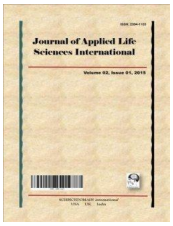

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Phytochemical Assessment and Oxidative Stress Biomarkers Evaluation in Swiss Albino Mice Treated with Methanol Leaf Extract of *Securidaca longipedunculata* (Polygalaceae)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SSA and SNA designed the study. Authors RAA, YAT and HDM managed the collection and preparation of plant extract. Authors PPM and KA managed the laboratory work. Authors TFMA and AUA managed the literature searches while authors NSE and CNC managed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was designed to evaluate the phytochemical constituents, lipid peroxidation and superoxide dismutase activity of methanol leaf extract of *Securidaca longepedunculata* (polygalaceae) (MLESL).

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Study Design: This is an experimental study. A total of twenty four (24) mice were used for this study. Twenty four mice were divided into four (4) groups consisting of six animals each (n= 6) and treated as follows: group one was administered normal saline (10 ml/kg) and served as control, groups two, three, and four were treated orally with the extract (MLESL) at doses of 60, 120 and 240 mg/kg respectively.
Methodology: Administration was carried out orally for a period of twenty eight days (28). Sera obtained from the study at the end of the experiment were used to assess for serum malondialdehyde (MDA) and superoxide dismutase (SOD) concentrations. The extract was screened for the presence of alkaloids, glycosides, saponins, flavonoids, cardiac glycosides, tannins, anthraquinone derivatives and carbohydrates.
Results: The phytochemical screening revealed the presence of carbohydrates, glycosides, cardiac glycosides, triterpene, steroids, saponins, flavonoids, tannins and alkaloids. There was a significant increase ($P< 0.05$) in serum MDA in the group treated with 240 mg/kg (1.65±0.10) when compared to the control. Although serum superoxide dismutase (SOD) was decreased in all the treated groups compared to the control, it was however not significant.
Conclusion: Methanol leaf extract of *Securidaca longepedunculata* (polygalaceae) in this study increases lipid peroxidation with increase in dosage.

Keywords: Securidaca longepedunculata (polygalaceae); tannins; saponins; superoxide dismutase; malondialdehyde and oxidative stress.

1. INTRODUCTION

Securidaca longepedunculata is a semi-deciduous shrub or small tree that grows between 6 to 12 metres high. It has a pale grey, smooth bark with leaves that grow in clusters. The branches are small and have very fine hairs when young but loss them as they mature. The leaves are variable in size and shape, alternate, often in cluster [1]. The plant's geographical spread over Africa includes South Africa, Mozambique, Kenya, Tanzania, Zimbabwe, Nigeria, Sudan and Ethiopia. It is highly regarded medicinal and magical tree [2]. Reactive oxygen species (ROS) are chemically reactive molecules produced by living organisms as a result of normal cellular metabolism. At low to moderate concentrations, they function in physiological cell processes. However, when in high concentrations, ROS produce adverse modifications to cellular components, such as lipids, proteins, and DNA [3]. Oxidative stress occurs when this balance is disrupted by excessive production of reactive oxygen species, including superoxide, hydrogen peroxide and hydroxyl radicals, and/or by inadequate antioxidative defences. Antioxidants are the molecules that have the ability to counterbalance the effect of oxidants before they attack the cells [3]. Therefore this study was aimed at evaluating the effect of methanol leaf extract of *Securidaca longipedunculata* on serum MDA, SOD and assessment of phytochemical constituents.

2. MATERIALS AND METHODS

2.1 Animals

A total of twenty four (24) apparently healthy Swiss Albino mice of both sexes were sourced from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria for the study. The animals were housed under standard laboratory conditions of temperatures (25±2°C), and light approximately 12/12 hours light/dark cycle and fed on a standard diet and given water *ad libitum*. The animals were observed for any sign of discomfort or anxiety for one week prior to the study to acclimatise.

2.2 Experimental Site

This study was carried out in the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. It is located between latitudes 11° and 3´ N, and between 7° and 42´ E, at an altitude of 670 m above the sea level and 664 km away from the sea, in the Northern Guinea Savanna zone [4].

