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Haematological and Toxicological Investigation of Ethanol Extract of *Telferia Occidentalis* Using Some Enzymes as Biochemical Markers in Wistar Rats

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ABSTRACT

Leaves of *Telferia occidentalis* plant was investigated for toxic and haematological potentials using some serum enzymes as biochemical markers in Wistar rats. Sixteen rats were used for the investigation and were grouped into four group of four rats each. Group 1 was the control group and received distilled water. Group 2, 3 and 4 were the treatment groups which received 200, 300 and 400 mg/kg body weight of the extract respectively. The rats were dosed for 14 days, thereafter were sacrificed by cardiac puncture and blood collected for analysis. Enzyme immunosorbent assay (ELISA) and haematological tests were used to determine AST, ALT, ALP and haematological results respectively. Results of the treatment groups (2 ,3 ,and 4) were compared with the control (Group 1). All results were compared with the highest dose of the extract as the response increased or decreased as the doses increased to 400 mg/kg body weight. The statistical confidence was set at 95% ($p<0.05$). PCV, RBC, Hb,WBC statistically decreased when compared with the control. MCV, MCH was elevated with reduced MCHC. Differential leucocyte count showed lymphocytosis, neutropaenia, eosinopaenia, monocytopaenia but no significant change in the basophils counted. ALT, AST, ALP was elevated. Albumin, Total protein, K^+, Na^+ was also elevated. There was no significant increase in Urea and Creatinine level, indicating kidney safety. Thus the extract caused reduction in haematological parameters (anaemia) and increased biochemical serum markers suggesting hepatocyte damage.

Keywords: Toxicity, *Telferia occidentalis,* Haematology, Serum enzyme, ELISA.

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INTRODUCTION

T. occidentalis (Fluted Pumpkin) (Plate 1) is a plant with dark green three-bladed leaves. Bear an inedible pod containing edible seed belonging to the family of *Cucurbitacaea* (Nkang et. al, 2003). It is called *Ugu* in Igbo, *Ikong* by Efik, *Krobonlo* by the Ghanaians (Buckett, 1968). It is medical plant pharmacologically used traditionally for the treatment of liver and high blood sugar problems (Adaramoye et al., 2007), convulsion (Gbile,1986), and reproductive and infertility issues (Nwangwa et al., 2007). It contains oxalates, phytates, saponins, glycosides, flavonoids, alkaloids and resin, (Akubue et al., 1980) and blood enriched minerals like iron, potassium, sodium, Phosphorus (Kayode and Kayode, 2010), and some vitamins like riboflavin, nicotinamide, ascorbic acid and phytochemicals (Nwanna and Oboh, 2007). Phyto compounds in *T. occidentalis* was investigated using GC-MS analysis (Igwe et al., 2016).

In Nigeria, the herbal preparation of the plant has been employed in the treatment of anaemia, chronic fatigue and diabetes (Alada, 2000; Dina et al., 2000; Aderibigbe et al., 1999). The leaves of *T. occidentalis* are rich in iron and play a key role in the cure of anaemia. They are also noted for lactating properties and are in high demand for nursing mothers (Okoli and Mgbeogu, 1983).

Plate 1. Leaves of *Telferia occidentalis.*

Aqueous extracts of *T. occidentalis* had been reported to reduce blood glucose level and also have antidiabetic effects in glucose-induced hyperglycemic and streptozotocin (STZ) induced diabetic mice (Aderibigbe et al., 1999), while in normoglycemic mice, the glucose level was not altered (Salman et al., 2008) also reduced blood glucose level by *T. occidentalis* leaves in male rats was reported. Several studies as also reported hypoglycemic effects (Aderibigbe et al., 1999; Eseyin et al., 2007; Nwozo et al., 2004).

Natives are speculating that that extract of *T. occidentalis* could cause liver and cardiac problems, when the extract is squeezed and drank raw in water. The enzymes of liver, heart muscle, kidney and lungs were subjected to investigation to authenticate this claim.

The plant *T. occidentalis* is one of common leaf widely consumed vegetables due to its unsubstantial claims that it enhances blood and immune system when squeezed raw and drank in cold water. The present study seeks to confirm or refute these claims by natives scientifically.

MATERIAL AND METHODS

Plant Materials

Fresh leaves of *T. occidentalis* were collected from the University environment in Umudike, Nigeria and was identified by Prof. M. C. Dike at the Taxonomy section of College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Nigeria.

