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Hepatotoxicity of *Telfaria occidentalis* root extracts on wistar albino rat

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ABSTRACT

The present study investigates the toxicity of *Telfaria occidentalis* root extracted with different solvents. This was with a view to validating its widely acclaimed use as poison in traditional parlance. Air-dried powdered root of *Telfaria occidentalis* was extracted separately with distilled water, methanol and diethyl ether. Twenty albino rats were randomly placed into four groups of five animals per group: Group I served as the positive control and were administered distilled water only while groups II, III and IV animals were administered 50 mg/kg body weight of aqueous, methanolic and diethyl ether extracts respectively for two weeks. Activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), superoxide dismutase and catalase were assayed in the liver homogenate and serum. Levels of total cholesterol, HDL-cholesterol, LDL-cholesterol as well as lipid peroxidation were also measured in the liver and serum of animals. Results showed that the level of AST, ALT, ALP as well as LDL-cholesterol and HDL-cholesterol in the serum of group IV animals increased significantly relative to the control group. Antioxidant enzyme biomarkers such as catalase and superoxide dismutase as well as lipid peroxidation were significantly increased in the serum of group IV animals relative to the control. The study concluded that toxicity of root extract of *Telfaria occidentalis* is solvent-dependent.

1. Introduction

Fluted pumpkin (*Telfaria occidentalis*) is a leafy vegetable commonly used in folk medicine (Mensah et al., 2008). Evidence from several epidemiological studies concerning the use of its bioactive substances in a number of animal models, cell culture studies and clinical trials validate its immense pharmacological potentials (Ukwuoma and Muanya, 2005; Olaniyan and Adeleke, 2005; Oboh et al., 2006; Eseyin et al., 2014). *Telfairia occidentalis* is popularly used in ethnobotany as antidiabetic, antihypertensive, antitumouric, antioxidant, immunodulator, antibacterial, antihypercholesterolemic, antiparasitic, anti-inflammatory and in the treatment of central nervous system-related disorders including convulsion (Nwozo et al., 2004; Oyewole and Abalaka, 2012; Igbeneghu and Abdul, 2014). Its leaves have also been reported to exhibit chemo-suppressive properties (Igbeneghu and Abdul, 2014). Aqueous extract of *T. occidentalis* leaves have been shown to offer hepatoprotection against garlic-induced oxidative stress (Olorunfemi et al., 2005; Oboh, 2006; Oboh et al., 2006), while both its aqueous and ethanolic extracts have demonstrated hypoglycemic properties both in normoglycemic and alloxan-induced diabetic rats (Zhang, 2001; Zhang and Yao, 2002; Salman et al., 2008).

Relative to most vegetables, its protein content is very high (Ajibade et al., 2006; Aregheore, 2007). According to Kayode and Kayode (2011), leaves of *Telfairia occidentalis* are rich in minerals, antioxidants and vitamins such as thiamine, riboflavin, nicotinamide and ascorbic acid. Its young leaves are rich in magnesium and iron (Akwaowo et al., 2000) and can be used for treating anaemia due to its heamatinic properties (Ajibade et al., 2006; Eseyin et al., 2014).

Despite the vast potential usefulness of *Telfairia occidentalis,* its roots have been reported to be toxic both in the traditional parlance and from scientific reports (Akubue et al., 1980; Abiose, 1999; Ogbonnaya and Uadia, 2016).). There is therefore a dire need to unravel the role of extraction solvent in its toxicity.

2. Materials and methods

2.1. Materials

2.1.1. Collection of plant materials

Roots of *Telfaria occidentalis* were collected from Ora Ekiti, Ido/Osi

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Local Government Ekiti State, Nigeria. The plant material was washed, chopped, air dried and powdered using Master Chef Warring blender model MDL-MC-B919A from Pegasus Limited, China. The powdered root was kept in tightly closed containers and kept in the refrigerator prior to use.

2.1.2. Animals

Adult male wistar rats (200–250 g) from our own breeding colony were used. Animals were kept in separate animal cages, on a 12-h light:12-h dark cycle, at a room temperature of 22–24 °C, and with free access to food and water. The animals were used according to the National Institutes of Health guide for the care and use of Laboratory animals (National Research Institute, 2011). Ethical approval was obtained from the Committee on Care and Use of Experimental Animals, Department of Medical Biochemistry, College of Medicine, Ekiti State University, Ado Ekiti, Nigeria. Animals were placed into four groups of five rats each: Group I served as the positive control and were administered distilled water only while groups II, III and IV animals were administered 50 mg/kg body weight of aqueous, methanolic and diethyl ether extracts respectively for two weeks by oral gavage. At the end of the experiment, animals were fasted 24 h before sacrifice.

