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

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Synsepalum dulcificum **is believed to have medicinal and nutritional potentials. The objective of this study was to determine the beneficial effects of the methanol extract of the plant on some biochemical parameters such as liver function tests (alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate transaminase (AST), total bilirubin, total protein, albumin and globulin), blood glucose levels and histology of the liver in albino rats. In this study, rats were orally administered (gavage) methanol extracts of *S. dulcificum* pulp at doses of 0 (as normal group), 100 mg/kg (Group 2), 200 mg/kg (Group 3) and 500 mg/kg (Group 4) body weight/day for 14 and 28 days. Acute toxicity study showed that the methanol extract is not toxic to mice up to 5000 mg/kg. From the results, the 100 mg/kg doses of the extract significantly ($p<0.05$) reduced serum levels of bilirubin, ALT and glucose after the 14-day study when compared with the 28-day study. However, no significant difference ($p<0.05$) was observed across the groups in their serum ALP, AST, albumin and globulin levels on the 14th day when compared with the 28th day. A significant difference ($p<0.05$) was observed in the serum protein concentration in the 500 mg/kg test group while glucose concentration decreased significantly ($p<0.05$) in the 100 and 500 mg/kg test group after the 14 day study when compared with the 28 day study. Histopathological examination shows normal liver architecture across the groups at 100, 200 and 500 mg/kg.**

Key words: *Synsepalum dulcificum*, methanol extract, biochemical parameters, histology, rats.

INTRODUCTION

Synsepalum dulcificum is an evergreen plant that produces small orange like fruits (Duke and Ducellier, 1993). The seeds are about the same size as coffee beans. The plant is also known as *Richardella dulcificum* (old name), miracle fruit, magic fruit, miraculous or flavor fruit (Duke and Ducellier, 1993). The miracle fruit plant (*S. dulcificum*) produces fruits or berries that when eaten, causes sour foods (including lime and lemon) consumed

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later to taste sweet (Joseph et al., 2009). The berry contains an active glycoprotein molecule with some trailing carbohydrate chain called miraculin (Forester and Waterhouse, 2009). 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 (Aaron, 2006). This effect can last for 10 min to 2 h (Joseph et al., 2009).

In tropical West Africa where this specie originates, the fruit pulp is used to sweeten palm wine (Joseph et al., 2009). Attempts have been made to make a commercial sweetener from this fruit with an idea of developing this for patients with diabetes (Joseph et al., 2009). Fruit cultivators also report 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 (Aaron, 2006). This claim has not been researched scientifically. In Japan, miracle fruit is popular among patients with diabetes and dieters (Aaron, 2006).

Even though work has been done on the anti-diabetic effects of miracle fruit (Chen et al., 2006), there is really no information on this fruit as regards its nutritive and antinutritive composition. There is also paucity of information on its phytochemical constituents. Furthermore, no information has been documented on the effect of the extract on such biochemical parameters as liver function biomarkers, renal function biomarkers and lipid profile parameters. This study therefore aimed to determine the beneficial effects of the pulp methanol extract on some biochemical parameters such as liver function enzymes, serum total protein, serum total albumin, blood glucose levels and histology of the liver using rats as the animal model for the research.

MATERIALS AND METHODS

Plant materials

S. dulcificum were collected from Uke town in Anambra State, Nigeria and was identified by Mr. Alfred Ozioko, a taxonomist at the Bioresource and Development Conservative Programme (BDCP), Nsukka, Nigeria. The fruit was cleaned, washed and the pulp was removed.

Experimental animals

Adult albino rats of about 12 weeks old were purchased from the Faculty of Biological Science Animal House, University of Nigeria, Nsukka, Enugu State, Nigeria. The animals were kept under standard conditions for 7 days 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 Nigeria Limited) and water *ad libitum* at room temperature. Albino mice of average weight, 20.50±4.27 g were used in determination of median lethal dose (LD_{50}).

