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Phytochemical Screening And Hepatoprotective Properties Of The Aqueous Root Bark Extract Of Sarcocephalus latifolius (Smith) Bruce (African Peach)

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Objective: The hepatoprotective properties of the aqueous extract of Sarcocephalus (S). latifolius (Smith) Bruce was studied by investigating its effect on some serum biochemical parameters in rats treated with Carbon tetrachloride (CCl4).

Methods: Twenty Wister albino rats weighing 200-230g were divided into five groups of five rats each (Group A, B, C, D and E) by random selection.Rats in groups B, C and D were orally administered 100mg/kg, 200mg/kg and 300mg/kg body weight aqueous extracts daily for 20 days, while group A (control) was injected with 5ml/kg body weight normal saline for the same period. The animals in groups B, C, D and E were injected intraperitoneally with 3mg/kg body weight of CCl4; on the last day and observed for 24hours after which they were sacrificed by decapitation and blood collected for biochemical and haematological analysis.

Result: Phytochemical analysis of the aqueous extract showed the presence of flavonoids, alkaloids, carbohydrates, tannins and saponins. Alanine transaminase (ALT), Aspartate transaminase (AST), total and conjugated bilirubin levels were significantly decreased ($P<0.05$) across all the groups treated with extract and the toxin (CCl4). The decrease was dose dependent.

Conclusions: The decrease observed in the biochemical parameters, suggest that the aqueous extract of *S. latifolius* possess hepatoprotective properties.

Key words: Sarcocephalus latifolius, aqueous extract, phytochemicals, liver function, hepatoprotective, rats.

Introduction

The plant African peach (*S.latifolius*) is of the family Rubiaceae. It is a multistemmed tree or shrubs up to 12m found in undisturbed fringing forest and close savannah woodland (1). Its generic name is derived from the Greek word *sarco* (fleshy) and *cephalus* (headed) in reference to the flowers. The specific epithet is derived from the latin word *lati* (broad) and *folius* (leaved) (2). A hermaphrodite tree flowering from April-June and fruits ripen from July-September (3). The grey baboon (*Papio anubis*) disperses its seed (4). "Earlier reports on the various medicinal uses of this plant have been reported by many traditional medicine practitioners to be effective in the treatment and management of many ailments such as febrile illnesses, stomach disorder, cough, malaria fever and jaundices (5). Others includes constipation, dysmenorrhoea, abscesses, vomiting and threatened abortions (6).Clinically *S.latifoliues* has been shown to paralyses *Trichostrongylus columbriformis* larvae in a concentration dependent manner (7), lower blood glucose level in normal and alloxan induced rats (8).It has also demonstrated high anti fungal activity against *Macrophomina phaseolina*, the causal agent of dry root decay in pawpaw(9)".

Materials and Methods

Sample collection and preparation: The plant material was collected from Gyella, Mubi South Local Government Area of Adamawa State and authenticated by Prof. S.S. Sanusi of the Department of Biological Sciences, University of Maiduguri, Borno State, Nigeria. The sample was washed, air-dried and pulverized using mortar and pestle. The pulverized sample (250g) was boiled with distilled water (2 litres) for one hour. The extract obtained was concentrated in *vacuo* at 40°C and stored at 40°C until used.

Phytochemical Analysis

Phytochemical test was carried out to determine the presence of carbohydrates, tannins, saponins, alkaloids, steroidal glycosides and flavonoids by simple qualitative standard methods (10), (11),(12).

Animals

White albino rats of mixed sexes weighing 200-230g were obtained from the Department of Human Anatomy Animal House, University of Maiduguri. They were housed in standard cages and fed with growers mash (Sander Nigeria Ltd) and water ad libitum.

Experimental Procedure

The rats were weighed and assigned into five groups of five animals each. Group A was orally fed with a single dose of normal saline (5ml/kg body weight) daily and served as control. Animals in group B, C and Dwere orally fed with 100mg/kg, 200mg/kg and 300mg/kg aqueous root bark extract respectively. This was administered to the rats daily for 21 days using feeding tube. Group E was introduced on the last day of treatment and injected intraperitoneally alongside other groups (, B, C, D and E) with equal dose of (3mg/kg body weight) carbon tetrachloride (CCl4) to induce liver toxicity. The rats were sacrificed by humane decapitation 24hrs after injecting the toxin (CCl4). Blood was allowed to clot for 30 minutes and then centrifuged at 2500 rpm for 15 minutes and serum harvested (13). Liver function tests for alanine transferase (ALT), aspartate transaminase (AST), total and direct bilirubin were done calorimetrically using reagent kits (Randox lab. Ltd) (14).

Statistical Analysis

Data collected from the biochemical parameters assayed were summarized as Mean ± S.E.M. Difference between individual groups was assessed by the student t-test. P value less or equal to 0.05 was considered statistically significant (15).

