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Effect of methanol extract of *Synsepalum dulcificum* pulp on some biochemical parameters in albino rats

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PEER REVIEW

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Comments

The paper is really interesting. The experimental design is accorded with the objectives. The methodology is modern and adequately used.
(Details on Page)

ABSTRACT

Objective: To determine the beneficial effects of the methanol extract of *Synsepalum dulcificum* on some biochemical parameters.

Methods: In this study, rats were orally administered (gavage) with methanol extracts at doses of 0 mg/kg (Group 1, as normal group), 100 mg/kg (Group 2), 200 mg/kg (Group 3) and 500 mg/kg (Group 4) body weight per day for 28 d.

Results: Acute toxicity study showed that the methanol extract was not toxic to rats up to 5 000 mg/kg. From the results, the 100 mg/kg doses of the extract significantly (P<0.05) reduced serum levels of bilirubin, low density lipoprotein, alanine aminotransferase and glucose after 14 d compared with those after 28 d. A significant difference (P<0.05) was observed in the malondialdehyde and serum protein concentration in Group 4 while glucose concentration decreased significantly (P<0.05) in Group 1 and Group 4 after 14 d compared with 28 d. The high density lipoprotein significantly increased (P<0.05) in Group 3.

Conclusions: The fruit has no negative effect on some biochemical parameters in albino rats.

KEYWORDS

Synsepalum dulcificum, Methanol extract, Biochemical parameters, Histology, Rats

1. Introduction

Synsepalum dulcificum (*S. dulcificum*) is an evergreen plant that produces small orange-like fruits[1]. The seeds are about the same size of coffee beans. The plant is also known as *Richardella dulcificum* (old name), miracle fruit, magic fruit, miraculous or flavor fruit[1]. The miracle fruit plant (*S. dulcificum*) produces fruits or berries that, when eaten, cause sour foods (such as lime and lemon) consumed later to taste sweet[2].

The berry contains an active glycoprotein molecule, with some trailing carbohydrate chain called miraculin[3]. When the fleshy part of the fruit is eaten, the molecule binds to the tongue's taste buds, causing sour foods to taste sweet. While the exact cause of this change is unknown, one theory is that the glycoprotein, miraculin works by distorting the shape of sweetness receptors so that they become responsive to acids, instead of sugar and other sweet things[4]. This effect can last for 10 min to 2 h[2].

In tropical West Africa where this specie originates, the fruit pulp is used to sweeten palm wine[2]. Attempts have been made to make a commercial sweetener from this fruit with an idea of developing it for patients with diabetes[2]. Fruit cultivators also reported a small demand from cancer patients, because the fruit allegedly counteracts a metallic taste in the mouth that may be one of the many side effects of chemotherapy[4]. This claim has not been

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researched scientifically. In Japan, miracle fruit is popular among patients with diabetes and dieters[4].

Previous studies on this plant were mainly on its nutritional and medicinal potentials. No former studies have been reported on the toxicity and the biochemical effects of the plant. The major objective of this work is to determine the beneficial effects of the plant extract on some biochemical parameters such as liver function enzymes, kidney function parameters, serum total protein, serum total albumin, blood glucose levels, serum lipid profile and lipid peroxidation of rats as our animal model for the research.

2. Materials and methods

2.1. Plant materials

S. dulcificum were collected from Uke Town in Anambra State, Nigeria and was identified by Mr. Alfred Ozioko of Bioresource and Development Conservative Programme, Nsukka, Nigeria through comparison with voucher specimen presented in their herbarium. The fruit was cleaned, washed and the pulp was removed from the fruit.

2.2. Experimental animals

Adult albino rats of about 12 weeks old were obtained from the Faculty of Biological Science Animal House, University of Nigeria, Nsukka, Enugu State, Nigeria. The animals were kept under standard conditions for 7 d with free access to water and food before starting the experiment. The animals were housed in separate standard cages and provided with palletized feed (Grand Cereals and Oil Mills Limited, Nigeria) and water *ad libitum* at room temperature. Albino rats with average weight of (20.50±4.27) g were used in determination of median lethal dose (LD_{50}). The procedures in this study were performed in accordance with the National Institute of Health Care Guide for the Care and Use of Laboratory Animals and approved by the ethics committee of the institution. All efforts were made to minimize suffering of animals and to reduce the number of animals used in the experiment.