Preparation of methanol extract of *S. dulcificum*

The air dried pulp (1000 g) was soaked for 12 h in methanol (3 L) at room temperature. The residue was extracted with hot methanol under reflux 3 times (each 1500 ml) 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°C until use.

Experimental design

A total of twenty-four (24) albino rats were used. They were acclimatized for a period of one week, all rats had access to commercial poultry feed and water. They were randomly distributed into four (4) groups of six (6) animals each. The study lasted for 14 days for the 1st phase and 28 days for the 2nd phase. The experimental groups were as follows: Group I (Control): Rats were administered 0.2 ml of normal saline (0.9% NaCl); Group II: Rats were administered 100 mg/kg of *S. dulcificum* pulp extract; Group III: Rats were administered 200 mg/kg of *S. dulcificum* pulp extract; Group IV: Rats were administered 500 mg/kg of *S. dulcificum* pulp extract.

The first phase of the animal experiment lasted for fourteen (14) days. On the 15th day, blood samples (2 ml) were collected from three (3) animals in each group, emptied into EDTA bottles and mixed thoroughly for analysis of biochemical parameters such as liver function enzymes, serum total protein, serum total albumin and blood glucose levels. Thereafter, they were anaesthetized with chloroform, sacrificed and their liver removed for histopathological studies.

The experiment continued with the remaining animals in the groups for another fourteen (14) days. Blood samples were collected from the remaining animals via ocular puncture on the 29th day and used for same biochemical analyses; they were anaesthetized in chloroform and sacrificed. The internal organ, liver, were removed and used for histopathological studies as well.

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

Acute toxicity studies of the methanol extract of *S. dulcificum* pulp was carried out by the Lorke (1983) method. A total of twenty-two albino mice were used for the determination. The studies were conducted in two phases. In phase I, three groups of nine (9) mice per group were administered one dose of the extract daily using the oral route, by means of polythene cannula, 10, 100 and 1000 mg/kg, respectively, for each group. The mice were monitored for 24 h for mortality and general behaviour. In phase II, after 24 h, three (3) mice each were given different concentrations (1,600 and 2,900 mg/kg, respectively) orally, by means of polythene cannula based on the findings from phase 1. The fourth mice received distilled water which served as control. The mice were monitored for 24 h for lethality and general behaviour.

Determination of biochemical parameters

Assay of alanine aminotransferase (ALT) activity

The activity of (ALT) was determined by the Reitman-Frankel colorimetric method (1957) for *in vitro* determination of GPT/ALT in serum using a Quimica Clinica Applicada (QCA) test kit.

Assay of aspartate transaminase (AST) activity

GOT (AST) determination by the Reitman-Frankel (1957) colorimetric method was used for *in vitro* determination of GOT/AST in serum using a Quimica Clinica Applicada (QCA) test kit.

Assay of alkaline phosphatase (ALP) activity

Phenolphthalein monophosphate method (Klein et al., 1960) was used for the *in vitro* determination of alkaline phosphatase in serum using Quimica Clinica Applicada (QCA) test kit.

Determination of total bilirubin concentration using colorimetric method

Total serum protein was determined by the Biuret method of Lubran (1978) and serum albumin was determined by the method of Doumas (1975). Blood glucose was determined using the glucose oxidase method of Marks and Dawson (1965)

Statistical analysis

One way analysis of variance (ANOVA) and Fisher's least significant difference (F-LSD) were used to separate the means. Results were presented as mean ± standard deviation of all parameters determined.

RESULTS

Acute toxicity (LD_{50})

The results of the acute toxicity study shows that the extract was not toxic to mice at the tested concentrations.