2.3 Chemicals and Drugs

All drugs and chemicals used are of analytical grade and were purchased from a registered company. Some of which include; Extraction solvent; absolute alcohol (Sigma-Aldrich, USA), methanol (Sigma-Aldrich, USA), glacial acetic

acid solution (Seaarle Essex, England), Distilled water, Concentrated hydrochloric acid (BDH Ltd Poole, England), formaldehyde (BDH Ltd. Poole, England), piroxicam (Neimeth, Nigeria), normal saline (0.9% normal saline Isotonic Solution), lead acetate (FERZMERK India chemicals, India), morphine, potassium hydroxide pellets (NAAFCO London, England), fehling solution A (Prolabo, England), fehling solution B (Prolabo, England chloroform (Sigma Chemical Co. USA), picric acid reagent (BDH Ltd Poole, England), acetic anhydride (BDH Ltd Poole, England) and acetic acid anhydride (BDH Ltd Poole, England).

2.4 Plant Identification

The leaves of *Securidaca longepedunculata* were collected from Maraban Yakawada, Giwa LGA of Kaduna State. It was identified and authenticated in the Herbarium unit of the Department of Biological Science, Ahmadu Bello University, Zaria and voucher number identified as 900149 was given by comparing with deposited specimen available as reference.

2.5 Preparation of Plant Extract

The leaves were pruned while fresh, air dried at room temperature for two weeks, pulverised in a mortar, and sieved to fine powder. Five hundred grams of the powdered material was extracted with methanol for 72 hours. The extract was concentrated using rotary evaporator. This gave a yield of 126 g (25.2% w/w). The product which was coffee brown in colour was regarded as the methanol leaf extract of *Securidaca longepedunculata* (MLESL) and was stored in desiccator for use.

3. PHYTOCHEMICAL SCREENING

Phytochemical screening of the methanol leaf extract of *Securidaca longepedunculata* (MLESL) was performed according to the methods of [5] and [6]. The extract was screened for the presence of alkaloids, glycosides, saponins, flavonoids, cardiac glycosides, tannins, anthraquinone derivatives and carbohydrates.

3.1 Test for Carbohydrate

3.1.1 Molish test

4 millilitres of Molish reagent was added to 5 ml of the methanol leaf extract in a test tube and 3 ml of concentrated sulphuric acid was allowed to run down the side of the test tube to form a lower purple to violet colour at the interface. This was indicative of the presence of carbohydrate [7].

3.1.2 Fehlings test

To 2 ml of the extract, 5 ml of a mixture of Fehling solution A and B in the ratio of 1:1 was added and the mixture was boiled for few minutes. A brick red precipitate indicated the presence of free reducing sugar [6].

3.1.3 Test for glycosides

Two (2) millilitres of concentrated sulphuric acid was added to 0.5 g of the extract and boiled for 15 min. This was then cooled and neutralised with 20% potassium hydroxide and was divided into two portions. Another part of the extract was dissolved in distilled water to serve as a control without acid hydrolysis.

3.1.4 Fehlings solution test

Fehling's solution A and B was added to one of the portions above and boiled for few minutes between 5 – 10 mins. The appearance of brick red precipitate was indicative of the presence of glycone as a result of hydrolysis of glycoside [7].

3.1.5 Ferric chloride test

Three drops of ferric chloride was added to the second portion. Green to black precipitate indicated the presence of phenolic aglycones as a result of hydrolysis of glycosides [7].

3.2 Test for Anthraquinone Derivatives

3.2.1 Test for free anthraquinone derivatives (Borntrager's test)

Unto 10 ml of benzene, 0.5 g extract was weighed, shaken and filtered. 5 ml of 10% ammonia solution was added to the filtrate and stirred. The absence of a pink red or violet colour was indicative of the absence of free anthraqinones [8].

3.2.2 Test for combined anthracene (Modified borntrager's test)

Unto 5 ml of 10% hydrochloric acid, 0.5 g of extract was weighed and boiled for 3 minutes. This caused the hydrolysis of the glycosides to yield aglycones, which were soluble in hot water only. The solution was filtered hot; the filtrate was cooled and extracted with 5 ml of benzene. The

benzene layer was filtered off and shaken with half its volume of 10% ammonia solution. The absence of rose pink or a cherry colour appearance was indicative of the absence of combined anthracene [6].

