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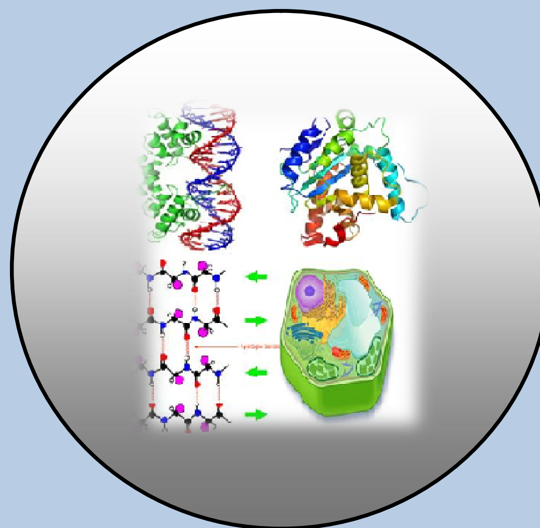

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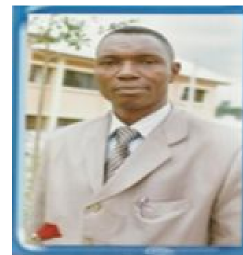

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RESEARCH PAPER

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ABSTRACT

The effects of ethanol extract of root of Sarcocephalus latifolius on biochemical indices commonly associated with kidney functions were studied. Eighteen wistar rats divided into three groups of six animals each and administered the extract for thirty days were used to study the effects of the extract on serum creatinine and urea. Animals in group A were administered with a daily oral dose of 500mg/kg body weight of the extract of Sarcocephalus latifolius. Animals in group B were given a daily dose of 800mg/kg body weight. Animals in group C were kept as control and were not administered with the extract but they were sustained on normal feed (and given tween-80 in water). From the two dose schedules, very small increases were observed for creatinine ($p > 0.05$) and apparently no changes were observed in the mean urea levels. Another set of twelve animals were used to monitor the possible acute effect of Sarcocephalus latifolius on these parameters as well as uric acid. The animals were divided into three groups (A, B, and C) of four animals each. Group A was given 2000mg/kg body weight, group B was given 1500mg/kg body weight of the extract while group C was sustained on normal diet to serve as control. *Contd.......*

All the animals were sacrificed on day four. Creatinine, urea, and uric acid were all decreased significantly ($p < 0.05$) by the root extract of S. latifolius at each single acute dose. However, reduction in serum urea at 1500mg/kg body weight was nonsignificant ($p > 0.05$). Thus, ethanol extract of root of Sarcocephalus latifolius does not possess substances that negatively affect the parameters under study and therefore any toxicity to the kidney is not related to these parameters.
Keywords: Sarcocephalus Latifolius, Biochemical Indices and Kidney Function.

INTRODUCTION

The recognition and application of medicinal plants for healthcare delivery has continued to widen globally with certification by relevant regulatory agencies. The use of herbal medications however may be logically viewed as an alternative means of applying the contemporary medications since several modern medicines were developed from natural sources. Some opinions actually believe that more than 50 percent of all modern clinical drugs are of natural products origin (Suffness et al, 1982) and natural products play an important role in drug development programmes of the pharmaceutical industry (Baker et al, 1995).

Sarcocephalus latifolius is prominent in the list of medicinal plants common within the vegetation in Nigeria, and literature shows it has a wide spread across Africa and many parts of the world. It is a perennial shrub with numerous stems. Its leaves are densely populated, broad and deep green in colour.