Biochemical parameters

Table 2 shows the effect of the methanol pulp extract on some biochemical parameters in rats. Significant difference ($p<0.05$) in ALP concentration was observed at the end of the 28 days feeding in the groups fed with 100 mg/kg (low dose) of the extract when compared with the control. A significant difference ($p<0.05$) was observed only in the group that had 100 mg/kg of the extract between the first initial 14 days of experiment and the final 14 days of experiment. Only the groups fed 100 mg/kg of extract (low doses) significantly decreased ($p<0.05$) the serum concentration of ALT at the end of the 28 days study when compared with the control. A significant difference ($p<0.05$) in AST concentration was observed only in the group that had 100 mg/kg of the extract between the two phases of the experiment (14 and 28 days). A decrease in bilirubin concentration was observed in all the test groups when compared with the control after 28 days of the experiment. A significant decrease ($p<0.05$) in bilirubin concentration was observed in the groups administered 100, 200 and 500 mg/kg of the extract when compared with the control after the 28 days study. 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 days) of administration of *S. dulcificum* extract relative to the control. Protein and globulin concentrations in the blood increased significantly ($p<0.05$) in all the test groups (Groups 2, 3 and 4) administered 100, 200 and 500 mg/kg of *S. dulcificum* extract as compared to the animals in the control group after 28 days experiment. A significant difference ($p<0.05$) in the protein and globulin concentrations were observed between the first (14 days) and second (28 days) feeding with the pulp extract. However, no significant difference ($p>0.05$) was observed in the albumin concentration across the groups administered 100, 200 and 500 mg/kg of the *S. dulcificum* extract when compared with the control that had normal saline at the end of the 14 days feeding. 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 days) of administration of *S. dulcificum* extract relative to the control. The 200 and 500 mg/kg doses significantly decreased ($p<0.05$) the blood glucose level when compared with the control at the end of the 14 days experiment. During the second phase of the experiment, the blood glucose of the groups fed with 100 and 500 mg/kg doses significantly decreased ($p<0.05$) the blood glucose level when compared with the control at the end of the 28 days experiment. In the mean blood glucose concentration of all the groups between the 14 and 28 days of experiments, 100 and 500 mg/kg doses of the extract significantly decreased ($p<0.05$) at the end of the 14 days experiment when compared with the 28 days experiment.

DISCUSSION

Safety profile assay of the extract using mice 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 (OECD) guideline 423 for testing of chemicals (OECD, 2008). The result of this study as

shown in Table 1 suggests that the pulp is relatively non-toxic since LD_{50} above 5 g/kg body weight is of no practical significance (Lorke, 1983). This is expected

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

Group	No. of animals	Dosage (mg/kg)	Mortality
Stage I			
Group I	3	10	0/3
Group II	3	100	0/3
Group III	3	1000	0/3
Stage II			
Group I	1	1600	0/1
Group II	1	2900	0/1
Group III	1	5000	0/1
Control	1	-	0/1

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

Parameter	Day 14				Day 28			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
ALP (IU/L)	63.33±8.08	58.33±9.07	57.67±4.93	62.33±4.93	53.67±7.02*	31.67±3.51	51.00±5.20	50.67±3.06
ALT (IU/L)	49.33±231	42.00±0.00	44.00±12.49	44.00±4.00	29.33±4.16	16.67±4.16*	24.67±3.06	24.00±5.29
AST (IU/L)	39.00±6.56	34.33±2.08	35.33±3.06	38.00±2.00	41.00±10.00	33.00±6.08	40.00±1.00	39.00±5.29
T. Bil (mg/dl)	0.70±0.24	0.61±0.11	0.67±0.19	0.67±0.20	0.44±0.11	0.19±0.01*	0.25±0.11	0.26±0.11
Protein (g/dl)	5.00±0.20	5.27±0.75	5.43±0.31	5.57±0.60	5.47±0.45	6.10±0.26	6.17±0.06	6.43±0.40*
Globulin (g/dl)	1.4±0.8	1.53±1.2	1.63±0.67	1.7±0.66	1.83±0.15	2.4±0.2	2.47±0.23	2.67±0.72
Albumin (g/dl)	3.60±0.60	3.73±0.45	3.80±0.36	3.87±0.15	3.63±0.31	3.70±0.10	3.70±0.26	3.77±0.32
Glucose (g/dl)	261.00±32.74	231.00±29.10	136.00±27.22	114.33±8.02	100.33±3.51*	82.67±7.02*	74.33±10.50	64.33±4.04*

