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Toxicological Evaluation of Methanol Extract of *Securidaca longepedunculata*

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The inadequate information on the phytochemistry, toxicity and pharmacological profiles of many medicinal plants has been a major challenge. The present study was undertaken to ascertain the level of toxicity of *Securidaca longepedunculata* in order to have an informed knowledge of this medicinal plant thereby avoiding the deleterious effects often associated with some of these herbal remedies. Thirty two rats divided into four groups of eight rats each were used for the toxicity study. Two rats were picked for the assays from each group in weeks 1, 2, 3 and 4. Weeks 1 and 2 served as acute phase while weeks 3 and 4 served as the chronic phase. The phytochemical analysis revealed the presence of tannins, flavonoids, alkaloids, reducing sugar, glycosides, saponin, steroid, oil and protein. The extract solution showed a maximum absorption at wavelength ($lambda_{max}$) of 285nm-thus indicating that subsequent investigations using the extract would be better at UV region in the absorption spectra since its active constituents are likely going to absorb at this region. The mean lethal dose (LD_{50}) investigation showed no death. Different doses- 100, 200 and 500 mg/kgbw administered to albino rats revealed that the aspartate aminotransferase (AST) showed a significant ($p< 0.05$) decrease in week one and a general increase in other weeks. The alanine aminotransferase (ALT) showed a significant ($p< 0.05$) decrease in weeks one, three and four while week two showed a non-significant ($p> 0.05$) increase. The serum alkaline phosphatase (ALP) showed mainly significant ($p< 0.05$) decrease at weeks one to three, but week four showed a significant ($p< 0.05$) increase. Both conjugated and unconjugated bilirubin of groups two to four of weeks two to four showed significant ($p< 0.05$) increase compared to that of the control. The serum sodium level showed a significant ($p< 0.05$) increase in groups two and three of week one. Weeks two and three showed a non-significant ($p> 0.05$) increase compared to that of the control group. Serum potassium and chloride ion concentrations showed a significant ($p< 0.05$) decrease in weeks one to three and significant ($p< 0.05$) increase in week four. The serum urea showed predominantly significant ($p< 0.05$) decrease compared to that of the control group. The serum creatinine showed a significant ($p< 0.05$) increase at the acute phase (weeks one and two), and a level of significant ($p< 0.05$) decrease at the chronic phase. Catalase activity significantly ($p< 0.05$) increased at the first week, while in weeks two and three the catalase activity decreased ($p< 0.05$) significantly. These results seemed to suggest rich phytochemical constituents

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and a relatively safe level at acute phase (within 14 days) and a visible damage (moderate toxicity) at chronic stage.

Key words: *Securidaca longepedunculata*, toxicity, phytochemistry, rats, acute, chronic

Introduction

Phytomedicinal research of indigenous plant parts is presently gaining more grounds than ever as the majority of people are now patronizing herbal medicinal treatment which is considered to be more easily accessible and cheaper than orthodox medical treatment.[1-2] The usefulness of medicinal plants is directly linked to the wide range of chemical compounds synthesized in various biochemical pathways; which are classified as secondary metabolites.[3] The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body.[4-5] The outstanding disadvantages of herbal medicinal treatment is that the toxicity of the concoctions taken by patients are not determined nor the active principles standardized. This could be as a result of little or no information available on the toxicity of the decoctions usually used in the folk medicine. In addition, such plant could contain some toxic materials that could militate against the use of the extract of such plant parts as beneficial drug. The measurement of enzyme activities in the body fluid aids the diagnosis of assault on organs and tissues and also assists the determination of the toxicity of chemical compounds or drugs.[2] The levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum can help diagnose the injury of body tissues or organs especially the heart and the liver.[6] AST and ALT are enzymes found mainly in the liver, but also found in red blood cells, heart cells, muscle tissue and other organs, such as pancreas and kidney. The normal concentrations in the blood are from 5 to 40 U/L (unit/litre) for AST and from 5 to 35 U/L for ALT. When body tissues or an organ such as the liver or heart is diseased or damaged, additional AST and ALT are released into the blood stream, causing the levels of the enzyme to rise. Therefore, the amount of AST and ALT in the blood is directly related to the extent of the tissue damage.[7] Alkaline Phosphatase (ALP) catalyzes the hydrolysis of phosphate esters in an alkaline environment, resulting in the formation of an organic radical and inorganic phosphate. In mammals, this enzyme is found mainly in the liver and bones. Marked increase in serum ALP levels, a disease known as hyperalkalinephosphatasemia, has been associated with malignant biliary obstruction, primary biliary cirrhosis, primary sclerosing cholangitis, hepatic lymphoma and sarcoidosis.[8]

