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Full Length Research Paper

Evaluation of acute and subchronic toxicity of *Stachytarpheta angustifolia* (Mill) Vahl (Fam. Verbanaceae) extract in animals

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Stachytarpheta angustifolia **is an important and a highly valued medicinal plant ethnobotanically used in the treatment of various diseases. The present study was carried out to evaluate acute and subchronic toxicity in animals and also evaluate the phytochemical profiles of hydroethanolic extract of *S. angustifolia* (Mill) Vahl plant. *S. angustifolia* attracted the attention of the researchers because of its uses as an anti-infective, antidiabetic in folkloric medicine and also as soap by local farmers. The aqueous ethanol (80%) extract of the powdered dried plant was obtained by maceration. Evaluation of acute and subchronic toxicity and phytochemical profiles of the plant extract was performed using standard procedures. The median acute toxicity value (LD_{50}) of the extract of *S. angustifolia* was determined to be 8.721g/kg body weight. The extract lowered blood plasma glucose and low density lipoprotein (LDL-cholesterol) levels but raised high density lipoprotein (HDL-cholesterol) level. The protein, creatinine, and phosphorous levels were significantly affected only by the highest dose of the extract while calcium level was not affected by all the doses used. The extract contained triterpenoid saponins as the major bioactive constituent. The LD_{50} value indicated the drug as being slightly toxic. The extract did not produce any toxic effect in the animals' tissues at low and moderate doses but could cause kidney damage in higher doses. Lowering of plasma glucose level and the positive effects of the extract on the cardiovascular risk factors were an indicator that the extract could have some good antidiabetic activity.**

Key words: *Stachytarpheta angustifolia*, acute, subchronic, toxicity, phytochemical profile.

INTRODUCTION

Plant derived compounds, apart from their nutritional values, could serve as important therapeutic weapons to fight various human and animal diseases, thereby making them indispensable to human and animal lives. Plant drugs, popularly known as herbal medicine, have since been unabatedly used to treat various diseases. They are exclusively employed in some Nigerian communities as well as in the other developing countries for the treatment of certain disease conditions. Medicinal plants, therefore, remain the main source of the active drugs from a natural source and are still indispensable in traditional medicine for treating a number of diseases.

Traditional or folkloric medicines are used by about 60% of the world population both in the developing and developed countries where modern medicines are predominantly used (Mythilypriya et al., 2007). The herbal recipes are prepared most often from a combination of two or more plant products which many a time may contain active constituents with multiple physiological activities and could be used in the treatment of a variety of disease conditions (Pieme et al., 2006). They are administered in most disease conditions over a long period of time without proper dosage monitoring and consideration of toxic effects that might result from such prolonged usage.

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Stachytarpheta angustifolia (Fam. Verbanaceae) is a very important and highly cherished medicinal plant employed in the traditional medicine in many Nigerian communities. Remedy prepared with *S. angustifolia* is used locally as an anti-infective in the treatment of bacterial infections and is much valued in the treatment of protracted sexually transmitted diseases (STDs) (Personal communication with a herbalist Ugboaja N in September 17, 2007 in Atani town,Amanbra State of Nigeria, on the traditonal use of *S. angustifolia*). *S. angustifolia* herbal preparation is also used in the treatment of other diseases such as diabetes, where it is administered over a long period of time (Isah et al., 2007). The warning regarding the potential toxicity of this therapy and other herbal therapies employed in the treatment of diseases over a long period of time demands that the practitioners should be kept abreast of the reported incidence of renal and hepatic toxicity associated with the ingestion of medicinal herbs (Tédong et al., 2007). *S. angustifolia* is a seasonal plant growing mostly along the banks of rivers, streams and in farm-lands during the rainy seasons, especially in southern Nigeria.

The preliminary phytochemical analysis of the extract indicated positive results for polyphenols and triterpenoid saponins. For a plant or herbal preparation containing active organic principles to be identified for use in the traditional medicine especially for long term treatment, a systemic approach is required for the evaluation of efficacy and safety through experiment and clinical findings (Mythilypriya et al., 2007). There is need, therefore, for scientific evaluation of the toxic effects of this useful medicinal plant widely employed by the traditional medical practitioners in the long term treatment of various diseases.

