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Evaluation of The Renal Oxidative Stress Indices of Male Wistar Rats Exposed to Tetrorchidium Didymostemon Extracts

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Abstract

Introduction:*This project is to evaluate the renal oxidative stress indices of male Wistar rats exposed to extracts of Tetrorchidium didymostemoom. Tetrorchidium didymostemon has long been used for the treatment of several ailments and diseases.*

Objectives: *The aim of this study was to evaluate the biochemical changes associated with the administration of T. didymostemon methanol leave extracts for 14 days.*

Methods: *Four groups of seven rats each was used for this study and they were given carboxyl methyl cellulose (control), 100, 300 and 600 mg/kg b. wt. of plant extract for 14 days. During these 14 days of treatment, the rats were observed daily for toxicity symptoms. Following the 14-day treatment, the rats were sacrificed for biochemical studies. Kidney reduced glutathione (GSH), superoxide dismutase (SOD), malondialdehyde (MDA), and organ body weight ratio were evaluated.*

Results: *There was significant decrease in MDA level and increase in GSH concentration of the group given T. didymostemon leave extract compared to the control. However, the changes in SOD activity and the organ body weight ratio of the group given extracts was not significantly different from control.*

Conclusion: *Thisfindings suggest that the leave extract may have protective potential on the kidney and may be considered relatively safe for therapeutic purposes.*

Keywords: Evaluation, Renal oxidative stress, Male, Wistar rats, Exposed, Tetrorchidium didymostemom, Extracts.

Introduction

Background of The Study

Medicinal plants have been used in virtually all cultures as a source of medicine. Assurance of the safety, quality, and efficacy of medicinal plants and herbal products has now become a key issue in industrialized and in developing countries (Singh, 2015). The widespread use of herbal remedies and healthcare preparations is described in the Vedas and the Bible. Plant is an important source of medicine and plays a key role in world health (Sandberg and Corrigan, 2001). Medicinal Plants have been used for thousands of years to flavor and conserve food, to treat health disorders and to prevent diseases including epidemics (Singh, 2015). Medicinal herbs or plants have been known to be an important potential source of therapeutics or curative aids. The knowledge of their healing properties has been transmitted over the centuries within and among human communities (Singh, 2015). Many countries in the world, that is, two-third of the world's population depends on herbal medicine for primary health care. From records, most of the used drugs contain plant extracts. Some contain active ingredients (bioactive components or substances) obtained from plants. Active compounds produced during secondary metabolism are usually responsible for the biological properties of plant species used throughout the globe for various purposes, including treatment of infectious

diseases (Singh, 2015). In recent years, many researchers have focused on medicinal plants derived from natural products due to their wide range of pharmacological significance (Shukla et al., 2010). Moreover, natural resources of vegetable origin represent an important source of drugs in the process of developing new pharmacologically active compounds (Vieira et al., 2014). The World Health Organization established that, in many developing countries, traditional medicine plays an important role in meeting the primary health care needs of the population, and highlights specific types of this medicine (WHO, 2014).

Tirocinium midmotion is an evergreen shrub or a tree distributed in west and central Africa, sometimes adopting a climbing habit; it can grow up to 25 metres tall, with drooping branches (Burkil, 2004). The tree is harvested from the wild for local use as a medicine and source of materials. Throughout its distribution area the latex from the stem bark is used as eye drops to treat filariasis, and is also applied to abscesses, leprous sores and glandular swellings. Leaf sap is applied to wounds as a haemostatic (Burkil, 2004).

The leaf sap in water or rum, or a stem bark decoction, is commonly taken as a purgative and to treat fever. To treat constipation or enlarged spleen in babies, leaf sap is applied to nipples of nursing mothers or to scarifications. A stem bark infusion is rubbed on to rheumatic and painful limbs, painful kidneys and to treat oedema (Burkil, 2004).