In Nigeria, the plant is widely distributed in the South-east and South-south regions of the country. *Sarcocephalus latifolius* is reported to have a wide range of medicinal properties and its medicinal uses vary from one traditional setting to another; common traditional uses include fever, pain, dental caries, septic mouth, malaria, hypertension, dysentery, diarrhea, and diseases of the central nervous system such as epilepsy (Amos et al., 2005; Ngo Bum et al., 2009; Abbah et al., 2010). Anticonvulsant, anxiolytic and sedative properties of *Sarcocephalus latifolius* roots decoction had also been reported (Ngo Bum et al., 2009). It is among those medicinal plants strongly associated with antimalarial potency and widely used as such (Zirihi, et al, 2005; Asase et al, 2009). Almost all the parts of *Sarcocephalus latifolius* have been found medicinally useful with different parts linked to particular pharmacological activities. The capacity of the root extract to reduce blood pressure in both hypertensive and normotensive rats was reported (Nworgu et al, 2008). The root extract also exhibited antimicrobial activities (Omer et al., 1998; Hussain and Deen, 1991; Tona et al., 1999; El-Mahmood, et al, 2008). The root and stem bark have reportedly been applied in the control of preterm contractions in pregnant women (Duke, 2008). The root extract of the plant have been shown to produce a significant decrease in oxytocin-induced contraction of rat uterus in a dose dependent manner (Nworgu, et al, 2010). The ethanol root extract has been shown to significantly depress serum Na^+, an indication of possible antihypertensive capacity (Enemor and Okaka, 2013).

Products of herbal medicine are consumed as dietary supplements, being sold as tablets, capsules, powders, teas, extracts and fresh or dried plants. Owing to the diverse nature of plant components, some may complicate health problems or be the reason for a health challenge. There may also be a problem of interaction with other drugs leading to alteration of activities. These uncertainties culminate in toxicity associated with certain products. This study therefore seeks to monitor the possible effects of root extract of *Sarcocephalus latifolius* on the kidney function by analysis of certain kidney function indices.

MATERIAL AND METHODS

Collection and processing of plant specimen

Sarcocephalus latifolius was identified by P.O. Ugwuozor, a taxonomist in the Department of Botany, Nnamdi Azikiwe University, Awka, in the South East of Nigeria. Roots were harvested fresh and washed in clean water. They were then cut into smaller pieces and dried under shade. The dried roots were ground to very fine texture.

Preparation of extract

About 1kg of the powdered sample was soaked in ethanol for up to 48 hours. The liquid was then thoroughly filtered using whatman filter paper. Concentration of the sample was done by evaporation in a rotary evaporator RE 52–2 (Searchtech Instruments). The concentrate (extract) was stored in the refrigerator and later used for administration to the experimental animals.

Experimental animals

Wistar albino rats used for this work were obtained from a privately operated commercial animal house in the town of Nsukka, Enugu State, Nigeria. The animals were housed in cages kept in an adequate animal house environment and were acclimatized for three days. They were adequately fed with appropriate feed formula.

Sub-acute determination of creatinine and urea

Mature wistar albino rats were used for these studies. A total of eighteen rats were used, divided into three groups of six animals each and labelled groups A, B, and C.

Administration of extract

The extract was solubilized with 1% tween-80 in normal saline. Groups A and B served as test animals whereas animals in group C were used as control. Animals in group A were administered with a daily oral dose of 500 mg/kg body weight of the ethanol extract of *Sarcocephalus latifolius.* Animals in group B were given a daily oral dose of 800 mg/kg body weight. Administration lasted for thirty days. Animals in group C were kept as control and were not administered with the extract but they were given tween-80 in water.

Preparation of serum sample

All the animals were sacrificed according to their groups about 24 hours after the last administration (Enemor et al, 2013). The blood was drained from each animal by cardiac puncture with sterile syringes and needles and emptied into labeled centrifuge tubes for serum separation. They were left standing at room temperature for thirty minutes before centrifuging at 4000 rpm for five minutes in an 80 – 1 electric centrifuge (B – Bram Scientific and Instrument Co., England). The resulting sera were used for analysis of serum creatinine and urea.

