



Effect of aqueous methanol extract of *Sarcocephalus latifolius* fruit on carbon tetrachloride induced toxicity in albino rats

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Abstract

In recent times, interest in the use of medicinal plants (ethnomedicine or ethnobotanical medicine) as alternative to modern medicines is increasing globally, especially in developing countries where traditional beliefs and high cost of, or limited access to conventional medical treatment may constitute important factors. The use of *Sarcocephalus latifolius* for the control of various illnesses, especially in West Africa, is widely documented. This study therefore, was designed to highlight the protective potential of *Sarcocephalus latifolius* fruit extract on body organs such as the liver and kidney by monitoring some biochemical parameters. The plant material was collected from Kadale village in Gwaram Local Government Area of Jigawa State, Nigeria. It was extracted with aq. methanol in a Soxhlet extractor. Acute toxicity examination was carried out on the extract in line with standard methods with the lethal dose obtained to be above 2000mg/kg body weight. The percentage protection of the fruit of *S. latifolius* on CCl₄ induced toxicity was dose dependent. TLC examination of the column fractions (Fr 1-Fr 7) revealed that Fraction 1,2,3 could be separated easily and that the chemical components are similar.

Keywords: Proximate analysis; Hepatotoxicity; Carbon tetrachloride; Mineral content; *Sarcocephalus latifolius*

INTRODUCTION

Medicinal plants have been used since antiquities to maintain health and treat diseases (Amresh *et al.*, 2004). Therefore, plants which show efficacy and are frequently used may contain compounds that are potential source of drugs and could be recommended for further investigation (Igoli *et al.*, 2005). *Sarcocephalus latifolius* is a savannah tree or shrub up to 12m. *S. latifolius* has been used for several economic and medicinal purposes. The fleshy fruits are edible, live-stock feed on the stalk and leaves,

flowers provide nectar and pollen to bees, wood is termite-resistant and bark yields tannins used in dyeing (Arbonnier, 2002). Despite its use as food and medicine in this region, there has been little or no report on its protective effect on body organs especially the liver and kidney which are essential body organs. Many biochemical parameters tend to have specificity for an organ and/or a limited range of pathological processes (Lee, 2009). Investigative biochemical profiles are designed to provide all the data necessary for a broad investigation of internal disease.

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Profiles with limited data are best used for monitoring an established diagnosis for which the results of a wider ranging profile have already been obtained (Gamzi *et al.*, 1999). Therefore, this work is aimed at evaluating the hepato-protective effect of *S. latifolius* fruit obtained from Kadale village, Gwaram local government area of Jigawa State with the hope of encouraging the consumption of this forest fruit as an alternative food source.

EXPERIMENTAL

Sample collection and preparation. The matured fruits of *S. latifolius* were randomly sampled from different branches of the tree growing in areas around Kadale village, Gwaram local Government area, Jigawa state, Nigeria, West Africa. The samples were transported to the laboratory in air-tight polyethylene bags. The samples were air dried and pulverised with porcelain mortar and pestle to fine particles and stored in plastic containers. The pulverised sample was extracted in a Soxhlet extractor using 80% methanol for about 8 hours. The extract obtained was concentrated *in vacuo* at 40°C and stored until used.

Chemical investigation. Test was carried out to determine the presence of phyto-active principles such as carbohydrate, tannins, saponins, alkaloids steroidal glycosides, flavonoids etc. By simple qualitative standard methods. Chromatographic examination using Column and T.L.C technique was also carried out in line with standardized procedure.

Biological investigation

Animals. White albino rats of mixed sexes weighing 160-220g were obtained from faculty of Pharmacy animal house, University of Maiduguri. They were housed in standard cages and fed with growers' mash and water *ad libitum*.