2.3. Preparation of methanol extract

The air dried pulp (1 000 g) was soaked for 12 h in methanol (3 L) at room temperature. The residue was extracted 3 times after vacuum filtration. All solvent was evaporated under vacuum and extract was then concentrated to dryness to yield a residue which was stored at 20 ℃ until use.

2.4. Animal study

A total of 24 albino rats were used. They were acclimatized for one week, and all rats had access to commercial poultry feed and water. They were randomly distributed into 4 groups with 6 animals in each group. The study lasted for 28 d.

The experimental groups were as follows: Group 1 (control): rats were administered with 0.2 mL of normal saline (0.9%, NaCl); Group 2: rats were administered with 100 mg/kg of *S. dulcificum* pulp extract; Group 3: rats were administered with 200 mg/kg of *S. dulcificum* pulp extract; Group 4: rats were administered with 500 mg/kg of *S. dulcificum* pulp extract.

The first phase of the animal experiment lasted 14 d. On the 15th day, blood samples (a quantity, 2 mL) were collected from 3 animals in each group, emptied into ethylenediaminetetraacetic acid bottles and mixed thoroughly for analysis of biochemical parameters such as liver function enzymes, kidney function parameters, serum electrolyte levels, serum total protein, serum total albumin, blood glucose levels, serum lipid profile and lipid peroxidation. Thereafter, the rats were anaesthetized with chloroform, sacrificed and then their organs removed for histopathological studies.

The experiment continued with the remaining animals in the groups for another 14 d. Blood samples were collected from the remaining animals via ocular puncture on the 29th day and used for same biochemical analyses. They were anaesthetized with chloroform and sacrificed. The internal organs were removed and used for histopathological studies as well.

2.5. Acute toxicity studies and lethal dose (LD_{50}) test

Acute toxicity studies of the methanol extract of *S. dulcificum* pulp were carried out by the method of Lorke[5]. A total of 22 albino rats were used for the determination. The studies were conducted in two phases. In phase I, three groups of 9 rats were orally administered with the extract daily (10 mg/kg, 100 mg/kg, and 1 000 mg/kg respectively for each group) by means of polythene cannula. The rats were monitored for 24 h for mortality and general behaviour. In phase II, after 24 h, 3 rats each were given different concentrations (1 600 mg/kg, 2 900 mg/kg and 5 000 respectively) orally, by means of polythene cannula based on the findings from phase I. The fourth rats received distilled water which served as control. The rats were monitored for 24 h for lethality and general behaviour.

2.6. Statistical analysis

One way analysis of variance and Fisher's least significant difference were used to separate the means. Results were expressed as mean±SD of all parameters determined.

3. Results

The effect of the methanol extract of the pulp of *S. dulcificum* on some biochemical parameters was investigated to determine the

beneficial effects.

3.1. Acute toxicity (LD_{50})

The results of the acute toxicity test shows that the extract was not toxic to rats at the tested concentrations (Table 1).

Table 1
Result of the acute toxicity (LD_{50}) test for the methanol pulp extract of *S. dulcificum*.

Groups		Numbers of animals	Dosage (mg/kg)	Mortality
Phase I	Group 1	3	10	0/3
	Group 2	3	100	0/3
	Group 3	3	1000	0/3
Phase II	Group 1	1	1600	0/1
	Group 2	1	2900	0/1
	Group 3	1	5000	0/1
	Control	1	-	0/1