Assay of creatinine and urea

The reactions for the determination of creatinine and urea begin with a deproteinization (protein precipitation) step. Test tubes were arranged corresponding to the number of samples. To each sample tube was added 1.5 ml of water, 0.5ml of sulphuric acid, 0.5 ml of sodium tungstate. Then 0.5ml of each sample was transferred to the corresponding test tube. The blank tube contained 2.0 ml, 0.5 ml, and 0.5 ml of water, sulphuric acid, and sodium tungstate, respectively. The sulphuric acid and sodium tungstate served to deproteinize the mixtures. The contents of the tubes were mixed thoroughly and spun for 10 minutes at 4000 rpm using MPW 203E table top centrifuge to precipitate the proteins. The supernatants were finally used to determine the concentration of creatinine and urea in the samples.

Creatinine concentration was finally determined (Fabing et al, 1971) by mixing 150 µl of each supernatant with 0.5 ml of sodium hydroxide and 0.5ml of picric acid. The reaction mixtures were mixed and left standing at room temperature for 20 minutes. The absorbance was read at 520nm using Spectrumlab 22 spectrophotometer. The concentrations were resolved from the expression

$$\text{Creatinine conc. (µmol/L)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

For urea determination (Vijayalakshmi et al, 2000), 50µl of each sample supernatant was mixed with 1ml each of water, mixed acid reagent and mixed colour reagent. The tubes were plugged with cotton wool and were incubated in boiling water bath for 20 minutes resulting to a clear deep pink colour. The contents were allowed to cool and then the absorbance was read at 520nm using spectrumlab 22-spectrophotometer. Urea concentration was finally resolved from the expression

$$\text{Urea concentration (mmol/L)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

Acute determination of creatinine, urea and uric acid

Administration of Extract and serum preparation

Twelve adult rats were used for these studies. The animals were divided into three sets (A, B, C) of four rats each. Animals in group A were each administered with a single oral dose of 2000 mg/kg body weight of the extract; those in group B were each administered with a single oral dose of 1500 mg/kg body weight, while those in group C were not administered with the extract but sustained on normal diet and water.

The sera, prepared as described before, were appropriately stored in the refrigerator, for analysis of creatinine, urea and uric acid.

Determination of creatinine and urea

The assays were done as described under sub-acute studies.

Uric acid assay

The method is as described by Giugliani et al (1985). The protein precipitation as described for creatinine and urea applies. The resulting supernatant fluids were mixed with appropriate portions of sodium carbonate and phosphotungstate and left to stand at room temperature for 15 minutes. The absorbance was then read for each sample at 700 nm. Uric acid concentration was resolved as follows

$$\text{Concentration of Uric acid } (\mu mol/L) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

Statistical analysis

Data were analysed using one-way analysis of variance (ANOVA) at 0.05 level of significance, using the statistical package for social sciences (SPSS), version 17.0 for windows software package.

RESULTS

Figure 1 represents the effects of high concentrations of ethanol extract of the root of *Sarcocephalus latifolius* on creatinine and urea levels at doses of 500 mg/kg and 800 mg/kg body weight, comparative to the control. There were non significant increases ($p > 0.05$) in creatinine concentrations. There were apparently no variations in the concentrations of urea. Figure 1 therefore suggests that there were no significant changes in the concentrations of serum creatinine and urea when both 500 and 800 mg/kg body weight were administered to the rats.

Figures 2 and 3 show that higher doses of the root extract of *Sarcocephalus latifolius* affected the concentrations of both creatinine and urea, apparently in a dose-dependent manner, with the depression more significant on urea than on creatinine. This suggests that increasing concentrations of the extract will have more noticeable effect even at single dose applications.

Figure 4 presents the effect of extract on serum uric acid concentration. The root extract of *Sarcocephalus latifolius* significantly ($p < 0.05$) depressed the serum concentration of uric acid in wistar albino rats, comparative with that of the control.

DISCUSSION

The very wide application of *Sarcocephalus latifolius* (synonym *Nauclea latifolia*) plant preparations in the control and management of a wide range of health challenges makes it imperative to critically study the effects on essential organ parameters. Drugs interact directly with organs such as liver and kidney and toxicities rapidly manifest in them.