2.2. Methods

2.2.1. Extraction of active principles

Dried pumpkin root weighing 137.3 g each was extracted with 250 ml each of methanol, distilled water and diethyl ether respectively for 72 h. Thereafter, the resulting mixture was filtered in sequence using a muslin cloth and Whatmann's filter paper, No. 1.0. The filtrates were left opened for 72 h to allow for complete evaporation of the solvents of extraction. The crude extract recovered after evaporation was stored in an airtight container in the refrigerator prior to use. The crude extracts were then reconstituted in distilled water and administered to the animals orally for two weeks.

2.2.2. Preparation of tissue homogenate

Animals weighing (230 g) were decapitated and dissected to obtain the liver. Ten percent homogenate of the liver was then prepared (by weighing 8.2 g of liver tissue in 82 ml of the homogenate) in 6.7 mM potassium phosphate buffer, pH 7.4, using Teflon homogenizer model 3431E10EA, Thomas Scientific, India. The liver homogenate was centrifuged at 10,000 rpm for 10 min at 4 °C. to obtain a clear supernatant which was kept at 8 °C. for subsequent analysis. Whole blood was collected by cardiac puncture into a serum bottles and the serum was obtained by centrifugation at 3000 rpm for 15 min.

2.2.3. Determination of total cholesterol

Total cholesterol in the serum and tissue homogenates were routinely determined following the method of Richmond (1973) using standard Fortress kit from England. Ten microliter (10 μl) of tissue homogenate was thoroughly mixed with 1 ml of working reagent (4-aminoantipyrine, cholesterol oxidase, cholesterol esterase and peroxidase dissolved in 50 ml of pipes buffer and phenol). The reaction mixture was incubated for 10 min at 25 °C. The absorbance of the sample against the blank was read at 546 nm. Total cholesterol (mM) was then calculated.

2.2.4. Determination of alkaline phosphatase (ALP)

Alkaline phosphatase was assayed following the method described by Reitman and Frankel (1957) using standard Fortress kit from England. Five hundred microliter (500 μl) of substrate was equilibrated at 37 °C for 3 min. Fifty microliter (50 μl) of sample was added to the equilibrated substrate and incubated for 10 min at 37 °C. 2.5ml of colour reagent (mixture of NaOH, 0.09 M and Na_2CO_3, 0.1 M) was added. This mixture was thoroughly mixed. Absorbance of the sample against the blank was read at 590 nm. ALP (IU/L) was then calculated.

2.2.5. Determination of aspartate transaminase (AST)

Aspartate transaminase AST was assayed following the method described by Reitman and Frankel (1957) using standard Fortress kit from England. Briefly, 100 μl of homogenate was added to 500 μl of AST buffer, thoroughly mixed and incubated for 30 min at 37 °C. Thereafter, 0.5 ml of dye reagent (2,4-dinitrophenyl hydrazine, (2.0 mM) was added and the mixture was thoroughly mixed and allowed to stand for 20 min at 20 °C. Five milliliters (5.0 ml) of 0.01 M NaOH was then added and thoroughly mixed. Absorbance of the sample against the blank was read at 546 nm.

2.2.6. Determination of alanine transferase (ALT)

ALT was measured using standard Fortress kit from England according to the method of International Federation of Clinical Chemistry (1986) using standard Fortress kit from England. One hundred microliters (100 μl) of homogenates was added to 500 μl of ALT buffer, thoroughly mixed and incubated for 30 min at 37 °C in water bath. Five hundred microliters (500 μl) of dye reagent (2, 4 - dinitrophenyl hydrazine, 2.0 mM) was then added. This mixture was thoroughly mixed and allowed to stand for 20 min at 20 °C. Thereafter, 5.0 ml of diluted NaOH was then added and thoroughly mixed. Absorbance of the resulting mixture sample against the blank was read at 546 nm.

2.2.7. Determination of catalase activities (CAT)

Catalase activity in the serum and tissue homogenates were determined as described by Sinha (1972) using standard Fortress kit from England. Two hundred microliters (200 μl) of tissue homogenates was mixed with 0.8 ml distilled H_2O to give 1 in 5 dilution of the sample. Five hundred microliters (500 μl) of tissue homogenate was rapidly mixed with the reaction mixture at room temperature by a gentle swirling. One milliliter (1 ml) portion of the reaction mixture was withdrawn and blown into 1 ml dichromate/acetic acid reagent at 60 s intervals. The hydrogen peroxide content of the withdrawn sample was determined by the method described above at 570 nm.