Experimental procedure. The rats were weighed and assigned into five groups of four

animals each. Group A was orally fed with a single dose of normal saline (5 ml/kg body weight) daily and served as control. Animals in group B, C and D were orally fed with 100 mg/kg, 200 mg/kg 400 mg/kg and 800 mg/kg aq. methanol fruit extracts respectively. This was administered to the rats daily for 14 days using and intubator. Group E was introduced on the last day of treatment and injected intraperitoneally alongside other groups (B, C, D and E) with equal doses of 50% carbon tetrachloride (CCl₄) mixed with 50% olive oil (3ml/kg body weight). CCl₄ was used to induce liver toxicity. The rats were sacrificed by humane decapitation 24 h after injecting the toxin (CCl₄). The Blood was allowed to clot for 30 minutes and then centrifuged at 2500rpm for 15 minutes and then serum harvested. Liver and kidney function test were done colorimetrically using reagent kits

Statistical analysis. Data were expressed as mean \pm standard error of the mean (Mean \pm SEM) where applicable.

RESULTS AND DISCUSSION

The phytochemicals reported in the study include flavonoids, tannins, terpenes, saponins, anthraquinones, steroids and alkaloids. These phytochemicals have been reported to possess suitable pharmacological activity (Evans, 2009). Although, similar phytochemicals had been reported from the fruit obtained from a different location, tannins, anthraquinones and alkaloids were not reported earlier. Perhaps, difference in the fruits geographical location may be responsible for this observation.

The various fractions [EA (50), EA/NB (40/10), (30/20), (25/25), (20/30), (10/40), NB (50)] from column chromatography subjected to TLC using known solvents systems such as *n*-butanol: acetic acid: water showed several bands and colours on spraying with 5% sulphuric acid. These colours of bands were characteristic of different class of compounds principally

terpenoids present in the fruit sample. 1% FeCl₃ was also used to spray the developed plate. This yielded green spots from four different fractions suggesting the presence of phenolic group (Table 3). The biological potential of the plant in preventing and controlling hepatic damage to the liver and kidney was established in the study (Table 4-10). Carbon tetrachloride is one of the most commonly used hepatotoxin in experimental studies of liver diseases (Clementine and Tar, 2010). The first metabolite of CCl₄; trichloromethyl free radical, is believed to initiate the biochemical processes leading to oxidative stress, which the direct cause of many pathological conditions such as diabetes mellitus, cancer, hypertension, kidney damage, liver damage death (Pohl *et al.*, 1984; Guven and Gumez, 2003). These activated radicals bind to micro molecules and reduce lipid peroxidative degradation of poly unsaturated fatty acids. This leads to the formation of lipid peroxides, which in turn gives products like malonylaldehyde that

cause damage to membranes (Ulicna *et al.*, 2003). The lipid peroxidative degradation of bio-membranes is one of the main causes of the hepatotoxicity by CCl₄. This is usually evidenced by a rise in serum marker enzymes of the liver namely ALP, AST and ALT. In this study, the aqueous methanol fruit extract of *S. latifolius* demonstrated protection against liver toxicity induced by CCl₄ in a dose dependent manner. The protection observed could be linked to the type of phytochemicals present. Phytochemicals such as flavonoids are potent antioxidants because of their ability to scavenge hydroxyl radicals, superoxide and lipid peroxy radicals (Farombi *et al.*, 2001; Lilito and Frei, 2006). In the study an elevation in the levels of end products of lipid peroxidation of liver of rats treated with CCl₄ was observed. The increase in AST and ALT suggests enhance lipid peroxidation giving rise to liver damage and failure of antioxidant defence mechanism to prevent formation of an excessive free radicals (Farombi *et al.*, 2001).