T. didymostemon is widely distributed in Africa and there are known medicinal uses of these herb. Despite the traditional folk use of this herb in the management of several ailments and diseases that has been perpetuated along several generations, there are still limited information on the pharmacological uses and toxic effect of this herb. Hence this study was designed to investigate the effect of T. didymostemon methanol leaves extract on antioxidant status of the kidney of Wistar rats after oral administration.

Medicinal plants from time immemorial have been used in virtually all cultures as a source of medicine (Cragg and Newman, 2001). The World Health Organization estimated that perhaps eighty percent of the inhabitants of the world rely chiefly on traditional medicines. It, therefore, approved the use of herbal products for national policies and drug regulatory measures in order to strengthen research and evaluation of the safety and efficacy of herbal products.

The use of traditional medicine and medicinal plants in most developing countries, as a basis for the maintenance of good health, has been widely observed by (UNESCO, 1996). Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies (UNESCO, 1996). Herbs are supposed to be safe but many unsafe and fatal side effects have been reported (Whitto et al., 2003). Phytotherapeutic products are many times, mistakenly regarded as less toxic because they are 'natural' (Gesler, 1992). Nevertheless, those products contain bioactive principles with potential to cause adverse effects (Bent and Ko, 2004). Thus, all the "natural" products used in therapeutics must be submitted to efficacy and safety test by the same methods used for new synthetics drugs (Talalay and Talalay, 2001). Hence, this study was designed to address the possible toxic effect of T. didymostemon leave extract since it's a medicinal plant with known therapeutic uses.

This study aimed at evaluating the effects of Tetrorchidium didymostemon methanol leaves extract on the antioxidant status of the kidney of Wistar rats after oral administration for 14 days.

The following were the objectives of this study

- Investigate the effects of T. didymostemon methanol leaves extract on oxidation stress indices of the kidney.
- To determine the level of lipid peroxidation in the kidney after administration of T. didymostemon methanol leaves.
- Organ body weight ratio of the kidney, liver, spleen, brain and testis was also investigated.
- The significance of the study is to provide scientific validation on the sub-acute effects of T. didymostemon leaves extract in wistar rats after oral administration for 14 days.

Origin and Geographic Distribution

Origin and geographic distribution T. didymostemon occur from Guinea Bissau east to Uganda and south to Tanzania and Angola. is an evergreen shrub or a tree, sometimes adopting a climbing habit; it can grow up to 25 metres tall, with drooping branches. The tree is harvested from the wild for local use as a medicine and source of materials. It is potentially useful as a pioneer species for restoring woodland (Burkil, 1985).

Medicinal uses of t Didymostemon

Throughout its distribution area the latex from the stem bark is used as eye drops to treat filariasis, and is also applied to abscesses, leprous sores and glandular swellings. Leaf sap is applied to wounds as a haemostatic. The leaf sap in water or rum, or a stem bark decoction, is commonly taken as a purgative and to treat fever. To treat constipation or enlarged spleen in babies, leaf sap is applied to nipples of nursing mothers or to scarifications. A stem bark infusion is rubbed on to rheumatic and painful limbs, painful kidneys and to treat oedema. In Gabon beaten stem bark is taken as a mouth wash to treat toothache.

In Congo and DRCongo the leaves, crushed together with the stem bark of Cola ballayi Cornu ex Hack., are applied to broken limbs as an embrocation to treat swellings. Young leaves are cut and cooked with fish and eaten to treat enlarged spleen. Leaf sap or stem bark latex, sometimes with banana or in palm wine, is taken to treat stomach-ache, gonorrhoea, intestinal worms, coughing fits and food poisoning. Bark scrapings are applied as an enema to treat malaria and backache. A maceration of the stem bark is applied as a wash to treat hernia and urinary infections. The twig or root bark latex mixed with palm oil is applied as a lotion to treat measles. A root decoction is drunk as an emetic. The ash from the stem bark or root bark mixed with palm oil is applied to kill lice. A bark extract is rubbed on the body as a mosquito repellent. Bark latex is applied to snakebites. A stem bark decoction is drunk as an emetic and antidote against

Erythrophleum suaveolens (Guill. & Perr.) Brenan poisoning in ordeal by poison. Cows are given leaf sap with salt to increase milk production (Burkil, 1985).

