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Effect of ethanol extract of *Telfairia occidentalis* leaf on some biochemical parameters of 2,4-dinitrophenyl hydrazine induced oxidative stress in albino rats

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Abstract. In this study, we attempt to verify the claim that the leaf-extract of *Telfairia occidentalis* can remedy oxidative damage condition as well as assess its phytochemical content. Fifteen male albino rats weighing 180 g to 240 g were randomly divided into three groups of five rats each. Group A was designated the control group while group B and C were both induced with 40 mg/kg body weight 2,4-dinitrophenyl hydrazine. Group C was subsequently treated with 200 mg/kg body weight of ethanol extract of *T. occidentalis* leaf for 21 days. At the end of the treatment, the animals were sacrificed, and serum of the samples were subjected to relevant tests. Result shows that the plant leaf contained saponin, tannins, alkaloids, flavonoids and phenols whereas, terpenes, steroids and anthraquinones were not detected. The serum enzymes alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were significantly elevated from 17.43 u/L and 28.40 u/L to 21.60 u/L and 34.27 u/L respectively. These were significantly lowered in the group C to 18.37 u/L and 29.23 u/L respectively for ALT and ALP. Also, a significant lowering of superoxide dismutase (SOD) activity was observed in the treated group (54.33 u/mg) from 79.40 u/mg recorded in the intoxicated group. Similarly, a significant decrease in malondialdehyde was observed in the treated group (25.80 u/mg) relative to the intoxicated group (35.87 u/mg). Moreover, catalase activity in the treated group (7.43 u/mg) was significantly lower compared with the intoxicated group. Our observation confirmed that ethanolic leaf extract of *T. occidentalis* reversed the oxidative damage condition in albino rats. The result confirms the ethnomedicinal use of the plant in the management of oxidative stress related diseases.

Keywords: ethnomedicine, phytochemicals, malondialdehyde, oxidative stress, antioxidant.

1. Introduction

The plant kingdom comprises vast natural resources whose potential have not been adequately utilized. The search for alternative medicine has led to the acceptance of ethnomedicine which is more a form of cultural approach to healthcare delivery [1]. Ethnomedicine, a part of holistic medicine, provides cheap but somehow crude medical practice for the majority of rural folk especially in Nigeria. Also, the emergence of new and drug-resistant pathogens is directing attention to herbal alternatives. Moreover, the concrete result from the treatment of infertility and other difficult health challenges is calling for concerted scientific investigation to authenticate the validity of the ethno-pharmaceutics [2, 3]. Many prophylactics have been developed from plants with high therapeutic potency [4].

Telfairia occidentalis (fluted pumpkin) is one of such plants with numerous medicinal and nutritional benefits. The leaf, seed, root and entire shoot of *T. occidentalis* have been subjected to scientific investigation purposely to harness the nutritional benefits of the plant as well as verify the health impact attributed to it in haematinic properties [5-7].

T. occidentalis is among the most popular vegetable crops being propagated in the West African rainforest zone for its green leafy vegetable and ellipsoidal fruit which is very nutritious [8]. Oluwole *et al*. [9] reported that *T. occidentalis* has anti-inflammatory properties, Odoemena and Essien [10] documented the antibacteria attributes of the plant while Kayode *et al*. [7] reported that *T. occidentalis* leaf-supplemented protein diet is more effective than only protein meal for recovery of rats suffering from protein energy malnutrition induced by oxidative damage. The various extract of the plant has also been claimed to exhibit anti-diabetic anti-cholesterolemic, anti-hyperglycemic and anti-anaemic properties [11-15].

Moreover, the consumption of the leaf of *T. occidentalis* was reported to elicit hypolipidemic effect and can help to reduce the risk of cardiovascular disease [5, 16]. Therefore, the present study was designed to assess the phytochemical constituents and the effect of *T. occidentalis* leaf extract on induced oxidative stress in albino rats.

2. Experimental

2.1. Materials

All the reagents used in this study were of analytical grade and they were sourced from Sigma-Aldrich Inc (Missouri, USA). The reagents were used as purchased without further purification or alteration.