2.2.8. Determination of HDL-cholesterol

HDL-cholesterol was measured following the method described by Assmann and Schulte, 1990 using standard Fortress kit from England. Briefly, 5 μl of homogenate was added to 450 μl of Good's buffer (4-amino antipyrine, POD, ascorbic oxidase and anti-human lipoprotein Ab). This mixture was mixed well and incubated at 37 °C in water bath for 5 min. Thereafter, 150 μl of enzyme reagent (Good's buffer I, cholesterol esterase and cholesterol oxidase) was later added to the mixture and incubated for 5 min at 37 °C. Absorbance of the resulting mixture was then read against the blank at 600 nm.

2.2.9. Determination of LDL-cholesterol

Low density lipoprotein-cholesterol (LDL-chol) was determined by the method earlier described by Armstrong and Seidel (1985) using standard Fortress kit from England. Briefly, 5 μl of tissue homogenate was added to 450 μl of buffer solution (Good's buffer, cholesterol oxidase, cholesterol esterase, catalase, ascorbate oxidase, and peroxidase). This mixture was mixed well and incubated at 37 °C in water bath for 5 min. Absorbance 1 was measured at 600 nm. One hundred and fifty microliters (150 μl) of enzyme reagent R2 (Amino antipyrine and POD) was later added to the mixture and incubated for 5 min at 37 °C. Absorbance 2 of the sample against the blank was read at 600 nm.

2.2.10. Estimation of lipid peroxidation assay (TBARS)

Levels of thiobarbituric acid reactive species (TBARS) in the tissues and serum homogenates were measured according to the method of Varshney and Kale (1990) using standard Fortress kit from England. An

aliquot of 0.4 ml of serum and other tissue homogenates was mixed with 1.6 ml of Tris-KCl buffer to which 0.5 ml of 30% trichloroacetic acid (TCA) was added. Thereafter, 0.5 ml of 0.75% thiobarbituric acid (TBA) was added and incubated for 45 min at 80 °C. The resulting mixture was then cooled on ice and centrifuged at 3000 *g*. The clear supernatant was collected and absorbance measured against a reference blank at 532 nm. The MDA level was calculated according to the method of Adam-vizi and Seregi (1982).

2.2.11. Superoxide dismutase (SOD)

Superoxide dismutase activity in both serum and tissue homogenates was determined by the method of Misra and Fridovich (1972) using standard Fortress kit from England. An aliquot of the serum and/or tissue homogenates was added to 2.5 ml of 0.05 M carbonate buffer, pH 10.2 and the mixture was allowed to equilibrate for 2 min in a spectrophotometer. The reaction was then started by the addition of 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained all other assay components except the homogenates (s) and/or serum which was replaced with distilled water. Increase in absorbance at 480 nm was monitored every 30 s for 150 s.

2.2.12. Determination of total protein

Total protein content was determined using the Biuret method (Gornall et al., 1949) using standard Fortress kit from England. Briefly, 20 μl of homogenate was thoroughly mixed with 1 ml of Biuret reagent and incubated at room temperature for 10 min. Absorbance of the resulting mixture was read at 546 nm against the blank.

2.2.13. Statistical analysis

All values obtained were expressed as mean ± SD. The data were analyzed by appropriate ANOVA followed by Duncan's multiple range tests where appropriate and this is indicated in the text of results. The differences were considered significant at 95% confidence limit.

3. Results

3.1. General observation

Tables 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 show the effects of root extracts of *Telfaria occidentalis* on animal weight, liver and serum of experimental animals. The extract caused an increase in body weight of animals relative to the control group (Table 1). Similarly there was an increase in liver weight/animal weight relative to the control group (Table 2). Meanwhile, there was a decrease in liver AST activity regardless of the solvent used for extraction (Table 3). However, there was an increase in AST activity in the serum relative to the control animals (group I) that were given distilled water only (Table 3). Table 4 revealed a decrease in ALT activity in the liver homogenate of experimental animals relative to the control (group I). ALP activity was significantly decreased in the liver of experimental animals but increased in the serum (Table 5). Total cholesterol was significantly ($P = 0.01$) decreased both in the liver homogenates and serum of experimental animals relative to the control group (Table 6). A significant decrease in HDL-cholesterol was observed in experimental animals relative to the control animals (Table 7). On the other hand, a significant ($P = 0.05$) increase in HDL-cholesterol was observed in the serum relative to the

Table 1
Effect of *T. occidentalis* extracts on weight of animals.