Preparation of Plant Extract

The identified leaves of *T. occidentalis* was shade dried for 10 days and pulverized to a coarse powder using a mechanical grinder. The plant extract was prepared using Soxhlet method as described by Jensen (2007). Thirty-five gram (35 g) of coarse powdered sample was introduced into the extraction chamber of the Soxhlet extractor using ethanol as solvent. Temperature was maintained at 70^0C throughout the extraction period of 48 h. At the end of the extraction period the extract was concentrated using oven at 30^0C to obtain dried extract which was weighed and kept in a well labelled sterile specimen bottle.

Different doses of 200, 300 and 400 mg/kg body weight was prepared and administered to rats in group 2, 3, and 4 respectively *per os* by gastric lavage daily for 14 days. These doses were calculated from a stock solution dissolved in distilled water.

Experimental Animals

Wistar rats weighing between 125 to 135 g of about seven weeks old were purchased from College of Vet Medicine laboratory animal house and ethical approval obtained from the College of Vet Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria. Approval was in line with guidelines for the care and use of laboratory animals as given by the National Research Council (NRC,1985).The rats were allowed to acclimatized for two weeks, thereafter were divided into different groups. All rats had access to food and water *ad libitum.*

Experimental Design

Sixteen rats grouped into four of four rats each was used for the study. Group 1 was the negative control group and distilled water was administered. Groups 2, 3, and 4 were the treatment groups which received 200, 300 and 400 mg/kg body weight of the *T. occidentalis* extract respectively *per os* by gastric lavage daily for 14 days. The rats were treated for 14 days, thereafter was

Table 1: Effect of extract on the haematological parameters of Wistar albino rats.

Parameters	Distilled water	200 mg/kg extract	300 mg/kg extract	400 mg/kg extract
PCV (%)	36.00 ± 0.57^{a}	33.66 ± 0.33^{bc}	32.00 ± 0.57^{c}	35.00 ± 0.58 ^{ab}
Hb (g/dl)	13.30 ± 0.05^{a}	12.26 ± 0.08^{b}	11.13 ± 0.14^{c}	12.63 ± 0.14^{b}
RBC ($\times 10^{12}$)	9.30 ± 0.05^{a}	4.80 ± 0.05^{c}	5.26 ± 0.08^{b}	5.23 ± 0.08^{b}
WBC ($\times 10^{9}$/L)	9.33 ± 0.06^{a}	7.30 ± 0.05^{c}	8.70 ± 0.05^{b}	5.23 ± 0.08^{d}
MCV (FL)	57.00 ± 0.57^{c}	64.00 ± 0.57^{a}	65.00 ± 0.57^{b}	66.00 ± 0.57^{b}
MCH (pg)	21.33 ± 0.33^{bc}	20.00 ± 0.57^{a}	21.00 ± 0.57^{c}	23.00 ± 0.57^{b}
MCHC (g/dl)	37.00 ± 0.57^{a}	36.00 ± 0.57^{ab}	33.00 ± 0.57^{c}	35.00 ± 0.57^{b}

Values presented as mean ± S.EM; different superscript represents significant difference at p<0.05. PCV = Pack cell volume; Hb= Haemoglobin; TRBC = Total red blood cell; WBC= White blood cell; MCV= Mean corpuscular volume; MCH= Mean corpuscular Haemoglobin; MCHC= Mean corpuscular Haemoglobin concentration.

sacrificed by cardiac puncture and blood samples collected for analysis. The effect of *T. occidentalis* extract was checked on haematological parameters and some serum enzymes activities.

Haematology and Biochemical Investigation

Haematological investigations performed by manual methods included red blood cell count (RBC), white blood cell count (WBC), differential count (DC), Hemoglobin concentration (Hb), packed cell volume (PVC) or hematocrit as described by Cole (1986).
PCV was measured by the micro-hematocrit method using capillary tubes while RBC and WBC were measured manually using an improved Neubauer counting chamber.
The differential count was measured manually using a thin blood film stained with Leizhman stain. Haemoglobin concentrations were determined by cyanomathemoglobin method (Kachmar, 1970).
Using RBC, PCV and haemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were calculated using standard formulae (Lewis et al.,2006; Hoffbrand and Moss, 2011).

$$MCV = \frac{PCV}{RBC} \times 10 \quad fl$$

$$MCH = \frac{Hb}{RBC} \times 10 \quad pg$$

$$MCHC = \frac{Hb}{PCV} \times 100 \quad g/dl$$

Biochemical investigation was performed using ELISA reagent kits. The measure included alkaline phosphatase (ALP), alkaline aminotransferase (ALT) and aspartate aminotransferase (AST) enzyme levels to determine liver function(Klein et al.,1960), Urea (Fawcett and Scott, 1960), creatinine (Blass et al., 1974).
Albunin, globulin and total protein were determined. Biuret method was used to determine total protein as described by Luban (1978), albumin using the method of Doumas et al, (1971) and Doumas and Peters (1997). Samples were analysed immediately to avoid artifactual changes (Ihedioha and Onwubuche, 2007).