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2.2. Preparation of leaf extract

Fresh green vegetable of *Telfairia occidentalis*were bought at Oja-Oba, Owo, Ondo state, Nigeria, and brought to the Environmental Biology Laboratory of Rufus Giwa Polytechnic, Owo, for authentication where a voucher specimen was deposited. The leaves were washed with distilled water and shade dried for three weeks. The dried leaves were milled into powder form using a mechanical grinder. 250 g of the leaf powder were dissolved in 500 ml of ethanol for 72 hours with occasional mixing using sterile glass stirrer. The suspension was then filtered with Whatman No. 1 filter paper and the filtrate subjected to rotary evaporation at 50 °C to produce a sticky greenish substance which was later reconstituted to 200 mg/ml extract with normal saline.

2.3. Laboratory animals

The albino rats (*Rattus norvegicus*) weighing between 180 and 240 g used in this study were procured from the Biochemistry Department, University College Hospital, Ibadan. The rats were kept in wooden cages at the animal house of the Science Laboratory Technology Department, Rufus Giwa Polytechnic, Owo, Nigeria, with free access to pelletized feed and portable water to acclimatize.

2.4. Qualitative phytochemical screening

All the tests described below were carried out on the extracts using the methods of Trease and Evans [17] and Sofowora [18].

2.5. Toxicological study

After two weeks of acclimatization, the rats were randomly divided into three groups of 5 rats each. Group A was the control, groups B and C were induced with 40 mg/kg body weight 2,4-dinitrophenyl hydrazine. Group C was on the second day (24 h after inducement) treated on daily basis with 200 mg/kg body weight extract of *T. occidentalis* leaf till the 21st day of the experiment. All the rats were fasted overnight to the 22nd day and thereafter sacrificed through chloroform anesthesia and the blood collected through cardiac puncture. The blood was allowed to clot, and serum collected by centrifugation of the blood samples at 10,000 rpm for 10 min. The serum was kept in the refrigerator.

Determination of the liver enzymes. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were measured using Randox kits manufactured by Randox Laboratories, UK. The procedures were carried out as prescribed in the kits manual.

Determination of serum concentration of superoxide dismutase (SOD). The determination of superoxide dismutase was done using the procedure of [19] by assessing the inhibition of adrenaline at pH 10.2 at 30 ^{o}C.

Determination of malondialdehyde (MDA) level. Thiobabituric acid (TBARS) assay was done to measure the level of MDA according to the procedure of Rice Evan *et al*. [20].

Determination of catalase activity (CAT). Catalase activity in the serum was determined by the method described by Sinha [21] using standard Randox kit manufactured by Randox Laboratories, UK. The procedures were carried out as prescribed in the kit's manual.

2.6. Statistical analysis

Data were presented as mean ± standard error (SE). Significant difference between different groups was tested using one-way analysis of variance (ANOVA) and treatment means were compared with Duncan's New Multiple Range Test using SSPS window 7 version 21.0 software. At $p < 0.05$, the differences were considered significant.

3. Results and discussion

Medicinal plants have been described as those plants that produce active substances that may be used as cure of diseases or which can be used as precursor of new drugs [22].The major bioactive substances produced by plants are the secondary metabolites which are generally referred to as phytochemicals.

The qualitative phytochemical screening of ~~the leaf of~~ the *T. occidentalis* leaf extract revealed the presence of saponin, tannins, alkaloids, flavonoids and phenols whereas terpenes, steroids and anthraquinones were not detected in the plant material.

The array of phytochemicals present in the leaf extract of *T. occidetalis* in this study, such as saponin, tannins, alkaloids, flavonoids, and phenols, have been reported earlier in different extracts of the plant leaf [14, 15, 23]. The presence of these phytochemicals may explain why the leaf is highly regarded among traditional healers in Nigeria as a potent remedy against many ailments.

Table 1. Qualitative phytochemical screening of *T. occidentalis* leaf extract

Phytochemical	Present/Absent
Saponin	+
Tannin	+
Terpenes	-
Anthraquinone	-
Steroids	-
Flavonoids	+
Alkaloids	+
Phenols	+

Key: + = detected, - = not detected.

On intoxication, the serum enzymes ALT and ALP were significantly elevated from17.43 u/L and 28.40 u/L to 21.60 u/L and 34.27 u/L respectively. These were significantly lowered in the group treated with *T. occidentalis* leaf extract to 18.37 u/L and 29.23 u/L respectively for ALT and ALP. There was no significant difference in the AST level of all the groups (Table 1).

The values of these various serum biomarkers fall within the normal range in the control group when compared with range of values contained in the "Lewis and Dacie practical hematology" [24]. The induction of the rats resulted to the elevation of the levels of ALT and ALP significantly. This observation agrees with that of Toma *et al*. [25].