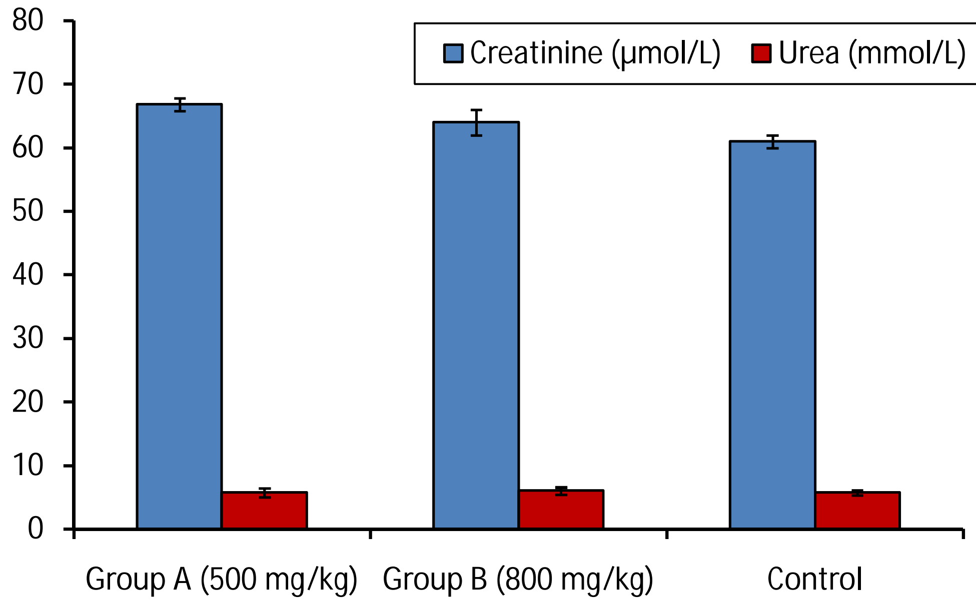

Figure 1. Mean creatinine and urea concentrations from sub-acute studies (Data represented as mean ± SEM).

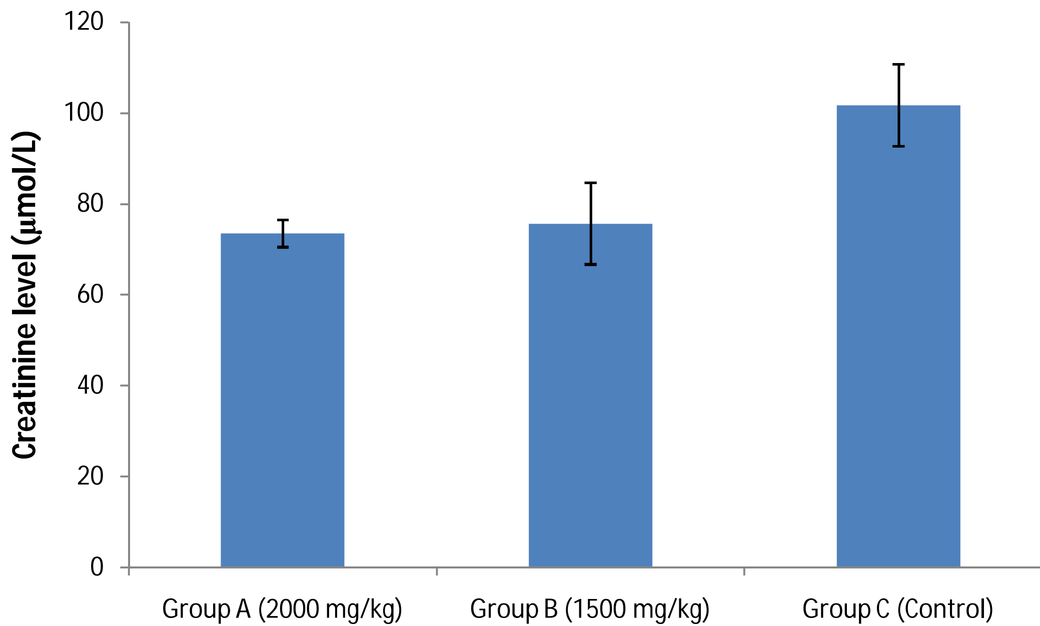

Figure 2. Mean concentrations of creatinine from acute studies (Data represented as mean ± SEM).