Results

Phytochemical analysis of the ethanol extract of *S.latifolius*, showed that tannins are present at high concentrations, while flavonoids, alkaloids, carbohydrates are present at moderate concentration and saponins, Cardiac glycosides, and Terpenoids/steroids were present in low concentration. The results obtained are shown in Table 1. The aqueous root bark extract of *S.latifolius* was found to significantly (P<0.05) inhibit the effect of CCl4 on the liver and the protection was dose dependent. For aspartate transaminase (Table 2) percentage protection were 0.67, 18.33 and 44.33% for groups treated with 100mg/kg, 200mg/kg and 300mg/kg of the aqueous extract and injected intraperitoneally with toxin (CCl4). For alanine transferase (Table 3) percentage protection were 0.5, 5.0 and 8.1% for group treated with 100mg/kg, 200mg/kg and 300mg/kg of the aqueous extract and injected intraperitoneally with toxin (CCl4). Percentage protection for total bilirubin (Table 4) were 3.67, 18.60 and 37.00% for groups treated with 100mg/kg, 200mg/kg and 300mg/kg of the aqueous extract and injected intraperitoneally with toxin (CCl4). Percentage protection for conjugated bilirubin (Table 5) were 19.0, 20.4 and 47.6% for groups treated with 100mg/kg, 200mg/kg and 300mg/kg of the aqueous extract and injected intraperitoneally with toxin (CCl4).

S/No.	Phytochemicals	Aqueous Extract
		Results
1.	Carbohydrates	++
2.	Phenolic Compounds (Anthraquinone)	–
3.	Tannins	+++
4.	Phlobatannins	–
5.	Saponins	+
6.	Cardiac glycosides	+
7.	Terpenoids/Steroids	+
8.	Flavonoids	++
9.	Alkaloids	++

Table 1: Phytochemical Screening Results of *S. latifolius* root bark

Key:
+ Present in low concentration.
++ Present in moderate concentration
+++ Present in high concentration.
- Absent.

Treatment group	Extract Dose	Mean no. of inhibition in 24Hrs	Percentage protection
Control	–	56.67+ 4.04	–
CCl4	3mg/kg	216.33±0.58c	0.00
Extract + CCl4	100mg/kg	214.67±1.15c	0.67
Extract + CCl4	200mg/kg	176.67±1.15b	18.33
Extract + CCl4	300mg/kg	126.67±1.15a	44.33

Table 2: Effect of *S. latifolius* aqueous root bark extract on mean Aspartate Amino Transferase (AST).

Mean + SD based on five observations.
Results with different superscript (a, b, c) on the same row are significantly different (P< 0.05).

Treatment group	Extract Dose	Mean no. of inhibition in 24Hrs	Percentage protection
Control	–	17.67+ 1.15	–
CCl4	3mg/kg	127.33±1.15c	0.0
Extract + CCl4	100mg/kg	126.67±1.15c	0.5
Extract + CCl4	200mg/kg	121.00±2.00b	5.0
Extract + CCl4	300mg/kg	117.33±1.53a	8.1

Table 3: Effect of *S. latifolius* aqueous root bark extract on mean Alanine Amino Transferase (ALT)
Mean + SD based on five observations.
Results with different superscript (a, b, c) are significantly different (P< 0.05)

Treatment group	Extract dose	Mean no. of inhibition in 24hrs	Percentage protection
Control	–	50.00+ 1.00	–
CCl4	3mg/kg	90.00±1.00d	0.00
Extract + CCl4	100mg/kg	86.70±1.53c	3.67
Extract + CCl4	200mg/kg	73.30±0.58b	18.60
Extract + CCl4	300mg/kg	56.70±1.53a	37.00

Table 4: Effect of *S. latifolius* aqueous root bark extract on mean Total Bilirubin (TB) _ 10
Mean + SD based on five observations.
Results with different superscript (a, b, c d) are significantly different (P<0.05).

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Treatment group	Extract dose	Mean no. of inhibition in 24Hrs	Percentage protection
Control	–	30.00+ 1.00	–
CCl4	3mg/kg	70.00±1.00c	0.0
Extract + CCl4	100mg/kg	56.70±1.15b	19.0
Extract + CCl4	200mg/kg	55.70±0.58b	20.4
Extract + CCl4	300mg/kg	36.70±0.58a	47.6

Table 5: Effect of *S. latifolius* aqueous root bark extract on mean Conjugated Bilirubin (CB) _ 10 Mean + SD based on five observations.
Results with different superscript (a, b, c) are significantly different (P<0.05)

Discussion

Carbon tetrachloride (CCl4) is one of the most commonly used hepatotoxins in experimental studies of liver diseases (16). The first metabolite of CCl4; trichloromethyl free radical, is believed to initiate the biochemical processes leading to oxidative stress, which is the direct cause of many pathological conditions such as diabetes mellitus, cancer, hypertension, kidney damage, liver damage and death(17 – 19). These activated radicals bind to micromolecules and reduce lipids peroxidative degradation of polyunsaturated fatty acids. This leads to the formation of lipid peroxides which in turn gives products like malonylaldehyde that cause damage to membranes (20). This lipid peroxidative degradation of biomembranes is one of the main causes of hepatotoxicity by CCl4. This is usually evidenced by a rise in the serum marker enzymes of the liver namely ALP, AST and ALT.