Values are presented as mean ± SD of triplicates. Values on the day 28 row followed by superscript letters differ significantly (*p<0.05) from the values on day 14 when compared. Group 1 = control; Group 2 = 100 mg/kg *S. dulcificum* methanolic pulp extract; Group 3 = 200 mg/kg *S. dulcificum* methanolic pulp extract; Group 4 = 500 mg/kg *S. dulcificum* methanolic pulp extract.

considering that the pulp is edible. Since serum total proteins, albumins and globulins are generally influenced by total protein intake (Onifade and Tewe, 1993), 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 (Apata, 1990). Awosanya et al. (1999) have demonstrated the dependence of blood protein on the quality and quantity of protein source. 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 (Johnston, 1999). For the duration of administration of the pulp extract, the results obtained for serum total protein, albumin and globulin as shown in Table 2

suggests 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, hypogammaglobulinemia/agammaglobulinemia; severe and loss through the urine in severe kidney disease and pregnancy. Prolonged destruction of the hepatic cells results in more hepatic releases to exacerbate hepatic dysfunction and causes decrease in the serum levels of total protein, albumin and globulin.

Failure to maintain blood glucose in the normal range leads to conditions of persistently high (hyperglycaemia) or low (hypoglycaemia) blood sugar (Sacher and Macpherson, 2001). The 200 and 500 mg/kg doses significantly decreased ($p<0.05$) the blood glucose level when compared with the control at the end of the 14 days experiment as shown in Table 2. Similarly, the 100 and 500 mg/kg doses significantly decreased ($p<0.05$) the blood glucose level when compared with the control at the end of the 28 days 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 contain flavonoids, saponins, tannins and alkaloids (Nkwocha et al., 2014). 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 (Trease and Evans, 1996).

Table 2 shows a significant decrease ($p<0.05$) in ALP and ALT concentrations at the end of the 28 day feeding in the groups fed with 100 mg/kg (low dose) of the extract when compared with the control. The value of the liver function test depends on the specificity for damage as well as their sensitivity (Okonkwo et al., 1997; Sodipo et al., 2009). 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 (Kachmar and Moss, 1976; Sodipo et al., 2009). The result of the present study in which AST and ALT concentrations were decreased at the two phases of the experiment (14 and 28 days) dose-dependently, therefore 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 (Kaneko and Cornelius, 1971; Wilkinson, 1976; Okonkwo et al., 1997; Nduka, 1997; Mayne, 1998; Atangwho et al., 2007; Sodipo et al., 2009). 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 days administration, there is no likelihood of liver damage by the methanol pulp extract of *S. dulcificum*.