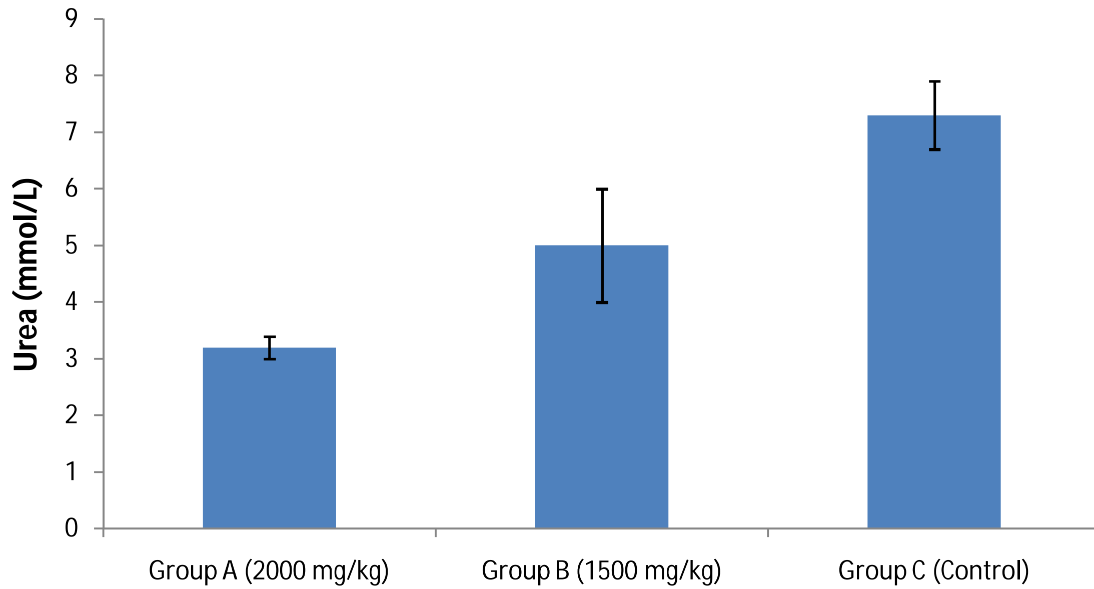

Figure 3. Mean concentration of urea from sub-acute studies (Data represented as mean ± SEM).