The aim of this study was to evaluate the safety of *S. angustifolia* extract by carrying out the acute and subchronic toxicity studies in the animals and also to carry out the preliminary phytochemical screening. Subchronic toxicity evaluation is required to establish potential adverse effects of this valuable medicinal plant.

MATERIALS AND METHODS

Plant material

The plants were collected from Atani, a town located at the bank of River Niger in Anambra State of Nigeria, and was authenticated at the Forestry Research Institute (FRIN) Ibadan, and voucher specimen (FHI81324) deposited at the Institute Herbarium. The aerial part of the plants were collected, washed, cut into small pieces and dried at an ambient temperature between 35 – 45°C in an oven for seven days, and powdered to coarse particles. 500 g of the powder was macerated with ethanol (80%) at room temperature for seven days with stirring at intervals. After filtration, the solvent was removed under reduced pressure in a rotary evaporator at a temperature below 50°C and dried to a constant weight of 29.45 g (5.89%, w/w yield).

Acute toxicity study

The toxicity study was carried out using thirty-five (35) male and female Swiss albino mice (each weighing 20 – 25 g) obtained from the Laboratory Animals Center, College of Medicine, University of Lagos. The animals were randomly distributed into: one control group and six treated groups, containing five animals per group. They were maintained on animal cubes (Feeds Nigeria Ltd), provided with water *ad libitum* and were allowed to acclimatize for seven days to the laboratory conditions before the experiment. After fasting the animals overnight, the control group received 0.3 ml of normal saline orally while each treated group received orally the hydroalcoholic extract prepared by dispersing 8.0 g in 10 ml volume with normal saline (80% solution), in the doses as follows: 1.0, 2.5, 5.0, 10.0, 15.0 and 20.0 g/kg. The animals were observed continuously for the first 4 hours and then for each hour for the next 24 hours and at 6 hourly interval for the next 48 hours after administering the extract to observe any death or changes in general behavior and other physiological activities (Shah et al., 1997; Bürger et al., 2005)

Subchronic toxicity study

Male and female Wistar albino rats weighing 160 ± 10 g were used. They were allowed to acclimatize to the laboratory conditions for seven days and were maintained on standard animal feeds and provided with water *ad libitum*. The animals were weighed and divided into five groups of five animals each. After fasting the rats overnight the control group received a dose of 0.5 ml of normal saline orally once a day for 30 days. The four treated groups received the following doses respectively: 50, 100, 250 and 500 mg/kg of the hydroalcoholic extract (prepared as in the above acute toxicity study) orally once a day for 30 days (Pieme et al., 2006; Joshi et al., 2007; Mythilypriya et al., 2007). The animals were then weighed every five days from the start of the treatment, to note any weight variation. At the end of the experiment, they were made unconscious by cervical dislodgement and blood collected via cardiac puncture in two tubes: one with EDTA for immediate analysis of haematological parameters and the other with heparin to separate plasma for biochemical estimations. The animals were sacrificed and liver, kidney and heart were dissected out, washed and transferred to ice-cold saline solution and weighed (Mythilypriya et al., 2007). The brains were also dissected out and weighed. The collected blood was centrifuged within 5 min of collection at 4000 g for 10 min to obtain plasma, which was analyzed for total cholesterol, total triglyceride, HDL-cholesterol levels by precipitation and modified enzymatic procedures from Sigma Diagnostics (Wasan et al., 2001). LDL-cholesterol levels were calculated using Friedwald equation (Crook, 2006). Plasma was analysed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine by standard enzymatic assay methods (Sushruta et al., 2006). Plasma glucose and protein contents were determined using enzymatic spectroscopic methods (Hussain and Eshrat, 2002). Haematocrit was estimated using the methods of Ekaidem et al. (2006). Haematocrit tubes were filled by capillary action to the mark with whole blood and the bottom of the tubes sealed with plasticide and centrifuged for 4 - 5 min using haematocrit centrifuge. The percentage cell volume was read by sliding the tube along a "critocap" chart until the meniscus of the plasma intersects the 100% line. Haemoglobin contents were determined using Cyanmethaemoglobin (Drabkin) method (Ekaidem et al., 2006).