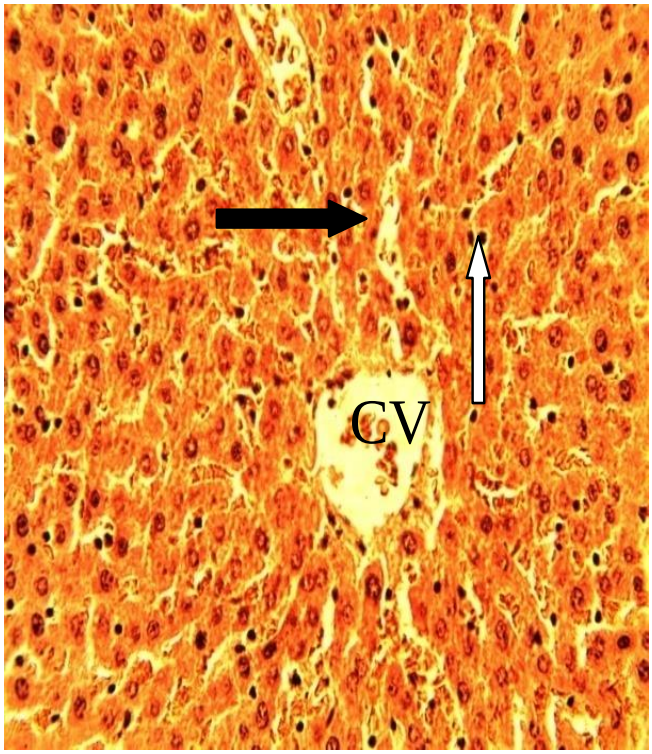
A significant decrease ($p<0.05$) was observed in bilirubin concentration in the groups administered 100, 200 and 500 mg/kg of the extract when compared with the control after the 28 days study. The decrease in bilirubin concentration which was significant after the second phase of the experiment was 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 drug-induced reactions (Mukherjee, 1998; Odutola, 1992, Sood, 2006). However, if the AST and ALT values are normal as in the present study, the diagnosis of hepatocellular damage cannot be confirmed (0dutola, 1992).

A statistically significant decrease ($p<0.05$) in ALP value as obtained in the group administered 100 mg/kg of extract after the 28 day study is not of much clinical significance (Atangwho et al., 2007; Sodipo et al., 2009). Even if there had been an elevation in ALP upon extract administration, it could still not have confirmed liver damage because according to Odutola (1992), ALP and AST originate 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 that of toxicity.

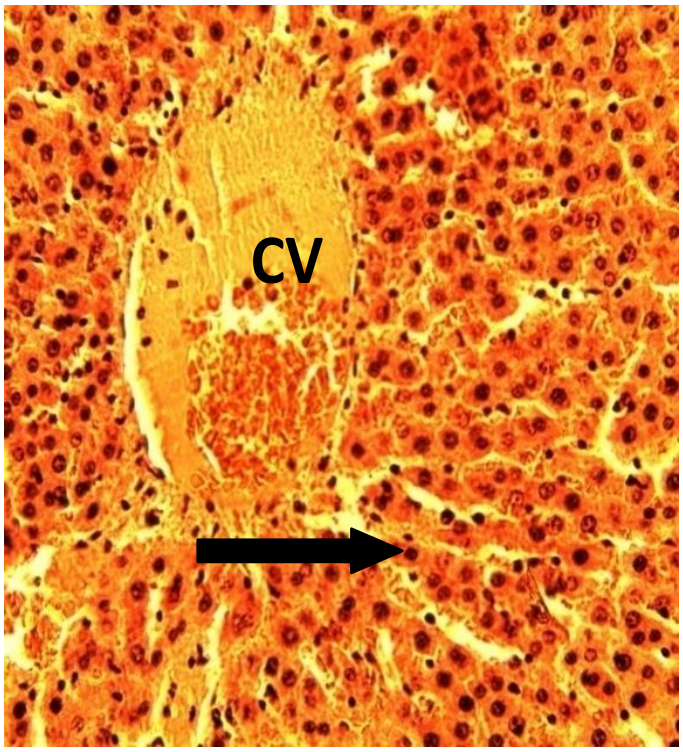
Histopathological examination of liver sections of rats 14 days post administration with *S. dulcificum* methanol extract as shown in Figure 1 shows normal liver architecture. In this study also, a non-significant effect of the methanol extract of *S. dulcificum* pulp on the morphological architecture of the liver tissues is reported (Figure 2).