The wood is used as firewood and for charcoal making. The stems are used as poles for making huts and sometimes also in carpentry. In Gabon the wood is used for plywood production. In West Africa the twigs are used as chew sticks. In Benin, Gabon and Congo the beaten stem bark is used as soap for washing clothes (Burkil, 1985).

Botanical description of t. Didymostemon

Scientific classification

Kingdom	Plantae
Division	Angiosperms
Subdivision	Eudicots
Class	Rosides
Order	Rosales
Family	Euphorbiaceae
Genus	Tetrorchidium
Species	Tetrorchidium didymostemon (Baill.) Pax & K. Hoffm

Description

Dioecious, evergreen, glabrous liana, shrub or small tree up to 12 m tall, sometimes a medium-sized tree up to 25 m tall, with drooping branches; latex usually white, sometimes reddish or colourless; bark smooth or minutely fissured, brown; twigs slightly zigzag, prominently scarred at the nodes. Leaves alternate on flowering shoots, opposite on other branches; stipules small; petiole 0.5–1 cm long, channelled; blade obovate to elliptical-oblanceolate or elliptical, (4–)7–12(–17) cm × (2–)3–6(–8) cm, base cuneate, apex abruptly acuminate, margins entire or sometimes shallowly and remotely toothed, firmly papery, pinnately veined with 5–9 pairs of lateral veins. Inflorescence axillary to leaf-opposed, male inflorescence a densely flowered spike 2.5–8 cm long, female inflorescence a 3–5-flowered false umbel 1–1.5 cm long; peduncle 0.5–1 cm long. Flowers unisexual, 3-merous, petals absent; male flowers sessile, sepals broadly ovate, c. 0.7 mm long, minutely fringed, greenish yellow, stamens short; female flowers with pedicel 2–4 mm long, sepals triangular-ovate, c. 1 mm long, minutely fringed, greenish, disk glands petal-like, triangular-ovate, up to 1 mm long, yellowish green, ovary superior, almost globose, 1.5–2 mm in diameter, 3-celled, smooth, styles 3, forming a cap c. 1 mm in diameter. Fruit a 3-lobed capsule c. 5 mm × 6 mm, smooth, green becoming brownish green, 3-seeded. Seeds compressed ellipsoid, 4–5 mm × 3–4 mm, orange-red, pitted (baill0 pax and K.Hoffm, 1919).

Leaves of t. Didymostemon showing its fruiting branch
Source: Toirambe, (2008)

Materials and Methods

Chemicals and Reagents

Laboratory Apparatus and Reagents

The following apparatus were used for the experiment: Plain and Universal Containers, EDTA containers, syringes, ph meter, test tubes (pyrex, england), test tube racks, spectrophotometer (spectrumlab), cuvette, centrifuge (harris england), beakers, water bath (hh-4), gavage, cotton wool, foil paper, spatula, funnel, weighing balance (s. mettler). Stopwatch, glass rod stirrer, magnetic stirrer, pestle and mortar, measuring cylinder, micropipettes.

The reagents used for this researched were of analytical grade. They include: reduced glutathione (GSH), 2,2-diphenyl-1-picryl-hydrazyl (DPPH), 2,4,6-tripyridylstriazine (TPTZ), Thiobarbituric acid (TBA), 5,5-dithiobis-2-nitrobenzoic acid (DTNB, hydrochloride was bought from Sigma Aldrich (Merck), 0.9% Normal saline, Chloroform (May and Baker, Dagenham, England and Formalin.