Phytochemical evaluation of the crude extract

Phytochemical screening of the extract for the presence of secondary metabolites was performed using the following reagents and chemicals: alkaloids with Mayer's reagent and Dragendorff's reagent (Farnsworth, 1966; Harborne, 1998), flavonoids with the use of Mg and HCl (Silva et al., 1993; Houghton and Raman, 1998); tannins with 1% gelatin and 5% ferric chloride solution and sapo-

nins with ability to produce suds (Houghton and Raman, 1998). Liebermann-Buchard test consisting of a mixture of glacial acetic acid and sulphuric acid (19:1) was used to differentiate the types of saponins and steroidal nuclei present (Farnsworth, 1966).

Statistical analysis

All data collected were summarized as mean ± SEM. Significant differences were determined using a Student's t-test and the differences were considered significant if $p<0.05$.

RESULTS

In acute toxicity study (Table I), 100% death was recorded for all the animals that received 20.0 g/kg b.wt of the extract while 37.5 and 12.5% deaths were recorded for the animals that received 12.0 and 9.0 g/kg b.wt of the extract, respectively. There was no death in the animals that received 6.0 g/kg b.wt or less. LD_{50} of the extract was calculated to be 8.721 g/kg b.wt. The result of the effect of the extract on the body weight of the animals compared with the control is shown in Table 2. There was significant increase ($p<0.05$) in the body weights of the treated animals compared with the control except for the group treated with 250 mg/kg b.wt where there was decrease in the body weight between day 10 and 15 and a dramatic increase was witnessed from day 20 to the end of the experiment. The reason for this anomaly: reduction and sudden increase in weights can not be easily deduced. The mean percentage increase in the weights of all the treated animals compared with the control was very significant and is shown in Figure 1.

The result of the effect of the extract on the organs was presented in Table 3. The macroscopic examinations did not show any changes in colour of the organs of the treated animals compared with the control. Also, significant changes ($p<0.05$) in the various organs weights were observed only in the animals treated with the highest dose of the extract compared with the control. Table 4 summarized the results of the extract's effects on the biochemical parameters. There was significant decrease ($p<0.05$) in the plasma glucose level especially in the rats treated with the highest dose compared with control. A significant decrease in the plasma protein levels and also, a significant increase ($p<0.05$) in the plasma creatinine and AST levels were observed only in the animals treated with the highest dose of extract (500 mg/kg.bwt.). A significant decrease in ALT level was observed in all groups treated with the extract compared with the control. There were significant decreases ($p<0.05$) in the plasma total cholesterol (TC), triglyceride (TG) and LDL-cholesterol levels while significant, increase ($p<0.05$) in HDL-cholesterol levels were observed in all the treated animals compared with the control.

Significant changes ($p<0.01$) were observed in the hemoglobin contents, in the packed cell volume (PCV), red blood cells (RBC), and white blood cells (WBC) contents of the treated animals compared with the control (Table 5). There was no significant change in the calcium level in all the treated animals compared to control, while a significant increase ($p<0.01$) in the level of phosphorus was observed only in the animals treated with the highest dose of the extract.

Table 1. Acute toxicity study of the aqueous ethanol extract of *S. angustifolia* in mice.

Number of mice (n)	Doses of extract (g/kg)	Number of mice dead	Percentage cumulative of mice dead
5	0.60	0	0
5	1.5	0	0
5	3.0	0	0
5	6.0	0	0
5	9.0	1	12.5
5	12.0	2	37.5
5	20.0	5	100.0

Control group received a dose of 0.3 ml normal saline.

The results of the phytochemical screening of the plant extract (Table 6) revealed the presence of saponins as the major active secondary metabolite, while free anthraquinones and polyphenols other than flavonoids were the minor constituents. Alkaloids and organic bases were conspicuously absent.

DISCUSSION

In recent times there is an increasing awareness and interest in medicinal plants and their preparations commonly known as herbal medicines. Consequently, herbal medicines have received greater attention as alternatives to clinical therapy, leading to increase in their demand (Mythilypriya et al., 2007). The exclusive use of herbal drugs, prepared and dispensed by unscientifically trained herbalists, for treatment of diseases is still very common in rural Nigerian communities. Experimental screening method is, therefore, important in order to ascertain the safety and efficacy of herbal products as well as to establish the active components of these herbal remedies.