3.3 Test for Cardiac Glycosides

3.3.1 Kella-killiani test

Unto glacial acetic acid containing traces of ferric chloride, 0.5 g of crude extract was dissolved. The tube was held at an angle of 45 degree and 1 ml of concentrated sulphuric acid was added down the side. A purple ring colour at the interface was indicative of the presence of cardiac glycoside [6].

3.3.2 Salkwoski's test

To 2 ml of chloroform, 0.5 g of extract was dissolved and few drops of concentrated sulphuric acid were added to form a lower layer. A reddish brown colour at the interface was indicative of the presence of steroidal ring [6].

3.4 Test for Saponins (Frothling Test)

With 50 ml of 95% ethanol, 0.5 g of extract was mixed and boiled. The mixture was filtered and 2.5 ml of the filtrate was added to 10 ml of distilled water in a test tube. The tube was stoppered and shaken vigorously for about thirty seconds. It was then allowed to stand for half an hour. Persistent honeycomb froth was indicative of the presence of saponins [8].

3.5 Test for Steroids and Triterpenes (Lieberman-burchards Test)

Five millilitres of acetic anhydride was added to 5 ml of the extract. 1 ml of concentrated sulphuric acid was added down the side of the test tube. The colour change was observed immediately and later. Red colour was indicative of the presence of triterpenes while blue-green colour was indicative of the presence of steroids [6].

3.6 Test for Flavonoids

3.6.1 Shinoda test

Extract weight of 0.5 g was macerated in 50 ml of 1% hydrochloric acid and filtered. 4 ml of the filtrate was shaken with 5 ml of amyl alcohol. An orange colour indicated the presence of flavonoid aglycones.

3.6.2 Sodium hydroxide test

One millilitre of aqueous sodium hydroxide (NaOH) was added to 5 ml of extract. Formation of a yellow colour showed the presence of flavonoid [6].

3.7 Test for Tannins

Three grams of the crude methanol extract was boiled in 50 ml of distilled water for 3 minutes on a hot plate. The mixture was filtered and the resultant filtrate used for the following tests.

3.7.1 Lead sub-acetate test

One millilitre of the previously diluted (1:4) filtrate was mixed with lead sub-acetate solution and the formation of light brown coloured precipitate indicated the presence of tannins.

3.7.2 Ferric chloride test

Five millilitre of the filtrate was further diluted with distilled water in a ratio of 1:4 and a few drops of ferric chloride solution were added. An immediate green colour indicated the presence of tannins [7].

3.8 Test of Alkaloids

Three grams of the crude methanol extract was dissolved in 50 ml of methanol. The solution was evaporated to dryness and 0.5 g of the residue was mixed with 10 ml of 1% aqueous hydrochloric acid on a water bath. 1 ml portion was treated with a few drops of the following reagents.

3.8.1 Dragendoff's test

One millilitre of Dragendoff's reagent (potassium bismuth iodine solution) was added to the extract. Deep brown precipitate indicated the presence of alkaloids.

3.8.2 Wagner's test

One millilitre of Wagner's reagent (solution of iodine in potassium iodine) was added to the extract. Whitish precipitate was indicative of the presence alkaloids.

3.8.3 Meyers test

Three millilitre of the above reagent was added to sample of extract in a test tube. The formation of cream precipitate indicated the presence of alkaloids.

4. EXPERIMENTAL PROTOCOL

A total of twenty four (24) mice were used for this study. Twenty four (24) mice were divided into four groups of six animals each (n=6) and treated orally as follows: Group 1 served as the control and was administered normal saline (10 ml/kg), Groups 2, 3 and 4 were treated with methanolic leaf extract of *securidaca longepedunculata* (MLESL) at 60, 120 and 240 mg/kg respectively.

4.1 Assay of Serum Malondialdehyde (MDA)

The level of thiobarbituric-acid reactive substance, malondialdehyde (MDA), as an index of lipid peroxidation was evaluated. Quantitative measurement of lipid peroxidation of MDA was determined using NWLSSTM MDA assay kit (Northwest Life Sciences Specialities, Product NWK-MDA01, Vancouver WA, and Specificity: Malondialdehyde, sensitivity: 0.08 µM). The principle is based on the reaction of MDA with thiobarbituric acid (TBA), forming an MDA-TBA adducts that absorbed strongly at 532 nm.