3.2. Biochemical parameters

Table 2 shows the effect of the methanol pulp extract on some biochemical parameters of rats. Significant difference (P<0.05) in alkaline phosphatase (ALP) concentration was observed at the end of the 28 d in Group 2 (low dose) compared with Group 1. A significant difference (P<0.05) was observed only in Group 2 between the first initial 14 d of experiment and the final 14 d of experiment. Only Group 2 (low doses) significantly decreased (P<0.05) the serum concentration of alanine transaminase (ALT) at the end of the 28-day study compared with Group 1. A significant difference (P<0.05) in aspartate transaminase (AST) concentration was observed only in Group 2 between the two phases of the experiment (14 d and 28 d). A decrease in bilirubin concentration was observed in all the test groups when compared with Group 1 after 28 d of the experiment. Table 2 shows time and dose dependent increase in the protein concentration with increase in dose ranges across the test groups during the first phase (14 d) of administration of *S. dulcificum* extract compared to the control. Protein concentration in the blood increased significantly (P<0.05) in all the test groups (Groups 2, 3 and 4) when compared with the animals in Group 1 after 28 d experiment. A significant difference (P<0.05) in the protein concentration was observed between the first (14 d) and the second (28 d) phase of feeding with the pulp extract. A significant decrease (P<0.05) was observed in the concentration of cholesterol in the entire test groups in the second phase (28 d) compared with the first phase (14 d). Table 2 shows time and dose dependent increase in the concentration of high density lipoprotein (HDL) with increase in dose ranges across the test groups during the first phase (14 d) of administration of *S. dulcificum* extract compared to the control. There were both time and dose dependent decrease in low density lipoprotein (LDL) concentration across the groups. The LDL concentration of the test groups showed insignificant difference (P>0.05) compared with the control after the 14-day experiment. The second phase (28 d) of the experiment also showed a dose and time dependent relationship across the groups. There was a significant difference regarding the LDL concentration (P<0.05) in the test groups compared with the control group after the 28 d experiment. Similarly, when LDL was determined after the duration of 28 d, a significant difference (P<0.05) was observed across the groups compared with similar groups recorded after the 14 d administration. The triacylglycerol (TAG) concentration in the sera differed significantly (P<0.05) only in Groups 3 and 4 compared with Group 1 after the 14 d experiment. A significant difference (P<0.05) was observed in the concentration of malondialdehyde (MDA) in the entire test groups in the second phase (28 d) compared with the first phase (14 d). After 28 days of administration of *S. dulcificum* extract, the concentration of urea significantly decreased (P<0.05) in Group 2 compared with Group 1. The decrease in

Table 2
Levels of some biochemical parameters in rats administered methanol extract of *S. dulcificum* in albino rats.

Parameters	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	14 d after the experiment, n=3				28 d after the experiment, n=3			
ALP	63.330±8.080	58.330±9.070	57.670±4.930	62.330±4.930	53.670±7.020	31.670±3.510*	51.000±5.200	50.670±3.060
ALT	49.330±2.310	42.000±0.000	44.000±12.490	44.000±4.000	9.330±4.160	16.670±4.160*	24.670±3.060	24.000±5.290
AST	39.000±6.560	34.330±2.080	35.330±3.060	38.000±2.000	41.000±10.000	33.000±6.080	40.000±1.000	39.000±5.290
Bilirubin	0.700±0.240	0.610±0.110	0.670±0.190	0.670±0.200	0.440±0.110	0.190±0.010*	0.250±0.110	0.260±0.110
Protein	5.000±0.200	5.270±0.750	5.430±0.310	5.570±0.600	5.470±0.450	6.100±0.260	6.170±0.060	6.430±0.400*
Albumin	3.600±0.600	3.730±0.450	3.800±0.360	3.870±0.150	3.630±0.310	3.700±0.100	3.700±0.260	3.770±0.320
Globulin	1.400±0.800	1.530±1.200	1.630±0.670	1.700±0.660	1.830±0.150	2.400±0.200	2.470±0.230	2.670±0.720
Cholesterol	3.530±0.210	3.400±0.460	3.330±0.210	3.370±0.120	3.030±0.510	2.930±0.310	2.830±0.320	2.830±0.550
HDL	0.750±0.090	0.810±0.020	0.830±0.130	0.850±0.050	0.910±0.130	0.996±0.330	1.080±0.080*	1.170±0.130
LDL	2.170±0.250	2.000±0.500	1.800±0.260	1.900±0.100	1.670±0.780	1.400±0.640*	1.090±0.350	0.850±0.540
TAG	1.110±0.080	1.140±0.100	1.340±0.120	1.540±0.080	1.250±0.230	1.180±0.140	1.410±0.140	1.610±0.240
MDA	7.200±0.170	7.100±0.610	7.030±0.450	6.830±0.720	8.930±1.020	8.200±1.210	7.870±1.310	9.330±0.120*
Urea	24.670±4.510	21.670±2.080	23.670±1.150	23.330±3.510	26.670±4.930	18.670±1.150	23.000±2.000	25.330±4.510
Creatinine	0.900±0.100	0.700±0.100	0.670±0.150	0.6330±0.060	0.930±0.210	0.630±0.580	0.800±0.100	0.900±0.200
Na^+	111.000±5.300	116.330±4.160	100.670±4.040	77.000±6.560	118.000±3.000	113.670±15.010	131.330±13.800	147.000±20.810*
K^+	5.600±0.200	5.270±0.760	5.400±0.700	4.600±0.520	2.740±0.360*	1.570±0.720*	2.350±0.410*	2.590±0.620*
Cl^-	104.670±2.310	106.330±1.530	107.330±1.530	107.670±1.150	95.670±2.520*	99.670±0.580*	98.000±4.000	92.330±3.210*
Glucose	261.000±32.740	231.000±29.10	136.000±27.220	114.330±8.020	100.330±3.510*	82.670±7.020*	74.330±10.500	64.330±4.040*