Materials and Methods

Determination of Total Phenolic Contents

The total phenolics were determined using the method of Velioglu *et al.*[9] With slight modifications. 100µl of the extract (1mg/ml) dissolved in ethanol, was mixed with 750µl of folin-Ciocalteau's Reagent (previously diluted 10-fold with distilled water) and allowed to stand for 5 minutes; 750µl of Na_2CO3 (60 g/l) solution was then added to the mixture. After 90mins the absorbance was read at 725nm. Results were expressed as Gallic acid equivalents using the formula:

$$\frac{A_o \times m}{\overline{A \times m_o}}$$

Flavonoids

One gram (1g) of the extract was taken and macerated with 20ml of ethyl acetate for 10minutes. Five minutes centrifugation followed. Five milliliters (5ml) of the supernatant were each transferred into three test tubes into which 5ml of 1M ammonium hydroxide and shaken vigorously for 2 to 5 minutes. The upper layers were discarded, and the absorbance of the lower layer taken at 470nm. The blank was 1N ammonia solution. Calculation of flavoid was done using standard curve. The standard curve was prepared in the same way the test sample was done. The absorbance of the standard was plotted against concentration of the standard flavonoid. The slope was taken and used to calculate in the quantifying of flavonoid in the test sample using the following:

$$\frac{Abs\ of\ Sample}{Slope\ (Standard)}\ x\ dilution\ factor$$

Alkaloids

One gram (1g) of the extract was macerated with 10 ml of ethanol and 10 ml of 20% sulphuric acid for 10 minutes and centrifuged for 5minutes and then 0.5ml of the supernatant each were transferred into three test tubes. To the tubes were added 2.5 ml of 60% sulphuric acid and 2.5 ml of 0.5% formaldehyde in 60% sulphuric acid, mixed thoroughly and allowed to stand for 3hours. Absorbance at 565nm against the blank was read. The concentration of the alkaloid was extrapolated from the standard curve.

Tannin

One gram (1g) of the extract was macerated with 20ml of methanol for 10 minutes, followed by five minutes centrifugation. The supernatant (2ml) was poured into three test tubes. Methanol, (3ml) were each poured into the test tubes followed by 0.3ml 0.1M ferric chloride in 0.1M HCl and then mixed thoroughly. To the mixture, was added 0.3ml of 0.0008M potassium ferricyanate, and mixed. The absorbances of the solutions in each of the tubes were read against the blank at 750nm after 5 minutes, but not exceeding 30 minutes. The concentration of tannin was extrapolated from the standard curve (prepared as described earlier).

Alanine aminotransferase (ALT)

ALT was determined using the method of Reitman and Frankel[10] as outlined in Teco Kit.

Aspartate AminoTransferase (AST)

The AST conc. was determined using the method of Reitman and Frankel[10] as outlined in Teco Kit.

Alkaline Phosphatase (ALP)

The ALP was determined using Randox kit as recommended by Deutsche Gesellschaft fur Klinische Chemmie (GSCC) that is German Association of Clinical Chemistry.