4.2 Assay of Serum Superoxide Dismutase (SOD)

Activity of SOD in the rat serum was determined using NWLSS SOD assay kit (Product NWK-SOD02, Specificity: Cu/Zn, Mn and Fe Superoxide Dismutase, Sensitivity: 5 U/mL) according to manufacturer's manual.

4.3 Statistical Analysis

Results were expressed as Mean ± Standard Error of Mean. Data were analysed using one way analysis of variance (ANOVA) and repeated measure ANOVA test and were followed Tukey's post hoc test. Results were considered significant at $P \leq 0.05$. Statistical Package for Social Sciences (SPSS) version 20 was used.

5. RESULTS

The result of the study below shows the phytochemical constituents of methanol leaf extract of *Securidaca longepedunculata* and its effects on lipid peroxidation and serum superoxide dismutase concentration.

Table 1. Phytochemical screening of methanol leaf extract of *Securidaca longepedunculata*

Phytochemical constituents	Inference
Carbohydrates	+
Glycosides	+
Anthraquinone	-
Cardiac glycosides	+
Tritepenes	+
Steroids	+
Saponins	+
Flavonoids	+
Tannins	+
Alkaloids	+

Key: + positive, - negative

Table 2. Effect of methanol leaf extract of *Securidaca longepedunculata* on serum MDA concentration in Swiss albino mice

Treatment groups	Serum MDA concentration (µ/ml)
Normal saline (10 ml/kg)	1.22±0.08
Extract (60 mg/kg)	1.40±0.12
Extract (120 mg/kg)	1.50±0.04
Extract (240 mg/kg)	1.65±0.10*

Table 3. Phytochemical screening of methanol leaf extract of *Securidaca longepedunculata* on serum SOD concentration in Swiss albino mice

Treatment groups	Serum SOD concentration (IU/L)
Normal saline (10 ml/kg)	2.42±0.13
Extract (60 mg/kg)	2.05±0.08
Extract (120 mg/kg)	2.10±0.11
Extract (240 mg/kg)	2.13±0.14

6. DISCUSSION

The preliminary phytochemical screening of methanol leaf extract of *Securidaca longepedunculata* shown in Table 1 indicates the presence of carbohydrate, glycoside, triterpene, steroid, saponin, flavonoids and alkaloid. These phytochemicals were also reported in the findings of Ekpendu et al. [8] and Onyeche and

Kolawole [9]. In addition to anthraquinones, Ndamitso et al. [10] also reported the above phytochemical constituents in the methanol root extract of *Securidaca longepedunculata*. These phytochemical constituents are most likely responsible for the observed antioxidant activities of the extract. A growing body of evidence from epidemiological and laboratory studies have demonstrated that some edible plants as a whole or their identified ingredients have antioxidant properties [11,12]. Antioxidant property of plant products are mainly mediated by their content of phenolic compounds the examples of which include flavonoids, phenolic acids, tannins and phenolic diterpenes [13,14,15]. The result from this study as shown in Table 2 shows a statistically significant rise in the levels of malondialdehyde in the group of animals administered 240 mg/ dose of the extract. Serum malondialdehyde level is associated with increased peroxidation of the lipid bi-layer of cell membranes. This result could have been from increased reactive oxygen species production from metabolism of the extract, resulting in the formation of metabolites that are pro-oxidant in nature. The depletion of serum SOD observed from this study in Table 3 above is due to the consistent utilisation of this antioxidant enzyme in mopping up of the reactive oxygen species generated from the extract activity. However, although Table 3 shows a decrease in the level of SOD in the treated groups compared to the control, there seems to be a dose dependent increase in the groups treated with 120 and 240 mg/kg of the extract when compared to 60 mg/kg treated. This increase could have been as a result of a compensatory mechanism which allows increased endogenous release of SOD with increasing MDA concentration in other to help alleviate the reactive oxygen species consequently generated. This also suggests the activity of the polyphenolic compounds as shown in the phytochemical screening of the extract. Oyewale et al. [16] Found a statistically significant decrease in the activities of superoxide dismutase, catalase and glutathione peroxidase and a statistically significant increase in the levels of serum *Securidaca longepedunculata* treatment.

7. CONCLUSION

Methanol leaf extract of *Securidaca longepedunculata* caused a dose dependent lipid peroxidation with a corresponding increase in serum superoxide dismutase in Swiss albino mice.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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