Table 1. Phytochemical screening of the fruit extracts of *S. latifolius*

S/N	Phytochemicals	Test	Result	
			Aq. CH ₃ OH	H ₂ O
1	Carbohydrate	Molisch's	+	+
		Fehling's	+	+
2	Flavonoids	Shinoda's	+	-
		Lead acetate	+	+
3	Steroidal nucleus	Salkowski	+	+
4	Terpenoid	Liebermann-Burchard	+	+
5	Saponins	Frothing	+	+
6	Tannins	Ferric chloride	+	+
7	Alkaloids	Dragendorff	+	-
		Wagner	+	+

+ = Present - = Absent

Table 2. TLC results of column fractions of the fruit of *S. latifolius* sprayed with 5% H₂SO₄

Sample	No of Bands (Colour)	R _f	R _f × 100
Fr 1	No visible spot		
Fr 2	No visible spot		
Fr 3	3 (Br)	0.52, 0.70, 0.81	52, 70, 81
Fr 4	4 (Br)	0.34, 0.53, 0.72, 0.81	34, 53, 72, 81
Fr 5	6 (Br, P)	0.25, 0.36, 0.50, 0.63, 0.74, 0.81	25, 36, 50, 63, 74, 81
Fr 6	6 (Br, P)	0.25, 0.36, 0.50, 0.63, 0.74, 0.81	25, 36, 50, 63, 74, 81
Fr 7	6 (Br, P)	0.25, 0.36, 0.50, 0.63, 0.74, 0.81	25, 36, 50, 63, 74, 81

Solvent System (mobile phase) – *n*-Butanol: Acetic acid: Water (4:1:1) Br = Brown; P = Pink; B = Blue

Table 3. TLC results of column fractions of the fruit of *S. latifolius* sprayed with 1% FeCl₃

Sample	No of Bands (Colour)	R _f	R _f × 100
Fr 1	No visible spot		
Fr 2	No visible spot		
Fr 3	No visible spot		
Fr 4	4 (green)	0.53, 0.66, 0.83, 0.92	53, 66, 83, 92
Fr 5	4 (green)	0.53, 0.66, 0.83, 0.92	53, 66, 83, 92
Fr 6	4 (green)	0.53, 0.66, 0.83, 0.92	53, 66, 83, 92
Fr 7	4 (green)	0.53, 0.66, 0.83, 0.92	53, 66, 83, 92

Solvent System (mobile phase) – *n*-Butanol: Acetic acid: Water (4:1:1)

Table 4. Effect of *S. latifolius* aq. methanol fruit extract on mean Alkaline Phosphatase (ALK.P).

Treatment group	Dose	Mean no. of inhibition in 24hrs	% Protection
Normal Saline	-	404.33 ± 1.69	
CCl ₄	3ml/kg	693.67 ± 1.25	0
Extract + CCl ₄	100 mg/kg	482.00 ± 0.81*	30.4
Extract + CCl ₄	200 mg/kg	474.67 ± 1.75*	31.5
Extract + CCl ₄	400 mg/kg	461.00 ± 0.82*	33.5
Extract + CCl ₄	800 mg/kg	421.67 ± 0.47*	39.3

Mean ± SD; n = 3. * = P < 0.05; ** = P < 0.01

Table 5. Effect of *S. latifolius* aq. methanol fruit extract on mean Aspartate Amino transferase (AST).

Treatment group	Dose	Mean no. of inhibition in 24hrs	% Protection
Normal Saline	-	97.00 ± 1.41	
CCl ₄	3ml/kg	108.00 ± 0.82	0
Extract + CCl ₄	100 mg/kg	104.00 ± 0.47*	2.7
Extract + CCl ₄	200 mg/kg	103.00 ± 0.81*	4.6
Extract + CCl ₄	400 mg/kg	97.67 ± 1.23**	9.5
Extract + CCl ₄	800 mg/kg	97.33 ± 0.47**	10.2

Mean ± SD; n = 3. * = P < 0.05; ** = P < 0.01

Table 6. Effect of *S. latifolius* aq. methanol fruit extract on mean Alanine Amino Transferase (ALT)