Plant Collection and Authentication

The leaves and stem bark of Tetrorchidium didymostemon (Baill.) Pax & K.Hoffm were selected based on local claims by traditional healers in Southern Nigeria of its efficacy when used to treat malaria. T. didymostemon leaves were collected from the wild in Iguerobo village in Uhumwonde Local Government Area of Edo State. Thereafter, the plant was authenticated at the Department of Plant Biology and Biotechnology, University of Benin, Nigeria and voucher specimen of the plant UBHT439 was deposited at the herbarium of same department above.

Plant Extracts and its Preparation

Leaves of T. didymostemon were obtained fresh, washed and air dried in the laboratory. The dried leaves and stem bark were macerated and 500g of powder was soaked in 2.5 liters of methanol in air tight containers for 3 days and it was stirred occasionally. After which they were filtered using Whatman No 1 filter paper into a clean flask, the filtrates were concentrated using a rotary evaporator (RE 300, Bibby Scientific, UK) and final concentrate was obtained using silica gel. The extracts were thereafter stored in a sterile container and kept at 4oC till when needed [1-20].

Experimental Animals

Healthy male wistar rats weighing between 90 - 110 g were obtained from Department of Anatomy, University of Benin, Benin City and were used for the experiments. The rats were housed under standard conditions at temperature 27±2ºC, relative humidity 70% and at 12 hr day/night cycles in the Vivarium of the Department of Biochemistry, Michael and Cecilia Ibru University. They had free access to grower mash and water. The animals were allowed to acclimatize for one week before commencing the experiment. The experiments were conducted in strict compliance with internationally accepted principles fo r laboratory animals' use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review (Ernest et al., 1993).

Experimental Design

For the study under discourse, the rats were divided into 4 groups after the acclimatization period. The rats were randomly selected on the basis of their weight and each group had 7 rats. Treatment was done once daily for 14 days.

- Group 1 served as the control and was given 1ml Carboxyl Methyl Cellulose (0.7% CMC).
- Group 2 was administered 100 mg/kg b. wt. of T. didymostemon leaves extract dissolved in 1ml of 0.7% CMC.
- Group 3 was administered 300 mg/kg b. wt. of T. didymostemon leaves extract dissolved in 1ml of 0.7% CMC.
- Group 4 was administered 600 mg/kg b. wt. of T. didymostemon leaves extract dissolved in 1ml of 0.7% CMC.

Collection of Samples

Animals were fasted overnight and sacrificed by cervical dissection on 'day' 15 of the experiment, samples were then collected.

Preparation of Tissue Homogenates

Kidney tissues were harvested after sacrificing the animals and placed in plain containers which contained 10 ml of phosphate buffered saline and stored in ice (4oC). 1 g of kidney tissue was homogenized in 5 ml of ice-cold physiological saline to obtain homogenate. The resulting homogenates were centrifuged at 4500 rpm for 15 minutes and the supernatant obtained used for subsequent analysis [21-30].

Estimation of Malondialdehyde (MDA)

This assay was carried out using the Buege and Aust (1978) method.

Principle

This method depends on the formation of MDA as an end product of lipid peroxidation which reacts with thiobarbituric acid producing thiobarbituric acid reactive substance (TBEARS), a pink chromatogen which can be measured spectrophotometrically at 532nm.

Procedure

The following reagents were prepared:

- HCL of 0.25: A volume of 2.15 ml of HCL was added to 100 ml of distilled water.
- Stock of TCA-TBA-HCL: 15 g of trichloroacetate (TCA) and 0.375 g of thiobarbituric acid (TBA) was dissolved in the prepared 0.25N HCL. The solution was mildly heated to assist in the dissolution of TBA.
- A 1ml of homogenate was added to 2ml of TCA-TBA-HCL reagent followed by thorough mixing by swirling.
- The resulting solution was heated for 15 minutes in boiling water bath. The flocculent precipitate, after cooling, was removed via centrifugation at 1000 revolution for 10minutes.
- Absorbance of the clear supernatant was taken at 535nm against a reference blank.