Statistical Analysis

Data obtained were analysed using Statistical Package for Social Sciences (SPSS) version 20. All values were expressed as mean ± Standard error. Data were further subjected to one-way analysis of variance(ANOVA) to compare the different doses of 200, 300 and 400 mg/kg body weight with the control. Duncan post-hoc test was used to separate the mean with a significant difference.The statistical confidence was set at 95% (p<0.05).

RESULTS

The effect of ethanol extract of *T. occidentalis* on the haematological parameters in Wistar rats was evaluated and the results are shown in Table 1.
The result showed that there were significant (p<0.05) differences in all the parameters evaluated when the untreated group is compared with the treated groups. The packed cell volume (PCV), Haemoglobin (Hb), Red blood cell count (RBC), the white blood cell (WBC) count, and the Mean Corpuscular Haemoglobin Concentration (MCHC) were significantly (p<0.05) reduced across the treated groups when compared with the untreated group. Whereas the Mean Corpuscular Volume (MCV), and the Mean Corpuscular Haemoglobin (MCH) were significantly (p<0.05) elevated by the plant extract compared with the untreated group (Table 1).
The effect of ethanol extract of *T. occidentalis* on the differential white cell in Wistar rats was ascertained and the results are shown in Table 2.
The result showed that there were significant (p<0.05) difference in the percentages of Lymphocytes, Neutrophils, Eosinophil, and Monocytes counts, whereas the plant extract did not alter the percentage of the basophils count comparing the untreated group with the treated groups. Apart from the percentage value of the Lymphocytes which increased significantly (p<0.05) from

Table 2: Effect of extract on differential white blood cell count of Wistar albino rats.

Parameters	Distilled water	200 mg/kg extract	300 mg/kg extract	400 mg/kg extract
Lymphocytes (%)	67.00 ± 0.57^{d}	81.33 ± 0.33^{b}	83.00 ± 0.57^{c}	88.66 ± 0.88^{a}
Neutrophils (%)	29.00 ± 0.57^{a}	18.33 ± 0.88^{c}	22.66 ± 0.88^{b}	8.66 ± 0.33^{d}
Eosinophils (%)	1.90 ± 0.05^{a}	1.00 ± 0.00^{c}	1.00 ± 0.00^{b}	1.00 ± 0.00^{b}
Monocyctes (%)	2.00 ± 0.05^{a}	1.00 ± 0.00^{b}	1.00 ± 0.05^{b}	1.00 ± 0.00^{b}
Basophils (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Values presented as mean ± S.E.M; different superscript represent significant difference at p<0.05.

Table 3: Effect of extract on biochemical parameter of Wistar albino rats.

Parameters	Distilled water	200 mg/kg extract	300 mg/kg extract	400 mg/kg extract
AST (IU/L)	122.90 ± 0.15^{c}	178.83 ± 0.16^{a}	99.00 ± 0.57^{d}	171.33 ± 0.88^{b}
ALT (IU/L)	38.00 ± 0.57^{c}	55.00 ± 0.57^{a}	36.00 ± 0.57^{d}	44.00 ± 0.57^{b}
ALB (g/dl)	1.63 ± 0.08^{b}	1.70 ± 0.05^{b}	1.80 ± 0.05^{c}	2.30 ± 0.05^{a}
TP (g/dl)	1.30 ± 0.05^{a}	2.20 ± 0.05^{c}	2.70 ± 0.05^{b}	2.80 ± 0.05^{b}
Potassium (mmol/L)	2.80 ± 0.05^{c}	2.70 ± 0.05^{c}	3.53 ± 0.03^{b}	3.90 ± 0.05^{a}
Sodium (mmol/L)	126.00 ± 0.57^{c}	119.00 ± 1.15^{d}	132.00 ± 0.57^{b}	136.00 ± 0.57^{a}
Creatinine (mg/dl)	2.00 ± 0.05^{ab}	2.10 ± 0.05^{a}	1.86 ± 0.03^{b}	0.80 ± 0.05^{c}
Urea (mg/dl)	32.00 ± 0.57^{b}	28.00 ± 0.57^{d}	35.00 ± 0.57^{a}	30.00 ± 0.57^{c}
ALP (IU/L)	201.00 ± 0.57^{c}	211.00 ± 0.57^{b}	180.10 ± 0.57^{d}	233.90 ± 0.20^{a}

Values presented as mean ± S.E.M; different superscript represent significant difference at p<0.05. AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; ALB = Albumin; TP= Total protein; ALP = Alkaline phosphatase.