Bilirubin

The concentration of conjugated and unconjugated bilirubin was determined using the method of Jendrassik and Grof (1938) as outlined in Randox Kit.

Haemoglobin Estimation (Hb)

The Hb was determined using the method of Miale (1972).

To a 4ml Drabkins solution in a test tube was added 20µl of well mixed anticoagulated whole blood. The tube was mixed by inversion and incubated at 25 ^{0}C for 5 minutes. Absorbance of the

solution was read at 540nm against a reagent blank. The concentration of haemoglobin was calculated by multiplying the absorbance with a factor of 36.8

Creatinine

The serum creatinine was determined using the method of Bartels and Bohmer (1972) as outlined in Randox kit.

Principle

Creatinine in an alkaline solution reacted with picric acid to form a coloured complex. The amount of the coloured complex formed was directly proportional to the creatinine concentration.

Sodium Ion

Sodium ion was determined using the method of Trinder (1951) and Maruna (1958) as outlined in Teco Kit.

Chloride Ion Concentration

The concentration of Chloride ion was determined using the method of Skeggs and Hochstrasser (1964) as outlined in Teco kit.

Statistical Analysis

Data were reported as means ±SEM, where appropriate. One-way analysis of variance (ANOVA) and correlation analysis were used to analyze the experimental data and Duncan multiple test range was used to compare the group means obtained after each treatment with control measurements. Differences were considered significant when $P \leq 0.05$.

