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Effects of Saccharum officinarum Molasses on Hematology and Hepatic Functions of Male Wistar Rats

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Background: Sweeteners are a utile source of nourishment for cuisine preparation but are linked with the risk of occurrence of several diseases. Hence, the quest for healthier sweetening agents with lesser harmful effects cannot be ignored. *Saccharum officinarum* molasses, a natural nutritive sweetener, has become a popular substitute despite a dearth of knowledge on its healthiness.

Aim: To assess the effects of *Saccharum officinarum* molasses on hematology, serum electrolyte, lipid profile, liver histology, and redox status of male Wistar rats.

Methods: Blackstrap® *Saccharum officinarum* molasses (SOM) was fractionated to obtain *Saccharum officinarum* molasses methanol fraction (SOMMF) and *Saccharum officinarum* molasses aqueous fraction (SOMAqF). Seven groups (n = 5) of adult male Wistar rats received distilled water (Control); 1.0, 3.2, 10.0 g/kg SOMMF and 0.6, 2.0, 6.4 g/kg SOMAqF, respectively. Administrations were done daily via oral gavage for eight weeks. Full blood indices were determined with an automated hematology analyzer, serum electrolyte by monoliquid colorimetric test, and serum lipid profile using the enzymatic colorimetric test. Liver malondialdehyde and antioxidant levels were assayed by spectrophotometry. Liver histology was assessed using microscopy. Data were analyzed using ANOVA at $p < 0.05$ significance.

Results: SOM increased liver catalase activity and serum levels of iron and potassium. It reduced serum levels of lipid profile, zinc and sodium ions, hemoglobin concentration, red blood cell count, packed cell volume, superoxide dismutase activity of the liver, and concurrently disrupted liver cytoarchitecture.

Conclusion: *Saccharum officinarum* molasses adversely disrupted the hematological and hepatic functions of male Wistar rats.

Keywords: *Sweetener, Saccharum officinarummolasses, Hematology, Oxidant status, Histology, Liver*

Introduction

Sweeteners are a useful source of nutrients and epicurean facilitators for culinary preparation.[1] Their constituents improve and keep food texture and quality.[2] Refined sugar is a well-known non-lethal sweetener that is safe for use.[3] However, studies have reported that it adversely affects human health by causing several forms of diseases.[4,5]This brought the idea of substituting sugar intake by most consumers with artificial (non-nutritive) sweeteners, since they contain just a few

calories or are calorie-free but still duplicate the effect of sugar in taste.[6] Studies however revealed that these artificial sweeteners also contribute to ill-health by increasing the risk of occurrence of colorectal cancer, body weight gain, diabetes, and obesity.[7,8] As a result, a further search for suitable sweetening agents with lesser harmful effects, led to the discovery of other natural products believed to have improved nourishing value owing to their constituents.[9]

Saccharum officinarum molasses (SOM) is one of such natural sweeteners, gotten during the production of refined sugar from S. officinarum (sugarcane) juice. It is the main by-product obtained during the processing of sugarcane syrup.[10]*Saccharum* officinarum molasses from being used as a supplement energy source in animal feeds, for eradicating dust and reducing feed wastage[11]became a popular ingredient for sweetening beverages, basting, and flavoring of food products.[12] Previous studies on S. officinarum molasses showed that it possesses antioxidant properties that prevented bone destruction and deoxyribonucleic acid damage.[13,14] However, contradicting evidence correlated *in vivo* exposure of animals to S. officinarum molasses with immunosuppressive and endocrine-disrupting properties[15] resulting in some diseases. Thus, this study investigates the effects of S. officinarum molasses intake on hematology, serum electrolyte level, lipid profile, liver histology, and oxidant status of male Wistar rats.

Materials and methods

Ethical approval for this study (Ethical committee number UI-ACUREC/18/0074) was provided by the University of Ibadan Animal Care and Use Research Ethics Committee. All procedures comprising the use of animals were by the EU Directive 2010/63/EU for animal experiments and the study conformed to the Animal Research: Reporting of *in Vivo* Experiments (ARRIVE) guideline (2010).

The *Saccharum* officinarum molasses (SOM) (Blackstrap®, Old English Incorporated, USA) extraction was by the method of Gandhi, et al.[16] resulting in two portions; SOM methanol fraction (SOMMF) and SOM aqueous fractions (SOMAqF) administered to the rats.