The MDA concentration of sample is calculated using the extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$. So that mathematically,

$$MDA = \frac{AbxVx1000}{Axvx1xY}$$

Where Ab = Absorbance of sample test at 535 nm
V= Total volume of the reaction mixture =3 ml
A= Molar estimation co-efficient of product = 1.56 x 105m-1cm-1
l = Light path = 1 cm
v = Volume of sample used=1 ml
Y= Weight of tissue in the volume of sample used (g)

Determination of Superoxide Dismutase (SOD) Activity

The activity of SOD was determined by the method of Misra and Fridovich (1972).

Principle

The ability of SOD to inhibit the autoxidation of epinephrine at pH 10.2 makes this reaction a basis for a simple assay for this dismutase. Superoxide radical causes the oxidation of epinephrine to adrenochrome and the yield of adrenochrome produced per superoxide radical introduced increases with increasing pH and concentration of epinephrine [31-40].

Reagents

- A Carbonate buffer (pH 10.2) of 0.05M: 1.573 g of Na-2CO3.10H2O and 0.588 g of NaHCO3 were dissolved in 200 ml of distilled water. The pH was adjusted to 10.2 and then made up to 250 ml with distilled water.
- Epinephrine 0.3M: A mass of 0.05 g of epinephrine was dissolved in 200 ml of distilled water containing 0.5 ml of concentrated HCl (37%).

Procedure

A sample of 50 µl was added to 2.5 ml of 0.05M carbonate buffer (pH 10.2) and 0.3 ml of epinephrine in a cuvette, mixed by inversion and change in absorbance monitored every 30 sec for 2.5 min at 480 nm. The reference cuvette was the same as for the samples with water replacing the samples.

Calculation

% inhibition = 100 – (100 × Increase in absorbance per min for sample) / Increase in absorbance per min for blank

1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the auto-oxidation of epinephrine.

Enzyme activity = (% Inhibition)/(50 × Y)
Y = mg of protein in the volume of sample used

Estimation of Reduced Glutathione (GSH)

The method of Beutler et al. (1963) was followed in estimating the level of reduced glutathione (GSH).

Principle

The reduced form of glutathione comprises in most instances the bulk of cellular non-protein sulfhydryl groups. This method is therefore based upon the development of a relatively stable (yellow) colour when 5',5'– dithiois – (2-nitrobenzoic acid) (Ellman's reagent) is added to sulfhydryl compounds. The chromophoric product resulting from the reaction of Ellman's reagent with the reduced glutathione, 2 – nitro-5-thiobenzoic acid possesses a molar absorption at 412nm.

Reagents

- GSH stock solution: 40 mg of GSH was dissolved in 0.1 M phosphate buffer, pH 7.4 and made up to 100ml with the same.
- Phosphate buffer (0.1 M, pH 7.4): Dipotassium hydrogen phosphate trihydrate (2.098 g) and potassium dihydrogen phosphate (0.791 g) were dissolved in 120 ml of distilled water, the pH adjusted to 7.4 and the volume made up to 150 ml with distilled water.
- Ellman's Reagent: 60 mg of Ellman's reagent was in dissolved in 0.1 M phosphate buffer, pH 7.4 and made up to 150 ml with the same.
- Trichloroacetic Acid (10% solution): 10 g of trichloroacetic acid was dissolved in 100 ml of distilled water.

Procedure

The sample (0.2 ml) was added to 1.8ml of distilled water and 3ml of the precipitating solution was added. The rate of addition was not critical. The mixture was then allowed to stand for approximately 5 minutes and then filtered. At the end of the fifth minute, 1ml of filtrate was added to 4ml of 0.1M phosphate buffer. Finally, 0.5ml of the Ellman's reagent was added. A blank was prepared with 4ml of the 0.1M phosphate buffer, a 1ml of diluted precipitating solution (3 parts to 2 parts of distilled water) and 0.5ml of the Ellman's reagent. The optical density was measured at 412nm. GSH was proportional to the absorbance at that wavelength and the estimate was obtained from the GSH standard (1 mg/ml).