	Group I	Group II	Group III	Group IV
Initial weight of Animals (g)	230.21 ± 1.20	230.34 ± 2.15	231.55 ± 3.50	230.11 ± 5.30
Final weight of animals (g)	231.15 ± 3.22[a]	233.44 ± 5.25[a*]	235.68 ± 4.46[a*]	238. 25 ± 3.60[a*]

Data is expressed as mean ± standard deviation.
[a*] Represents significant difference from the control in each column at P = 0.05.

Table 2
Effect of *T. occidentalis* extracts on liver-animal weight ratio.

	Group I	Group II	Group III	Group IV
Liver weight (g)	6.01 ± 0.10[a]	8.16 ± 0.13[a*]	8.72 ± 3.50[a*]	10.11 ± 5.30[a*]
Liver weight/Animal body weight (%)	2.60[a]	3.50[a*]	3.70[a*]	4.24[a*]

Data is expressed as mean ± standard deviation.
[a*] Represents significant difference from the control in each column at P = 0.05.

Table 3
Effect of *T. occidentalis* extracts on AST activity of selected tissues.

	Group I	Group II	Group III	Group IV
Liver	21.88 ± 1.83[a]	16.25 ± 0.90[a*]	15.88 ± 0.95[a*]	15.99 ± 0.96[a*]
Serum	60.13 ± 6.67[a]	65.73 ± 7.29	69.89 ± 7.75[a*]	72.09 ± 8.00[a*]

Data is expressed as mean ± standard deviation
[a*] Represents significant difference from the control in each column at P = 0.05.

Table 4
Effect of *T. occidentalis* extracts on ALT activity of selected tissues.

	Group I	Group II	Group III	Group IV
Liver	25.06 ± 2.09[a]	20.77 ± 1.73[a*]	18.04 ± 1.50[a*]	20.18 ± 1.69[a*]
Serum	63.80 ± 18.32[a]	78.50 ± 18.96[a*]	79.07 ± 18.99[a*]	79.54 ± 19.01[a*]

Data is expressed as mean ± standard deviation.
[a*] Represents significant difference from the control.
[a] At P = 0.02.

Table 5
Effect of *T. occidentalis* extracts on ALP activity of selected tissues.

	Group I	Group II	Group III	Group IV
Liver	34.39 ± 2.87[a]	24.82 ± 2.07[a*]	17.24 ± 1.44[a*]	14.93 ± 1.24[a*]
Serum	58.69 ± 7.06[a]	60.99 ± 7.33	61.96 ± 7.45	62.22 ± 7.47

Data is expressed as mean ± standard deviation.
[a*] Represents significant difference from the control.
[a] At P = 0.05.

Table 6
Effect of *T. occidentalis* extracts on total cholesterol level of selected tissues.

	Group I	Group II	Group III	Group IV
Liver	12.95 ± 1.08[a]	9.51 ± 0.79[a*]	8.54 ± 0.72[a*]	8.08 ± 0.68[a*]
Serum	19.09 ± 2.29[a]	13.82 ± 1.66[a*]	11.05 ± 1.33[a*]	11.29 ± 1.36[a*]

Data is expressed as mean ± standard deviation
[a*] Represents significant difference from the control.
[a] At P = 0.01.

Table 7
Effect of *T. occidentalis* extracts on HDL-cholesterol level of selected tissues.

	Group I	Group II	Group III	Group IV
Liver	9.40 ± 0.78[a]	3.83 ± 0.32[a*]	2.62 ± 0.22[a*]	2.57 ± 0.22[a*]
Serum	16.51 ± 7.27[a]	71.40 ± 62.15[a*]	59.49 ± 51.23[a*]	78.79 ± 69.24[a*]

Data is expressed as mean ± standard deviation.
[a*] Represents significant difference from the control.
[a] At P = 0.05.

Table 8
Effect of *T. occidentalis* extracts on LDL-cholesterol level of selected tissues.

	Group I	Group II	Group III	Group IV
Liver	7.20 ± 0.60[a]	3.83 ± 0.32[a*]	3.72 ± 0.31[a*]	3.67 ± 0.31[a*]
Serum	8.48 ± 1.92[a]	13.15 ± 2.97	12.41 ± 2.81	14.36 ± 3.25[a*]

Data is expressed as mean ± standard deviation.
[a*] Represents significant difference from the control.
[a] At P = 0.02.

Table 9
Effect of *T. occidentalis* extracts on superoxide dismutase activity of selected tissues.

	Group I	Group II	Group III	Group IV
Liver	11.13 ± 0.40	16.51 ± 0.59[a*]	31.66 ± 1.13[a*]	28.78 ± 0.67[a*]
Serum	73.54 ± 5.21[a]	45.74 ± 3.24[a*]	54.73 ± 3.87[a*]	79.09 ± 14.02

Data is expressed as mean ± standard deviation.
[a*] Represents significant difference from the control.
[a] At P = 0.01.