Treatment group	Dose	Mean no. of inhibition in 24hrs	% Protection
Normal Saline	-	23.33 ± 0.53	
CCl ₄	3ml/kg	64.67 ± 0.47	0
Extract + CCl ₄	100 mg/kg	41.00 ± 0.82	35.9
Extract + CCl ₄	200 mg/kg	27.00 ± 0.82*	58.3
Extract + CCl ₄	400 mg/kg	24.00 ± 0.82**	62.8
Extract + CCl ₄	800 mg/kg	23.67 ± 0.94**	63.64

Mean ± SD; n = 3. * = P < 0.05; ** = P < 0.01

Table 7. Effect of *S. latifolius* aq. methanol fruit extract on mean urea level

Treatment group	Dose	Mean no. of inhibition in 24hrs	% Protection
Normal Saline	-	8.00 ± 0.082	
CCl ₄	3ml/kg	12.10 ± 0.082	0
Extract + CCl ₄	100 mg/kg	11.20 ± 0.082	8.04
Extract + CCl ₄	200 mg/kg	9.50 ± 0.082*	21.49
Extract + CCl ₄	400 mg/kg	8.17 ± 0.047**	32.47
Extract + CCl ₄	800 mg/kg	8.80 ± 0.082**	27.28

Mean ± SD; n = 3. * = P < 0.05; ** = P < 0.01

Table 8. Effect of *S. latifolius* aq. methanol fruit extract on mean creatinine

Treatment group	Dose	Mean no. of inhibition in 24hrs	% Protection
Normal Saline	-	106.00 ± 0.82	
CCl ₄	3ml/kg	127.67 ± 0.47	0
Extract + CCl ₄	100 mg/kg	134.00 ± 0.82	4.60
Extract + CCl ₄	200 mg/kg	116.00 ± 0.82	9.38
Extract + CCl ₄	400 mg/kg	96.00 ± 0.82*	25.00
Extract + CCl ₄	800 mg/kg	79.00 ± 0.82**	38.28

Mean±SD; n = 3. * = P<0.05; ** = P<0.01

Table 9. Effect of *S. latifolius* aq. methanol fruit extract on mean Total Bilirubin (TB)

Treatment group	Dose	Mean no. of inhibition in 24hrs	% Protection
Normal Saline	-	11.03± 0.12	
CCl ₄	3ml/kg	14.13 ±0.12	0
Extract + CCl ₄	100 mg/kg	11.80 ± 0.082*	16.49
Extract + CCl ₄	200 mg/kg	11.30 ± 0.082*	20.02
Extract + CCl ₄	400 mg/kg	10.50 ± 0.082**	25.69
Extract + CCl ₄	800 mg/kg	10.33 ± 0.047**	27.10

Mean±SD; n = 3. * = P<0.05; ** = P<0.01

Table 10. Effect of *S. latifolius* aq. methanol fruit extract on mean Conjugated Bilirubin (CB)

Treatment group	Dose	Mean no. of inhibition in 24hrs	% Protection
Normal Saline	-	1.37± 0.12	
CCl ₄	3ml/kg	3.06± 0.09	0
Extract + CCl ₄	100 mg/kg	2.50 ± 0.08	18.35
Extract + CCl ₄	200 mg/kg	2.20 ± 0.08*	28.15
Extract + CCl ₄	400 mg/kg	1.80 ± 0.08**	41.18
Extract + CCl ₄	800 mg/kg	1.53 ± 0.04**	50.10

Mean±SD; n = 3. * = P<0.05; ** = P<0.01

The group treated with *S. latifolius* fruit inhibit the changes (P<0.05) at higher concentrations (800 mg/kg > 400mg/kg > 200 mg/kg > 100mg/kg body weight respectively) compared to CCl₄ group. Thus, it is possible that the mechanism of hepato-protection of *S. latifolius* due to its antioxidant effect. Yesufu *et al.* (2014) had earlier reported the antioxidant property of the solvent partitioned portions of the fruit of *S. latifolius*.