According to Ramaiah [26] the ALP, AST and ALT serum enzymes are markers of health indices and are of importance as diagnostic tools clinical evaluation of the state of health of individuals. The liver and heart usually

produce ALT and AST when under severe stress such as oxidative stress and increase in their concentrations in serum are indicators of liver and heart damage. Therefore, the lowering of the ALP and ALT significantly in rats treated with the ethanol extract of the *T. occidentalis* leaf is a good pointer to its potential in the management of oxidative stress related diseases.

Table 2. Effect of *T. occidetalis* leaf extract on serum enzymes in albino rats

Parameters group	ALT (µ/L)	AST (µ/L)	ALP (µ/L)
(A) Control	17.43±0.03[a]	18.20±0.06[a]	28.40±0.06[a]
(B) Intoxicated	21.60±0.06[b]	19.00±0.12[a]	34.27±0.35[b]
(C) Treated	18.37±0.07[a]	18.53±0.03[a]	29.23±0.09[a]

Note. *Values are mean ± SEM, values with different alphabet along column are significantly different at p < 0.05.*

Also, a significant lowering of superoxide dismutase activity towards the level of the control group (36.67 u/mg) was observed in the treated group (54.33 u/mg) from 79.40 u/mg recorded in the intoxicated group. Similarly, a significant decrease in MDA was observed in the treated group (25.80 u/mg) relative to the intoxicated group (35.87 u/mg). Moreover, catalase activity in the treated group (7.43 u/mg) was significantly lowered down to a level close to the control group (7.20 u/mg) compared with the intoxicated group (8.10 u/mg).

Similar pattern of significant increase and reduction were observed in the value of SOD and MDA in the intoxicated and treated groups. These values imply that the toxicant must have propagated free radicals leading to lipid peroxidation in the animals. These results corroborate the view of Talwar and Srivastava [27] with respect to serum enzymes under oxidative damage conditions. The lowering of the SOD and the MDA indicates a decreased production of oxygen free radicals by the *T. occidentalis* leaf extract.

Table 3. Effect of *T. occidetalis* leaf extract on serum antioxidants in albino rats

Parameters group	SOD (µ/mg)	CAT (µ/mg)	MDA (µ/mg)
(A) Control	36.67±0.44[c]	7.20±0.12[b]	23.23±0.09[b]
(B) Intoxicated	79.40±0.31[a]	8.10±0.06[a]	35.87±0.18[a]
(C) Treated	54.33±0.88[b]	7.43±0.07[b]	25.80±0.40[b]

Note. *Values are mean ± SEM, values with different alphabet along column are significantly different at p < 0.05.*

SOD helps to remove superoxide anion radicals by turning them to hydrogen peroxide and oxygen. The increase in the serum SOD enzyme in the intoxicated group may be as a result of oxidative activation of enzyme protein or increased of their synthesis. Hence, the reduction of both SOD and MDA in the group treated with the *T. occidentalis* leaf extracts is indicative of a reduction in the accumulation of superoxide anion radicals with oxidative stress, which leads to the reduction in liver damage [28].

These reduction in the serum enzymes might be linked with the phytochemicals inherent in the *T. occidentalis* leaf which may be responsible for the antioxidant activities of the plant. Tannins are reported to be effective in the management of tissue inflammation in cancer prevention. Therefore, its presence in the leaf extract of the plant suggests that the plant may be a good source of active principles in the prevention and management of cancer [22].

Flavonoids have been reported to be efficient antioxidants and free radical scavengers, which help to prevent oxidative cell damage [29]. They have been identified to exert high level of antioxidant activity in man and their effects on human nutrition and health is considerable [30].

The presence of these phenolic compounds in the *T. occidentalis* leaf extract promotes their antioxidative activities which may have informed its use in African folklore medicine. Phenols are known to function as free radical chain reaction inhibitors which invariably confer the antioxidative potential on the plant material [31]. Phenols have also been reported as a having potential of combating oxidative stress syndrome.

4. Conclusions

The result and observation show that ethanol extract of *Telfairia occidentalis* is able to heal oxidative damage in animals exposed to such damage as shown in the lowering of various oxidative stress biomarker enzymes in this study. This activity may have been brought about as a result of the presence of phytochemicals such as saponin, tannins, alkaloids, flavonoids and phenols in the plant's leaf extract.

Conflict of interest

The authors declare no conflict of interest regarding the publication of this article.

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