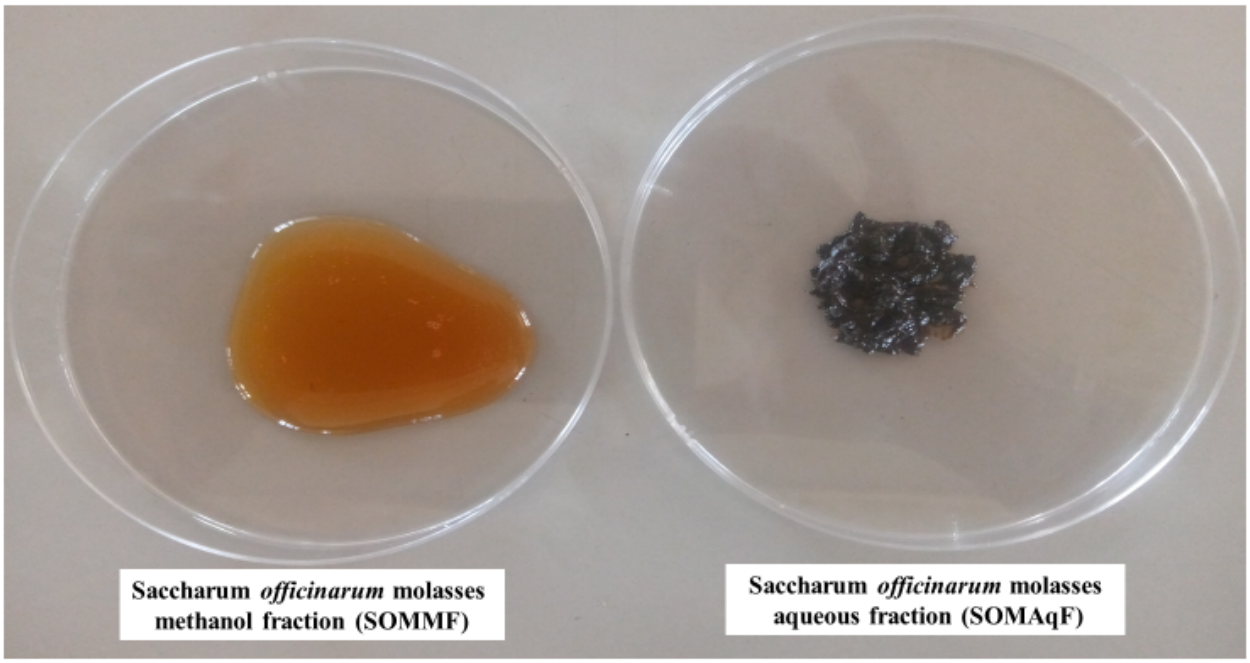

Figure 1.

Acute oral toxicity test

The dosage regime for S. officinarum molasses extracts was by the Limit test procedure.[17]

Experimental design

Thirty-five male Wistar rats (160-180) gotten from the Central Animal House, University of Ibadan, Ibadan, Nigeria, and acclimatized for two weeks to laboratory conditions, were allowed access to feed and water *ad libitum* before the experiment began. The rats were randomly divided into seven groups (n=5) which received 1.0 mL/kg distilled water (group 1 - control); 1.0, 3.2, 10.0 mL/kgSOMMF (groups 2, 3, and 4) and 0.6, 2.0, 6.4 g/kg SOMAqF (groups 5, 6 and 7) daily by oral gavage for eight weeks, respectively. The dosage regime was by the Organization for Economic Co-operation and Development (OECD) test guideline.[17] Distilled water was used as the vehicle for both extracts. Measurement of the body weights of the animals was done once a week and at sacrifice.

Blood collection and serum preparation

At the time of sacrifice, blood was collected through the cardiac puncture into EDTA and plain serum bottles. The blood in the EDTA bottle was for the determination of full-blood indices with an automated hematology analyzer (MSLAB45, China). The blood in the plain serum bottles was allowed to clot for about 45 minutes, and centrifuged at 3000 rpm for 15-minute afterward, to get the supernatant which was stored at −20°C for electrolytes and lipid profile assessment (Randox kits, UK). Afterward, thiopental anesthesia (40 mg/kg i.p.) was administered,[18,] and the liver was exposed and harvested by opening the linea alba of the anterior abdominal wall to the thoracic cavity. It was fixed in 10 % formalin for histological examination.

Electrolyte assessment

The serum electrolyte level assessment was by monoliquid colorimetric test using Randox kits (UK) in line with the manufacturer’s guide. The serum levels of zinc, sodium, potassium, Iron, and Inorganic phosphate were measured using a monoliquid colorimetric test. Calcium ion assessed with methylthmol blue (MTB) test.

Lipid profile assessment

The assessment of serum lipid profile level (triglycerides, total cholesterol, and HDL cholesterol) was by enzymatic colorimetric test using Randox kits (UK) and low-density lipoprotein level (LDL) cholesterol was determined by Friedewald formula.