Table 10
Effect of *T. occidentalis* extracts on catalase activity of selected tissues.

	Group I	Group II	Group III	Group IV
Liver	13.95 ± 2.72[a]	10.48 ± 2.04[a*]	11.43 ± 2.23[a*]	10.31 ± 2.01
Serum	15.47 ± 3.02[a]	11.59 ± 0.48	14.46 ± 0.61	18.84 ± 0.58

Data is expressed as mean ± standard deviation.
[a*] Represents significant difference from the control.
[a] At P = 0.02.

Table 11
Effect of *T. occidentalis* extracts on malonyldialdehyde level of selected tissues.

	Group I	Group II	Group III	Group IV
Liver	11.74 ± 3.35[a]	28.66 ± 5.49[a*]	23.99 ± 5.04[a*]	20.47 ± 4.54[a*]
Serum	16.51 ± 4.71[a]	19.26 ± 5.49	17.01 ± 5.04	15.93 ± 4.54

Data is expressed as mean ± standard deviation.
[a*] Represents significant difference from the control.
[a] At P = 0.03.

control group (Table 7). Table 8 showed a significant decrease and increase in the liver and serum respectively relative to the control animals (group I). A significant (P = 0.01) increase and decrease in superoxide dismutase activity was observed in the liver and serum respectively relative to the control animals (Table 9). Table 10 showed a decrease in catalase activity both in the liver and serum of experimental animals relative to the control. An increase in MDA was observed (Table 11) both in the serum and liver homogenates of experimental animals relative to the control group. Generally, among the three solvents used for extraction, diethyl ether extract showed the highest effect in all parameters determined (Tables 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10). The only exception was in lipid peroxidation where highest MDA was observed in the aqueous extract relative to the control (Table 11).

4. Discussions

In the struggle for continuous survival in a given habitat, organisms have evolved multiple adaptive strategies to ward off their predators and/or other stressors within the ecosystem. One of these strategies is the synthesis of compounds that would be hazardous to potential predators in different plant parts depending on their vulnerability to attack. Perhaps, this may explain the differential edibility and/or otherwise of different parts of plants. Specifically, the leaves and seeds of fluted pumpkin is widely consumed due to its potential health benefits (Oyewole and Abalaka, 2012). However, the root of the same plant is toxic and has been used traditionally as rodenticide (Akubue et al., 1980; Togun et al., 1994; Abiose, 1999; Ajibesin et al., 2002). Several excellent reports have been made on the fruit and leaves of the plant (Oboh, 2006; Kayode and Kayode, 2011), while reports on the toxicity of its root is scanty.

Administration of the root extract of *Telfaria occidentalis* resulted in a solvent-dependent increase in body weight of experimental animals and liver/animal weight ratio respectively relative to the control (Tables 1 and 2). Hepatic damage is routinely monitored by assaying for specific liver enzyme biomarkers such as AST, ALT and ALP in the liver and serum of experimental animals (Shivaraj et al., 2009). In a healthy animal, these enzymes are normally located in the cytoplasm of the hepatocytes. However, they are released into circulation following hepatic damage. Expectedly, levels of these enzymes should rise in the serum but decreased in the tissues where they were originally localized prior to damage (Vermeulen et al., 1992). Level of AST was significantly increased in the serum of animals administered with the extract relative to the control animals (Table 3). This observation can be attributed to the damage caused by the root extract of *Telfaria occidentalis* on the liver cells causing the leakage of the enzyme from the hepatocytes to the blood consequently leading to a surge in its level in the blood. On the other hand, there was also a concomitant decrease in AST activity in the liver homogenate (Table 3). This was due to leakage of the enzyme from the hepatocyte, leading to a decrease in its activity. Arguably, leakage of an enzyme from a localized compartment should lead to a decrease in its amount while its level in the other compartment (recipient) should increase. Worthy of note however, is the fact that the damage was higher in the animals administered with diethyl ether extract when compared with methanol and aqueous extracts. The order of hepatic damage involving AST was: diethylether > methanol > water. This is an indication that more active toxic principles in the root are not water-soluble. Comparatively, diethyl ether is the most non-polar of the three solvents used for the extraction, hence, it can be inferred that most of the toxic principles causing the hepatic damage are non-polar. The same pattern of results was obtained for ALT (Table 4) and ALP (Table 5) in the serum and liver homogenates of the experimental animals for similar reasons.