GSH concentration was proportional to the absorbance at 412 nm. The readings were taken before five minutes. This is because the colour is stable for at least five minutes after the addition of Ellman's reagent. After ten minutes of standing, there is frequently a loss of 1 to 2 % of the colour. However, an additional delay of five to fifteen minutes will result in only a small error. Each sample was prepared in duplicate. A graph of optical density against concentration (10 – 100 µg/ml) was plotted.

Statistical Analysis

The statistical analyses of the results were carried out. The various results obtained from this study were expressed as Mean ± SEM. Oneway analysis of variance (ANOVA) followed by LSD and Duncan test was used to determine significance of the differences between the groups. Statistical significance was declared when P value was less than 0.05. The statistical analysis was performed using the statistical package for social science (SPSS) for windows, version 16.0 (SPSS Inc., Chicago, IL, USA) [41-60].

Results

Mda level of the kidney after administration with t. Didymostemon leaves extract

The MDA level of T. didymostemon leave extract after oral administration is shown in fig. 4.1. The MDA level was dose dependent as it decreased as concentration increased. This decrease in MDA of the groups administered 300 and 600 mg/kg b. wt. was significantly ($p < 0.05$) different from the control.

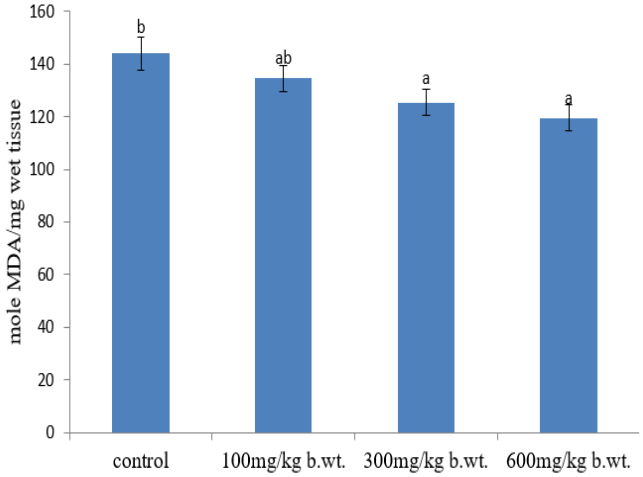

Figure 4.1: MDA levels of liver homogenate after T. didymostemon administration.
Values expressed as mean ± SEM. Columns with different letters on the chart are significantly ($p < 0.05$) different, while those with the same letters are not significantly different.

Superoxide dismutase (SOD) activity of the kidney after administration with T. didymostemon leave extract

The effect of T. didymostemon leave extract after oral administration is shown in fig. 4.2. The SOD activity was dose dependent as it increased nonsignificantly as concentration increased.

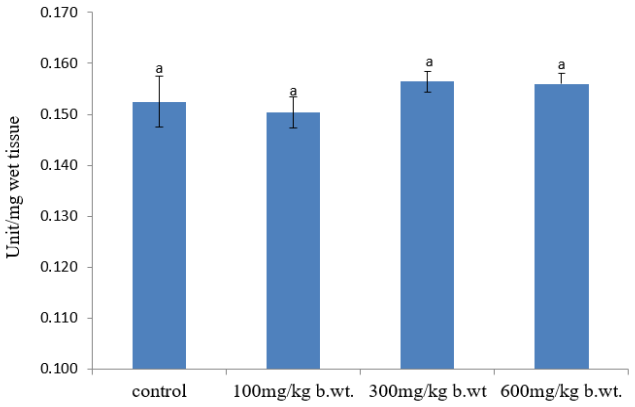

Figure 4.2: SOD activity of kidney homogenate after T. didymostemon administration.

