

<http://dx.doi.org/10.48198/NJPAS/20.A15>

## Effects of single oral dose of ethanol extract of root of *Sarcocephalus latifolius* (African peach) on liver function markers in wistar albino rats

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### ABSTRACT

Amongst numerous plants and plant products that find use in alternative healthcare as herbal medicines, *Sarcocephalus latifolius* has been attracting much attention as a plant whose different parts are invaluable for treatment and management of several disease conditions. This study was intended to evaluate the possible effects of a high single dose of *Sarcocephalus latifolius* root extract on liver enzymes, bilirubin, cholesterol, and triglyceride of rat serum. A total of thirty-six adult rats were used for the studies. The animals were divided into three sets of twelve rats each. Each set was subdivided into three groups (A, B, C) with each group consisting of four animals. The first set was used to study serum activities of alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and gamma-glutamyltransferase; the second set was used to evaluate the levels of serum bilirubin while the third set was used to assay serum concentration of cholesterol and triglyceride. For each set, the animals in group A orally received 2000mg/kg body weight single dose of the extract, and those in group B received 1500mg/kg single dose administered orally. Group C animals, fed normal diet and water were used as the control in all cases. All analyses were spectrophotometric. There were nonsignificant reductions ( $p > 0.05$ ), in the activities of ALP, ALT and AST for both dose schedules, whereas statistically nonsignificant increases in activity were recorded for GGT for the two dose schedules. The total bilirubin was found to decrease nonsignificantly ( $p > 0.05$ ), while conjugated bilirubin was found to decrease significantly ( $p < 0.05$ ) for both doses when compared with the control. Both cholesterol and triglyceride were nonsignificantly decreased for both dose schedules. Thus, *Sarcocephalus latifolius* root extract has the potential to conserve the integrity of the liver (non-hepatotoxic), and possesses cholesterol-lowering potentials.

**Keywords:** *Sarcocephalus latifolius*, cholesterol-lowering, non-hepatotoxic, extract

### Introduction

The global popularity in acceptance and use of herbal remedies has continued to increase tremendously over the years and the benefits in healthcare delivery is rapidly becoming more evident (Okoh *et al.*, 2016). In Nigeria, our environments, like those of most other African nations and other regions of the world, are richly adorned with vegetation full of medicinal and/or pharmacological potentials.

Obviously, the answers for many health challenges including some that are health threatening, are largely contained in these natural vegetations, in addition to other natural sources from which drug substances can be discovered and developed. An important scientific step often is to discover the sources of pharmacologically active substances that will act singly or in combination/synergy to improve health. However, while tremendous health

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benefits are attributable to herbs and herbal products, some reports still suggest possible injuries to the liver and perhaps other organs of the body (Navarro *et al.*, 2017).

Several plants have been subjected to screening for possible concentration of pharmacologically active principles and presence or absence of toxicity. They can undergo screening either singly or in combination (Si-Yuan *et al.*, 2013). One of the plants that have widely attracted attention is *Sarcocephalus latifolius*, from the family Rubiaceae, and is often called by various names (Pin cushion tree, African peach, Guinea peach, Sierra Leone peach) in English and several vernacular names. Different parts of the plant have attracted several remedial attributes as antihypertensive (Nworgu *et al.*, 2008), antimalarial (Traore-Keita *et al.*, 2000), antimicrobial (El-Mahmood, *et al.*, 2008; Tona *et al.*, 1999; Omer *et al.*, 1998; Hussain and Deen, 1991). Other medicinal advantages reported include use as antidiabetic (Gidado *et al.*, 2009), control of uterine contraction (Nworgu, *et al.*, 2010), control of stomach upset, measles, cough, cold, and general weakness of the body (Gill, 1992). So many other medicinal potentials have been credited to various formulations of *Sarcocephalus latifolius* parts.