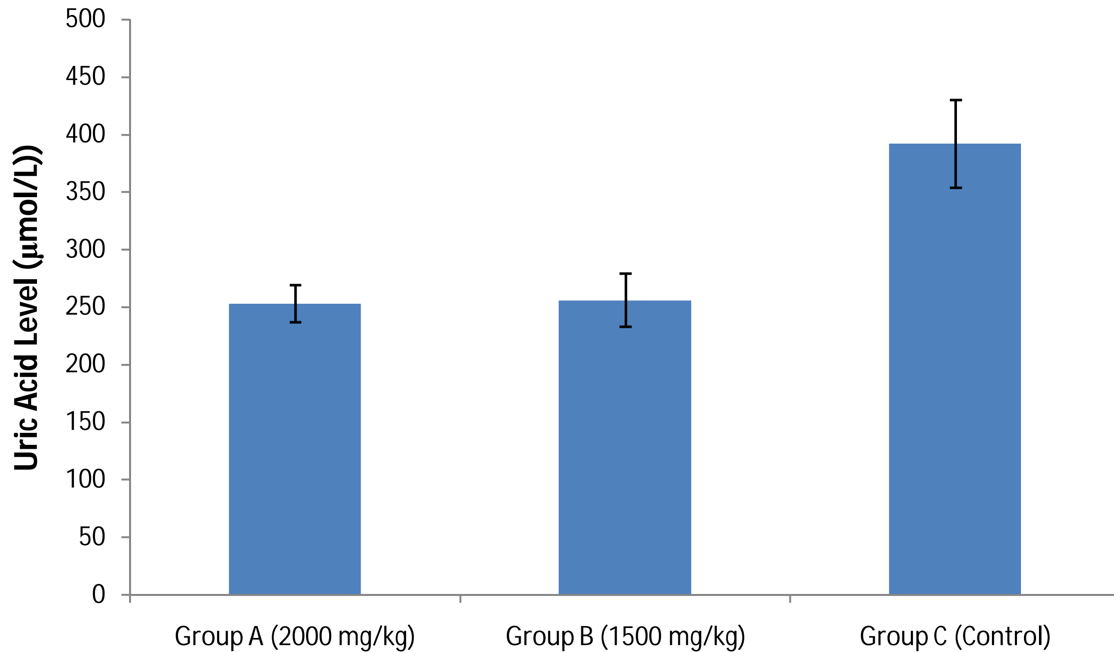

Figure 4. Mean concentration of uric acid from acute studies (Data represented as mean ± SEM).

Drugs, including herbal remedies, have strong ability to cause elevations or depressions in the status of various organ functional indices. Evidently, this work has suggested that the use of *Sarcocephalus latifolius* in healthcare delivery is nontoxic in reference to effects on creatinine, urea, and uric acid levels. Comparatively, from various reports some other plants extract do affect these parameters. Significant increases in creatinine levels have been reported (Lienou et al, 2007; LÃ©onard et al, 2007). There were also reports of significant decrease in serum creatinine with significant increase in serum urea (Ghasi et al, 2011).
Elevations in blood creatinine represent a sensitive indicator of kidney malfunction, because creatinine normally is rapidly removed from the blood and excreted (Champe et al, 2008). Kidney diseases, muscular cells damage, and drug actions can all result in elevations in creatinine levels (Lienou et al, 2007). Glomerular dysfunction was suggested to be a reason for increase in serum creatinine following administration of a leaf extract (Yakubu et al 2009).
The result of this study showed that serum uric acid was significantly decreased by root extract of *Sarcocephalus latifolius* for all the test dose schedules. Different sources have shown that extracts of different plants have varying levels of influence on blood uric acid levels (Balamurugan et al., 2009). Certain drugs contribute largely to the development of hyperuricaemia (Crook, 2006). A potentially serious effect of hyperuricaemia (elevated blood uric acid) is precipitation of urate in the kidneys and renal calculi, causing progressive renal damage. Crystallization in joints, especially those of the feet results in the formation of gout (Crook, 2006). The significant reduction in serum urate levels by ethanol extract of *Sarcocephalus latifolius* root may therefore be a strong indicator of its anti-inflammatory potential. Indeed, the combined qualities of *Sarcocephalus latifolius* to significantly reduce serum calcium (Enemor et al, 2013) and urate and the presence of saponins strongly mark the root of the plant as a sure potential source of very effective anti-inflammatory and analgesic agent.

CONCLUSION

The *Sarcocephalus latifolius* root extract apparently does not promote toxicities related to the parameters under study. The ability to reduce the concentration of such parameters places it as a good source for development of useful modern medications. Nevertheless, use of herbal preparations in crude form should remain strongly discouraged since some toxic potential may still remain concealed. Enemor et al (2013) had reported that *Sarcocephalus latifolius* could affect the balance of electrolytes in the body. Experiments will continue to be conducted on possible effects of this plant species (and other medicinal species) on various organ functional indices. More interest should focus on isolation of specific active principles with a view to developing them into modern medicines.

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