Values expressed as mean ± SEM. Columns with different letters on the chart are significantly ($p < 0.05$) different, while those with the same letters are not significantly different.

Reduced glutathione (GSH) level of the kidney after administration with T. didymostemon leave extract

The effect of T. didymostemon leave extract on GSH level after oral administration is shown in fig. 4.3. The groups given the extract had an increased GSH level relative to the control. This increase was significant in the groups given 300 and 600mg/kg b. wt. leaves extract.

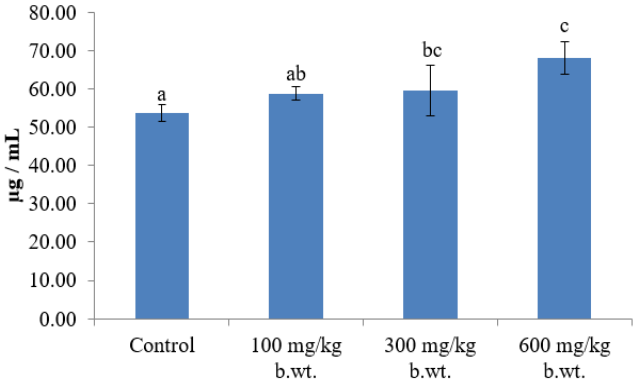

Figure 4.3: GSH level of kidney homogenate after T. didymostemon administration.
Values expressed as mean ± SEM. Columns with different letters on the chart are significantly ($p < 0.05$) different, while those with the same letters are not significantly different.

Organ / Body Weight

There was no statistically significant difference in the organ / body weight ratio of the treated groups relative to the control.

Table 4.1: organ/body weight ratio after 14 days administration of t. Didymostemon extract

Group	Liver	Kidney	Heart	Spleen	Testis
Control	0.038 ± 0.005a	0.007 ± 0.001a	0.004 ± 0.000a	0.004 ± 0.001a	0.014 ± 0.001b
100 mg/kg b.wt	0.029± 0.005a	0.006 ± 0.000a	0.004 ± 0.007a	0.004 ± 0.001a	0.012 ± 0.001ab
300 mg/kg b.wt	0.033 ± 0.002a	0.006 ± 0.000a	0.003 ± 0.000a	0.003 ± 0.001a	0.011 ± 0.001ab
600 mg/kg b.wt	0.032 ± 0.001a	0.006 ± 0.000a	0.007 ± 0.004a	0.003 ± 0.000a	0.009 ± 0.001a

Values expressed as mean ± SEM. Values in the same row with different letters are significantly ($p < 0.05$) different, while those with the same letters are not significantly different.

Discussion and Conclusion

Herbal medicine, also called botanical medicine or phytomedicine are generally cheaper, accessible or readily available and more culturally acceptable to many because of the belief that they cause less side effects than some synthetic drugs (Carlson, 2002; Dey and De, 2015). There are several available data on the toxic effect of medicinal plants. Evaluating liver and kidney function is pertinent in assessing the toxic effect of medicinal plants as they both play important role in the survival of organism (Olorunnisola, 2012).

Lipid peroxidation is a chain reaction initiated by free radical through the oxidation of polyunsaturated fatty acid (Badmus et al., 2014). Malondialdehyde (MDA) is an accumulated product of peroxidation of long chain fatty acids (Ceconi et al., 1992). Lipid peroxidation in biological systems is one of the major mechanisms of cell injury in aerobic organisms subjected to oxidative stress (Poli et al., 1987). The effect of T. didymostemon leave extract after oral administration is shown in fig 4.1. The result shows that oral administration of methanol extracts of T. didymostemon leave resulted in a dose dependent decrease in kidney MDA level. T. didymostemon leave (300 and 600 mg/kg b. wt.) significantly ($p < 0.05$) lowered the formation of MDA in rat kidney homogenate compared to the control. This implies that the leave extract was able to protect the integrity of the membrane of the kidney. The presence of antioxidants may have helped protected the kidney from free radical damage (Mattson and Cheng, 2006) [61-80].