Administration of *Telfaria occidentalis* root extracts caused a significant decrease in the level of serum cholesterol relative to the control animals (Table 6). Cholesterol is one of the major membrane lipids that are solely responsible for the structural integrity of the membrane. Within the limit of available data in the present study, some toxic principles of the plants root extract caused a deleterious alteration in the cholesterol of the liver cell membrane (Keely et al., 2018). As a consequence, membrane fluidity and integrity were compromised consequently leading to the leakage of the specific liver enzymes that should be localized within the liver hepatocytes. Similarly, the cholesterol level in the serum was also decreased relative to the control animals (Table 6) for a similar reason. Noteworthy is the fact that effect of the extract on total cholesterol was highest in the group treated with the diethyl ether extract (Group IV) buttressing the fact that more of the toxic principles were non-polar and got extracted in the most non-polar solvent.

High density-cholesterol (HDL) and low density - cholesterol (LDL) are often routinely measured as markers of toxicity especially if the mechanism involves derangement of the lipid profile. HDL-cholesterol is a 'good cholesterol' as it is involved in the transport of cholesterol away from cells back to the liver for metabolism and excretion. On the other hand, LDL-cholesterol is a 'bad cholesterol' as it distributes cholesterol to all parts of the body (Keely et al., 2018). HDL-cholesterol was significantly decreased in the liver relative to the control animals (Table 7). This is indicative of the toxicity of the root extract. Decrease in

HDL-cholesterol suggests that the transport of cholesterol from the various cells to the liver was probably impaired, hence, by implication, hyperlipidemia is expected which could further trigger the onset of several cardiovascular disorders associated with high cholesterol levels (Lee-Li et al., 2015). On the other hand, serum LDL-cholesterol was significantly increased relative to the control animals (Table 8) suggesting an increase in the 'bad cholesterol' which may indicate the likelihood of hypercholesterolemia and its associated pathological disorders. Worthy of mention, however, is the fact that the pattern of increase in LDL-cholesterol was diethyl ether > methanol > aqueous extracts. This further buttressed the fact that the toxic principles of the root extract of *Telfaria occidentalis* are essentially non-polar.

Superoxide dismutase is an antioxidant enzyme that alternately catalyzes the dismutation of superoxide radical into either ordinary molecular oxygen or hydrogen peroxide (Hayyan et al., 2016). This enzymic antioxidant was significantly elevated in the liver of experimental animals relative to the control animals (Table 9). This observation suggests that administration of *Telfaria occidentalis* root extracts triggered the generation of free radicals, specifically superoxide radicals, in the hepatocytes of animals. In order to mop up the radicals, there was a surge in the expression of superoxide dismutase in the liver to prevent the onset of oxidative stress. Since oxidative stress has been linked to the onset and progression of several pathological conditions, it suggests that free radical formation is the underlying mechanism of toxicity of the root extract of *Telfaria occidentalis*. It has been reported that superoxide dismutase is the major antioxidant enzyme offering protection against oxidative damage in the liver (Romao (2015). It is also plausible to mention that more radicals were generated when diethyl ether extract was administered because it is the most non-polar of the three solvents used for extraction.

Catalase is a preventive antioxidant enzyme located in the peroxisome of cell and plays an important role in the protection against deleterious effects of lipid hydroperoxide (Kirkman and Gaetani, 2007). In contrast to superoxide dismutase, catalase level was significantly decreased in the liver and serum of experimental animals (Table 10). This observation implies the presence of toxic principles in the extracts that adversely affected the activity of catalase. It should be noted also that the effect of *Telfaria occidentalis* root was highest in the group administered with ethyl acetate extract compared to other solvents. This observation also agreed with earlier fact that more of the toxic principles were extracted in the diethyl ether extract making it the most toxic.

Lipid peroxidation occurs naturally in small amount by the effect of reactive oxygen species (ROS) which attacks polyunsaturated fatty acids of the membrane, initiating a self-propagating chain reaction (Ali *et al.*, 2014). In the present study, there was a significant increase in hepatic lipid peroxidation in experimental animals when compared to the control animals (Table 11). This suggests that lipid peroxidation is one of the mechanisms through which the toxic principle of *Telfaria occidentalis* root extract elicits its toxicity in the liver. This partly explains the leakage of specific liver enzymes such as AST and ALT from the hepatocytes to the serum as earlier discussed. Noteworthy however, is the fact that lipid peroxidation was not highest in animals administered with diethyl ether extract. Rather, MDA level was highest in the aqueous extract suggesting that more of the toxic principles responsible for lipid peroxidation in *Telfaria occidentalis* root are water-soluble (Table 11).