Values on the Day 28 followed by superscript letters differ significantly (*P<0.05) from the values on Day 14 when compared.

concentration of creatinine was found to be significant (P<0.05) only in Group 2 compared with Group 1 after the 14 days of experiment. There was time and dose dependent decrease in the blood glucose concentration with decrease in dose ranges across the test groups during the first phase (14 d) of administration of *S. dulcificum* extract compared to the control. Group 3 and 4 significantly decreased (P<0.05) the blood glucose level compared with Group 1 at the end of the 14 d experiment. During the second phase of the experiment, the blood glucose of Groups 2 and 4 significantly decreased (P<0.05) the blood glucose level compared with Group 1 at the end of the 28 d experiment. The mean blood glucose concentration of Groups 2 and 4 were significantly decreased (P<0.05) at the end of the 28 d experiment compared with that of the 14 d experiment.

3.3. Histopathology

3.3.1. Histopathology of the liver after the 14 d administration of S. dulcificum extract

Photomicrographs of liver sections of rats 14 d post administration with *S. dulcificum* methanol extract showed normal liver architecture [central vein (CV), sinusoids (black arrow), plates of hepatocytes (white arrow)] (Figure 1).

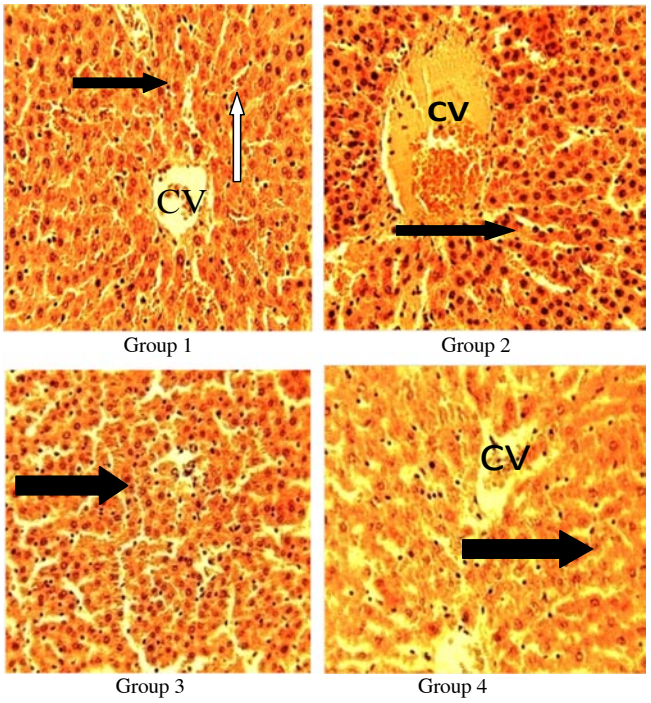

Figure 1. Photomicrographs of liver sections of rats 14 d post administration with *S. dulcificum* methanol extract.

3.3.2. Histopathology of the liver after the 28 d administration of S. dulcificum extract

Photomicrograph of liver sections of rats 28 d post administration with *S. dulcificum* methanol extract showed mild hepatocyte degenerations in Group 4 (arrows). Sections from Groups 1, 2 and 3 had no observable histologic changes (Figure 2).