Total and conjugated bilirubin were significantly increased in CCl₄ treated rats as compared with the control. Administration of the extracts (100 mg/kg, 200 mg/kg, 400 mg/kg and 800 mg/kg body weight) lead to a significant reduction (P<0.05) in their levels. Bilirubin is a major product of haemoglobin breakdown which rises when the liver injury or damage leading to discolouration of the skin and eyes known as jaundice (Sanjiv, 2002). Elevation of total bilirubin which

results from decreased up-take and conjugation of bilirubin by the liver is caused by liver cell dysfunction, while increased levels of direct and conjugated bilirubin is due to decreased secretion from the liver or obstruction of the bile ducts (Srivastav *et al.*, 1999). Reports of effects of extract of other plant species on these parameters abound; significant increases in creatinine were reported by Lienou *et al.* (2007) from their studies with *Aspilia africana*. Ghasi *et al.* (2011) also reported significant decrease in serum creatinine with significant increase in serum urea. The creatinine content of the urine falls when the muscle mass decreases for any reason such as reduction caused by paralysis or muscular dystrophy (Depner, 2001). It was also stated that any rise in blood creatinine is a sensitive indicator of kidney malfunction, because creatinine normally is rapidly removed from the blood and excreted

(Champe *et al.*, 2008). Also, Lienou *et al.*, (2007) stated that an increase in creatinine level can be observed in some kidney illnesses, due to loss of its normal excretive function of creatinine, when there is muscular cells damage or following incompatible medication interfering with normal functioning of the kidneys (Lienou *et al.*, 2007). Yakubu *et al.* (2009) stated that increased serum creatinine observed with the administration of the aqueous leaf extract of *Bambusa vulgaris* in their study could be an indication of glomerular dysfunction. The result of this study showed that serum urea was significantly decreased ($p < 0.05$) by the fruit extract of *Sarcocephalus latifolius*. The effect was dose dependent at 100mg-400 mg/kg body weight. The significant reduction in serum urate levels by the extract of *Sarcocephalus latifolius* fruit may therefore be a strong indicator of its anti-inflammatory potential. The presence of saponins strongly mark the fruit of the plant as a sure potential source of very effective anti-inflammatory and analgesic agent". Saponins isolated from about 50 plants have been shown to possess anti-inflammatory activities against several experimental models of inflammation in mice and rats (Ojewole, 2008). The report from our study showed that the aq. CH₃OH extract of the fruit of *S. latifolius* possess anti-hepatotoxic activity as demonstrated by its reduction of CCl₄ induced elevations of the levels of ALP, AST, Total and conjugated bilirubin, serum urea and creatinine. This hepatoprotective effect suggests that the plant may also possess antioxidant properties which was confirmed by its anti-radical effect on DPPH stained TLC plate. This may be responsible for the observed protection against CCl₄-induced oxidative stress in the liver. The ability of natural compounds to attenuate carcinogen-induced hepatotoxicity is believed to be related to their intrinsic antioxidant properties (Farombi, 2003). Phytochemical results from the study revealed

the presence of flavonoids, which has been reported to protect against toxicity induced by environmental toxicants such as CCl₄ (Farombi *et al.*, 2001). The chemoprotective activities of flavonoids are related to their ability to inhibit peroxidative damage caused by environmental toxicants (Chioma *et al.*, 2008). Interestingly, phytochemical screening of the current investigation has revealed that the fruit extract possesses at least four of the following classes of secondary metabolites; flavonoids, terpenoids, tannins, alkaloids and saponin hence may not only be hepatoprotective but useful as chemo therapeutic agent and therefore need to be tested for protection against hepatic pathogens.

Conclusion. The result from the study showed that the aq. methanol extracts of *S. latifolius* fruit possess hepatoprotective action, suggesting that the flavonoid content may have a major role in the action. In addition, assaying for more enzymes such as gamma-glutamyltransferase (GGT), alkaline phosphatase and lactate dehydrogenase 5 (LDH5) iso-enzymes would further justify the hepatoprotective effect of this plant.

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