From the foregoing, toxicity of *Telfaria occidentalis* root depends largely on the solvent used for its extraction. However, non-polar solvents extracted more toxic principles than their polar counterparts, hence, toxicity increased with increasing non-polarity of solvent.

Declarations

Author contribution statement

Temidayo Ogunmoyole: Conceived and designed the experiments; Wrote the paper.

Funmilola Comfort Oladele: Contributed reagents, materials, analysis tools or data.

Ayonbo Aderibigbe: Performed the experiments.

Olaitan Daniel Johnson: Analyzed and interpreted the data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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References

Abiose, S., 1999. Assessment of the extent of use of indigenous African foods, introduced foods and imported foods in hotels and other commercial eating places in southwestern Nigeria Pp 50-52. In: Baidu-Forson, J.J. (Ed.), African Natural Resources Conservation and Management Surveys. UNN/INRA, Accra.

Adam-vizi, V., Seregi, M., 1982. Receptor dependent stimulatory effect of noradrenaline on Na+/K+ ATPase in rat brain homogenate: role of lipid peroxidation. Biochem. Pharmacol. 31, 2231–2236.

Ajibade, S.R., Balogun, M.O., Afolabi, O.O., Kupolati, M.D., 2006. Sex differences in biochemical contents of *Telfairia occidentalis* Hook F. J. Food Agric. Environ. 4, 155–156.

Ajibesin, K.K., Bala, D.N., Ekpo, B.A., Adesanya, S.A., 2002. Toxicity of some plants implicated as poisons in Nigerian ethnomedicine to rats. Nig. J. Nat. Prod. Med. 6, 7–9.

Akubue, P.I., Kar, A., Nnachetta, F.N., 1980. Toxicity of extracts of roots and leaves of *Telfairia occidentalis*. Planta Med. 38, 339–343.

Akwaowo, E.U., Ndon, B.A., Etuk, E.U., 2000. Minerals and antinutrients in fluted pumpkin (*Telfairia occidentalis* Hook f.). Food Chem. 70, 235–240.

Aregheore, E.M., 2007. Voluntary intake, nutrient digestibility and nutritive value of foliage of fluted pumpkin (*Talfairia occidentialis*)-haylage mixtures by goats. Livest. Res. Rural Dev. 19 (4), 55–60.

Armstrong, V.W., Seidel, D., 1985. Evaluation of a commercial kit for the determination of LDL cholesterol based on precipitation of LDL with dextran sulfate. Arztliche Laboratorium 31, 325–330.

Assmann, G., Schulte, H., 1990. Relationship of HDL cholesterol to incidence of atherosclerotic coronary heart disease: the procam experience. Drugs affecting lipid metabol 2, 343–355.

Eseyin, O.A., Sattar, M.A., Rathore, H.A., 2014. A Review of the Pharmacological and Biological Activities of the Aerial Parts of Telfairia occidentalis Hook.f. (Cucurbitaceae). J.Pharm. Res. 13 (10), 1761–1769.

Gornall, A.G., Bardawill, C.J., David, M.M., 1949. Determination of serum proteins by means of the Biuret reaction. J. Biol. Chem. 177, 751.

Hayyan, M., Hashim, M.A., AlNashef, I.M., 2016. Superoxide ion: generation and chemical implications. Chem. Rev. 116 (5), 3029–3085.

Igbeneghu, O.A., Abdul, A.B., 2014. Multiple Anti-resistant bacteria on flute pumpkin leaves, a herb of therapeutic value. J. Health Popul. Nutr. 32 (2), 176–182.

International Federation of Clinical Chemistry, 1986. Methods for the measurement of catalytic concentration of enzymes. Part 3. IFCC method for alanine aminotransferase. J. Clin. Chem. Biochem. 18, 521–534.

Kayode, A.A., Kayode, O.T., 2011. Some medicinal values of Telfaria occidentalis: a review. Amer. J. Biochem. Molecular Biol. 1, 30–38.

Keely, B.K., Rachala, S., Nodzo, S.R., 2018. Centers for disease control and prevention (2017). guidelines for prevention of surgical site infections: review and relevant recommendations. Curr. Rev. Musculoskelet. Med. 11.

Kirkman, H.N., Gaetani, G.F., 2007. Mammalian catalase: a venerable enzyme with new mysteries. Trends Biochem. Sci. 32, 44–50.

Lee-Li, C., Li, W., Wen-Ching, H., Yi-Ju, H., Yi-Ming, C., Chi-Chang, H., 2015. Hypolipidemic effect of tomato juice in hamsters in high cholesterol diet-induced hyperlipidemia. Nutrients 7 (12), 10525–10537.