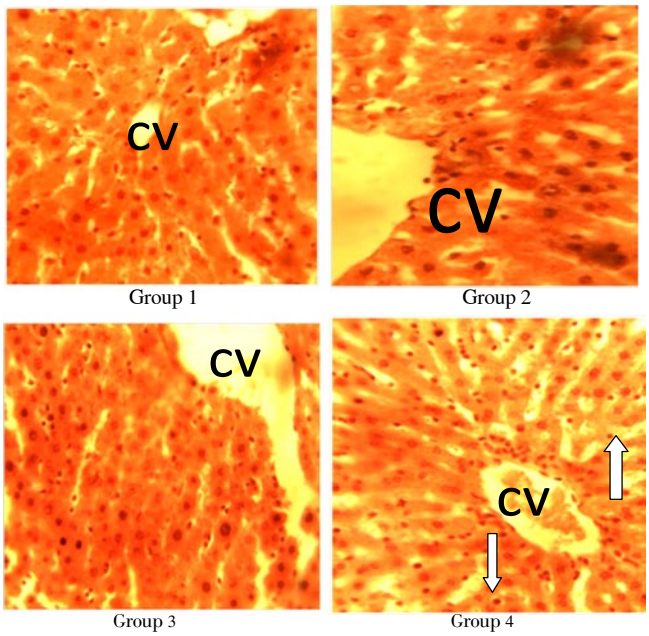

Figure 2. Photomicrographs of liver sections of rats 28 d post administration with *S. dulcificum* methanol extract.

3.3.3. Histopathology of the kidney after the 14 d administration of S. dulcificum extract

Photomicrograph of kidney sections of rats 14 d post administration of *S. dulcificum* extract showed normal glomerulus (G) and renal tubules (arrow) in Groups 1, 2, 3 and 4.

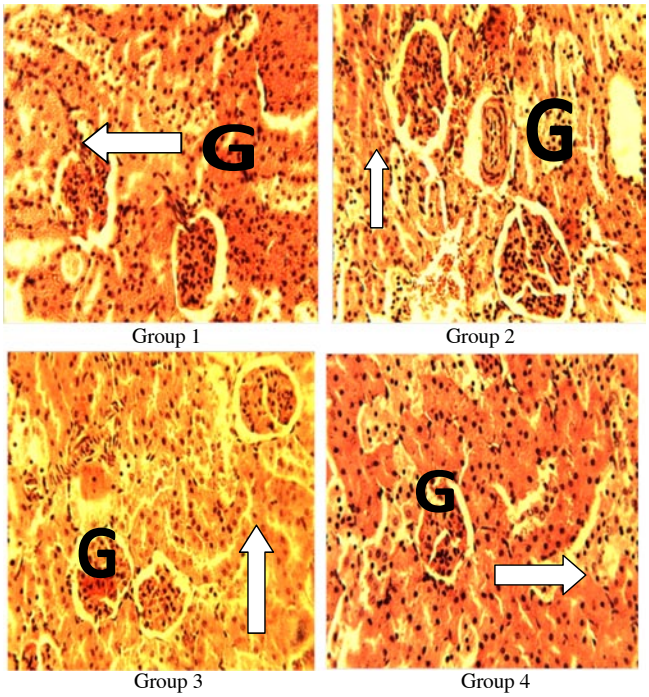

Figure 3. Photomicrograph of kidney sections of rats 14 d post administration of *S. dulcificum* extract.

3.3.4. Histopathology of the kidney after the 28 d administration of S. dulcificum extract

Photomicrograph of kidney sections of rats 28 days post administration with *S. dulcificum* methanol extract showed that all the groups had no observable histologic changes with the normal

glomeruli (G) and renal tubules (arrow).

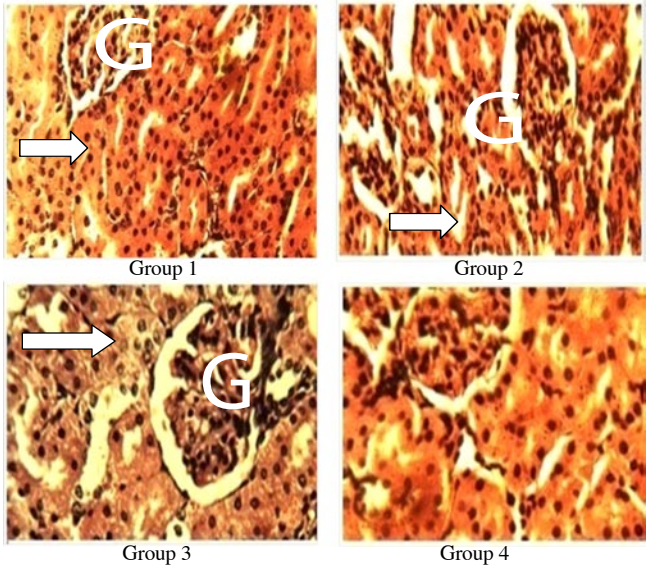

Figure 4. Photomicrograph of kidney sections of rats 28 d post administration of *S. dulcificum* extract (H and E, 400×).