An obvious imperative is to validate the safety of the plant for use in healthcare delivery. This study was designed to determine the possible development of toxicity due to a single (orally administered) dose of the ethanol root extract of *Sarcocephalus latifolius* in apparently healthy (non-induced) wistar albino rats by monitoring the serum status of liver enzymes, bilirubin, cholesterol and triglyceride, which often are common indices for estimating the margin of safety in the use of herbal remedies. Studies are a follow up on our earlier findings (Enemor *et al.*, 2013, 2014).

## Materials and Methods

### *Harvesting and processing of plant materials*

Identification of *Sarcocephalus latifolius* was done by a taxonomist in the Department of Botany, Nnamdi Azikiwe University, Awka, Nigeria. The roots were harvested fresh and dried under shade within the laboratory apartment of the Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, Nigeria. After drying under shade, until constant weight, the roots were pulverized to a very fine texture and used immediately.

### *Preparation of Extract*

About 1kg weight of the powdered sample was soaked in ethanol for about 48 hours after which the soaked sample was filtered by firstly squeezing through folds of muslin cloth and then carefully passing through whatman filter paper. Rotary evaporator, RE 522 (from Searchtech Instruments) was used to concentrate the extract. The resulting concentrate (*Sarcocephalus latifolius* root extract) was reconstituted in 1% tween-80 in normal saline and used for administration to the animals.

### *Experimental animals*

A total of thirty-six adult rats were used for these studies. The animals were divided into three sets of twelve rats each. Each set was subdivided into three groups labeled A, B, and C with each group consisting of four animals. The animals were kept in separately labeled cages in a conducive animal house and were well sustained on appropriate feed formula. They were acclimatized for four days prior to administration of the prepared root extract.

### *Administration of Extract*

Animals in group A were each administered with a single dose of 2000 mg/kg of the extract; those in group B were each administered with a single dose of 1500 mg/kg (doses below determined LD<sub>50</sub>, unpublished), while those in group C (control) were

not administered with the extract but sustained on normal diet and water.

### **Preparation of serum sample**

The animals were kept and observed until day four. They were sacrificed by draining the blood through cardiac puncture using syringes and needles. The blood from each animal was emptied into labelled centrifuge tubes and allowed to stand for about thirty minutes to allow proper separation of the serum. They were then centrifuged at 4000 rpm for ten minutes using 80 – 1 electric centrifuge (B – Bram Scientific and Instrument Co., England). Serum from each tube was mopped up using Pasteur pipette and emptied into labelled sample tubes. The sera were appropriately stored in the refrigerator, for analysis of selected liver and lipid parameters.

### **Biochemical analyses**

Alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed by means of reagent kits of the RANDOX Laboratories Ltd, UK. All the analyses were colorimetric and carefully performed according to the specifications of the Manufacturer. Serum ALP assay procedure was described by Plumer (1971), whereas ALT and AST assay were described by Reitman and Frankel (1957). Absorbance was read for ALP, ALT, and AST at 405nm, 550nm, and 546nm, respectively. Bilirubin was assayed by following its reaction with diazotized sulphanyl acid (Srivastav et al., 1999).

Determination of L- gamma-Glutamyltransferase (gamma-GT, GGT) was carried out following the method of Szasz (1969). The principle is based on the fact that the substrate, L-gamma-glutamyl-3-carboxy-4 nitroanilide, in the presence of glycylglycine is converted by gamma-GT in the sample to 5-amino-2-nitrobenzoate which can be measured at 405 nm. In the procedure, twelve test tubes were labeled corresponding to twelve serum samples. Then 0.10 ml of each serum sample was

transferred to a corresponding test tube, followed by addition of 1.0ml of working reagent (prepared by mixing one vial of L-gamma-glutamyl-3-carboxy-4-nitroanilide with 3.0 ml of glycylglycine buffer). The contents of each tube were adequately mixed (at room temperature) and the absorbance read at 405 nm at 1- and 3-minutes intervals. Measurement was done against air (no application of standard or blank).