67.00 ± 0.57 in the untreated rats to highest percentage value of 88.66 ± 0.88 in the rats that received 400 mg/kg dose of the plant extract, the percentage values of Neutrophils, Eosinophil, and Monocytes were significantly (p<0.05) decreased across the treated groups.

The toxic effect of ethanol extract of *T. occidentalis* was evaluated using the liver marker enzymes Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and alkaline phosphatase (ALP), and its effect on other biochemical parameters such as Total protein (TP), Albumin (Alb), Potassium (K), Sodium (Na), Creatinine and Urea in Wistar rats and the results are shown in Table 3.

The result showed that there were significant (p<0.05) difference in all the parameters evaluated when the untreated rats were compared with those rats treated with the plant extract. The concentrations of all the liver marker enzymes (ALT, AST, ALP), the Albumin, K, and Na electrolytes were significantly (p<0.05) elevated in the treated rats especially in those rats administered with the doses of 200, and 400 mg/kg, whereas, the concentrations of the kidney markers (TP, Creatinine, and Urea) were significantly (p<0.05) decreased in like manner. It is important to note that the TP significantly (p<0.05) increased in all the treated rats, while the creatinine significantly (p<0.05) decrease in value from 2.00 ± 0.05 mg/dl (untreated group) to 0.80 ± 0.05 mg/dl at the highest dose of 400 mg/kg.

DISCUSSION

The extract of *T. occidentalis* induced a mild decrease in activity in the treated rats. The slight reduction in PCV, Hb concentration and RBC counts was not significant. Alada (2000), established increase in heamatological activities of *T. occidentalis* in rats which slightly defer from the result of this study and can be attributed to the presence of antinutritional factors and may be responsible for the decrease in the heamatological data. Ekpenyong (2012) clearly reported antinutritional factors in *T. occidentalis* which includes tannin, hydrogen cyanide, phytic acid and oxalate. These phytochemicals in *T. occidentalis* leaves interferes with its vitamins and mineral content. Bonsi et al. (1995) observed that boiling destroys the antinutritional factors. The blood-boosting effect of *T. occidentalis* observed in some study might be as a result of cooking of the leaves before consumption, as a result, destroying the antinutritonal phytochemicals. The extract of *T. occidentalis* also produced significant decrease in WBC count except in lymhpocytes. The extract thus induced mild macrocytic mormochromic anaemia, eosinopaenia, neutropaenia, monocytopaenia and lymphocytosis. There was no significant change in the basophil counted. Macrocytic normochromic anaemia is seen in VitB_{12} and folic acid deficiency. This could be due to antinutritional factor-like phytic acid which could affect vitamins and minerals making them unavailable for haematopoiesis. This study recorded increase in albumin (ALB) and total protein (TP) which is similar to the results of Akorode (1990) and Kayode et al. (2010). Leaves of *T. occidentalis* has been reported to constitute a rich source of some amino acids like alanine, aspartate, glycine, glutamate, histidine, lysine, methionine, tryptophan, cysteinewereand leucine (Fasuyi, 2006), which could provide good starting material for protein biosynthesis. The rise in total protein

may also suggest inflammatory state as the plant extract may have stimulated synthesis of certain blood proteins by hepatocytes and immunoglobulins by *B*-lymphocytes as result of the study showed blood-boosting. Creatinine and urea reduced statistically, indicating no leakage of creatine kinase from the myocytes and protein breakdown indicating the safety of the kidneys cells. The nitrogenous waste product of protein breakdown, blood urea nitrogen (BUN) and creatinine which is formed due to catabolism of phosphorcreatinine (a compound that provides energy for muscle contraction is more accurate indicator of GFR as it is not affected by urine flow rate or by protein catabolism. In this study, there was an increase in the values of ALT, AST and ALP experimentally which was not significant and may not categorize extract of *T. occidentalis* as toxic which is in agreement with the study of Ekpenyong et al. (2012). The increased experimental values may be due to the reaction of the extract on the hepatocytes through elevation of ALT, AST and ALT which is an evidence of hepatic injury (Liz, 2003; Svetlivet, 2006; Dobbs, 2006) and may not provide any information on hepatic function. The magnitude of liver enzyme elevation may not correlate with prognosis. In this study, it was discovered out that there were no significant differences in values of AST, ALT and ALP; Albumin and Total protein. The serum levels of these parameters were statistically not significant even though the experimental groups (2, 3, and 4) recorded values higher than the control group.

Conclusion

The extract of *T. occidentalis* may not be toxic generally but may have possessed some antinutritional factors.

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