Conclusion

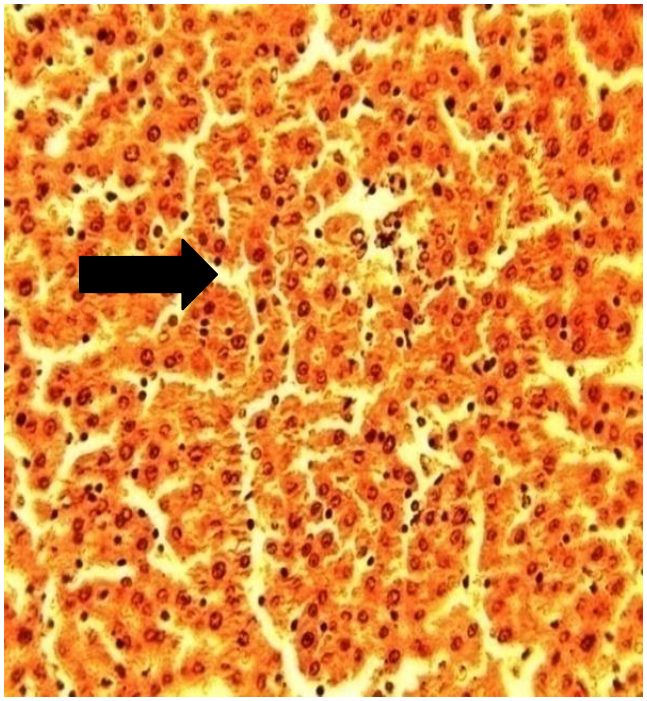
The findings indicate that the fruit which is popularly eaten as a sweetener and documented to be rich in



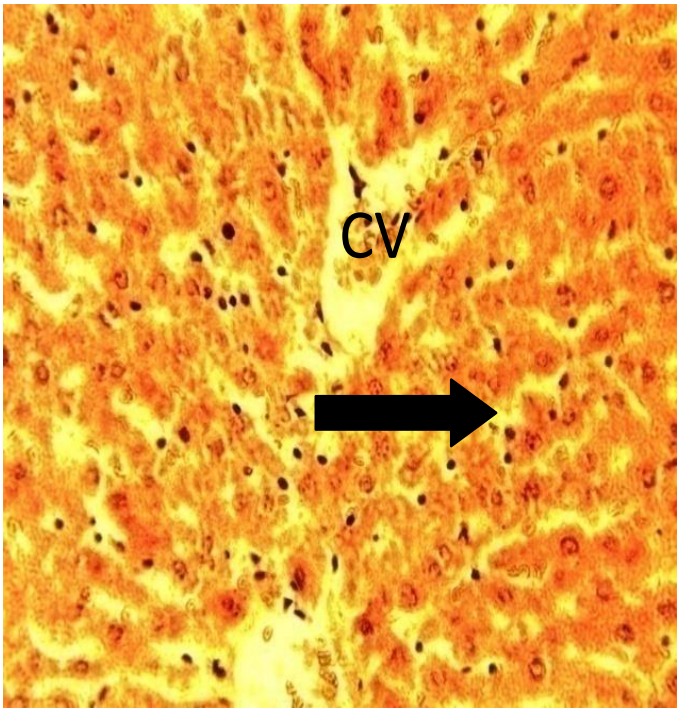
Group A (Control)



Group B (100 mg/kg)



Group C (200 mg/kg)



Group D (500 mg/kg)

Figure 1. Histopathology of the liver after the 14 days administration of *S. dulcificum* extract. Photomicrograph of liver sections of rats 14 days post administration of *S. dulcificum* methanol extract showing normal liver architecture (central vein- CV, sinusoids- black arrow, plates of hepatocytes- white arrow).