Mensah, J.K., Okoli, R.I., Ohaju-Obodo, K.E., 2008. Phytochemical, nutritional and medical properties of some leafy vegetable consumed by Edo people of Nigeria. Afr. J. Biotechnol. 7 (14), 2304–2309.

Misra, H.P., Fridovich, I., 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247 (15), 3170–3175.
National Research Institute, 2011. Guidelines for the Care and Use of Laboratory Animals, eighth ed. The National Academies Press, Washington D.C.
Nwozo, S.O., Adaramoye, O.A., Ajaiyeoba, E.O., 2004. Anti-diabetic and hypolipidemic studies of *Telifairia occidentalis* on alloxan induced diabetic rabbits. Niger. J. Natl. Prod. Med. 8, 45–47.
Oboh, G., 2006. Hepatoprotective property of ethanolic and aqueous extract of fluted pumpkin (*Telfairia occidentalis)* leaves against garlic-induced oxidative stress. J. Med. Food 8, 560–563.
Oboh, G., Nwanna, E.E., Elusiyan, C.A., 2006. Antioxidant and antimicrobial properties of Telfairia occidentalis (fluted pumpkin) leaf extracts. J. Pharmacol. Toxicol. 1 (2), 167–175.
Ogbonnaya, E.A., Uadia, P.O., 2016. Phytochemical screening and acute toxicity evaluation of *Telfairia occidentalis* aqueous extracts on rats *Pak*. J. Pharm. Sci. 29 (3), 913–917.
Olaniyan, M.F., Adeleke, A., 2005. A Study of the effect of pumpkin (ugu- *Telfaria occidentalis*) milk and raw egg mixture in the treatment of anaemic pregnant women in a rural area. Afr. J. Tradit., Complementary Altern. Med. 2 (3), 269–273.
Olorunfemi, A.E., Arnold, C.I., Emmanuel, O., Nkaima, N., Akeem, A., 2005. Hypoglycaemic activity of *Telfairia occidentalis* in rats. J. Pharm. Bioresour. 2, 36–42.
Oyewole, O., Abalaka, M.E., 2012. Antimicrobial activities of *Telfairia occidentalis* (fluted pumpkins) leaf extract against selected intestinal pathogens. J. Health Sci. 2 (2), 1–4.
Reitman, S., Frankel, S., 1957. Glutamic – pyruvate transaminase assay by colorimetric method. Am. J. Clin. Pathol. 28, 56–60.
Richmond, W., 1973. Preparation and properties of a cholesterol oxidase from *nocardia sp*. and its application to the enzymatic assay of total cholesterol in serum. Clin. Chem. 19 (12), 1350–1356.
Romao, S., 2015. Therapeutic value of oral supplementation with melon superoxide dismutase and wheat gliadin combination. Nutrition 31, 430–436.
Salman, T.M., Olayaki, L.A., Oyeyemi, W.A., 2008. Aqueous extract of *Telfairia occidentalis* leaves reduces blood sugar and increases haematological and reproductive indices in male rats. Afr. J. Biotechnol. 7, 2299–2303.
Shivaraj, G., Prakash, B.D., Vinayak, V.H., Avinash, A.K., Sonal, N.V., Shruthi, S.K., 2009. A review on laboratory liver function tests. Pan African Medical Journal 10, 55–60.
Sinha, A.K., 1972. Colorimetric assay of catalase. Anal. Biochem. 47, 389–394.
Togun, R.A., Animashaun, T., Kay, J.E., Aboderin, A., 1994. A galactose binding mitogenic lectin from the seeds of *Telfairia occidentalis*. Phytochemistry 35, 1125–1130.
Ukwuoma, B., Muanya, C., 2005. Fighting Degenerative Diseases with Vegetable Soup. www.guardiannewsngr.com/naturalhealth.article01/13075.
Varshney, R., Kale, R.K., 1990. Effects of calmodulin antagonists on radiation-induced lipid peroxidation in microsomes. Int. J. Radiat. Biol. 58, 733–743.
Vermeulen, N.P.E., Besems, J.G.M., de Straat, R., 1992. Molecular aspects of paracetamol-induced hepatotoxicity) and its mechanism-based prevention. Drug Metab. Res 24, 367–372.
Zhang, Y.J., 2001. Study on extraction and separation of pumpkin polysaccharide and its glucatonic effect. Food Sci. Technol. (Zur.) 5, 15–18.
Zhang, Y.J., Yao, H.Y., 2002. Revealing the effective ingredient in pumpkin for reducing blood sugar. J. Chinese Cereals and Oils Assoc. 17 (4), 59–62.