3.4. Mean body weights of animals

Table 3 shows that there was initial increase in weight in the animals at the end of the experimental period.

Table 3

The mean body weight (g) of rats administered with different doses of *S. dulcificum* methanol pulp extract.

Day	Group 1	Group 2	Group 3	Group 4
0 day	104.72	84.72	82.02	98.70
14th day	138.47	136.48	150.33	133.70
28th day	149.90	141.67	170.77	164.67

4. Discussion

Safety profile assay of the extract using rats revealed an oral median lethal dose (LD_{50}) greater than 5 g/kg body weight which is the maximum allowable dose by the Organization for Economic Co-operation and Development guideline 423 for testing of chemicals[6]. This result suggests that the pulp is relatively non-toxic since LD_{50} above 5 g/kg body weight is of no practical significance[5]. This is expected considering that the pulp is edible.

Since serum total proteins, albumins and globulins are generally influenced by total protein intake[7], the results obtained indicate nutritional adequacy of the dietary and the extract proteins. Abnormal serum albumin usually indicates an alteration of normal systemic protein utilization[8]. Awosanya *et al.* have demonstrated the dependence of blood protein on the quality and quantity of protein source[9]. Serum albumin is frequently utilized as an index of the hepatocyte's ability to carry out synthetic function. Serum albumin does not change in mild liver injury but readily declines in the face of submassive liver necrosis[10]. For the duration of administration of the pulp extract, the results obtained for serum total protein, albumin and globulin suggest that *S. dulcificum* pulp extract did not diminish the protein synthetic capacity of the liver. The total protein, albumin and globulin level may decrease due to liver dysfunction, malnutrition and malabsorption, diarrhoea, nephrosis, alpha-1-antitripsin deficiency, acute hemolytic anaemia and hypogammaglobulinemia/agammaglobulinemia and even loss through the urine in severe kidney disease and pregnancy. Prolonged destruction of the hepatic cells results in more hepatic releases to exacerbate hepatic dys-function and causes decrease in the serum levels of total protein, albumin and globulin.

LDL cholesterol is often designated bad cholesterol since high level of it in the plasma is linked with increased deposition of cholesterol in the arterial walls[11]. HDLs serve as acceptors of cholesterol from various tissues. They promote the removal of cholesterol from cells and its secretion into the bile by the liver[11]. The best single indicator of the likelihood of developing atherosclerotic heart disease is not total plasma cholesterol but rather the ratio of plasma LDL cholesterol to plasma HDL cholesterol. The value of crude fibre in the pulp may contribute to a reduction in the incidence of certain diseases, such as colon cancer, coronary heart disease, high blood pressure, obesity and other digestive disorders[12-16]. The effect of 14 d administration of *S. dulcificum* pulp methanol extract at different concentrations on lipid profile in rats showed no significant difference (P>0.05) across the test groups. The trend of result obtained on the lipid profile following the 28 d daily administration of methanol extract of *S. dulcificum* pulp is similar to that of the 14 d administration of the extract. The reduction in serum total cholesterol concentration following the repeated and prolonged administration of the extract is in agreement with the report of Obianime and Aprioku[17]. This will normally suggest a beneficial effect in pathologic conditions. The slight increase in TAG as observed in the results may predispose the liver to pathological risk[18]. Increase in TAG following administration of the extract could be due to decrease in lipolysis caused by the extract. LDLs transport cholesterol from its site of synthesis in the liver to the various tissues and body cells where it is separated and used by the cells. On the other hand, HDLs transport excess or unused cholesterol from the tissue back to the liver where it is broken down to bile acids and then excreted, which make HDL beneficial to health. Therefore, the increase in HDL concentration may impact on the function of the liver positively.

The reduction in cholesterol levels at the different doses may have contributed to the increase in level of HDL observed in the animals across the groups and at the two phases of animal study, about 30% of blood cholesterol is carried in the form of HDL cholesterol. HDL cholesterol can remove cholesterol from antheroma within arteries and transfer it back to the liver for its excretion or reutilization, thus high level of HDL-cholesterol protects against cardiovascular diseases[19]. The observed increase in HDL-cholesterol concentration upon the administration of the extract indicates that the extract doses have HDL-cholesterol boosting effect; this effect is concentration dependent for the methanol extract in this study.