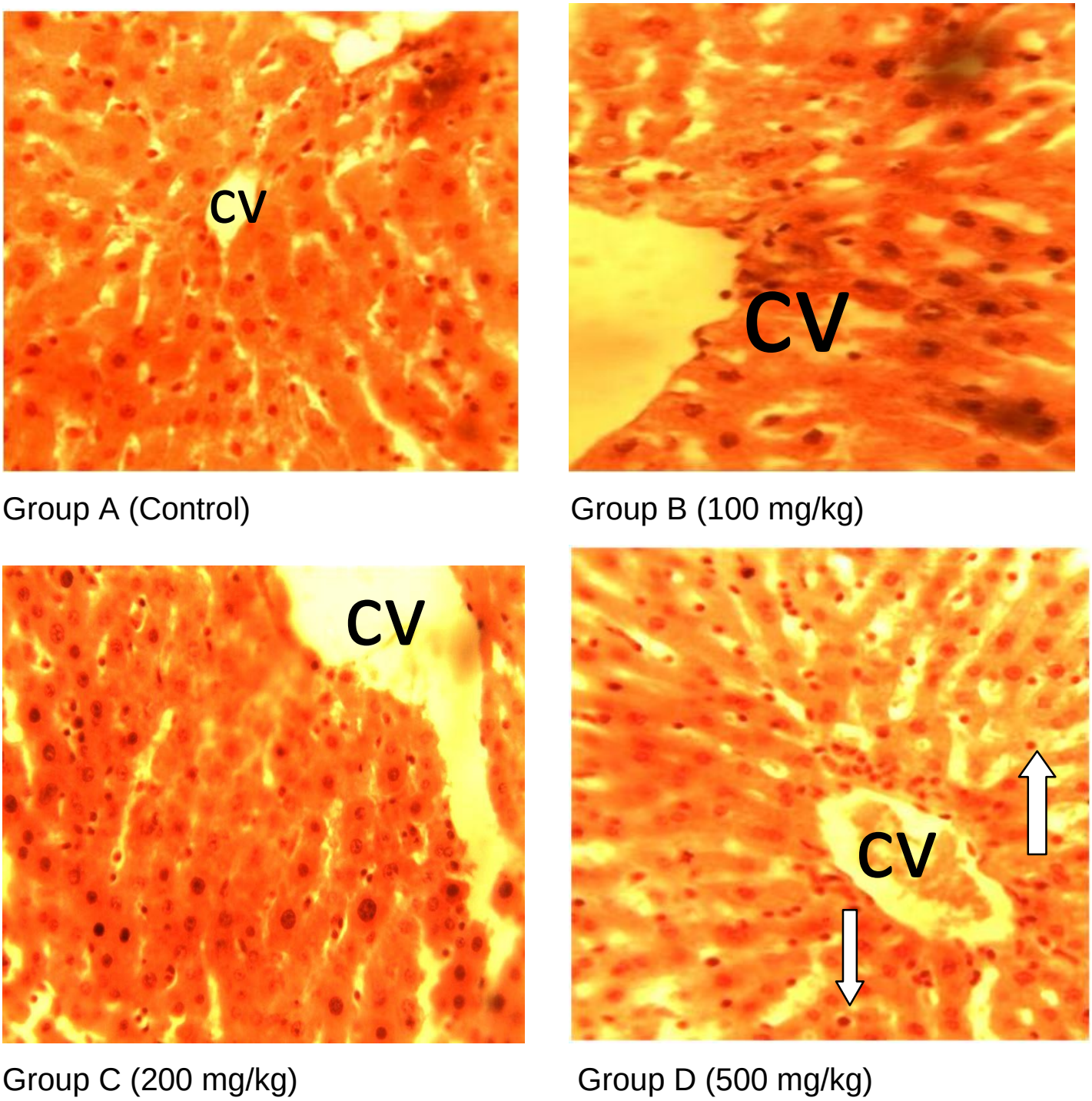


Figure 2. Histopathology of the liver after the 28 days administration of *S. dulcificum* extract. Photomicrograph of liver sections of rats 28 days post administration of *S. dulcificum* methanol extract. Note mild hepatocyte degenerations in Group D (arrows). Sections from Groups A (Control), B: 100 mg/kg (low dose) and C: 200 mg/kg had no observable histologic changes; CV: Central vein.

important food properties when compared with other fruits, has no negative effect on some biochemical parameters, at least in rats.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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