Superoxide dismutase (SOD) is an enzyme that alternately catalyzes the dismutation (or partitioning) of the superoxide ($O2^{-}$) radical into either ordinary molecular oxygen (O2) or hydrogen peroxide (H2O2) (Fridovich, 1997). Thus, SOD is an important antioxidant defense in nearly all living cells exposed to oxygen. SOD can be induced when cells are exposed to agents that stimulate oxidative stress (Zhu et al., 2004). The effect of T. didymostemon leave extract after oral administration is shown in fig. 4.2. There was increase in kidney SOD activity of the group given 300 and 600 mg/kg b. wt. compared to the control. However, this increase was not significant different from control. This observation may be as a result of the extracts inability to cause increase in superoxide anion that could induce the synthesis degeneration of SOD (Badmus et al., 2014).

Glutathione is the cell's natural antioxidant, which destroys free radicals formed in cells. GSH (□-glutamyl-cysteinyl-glycine or glutathione), a water-soluble tripeptide containing a thiol group, is a potent reducing agent (Townsend, 2003). GSH is used as a cofactor by multiple peroxidase enzymes to detoxify peroxides generated from oxygen radical metabolism (Strange, 2000). The effect of T. didymostemon leave extract on GSH level after oral administration is shown in fig. 4.3. The administration of the extract caused a significant ($p < 0.05$) increase in kidney GSH level when compared to the control. The extract was able to stimulate GSH synthesis in the kidney. Thus, the leaf extract may modulate glutathione metabolism via secondary plant metabolites (bioflavonoids and xanthones) (Badmus et al., 2014).

The relative organ weight is fundamental to diagnose whether the organ was exposed to the injury or not (Amresh, 2008). The liver is the major site of foreign compounds metabolism in the body (Dybing, 2002). Alterations in liver weight may suggest treatment-related changes which includes hepatocellular hypertrophy i.e enzyme induction or peroxisome proliferation (Greaves, 2000).

Organ body weight ratio after oral administration for 14 days is shown in table 4.1. There were no significant changes in the organ/body weight ratio of the liver, kidney, heart and spleen of the treated groups when compared to the control. The organ/body weight ratio of the testis was significant decreased at 600 mg/kg b. wt. relative to the control. Also, there was an observed decease in the organ body weight ration of the spleen and liver in the groups given plant extract when compared to the control. The extract may therefore play a role in reducing splenomegaly and hepatomegaly.

Conclusion

This study has provided information on the biochemical effects of T. didymostemon methanol leave extract. The extract showed no sign of toxicity to the kidney and it didn't affect the weight of organs significantly. It is recommended that prolonged study and investigation of other organs should be carried out. In addition, further work is required to clarify how this plant extract increases antioxidant enzyme activity. These findings support the safety and ethno medicinal uses of T. midmotion [81-122].

Author Contributions

P1A: Concept and design of the study, reviewed the literature, manuscript preparation , critical revision of the manuscript , collected data and review of study.

COA: Conceptualized study, literature search, prepared first draft of manuscript and critical revision of the manuscript.

AOO: Conceptualized study, literature search, prepared first draft of manuscript and critical revision of the manuscript.

LCA: Conceptualized study, literature search, prepared first draft of manuscript and critical revision of the manuscript.

NCA: Conceptualized study, literature search, prepared first draft of manuscript and critical revision of the manuscript.

MRTP: Conceptualized study, literature search, prepared first draft of manuscript and critical revision of the manuscript.

Work Attributed To

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2. Biological and Chemical Sciences Research Group, Department of Biological and Chemical Sciences, Michael and Cecilia Ibru University, Agbarha -Otor, Ughelli, Delta State, Nigeria.
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Conflict of Interest

None.

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