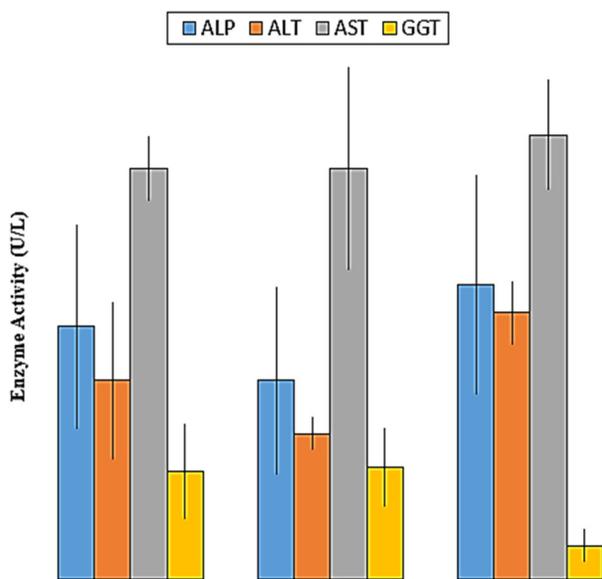
Total Cholesterol assay was performed following the method of Dixit et al., (1986). According to the principle cholesterol, in the presence of acetic acid and concentrated sulphuric acid, reacts with ferric chloride to give red coloured complex whose intensity is proportional to the concentration of cholesterol and is measured at 540 nm. Centrifuge tubes were labeled corresponding to the number of serum samples prepared for analysis. Exactly 0.1 ml of each sample was transferred into each tube using pipette, and then the addition of 3.0 ml of ferric chloride ( $\text{FeCl}_3$ ). The standard tube contained 0.1 ml of cholesterol standard and 3.0 ml of  $\text{FeCl}_3$ . Into the blank tube also was added 3.0 ml of  $\text{FeCl}_3$  and 0.1 ml of  $\text{H}_2\text{O}$ . The contents of each tube were thoroughly shaken (mixed) and then allowed to stand at room temperature for 25 minutes before centrifuging at 4000 rpm for 10 minutes. This allowed the precipitation of proteins in the sample. Then 2.0ml of each supernatant was transferred to a corresponding test tube and 1.0ml of concentrated  $\text{H}_2\text{SO}_4$  added. The contents were mixed and incubated at room temperature in the dark for 25 minutes. The absorbance for each sample was read at 540 nm, against the blank.

Serum triglyceride was assayed according to the method of Jacobs and Van Denmark (1960). Triglycerides are determined after enzymatic hydrolysis with lipases. Exactly 10  $\mu\text{l}$  of each sample was transferred to appropriate tube using micropipette and then 1.0 ml of enzyme reagent was added. The standard tube contained 10 $\mu\text{l}$  of triglyceride standard and 1.0 ml of enzyme reagent, while 10  $\mu\text{l}$  of water and 1.0ml of enzyme reagent

were added to the blank tube. The contents of the tubes were shaken to mix well and left standing for 10 minutes at room temperature. At the end of the ten minutes, the absorbance was read for sample and standard against blank at 546 nm.