Results

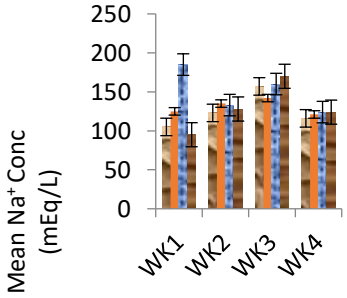

Fig.1 mean serum sodium ion concentration

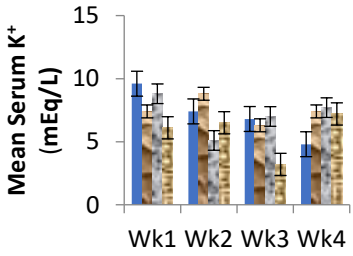

Fig.2 mean serum potassium ion concentration

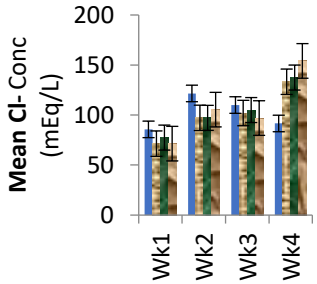

Fig.3 mean serum chloride ion concentration

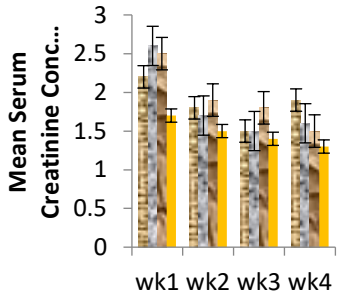

Fig.4 mean serum creatinine concentration

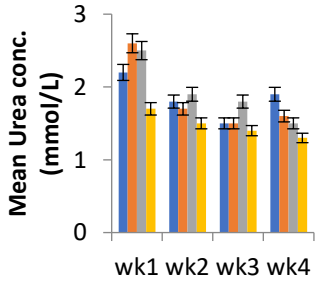

Fig.5 mean urea creatinine concentration

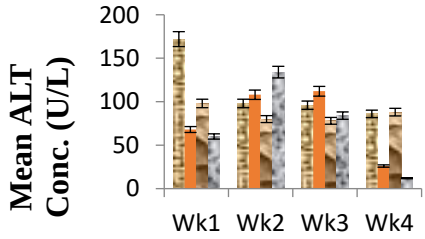

Fig. 6 mean serum ALT concentration

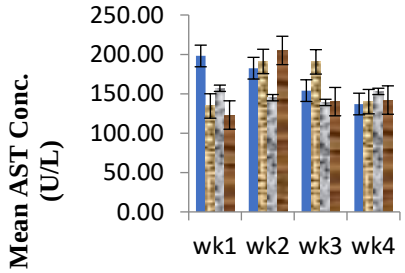

Fig.7 mean serum AST concentration

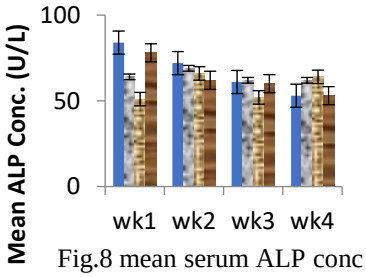

Fig.8 mean serum ALP conc

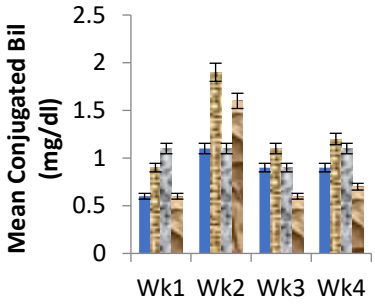

Fig.9 mean serum conjugated bilirubin concentration

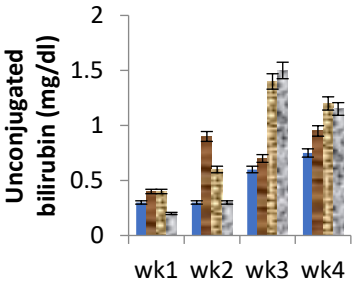

Fig.10 mean serum unconjugated bilirubin concentration

Discussion

The extract seemed to have caused some level of intra-cellular/inter-cellular ions imbalance as seen in the results of sodium, potassium and chloride ions (Figs 1-3). Extra-cellular fluid is characterized by high sodium and calcium ions, while chloride ion is the major anion.[11] Sodium ion is involved in acid-base balance and it is counteracted by

chloride ion.[12] A delicate balance, therefore, is needed to ensure normal cellular function. Potassium is the principal cation of the intra-cellular fluid. It is an important constituent of the extra-cellular fluid due to its influence on muscle activity. Its intra-cellular function is the modulation of acid-base balance and osmotic pressure, including water retention.[11] These imbalances in ions could be as a result of leakages due to membrane distortion. Changes in membrane structure can affect water balance and ion flux and therefore every process within the cell. For, normal cellular function depends on normal membranes.[11]

The increase in creatinine and urea at some point (Figs 4-5) suggested a possible defect with ultra-filtration. The hypo urea, seen in chronic phase (beyond week two), could be due to malfunctioning of the urea cycle, hence implicating the liver to be likely unwholesome.

The ALT, AST and ALP results pointed to a level of hepato-damage at the chronic phase (Figs 6-8) since these observed levels of these enzymes could be as a result of either liver damage or hepatocytes' membrane distortion. Besides revealing what happens in the liver, ALP, an ectoenzyme of the plasma membrane, is a marker enzyme, for the plasma membrane and endoplasmic reticulum. The loss of the enzyme from the tissues into the serum could be attributed to disruption of the ordered lipid-bilayer of the membrane structure, probably by peroxidation of the membrane polyunsaturated fatty acids (PUFA). Hydroxyl radical abstracts hydrogen (H) from PUFA, at the initiation stage of lipid peroxidation. The observed general increase in bilirubin (Figs 9-10), still pointed to these possibilities of either a distortion or total damage to the cell membrane/cells.

Conclusion

Securidaca longepedunculata has been reported by many researchers to possess different benefits, health-wise. This study elucidated some level of toxicity associated with the plant. It is pertinent, therefore, to be cautious while using it. No form of abuse is welcome. A prolonged exposure is seriously discouraged, since the chronic phase showed toxicity. Fractionation, isolation and structural elucidation of the active components of the plant for a better drug is highly desirable.

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