In the acute toxicity study of the extract, no change in the behavior and in the sensory nervous system responses was observed in the animals. Also no adverse gastrointestinal effects were observed in male and female mice used in the experiment. All the mice that received 20.0 g/kg dose of the extract died within 4 h after administration while the animals that received 6.0 g/kg dose survived beyond the 24 h of observation. The median acute toxicity value (LD_{50}) of the extract was determined to be 8.721 g/kg body weight. According to Ghosh (1984) and Klassen et al. (1995) the extract can be classified as being slightly toxic, since the LD_{50} was found to be between 5 - 15.0 g/kg b.wt.

Table 2. The effect of the extract on weight changes in the control and treated rats in the subchronic toxicity study.

Dose	Day 1	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
Control	170.01± 2.7	171.03±2.18*	172.06±1.80	173.51± 1.10	175.60±2.5	178.75±2.7	179.75±2.2
50 mg/kg	150.01± 2.1	151.20± 0.4*	154.30± 1.2*	160.06 ± 1.82*	161.51±0.20*	164.50±0.2*	167.30± 0.3 *
100 mg/kg	150.02±0.12	152.40±2.80	152.7±0.79**	153.04±1.4	155.41±1.30*	160.25±2.36*	160.20 ±2.3*
250 mg/kg	150.01±0.30	150.20±1.50*	148.02±7.5**	148.20±6.32	158.75±4.97*	160.61±1.2*	165.04±1.5*
500 mg/kg	160.20±6.12	161.25±6.92**	163.10± 4.8	159.75±4.97	160.75±4.97	166.05±2.25*	173.5±1.2*

Mean values of 5 animals ± SEM *p<0.05; ** p<0.01 vs. control group.
Control group received 0.5 ml normal saline.

Table 3. The effect of the extract on kidney, heart, liver and brain in the control and treated groups in the subchronic toxicity study.

Organ	Control	50 mg/kg	100 mg/kg	250 mg/kg	500 mg/kg
Heart (g)	0.62 ± 0.02	0.61± 0.08	0.61±0.02	0.60 ±0.01	0.65±0.06*
Kidney (g)	1.30 ± 0.10	1.25± 0.18	1.2±0.051	1.10 ±0.06	1.20±0.07*
Liver (g)	6.44 ± 0.07	6.42± 0.53	6.35±0.57	6.10 ±0.63*	7.25.±0.43*
Brain (g)	1.28± 0.14	1.23±0.08	1.29 ±0.081	1.30±0.04	1.34±0.08*

Mean ± SEM, (n = 5), *p<0.05; ** p<0.01 vs. control group.
Control group received 0.5 ml normal saline.

Table 4. Effect of daily administration of the extract for 30 days on biochemical profiles of the control and treated rats in the subchronic toxicity study.

Parameter	Control	50 mg/kg	100 mg/kg	250 mg/kg	500 mg/kg
Glucose (mg/dl)	90.6±0.2	83.65±0.12	81.18±0.32	76.83±0.52*	73.03±1.50*
Cholesterol (mg/dl)	37.62±0.60	29.24±0.03**	30.58±0.42	36.04±1.4	30.21±0.7
Triglyceride (mg/dl)	24.60±0.45	21.69±1.50	18.29±0.02*	18.37±0.02*	12.3±0.04*
HDL (mg/dl)	95.3±0.002	104.5±0.007*	143.67±0.07*	150.39±0.49*	253.03±0.39*
LDL(mg/dl)	81.44±2.50	32.48±4.75	25.60±0.60	21.77±3.50	16.70±0.50*
Protein (g/dl)	3.56±0.32	3.63± 0.25	3.26±0.60	3.68±0.20	2.88±0.15*
Creatinine (mg/dl)	2.51±0.003	2.17±0.001*	2.22±0.05	2.55±0.01	4.07±0.001*
AST (IU/L)	8.18±1.20	5.69±2.50**	8.14±0.02	8.5±2.30	19.01±0.60*
ALT (IU/L)	52.54±1.80	16.15±2.52**	57.20±2.6	27.00±3.6	38.50±2.40

Mean ± SEM (n = 5), *p<0.05; ** p<0.01 vs. control group.
Control group received 0.5 ml normal saline solution.

Table 5. Effect of the extract on haematological parameters of the control and treated rats in the subchronic toxicity study.

