ALB	TB	CB	ALT	AST	ALP
1	71.59± 1.65	34.21± 0.37	12.01± 0.61	4.20 ± 0.24	20.67± 0.58	30.67± 0.58	34.92± 0.87
2	71.59± 0.69	37.10± 1.12	10.53± 0.04	3.34 ± 0.32	73.50± 0.71	93.00± 1.41	36.92± 0.15
3	70.70± 0.84	31.49± 1.97	9.46 ± 0.50	3.43 ± 0.55	70.50± 0.71	90.00± 1.41	29.42± 0.59
4	75.60± 1.82	37.37± 0.33	10.90± 0.69	4.26 ± 0.06	13.50± 0.50	15.50± 0.50	41.3± 0.43
5	77.69± 0.83	38.75± 0.83	14.20± 0.12	4.22 ± 0.06	14.50± 0.50	13.00± 1.00	55.15± 1.61
6	81.41± 0.44	41.80± 1.40	6.34 ± 0.46	3.16 ± 0.07	11.00± 1.00	10.50± 0.50	37.09± 1.28

Values are expressed as mean ± SD, n = 3. TP = total protein; ALB = albumin; TB = total bilirubin; CB = conjugated bilirubin; ALT = alanine amino transferase; AST = aspartate amino transferase; ALP = alkaline phosphatase

4. DISCUSSION

Oral administration of the fresh and dry leaf extracts resulted in significant increase in PCV level in the infected and treated rats, with fresh leaf and dry leaf extracts and to prolonge their lifespan as compared to the infected but untreated rats. This is consistent with the observations made when rats were fed with the diet preparation of the air-dried leaves of T. occidental is for four weeks [9]; birds were fed with the dietary preparation of the sun-dried leaves of the plant [10]. The increases in the hematological indices observed following treatment with T. occidental is extract might not be unconnected with the chemical composition of the leaves of T. occidental is. The chemical composition had been shown to include proteins, fat, vitamin A, thiamine, riboflavin, nicotinamide, vitamin C and minerals such as zinc, iron, calcium and magnesium. The amino acid profile of T. occidental had also been shown to be very rich and includes alanine, aspartate, glycine, glutamine, histidine, lysine, methionine, tryptophan, cysteine, leucine, arginine, serine, threonine, phenylalanine, valine, tyrosine and isoleucine [11].

Some of these constituents are well-established haemopoietic factors that have direct influence on the production of blood in the bone marrow. Oral administration of the fresh and dry leaf extracts did not show much positive effect on the level of liver damage in the infected and treated rats, as seen from ALT and AST levels.

The reduced PCV and increased ALT, AST and ALP in the group 3 rats (infected and untreated) rats is an indication of anemia and tissue damage, which is a well-recognized and inevitable consequence of trypanosome infection in animals and man.

Conclusively, the fresh leaf extract proved to be more effective over the dry leaf extract. The wet leaf extract prolonged the life span of the infected rats longer than the dry leaf extract but they both increased the level of PCV in the infected rats. This has scientifically demonstrated the antitrypanosomal properties of the extract on T. brucei - infected rats.

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