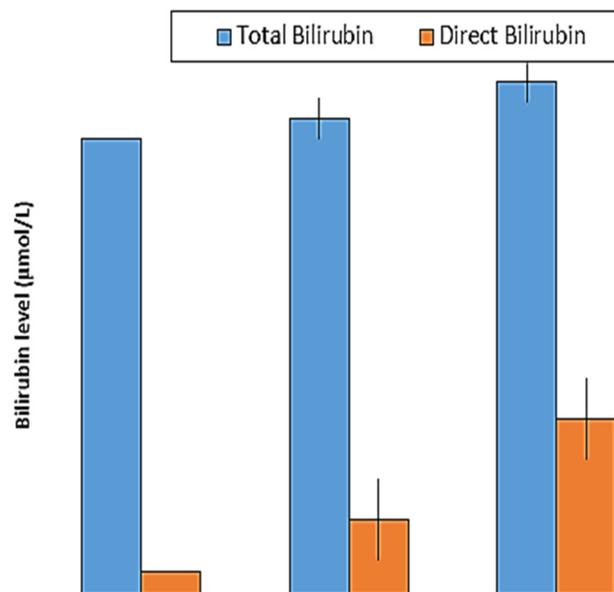
**Results**

Activities for alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-Glutamyltransferase (GGT) for a single dose of 2000mg/kg and 1500mg/kg, respectively, are represented in Figure 1. Values indicate nonsignificant reductions ( $p > 0.05$ ), in the activities of ALP, ALT and AST for the graded (two) doses. However, for GGT, there were nonsignificant increases in the activities of the enzyme for the two dose schedules.



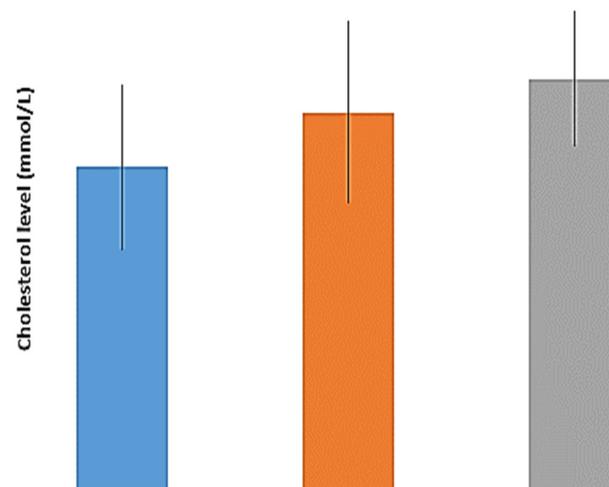
**Figure 1:** Mean alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and gamma-glutamyltransferase activities (Data represented as mean ± SEM)

Serum concentrations of total and conjugated bilirubin at doses of 2000 mg/kg and 1500 mg/kg and the control are represented in figure 2. Total bilirubin decreased non significantly ( $p > 0.05$ ), while conjugated bilirubin decreased significantly ( $p < 0.05$ ), comparative to the control.

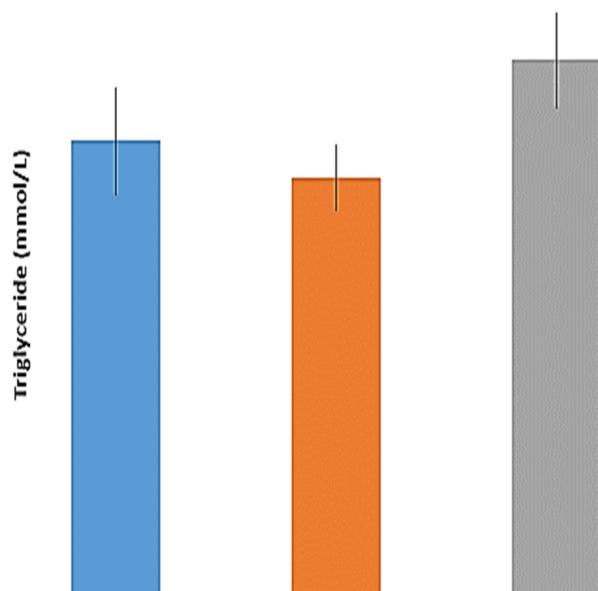


**Figure 2:** Mean bilirubin concentrations (Data represented as mean ± SEM)

The effect of the extract on serum cholesterol was evaluated (Figure 3). Nonsignificant reductions in blood cholesterol were recorded for both doses' comparative with the control.