LDL cholesterol transports cholesterol to the arteries where they

can be retained in the arteria proteoglycans starting the formation of plagues. LDL cholesterol possesses the risk of cardiovascular diseases when it invades the endothelium and becomes oxidized and the oxidized form is more easily retained by the proteoglycan, thus increase of LDL cholesterol is associated with artherosclerosis, heart attack, stroke, peripheral vascular disease[20]. The importance of this LDL cholesterol-lowering effect is that the extract may aid in the reduction or prevention of cardiovascular diseases.

Interest in oxidative stress with relation to the development of disease has gained large attention during the last decade. Lipid peroxidation is a major mechanism of cell injury in tissues and organs subjected to oxidative stress that has been studied extensively[21]. It is thought to be an important factor in the pathophysiology of a number of diseases and in the process of ageing. The control of lipid peroxidation is of special significance in biology because of its particular importance in relation to membrane damage[22]. After 28 d of *S. dulcificum* extract administration, there was no significant alteration (P>0.05) in the MDA concentration of test groups administered with 100 mg/kg, 200 mg/kg and 500 mg/kg extract compared with the control group. This indicates that the ability of the *S. dulcificum* methanol extracts can protect against lipid peroxidation, a major mechanism of cell injury in organisms exposed to oxidative stress when consumed over a short period of time. Similar observations have been reported by Arulselvan and Subramanian[23], and Ugochukwu *et al.*[24] on the respective effects of *Murraya koenigii* and *Gongronema latifolium* on diabetic rats.

Failure to maintain blood glucose in the normal range leads to conditions of persistently high (hyperglycaemia) or low (hypoglycaemia) blood sugar[25]. Groups 3 and 4 significantly decreased (P<0.05) the blood glucose level compared with Group l at the end of the 14 d experiment. Similarly, the Groups 2 and 4 significantly decreased (P<0.05) the blood glucose level compared with Group 1 at the end of the 28 d experiment. This finding is suggestive of a hypoglycaemic effect and this effect may aid in lessening the metabolic burden that would have been placed on the liver. The glucose-lowering effect of the extract may be ascribed to modifications in glucose uptake in the intestine. From the results of this investigation, hepatocellular function-enhancing effect of the methanol extract of *S. dulcificum* pulp is reported. Generally, analyses of the activities of some basic liver function enzymes in the plasma or serum can be used to indirectly access the integrity of tissues after being exposed to certain pharmacological agent(s). These enzymes are usually biomarkers whose plasma concentrations above the homeostatic limits could be associated with various forms of disorders which affect the functional integrity of the liver tissues. Preliminary phytochemical screening carried out in this study indicated that *S. dulcificum* pulp contains flavonoids, saponins, tannins and alkaloids. These phytochemicals are known to perform several general and specific functions in plants, and may exhibit different biochemical and pharmacological actions in different species of animals when ingested. These actions range from cell toxicity to cell protective effects[26].

A significant decrease (P<0.05) was observed in ALP and ALT concentrations at the end of the 28 d feeding in the groups fed with 100 mg/kg of the extract compared with the control. The value of the liver function test depends on the specificity for damage as well as their sensitivity[27,28]. Although serum levels of both AST and ALT become elevated when disease processes affect the liver integrity, ALT is the more liver-specific enzyme and therefore generally more specific to changes in activity levels than AST[28,29]. In the present study AST and ALT concentrations were decreased at the two phases of the experiment (14 and 28 d) and dose-dependently; therefore it suggests that the extract had no significant influence on the liver function. Also, AST is highly concentrated in several tissues including the heart, muscle, liver, skeletal muscle and kidney while ALT has its highest concentration in the liver[27,28,30-34]. Therefore, measure of ALT in serum is of greater diagnostic specificity in confirming or excluding liver damage. Since the decrease in ALT in this study was significant after 28 d administration, there is no likelihood of liver damage by the methanol pulp extract of *S. dulcificum*.

A significant decrease (P<0.05) was observed in bilirubin concentration in Groups 2, 3 and 4 compared with Group 1 after the 28 d study. The decrease in bilirubin concentration which was significant after the second phase of the experiment was caused by increasing doses of the extract. Increase in bilirubin concentrations may be caused by liver damage, excessive haemolytic destruction of the erythrocytes, obstruction of the biliary tract (obstructive jaundice) and in drug-induced reactions[35-37]. However, if the AST and ALT values are normal as in the present study, the diagnosis of hepatocellular damage cannot be confirmed[36].