Parameter	Control	50 mg/kg	100 mg/kg	250 mg/kg	500 mg/kg
Haemoglobin (mg/dl)	12.20±0.3	12.80±0.25**	13.60±0.40*	13.80±0.40*	14.79±0.40*
RBC(10^6/mm^3)	9.07±0.04	8.40±0.3	9.52±0.06	9.38±0.05	9.93±0.06
WBC (10^3/mm^3)	12.30±0.03	8.90±1.65	12.40±0.02	9.20±1.40	12.8±0.02
PCV (%)	39.0±2.20	38.72±2.3*	41.05± 1.52	40.85±2.50	44.55±0.50**
Calcium (mg/dl)	9.05±0.04	8.83±0.05	9.04±0.04	9.41±0.03	8.37±0.10
Phosphorus (mg/dl)	7.41±0.03	8.63±0.05	8.17±0.001	8.71±0.04	9.17±0.2

Mean ± SEM (n = 5),*p<0.05; ** p<0.01 vs. control group.
Control group received 0.5 ml normal saline solution.

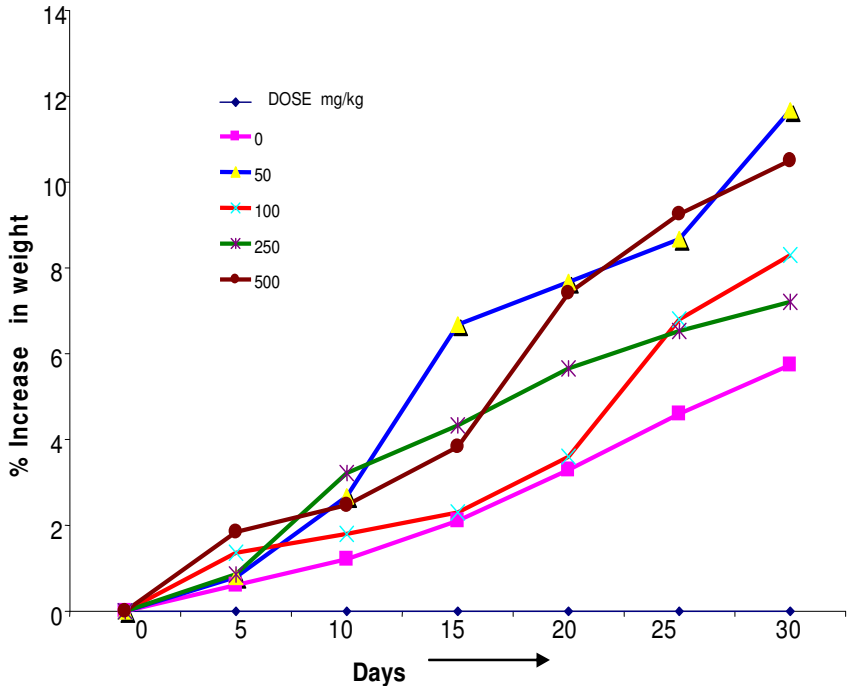

Figure 1. Mean percentage weight increase of the control and treated rats in the subchronic toxicity study.

Table 6. Phytochemical screening of the hydroethanolic extracts of *S. angustifolia*.

Phytochemical	Presence
Alkaloids	-
Cardiac glycosides	-
Anthraquinone glycoside	+ +
Free anthraquinone	+
Saponin glycosides	+++
Steriodal nucleus	-
Triterpenoid nucleus	+++
Cyanoglycoside	-
Polyphenols	++
Flavonoids	-
Red cell Haemolysis	+++

(-) Absent, (+) slightly present, (++) fairly present, (+++) abundantly present.

The gram equivalence of the LD_{50} in an average adult man would translate to 523.25 g dose of the extract. The very high equivalence for man makes it relatively safe for both internal and for external uses. The viscera of the animals did not show any macroscopic changes compared with the control that could point to the cause of the death. However, since the animals did not convulse before dying, it could be postulated that the extract did not kill the mice by some action on the nervous system (Ogwal-Okeng et al., 2003).

The weight increase in the animals was in consonance with the increase in the doses. The mean percentage increase in the weights of the treated animals compared with the control was very significant. This observed increase in the weights might be attributed to the stimulation of the animals' appetite by the extract which increased with the dose. The macroscopic examinations of the organs of the animals treated with various doses of the extract did not show any changes in colour compared with the control. Also, there were no significant changes in the organs weights of the treated animals compared with the control. Since no changes in animal behavior and in organs weights were observed at all doses used, the extract or its herbal formulation could be claimed to be non-toxic to the observed organs.