**Figure 3:** Mean total cholesterol concentration (Data represented as mean ± SEM)



**Figure 4:** Mean concentrations of triglyceride (Data represented as mean  $\pm$  SEM)

The effect of extract on serum triglyceride was determined (Figure 4). The result showed nonsignificant reductions in triglyceride levels by the extract from the two dose schedules.

## Discussion

The wide use of virtually all the parts of *Sarcocephalus latifolius* as effective remedies against several disease conditions has necessitated that the plant herbal extracts and products be subjected to intense screening to determine the toxicity status of the extracts and products. This is important in order to establish a margin of safety in the use of the plant and its products. Some analyses had earlier been conducted in line with this objective, including effects on serum electrolytes (Enemor and Okaka, 2013), sub-acute effects on kidney function markers (Enemor *et al.*, 2014), sub-acute effects on liver function markers (Enemor *et al.*, 2014). In this study we evaluate the possible effect of a relatively single high dose of the root extract for any sudden development of toxicity or a progressive development over a few days following the administration. Physical examination over a

four-day period showed the animals were stable without manifestations of physical abnormalities.

Nonsignificant reductions were recorded in the activities of ALP, ALT, and AST and this seem to suggest that the root extract of *S. latifolius* is relatively nonhepatotoxic with regard to these parameters, and seemingly impacts positively on the liver. The extract caused elevations in activities of GGT with the two dose schedules, comparative to that of the control. Since the increase in GGT activity is insignificant ( $p > 0.05$ ) the extract does not necessarily have hepatotoxic effect on the liver of the experimental rats in relation to GGT.

Some lipid parameters, cholesterol (total) and triglycerides were determined. Depressed serum cholesterol concentrations were recorded for both the 2000 mg and 1500 mg/kg body weight. Decreases in serum cholesterol were statistically insignificant ( $p > 0.05$ ) for both doses. Serum triglycerides also decreased insignificantly ( $p > 0.05$ ) for both doses of the extract.

However, many reports have highlighted marked influence of plant extracts on triglyceride and cholesterol levels in the blood. For example, significant decreases in both serum triglyceride and cholesterol in rats treated with *Moringa oleifera* leaf extract (Naznin *et al.*, 2008). Significant reduction in total cholesterol and triglyceride by aqueous leaf extract of *Chamaerops humilis* have equally been reported (Gaamoussi *et al.*, 2010). Significant increases in serum cholesterol and triglyceride levels in hypertensive patients were reported in other previous works (Saha *et al.*, 2006).

The dwindling action of *Sarcocephalus latifolius* root extract (and indeed any other plant extracts with similar actions) on serum cholesterol and triglyceride in this study is an indication of the involvement of the root extract in mechanisms involved in elimination of the lipids from the body, thus inferring its potential hypolipidaemic effects. Hypocholesterolemic activity of plant extract might be linked to predicted inhibition of HMG-CoA

reductase, as well as possible activation of cholesterol-7-alpha-hydroxylase (CYP7A1), which has the capacity to convert cholesterol to bile acids, and interference in the absorption of cholesterol from the intestine due to possible formation of complexes with compounds such as glycosides and saponins (Amin *et al.*, 1988; Yokogoshi and Oda, 2002; Chen *et al.*, 2004). *Sarcocephalus latifolius* root seems to be a good hypolipidaemic agent and could be the basis for its antihypertensive and antidiabetic properties.

While the search for solutions to global health challenges continues, the integration of medicinal plants and their products in mainstream healthcare delivery remains a focus of objective research. Our findings so far have continued to expose *Sarcocephalus latifolius* as relatively nontoxic and largely medicinally beneficial and reserves great potentials for development of modern medicines. Since organ toxicity has remained at the centre of controversy over the use of crude herbal extracts and herbal products, quantification and isolation (from this and other plants) of pharmacologically active metabolites with optimum potentials as lead substances for the development of modern medicines targeting various health challenges would represent a significant scientific breakthrough.

### Competing interests

Authors have declared no competing interests.

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