In this study, the aqueous root bark extracts 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. The terminal event in the attack on the liver by CCl4 is the production of highly reactive radicals leading to lipid peroxidation and inhibition of calcium pump of the microsome giving rise to liver lesions (21). However, antioxidants inactivate free radical reactions initiated by CCl4 by either blocking the initiation of free radicals or scavenge free radicals and terminate free radical damage(22). Photochemical such as flavonoids are potent antioxidant because of their ability to scavenge hydroxyl radicals, superoxide and lipid peroxy radicals (23, 24).

In the study, an elevation in the levels of the end products of lipids peroxidation of the liver of the rats treated with CCL4 was observed. The increase in AST and ALT suggests enhanced lipid peroxidation giving rise to liver damage and failure of the antioxidant defense mechanism to prevent formation of excessive free radicals.

The group treated with *S.latifolius* root bark inhibits the changes ($p<0.05$) at high concentrations (300mg/kg > 200mg/kg >100mg/kg body weight respectively), compared to CCl4 treated group, hence it is possible that the mechanism of hepatoprotection of *S latifolius* is due to its antioxidant effect.

Histopthological studies showed normal integrity of the hepatocytes in the control group. The liver of rats treated with 100mg/kg body weight of aqueous extract plus CCl4, showed severe congestion, necrosis, calcification of hepatocytes, mononuclear cell infiltration, with areas of vacoulation and interstitial haemorrhage; while rats treated with 300mg/kg body weight of the aqueous extract plus CCl4 shows minimal blood congestion, reduction in steatosis, fatty degeneration and peripheral hyalinazation of the hypatoctes of the liver tissue. This adds credence that physiologic recovery preceded obvious histological changes and the results may suggest that diets supplemented with *S.latifolius* will improve hepatoprotection against oxidative liver damage.

Total and conjugated bilirubin levels were significantly increased in the CCl4 treated rats as compared to control. Administration of the extracts (100mg/kg, 200mg/kg and 300mg/kg body weight) leads to a significant reduction ($P < 0.05$) in their levels. Report from Tirkey et al (25) also showed a marked rise in bilirubin levels after CCl4 administration.

Bilirubin, is a major product of haemoglobin breakdown which rises when there is liver injury or damage; leading to the discoloration of the skin and eyes known as jaundice (26).

Elevation of total bilirubin which results from decreased uptake and conjugation of bilirubin by the liver is caused by liver cell dysfunction, while increased levels of direct or conjugated bilirubin is due to decreased secretion from the liver or obstruction of the bile ducts (26). Reduction of CCl4 induced increases in total and conjugated bilirubin by *S. latifolius* extract further show its protective effect against CCl4 induced liver toxicity. The extract perhaps protects the liver cell from damage, thereby enhancing bilirubin uptake and conjugation by the liver and subsequent secretion into the bile ducts. These reports from our study show that the ethanol extract of *S. latifolius* possess antihepatotoxic activity as demonstrated by its reduction of CCl4 induced elevations in the levels of ALT, AST, total and conjugated bilirubin. This hepatoprotective effect suggests that the plant may also possess antioxidant properties that helped to combat the 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 (27). Phytochemical result from this study revealed the presence of flavonoids, which has been reported to protect against toxicity induced by environmental toxicants (28) such as CCl4. Alkaloids present in plants are known to have numerous beneficial pharmacological effects(10,29). Some bioflavonoids have been reported to possess antioxidant properties which help to combat free radical induced oxidative stress (24, 29). The chemoprotective activities of flavonoids are related to their ability to inhibit peroxidative damage caused by environmental toxicants.

Several secondary metabolites have been shown to have wide ranges of antimicrobial activities (30). In a study, by Kouassi Maximin et al(2007), *S. latifolius* was one of sixty-four extracts assayed from twenty-one plants used in the Malian traditional medicine that were found to be significantly active against the intracellular forms of Leishmania major (31). Similarly Abreu et al (2001), showed that *S.latifolius* displayed antiplasmodial activity against *P. falciparum* (32).

Interestingly, phytochemical screening of the current investigation has revealed that extracts from the root extract possess at least three to four of the following classes of secondary metabolites: flavonoids, terpenoids, tannins, alkaloids and saponin, hence may not only be hepatoprotective but useful as chemotherapeutic agents and need to be tested for protection against hepatic pathogens.

Conclusion

The results of this study show that the aqueous extracts of *S. latifolius* root park have hepatoprotective actions and suggest that flavonoids present in *S. latifolius* root bark may have a major role in this action. Future studies are planned to carry out quantitative analyses of the phytochemical parameters in order to have a more accurate picture about the possible mechanism of action of the components of these extracts. In addition, assaying for more enzymes such as gamma-glutamyltransferase (GGT), alkaline phosphatase and lactate dehydrogenase 5 (LDH5) isoenzymes would provide more clarification on the hepatoprotective effects of this plant.

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