Effects of the extract on the biochemical parameters clearly showed that there was a remarkable decrease in the plasma glucose level especially at higher doses in the treated rats compared with the control. This indicates the presence of hypoglycemic components in the extract and gives credence as to the use of *S. angustifolia* herbal preparation as a hypoglycemic agent. The decrease in the plasma protein levels may be a sign of impaired renal function (Kachmar and Grant, 1982), and the elevation in the plasma creatinine concentration observed suggested kidney damage, specifically by renal filtration mechanism (Wasan et al., 2001). The decrease in protein level and increase in creatinine level only occurred in the animals that received the highest dose of the extract (500 mg/kg body weight). The extract at this dose and beyond may

likely cause kidney damage.

The liver releases alanine aminotransferase (ALT) and an elevation in plasma concentration are an indicator of liver damage. The liver and heart release AST and ALT, and an elevation in plasma concentration are an indicator of liver and heart damage (Wasan et al., 2001; Crook, 2006). There was a significant increase in AST level only in the animals treated with the highest dose (500 mg/kg b.wt), while a significant decrease in ALT was observed in all treated groups compared with the control. It implied that the extract could not have caused some toxic effects on the liver and heart tissues at low and moderate doses but at a high dose extract might have some deleterious effects on the heart tissue (Crook, 2006). The decrease in the plasma total cholesterol (TC) and triglyceride (TG) levels might be attributed to the presence of hypolipidemic agents in the extract.

A significant increase in HDL-cholesterol levels and a reduction in LDL-cholesterol levels observed in all the treated animals are an indicator that the extract can reduce the cardiovascular risk factors which contribute to death of diabetic subjects (Barnett and O'Gara, 2003). The reduction of the cardiovascular risk factors gave further support to the traditional use of the herbal formulation of *S. angustifolia* as a hypoglycaemic agent.

The observed increase in the haemoglobin levels might be due to the increased absorption of iron. Also the increase in the haemoglobin level coupled with increase in the WBC count emphasized the beneficial effect of the extract to the general well being of the animals. There was no significant change in calcium level in all the treated animals while a significant increase ($p<0.01$) in the level of phosphorus was observed only in the animals treated with the highest dose of the extract. An increase in the level of phosphorus may be associated with renal failure (Tietz, 1982). Since there were significant increases in creatinine and phosphorus levels and a decrease in the protein level in the animals that received the highest dose of the extract (500 mg/kg b.wt,), it is most likely that the at this dose the extract could cause kidney damage, leading to renal failure (Tietz, 1982; Wasan et al., 2001; Crook, 2006).

Phytochemical screening helps to reveal the chemical nature of the constituents of the plant extract and the one that predominates over the others. It may also be used to search for bioactive lead agents that could be used in the partial synthesis of some useful drugs (Yakubu et al., 2005). The presence of saponins as the major active secondary metabolite contributes immensely to the bioactivity of the extract. Saponins are characterized by their surface-active properties and they dissolve in water to form foamy solutions. Drugs containing saponins have a very long history of usage (Jean, 1999). Saponins have been implicated as bioactive antibacterial agents of plants containing them (Mandal et al., 2005; Manjunatha, 2006). The exhibited antibacterial properties of *S. angustifolia* can be attributed to the presence of pentacyclic saponins and polyphenols in the plant. Polyphenols have been associated with antioxidant activities and were earlier reported to have some antibacterial activities (Tomas-Barberan et al., 1990). These might have complimented or potentiated the saponins in the antibacterial activities exhibited by *S. angustifolia* extract.

Conclusion

The high LD_{50} value (8.721 g/kg) obtained was a clear indication that *S. angustifolia* herbal preparations could be safe for both internal and external uses. The study showed that the extract had some hypoglycemic activity and good reducing effects on cardiovascular factors. The study also revealed that the extract at low and moderate doses did not provoke toxic effects to the animals' tissues, but higher doses could cause kidney damage leading to renal failure during a long term treatment. Kidney functions should therefore be monitored regularly during the long term management of diseases with *S. angustifolia* herbal preparations.

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