A statistically significant decrease (P<0.05) in ALP value as obtained in the group administered with 100 mg/kg of extract after the 28 d study was not of much clinical significance[28,34]. Even if there had been an elevation in ALP upon extract administration, it still could not have confirmed liver damage[36]; ALP and AST originates from different tissues such as the liver, bones, intestine and placenta. All these may show that the effect of the methanol extract of *S. dulcificum* pulp on the rats in this study was not of toxicity.

The kidney plays an important role in removal of metabolic wastes from the blood stream. Therefore, its functionality can be assessed among many others by determining the serum concentration of excretory constituents[38]. Measuring creatinine is a simple test and creatinine is the most commonly used indicator for renal function[39]. The decrease in concentration of creatinine was found to be significant (P<0.05) only in the groups with administered 100 mg/kg compared with the control after the 14 d experiment. The decrease in serum urea concentration of the extract was suggestive that urea may be converted to other products undetectable by the direct method of urea determination used in this study. After 28 days of administration of *S. dulcificum* extract, the concentration of urea significantly decreased (P<0.05) in Group 2 compared with the

control. Urea concentration is elevated in kidney damage, excessive protein intake and low fluid intake[40]. The normal creatinine levels of rats fed both control and test diets suggested that these diets did not alter protein metabolism in the rats[40]. Urea and creatinine levels are basically used to assess kidney function status. The normal levels of urea and creatinine of rats fed with *S. dulcificum* methanol extract strongly indicated that the diets have no adverse effects on kidney functions.

Histopathological examination of kidney sections of rats following the 14 d and 28 d post administration of *S. dulcificum* methanol extract showed that Groups 1, 2, 3 and 4 have a normal glomerulus and renal tubules. This suggested that the methanol extract did not have any negative effect on the kidneys at the tested concentrations and durations of the study. Histopathological examination of liver sections of rats 14 d post administration with *S. dulcificum* methanol extract showed normal liver architecture. In addition, in this study, a non-significant effect of the methanol extract of *S. dulcificum* pulp on the morphological architecture of the liver tissues is reported.

The mean body weight change in rats after 14 d and 28 d following administration of 100 mg/kg, 200 mg/kg and 500 mg/kg of *S. dulcificum* methanol pulp extract are presented. A general increase in physical activities, food and water intake were observed for all the animals during the feeding experiment. The increased weight could be due to increased feed and water intake observed all through the experimental period, which suggest that the extract could stimulate appetite.

In conclusion, the findings indicate that the fruit which is popularly eaten as a sweetener and documented to be rich in important food properties when compared with other fruits, has no negative effect on some biochemical parameters in albino rats.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Plants were used as medicine from ancient times all over the world. *S. dulcificum* produces a berry that contains a bioactive glycoprotein (among other bioactive compounds) called miraculin which is worth to be studied because of its probably use as sweetener for diabetic patients. Previous studies on this plant were mainly on its nutritional and medicinal potentials. The purpose of this work was to determine its influence on biochemical parameters such as liver function enzymes, kidney function parameters, serum total protein, serum total albumin, blood glucose levels, serum lipid profile and lipid peroxidation of plant extracts in rats.

Research frontiers

In the present research, the authors performed a detail analysis of the parameters to demonstrate the possible toxicity of the methanolic extracts of *S. dulcificum* pulp. The studies of acute toxicity of the extract were made with experimentation animals. Also, they showed the effect of different concentrations of the extract on some biochemical parameters.

Related reports

Although there are other studies on *S. dulcificum,* it is worth to take into account that the work of these authors made the relation between the traditional use of the plant as medicine and the scientific validation of its properties.

Innovations and breakthroughs

The paper demonstrates the possible use of *S. dulcificum* for the diabetes treatment for the first time. The absence of toxicity of the extract was demonstrated by *in vivo* experiments. Many biochemiscal parameters were analysed. I believe that the orientation of the research is important and well done.

Applications

As what was mentioned in the paper, extracts of *S. dulcificum* are important to develop medicines to alleviate the diabetes.

Peer review

The paper is really interesting. The experimental design is accorded with the objectives. The methodology is modern and adequately used.

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