Lipid peroxidation and antioxidant assessment

The liver lipid peroxidation assessment was done[19] where the level of malondialdehyde (MDA) produced during lipid peroxidation was measured. Liver catalase activity[20] Liver superoxide dismutase (SOD) activity[21] was determined. The reduced glutathione level of the liver was measured by a spectrophotometric assay kit (Oxford Biomedical Research, USA).

Histological assessment of Liver

The assessment of the liver was via routine techniques for histology. Samples underwent sectioning and staining with hematoxylin and eosin (H&E), the slides were cleared in xylene and mounted on

the microscope for examination. Photomicrographs were at 100×magnification.

Statistical analysis

Analysis of data was with GraphPad prism 5 and expressed as mean±SEM. The mean differences were compared by analysis of variance (one-way ANOVA) and $p<0.05$ was considered statistically significant.

Results

Effect of S. officinarum molasses on hematological indices

The result (Table 1) shows a significant decrease ($p<0.05$) in the packed cell volume (PCV) of groups treated with 3.2 mL/kg/day SOMMF, as well as 0.6 and 6.4 mg/kg/day SOMAqF when compared to the control, respectively. The 3.2 and 10.0 mL/kg/day SOMMF treated rats showed significant decreases ($p<0.05$) in red blood cell count relative to the control and SOMAqF treated rats. Significant decreases ($p<0.05$) were also noted in the hemoglobin concentration and mean corpuscular hemoglobin concentration of the SOMMF treated rats compared to the control, but the mean corpuscular hemoglobin concentration of the SOMAqF groups had a significant increase ($p<0.05$) as compared to the control. The mean corpuscular volume increased significantly ($p<0.05$) in 3.2 mL/kg/day SOMMF, nonetheless there were no significant differences in the mean corpuscular hemoglobin, white blood cell count, and platelet of all treated groups when compared to the control.

		SOMMF (mL/kg/Day)			SOMAqF (mL/kg/Day)		
Group	Control	1.0	3.2	10.0	0.6	2.0	6.4
PCV (%)	43.0±1.155	43.33±1.41	35.83±2.06[a]	42.5±1.18	37.0±1.27[a]	41.67±1.12	38.67±0.84[a]
HB (G/dL)	17.15±0.58	1 4.52±0.50[ab]	1 1.93±0.68[ab]	1 4.62±1.11[ab]	17.9±0.309	17.67±0.29	17.2±0.58
RBC (10^6 µL)	8.49±1.31	7.84±0.22	6.19±0.60[ab]	7.58±0.36[a]	8.38±0.09	8.26±0.81	7.95±0.23
WBC	4.662±0.23	5.275±0.52	5.417±0.70	4.775±0.70	4.45±0.23	4.57±0.51	4.583±0.32
MCV(FL)	50.67±1.09	55.67±2.98	5 9.23±3.31[ab]	54.72±4.79	43.48±1.28	50.47±1.72	48.82±1.62
MCH(fmol)	202.28±7.31	186.54±10.4	197.3±11.08	194.2±15.63	213.68±4.31	214.08±4.94	216.59±6.03
MCHC %	39.57±1.62	33.49±0.17[a]	33.3±0.03[a]	35.70±1.57[a]	48.57±1.34[a]	42.51±0.99[a]	44.67±2.15[a]
PLATELET	111500±415	110667±125	104833±984	109667±950	112167±430	109333±582	114333±277

Table 1. *Effect of Saccharum officinarum molasses on hematological parameters.*

Data are presented as mean±SEM, n=5. [a]$p<0.05$ compared with the control. [b]$p<0.05$ compared with SOMAqF. SOM= Saccharum *officinarum*molasses. SOMMF=Saccharum *officinarum*molasses methanol fraction. SOMAqF = Saccharum *officinarum*molasses aqueous fraction. PCV = packed cell volume. HB= Hemoglobin.RBC = Red blood cell. WBC=White blood cell. MCV=Meancorpuscular volume. MCH=Mean corpuscular hemoglobin. MCHC=mean corpuscular hemoglobin concentration.

Effect of S. officinarum molasses on lipid profile

The result (Figure 1) shows there was a significant decrease ($p<0.05$) in serum triglycerol levels of 1.0 and 3.2 mL/kg/day SOMMF treated rats when compared to the control. Also, serum total cholesterol level was significantly decreased ($p<0.05$) in 1.0 and 10.0 mL/kg/day SOMMF when compared to the control. There were no significant differences in both the serum high and low-

density cholesterol levels of all treated groups compared to the control.

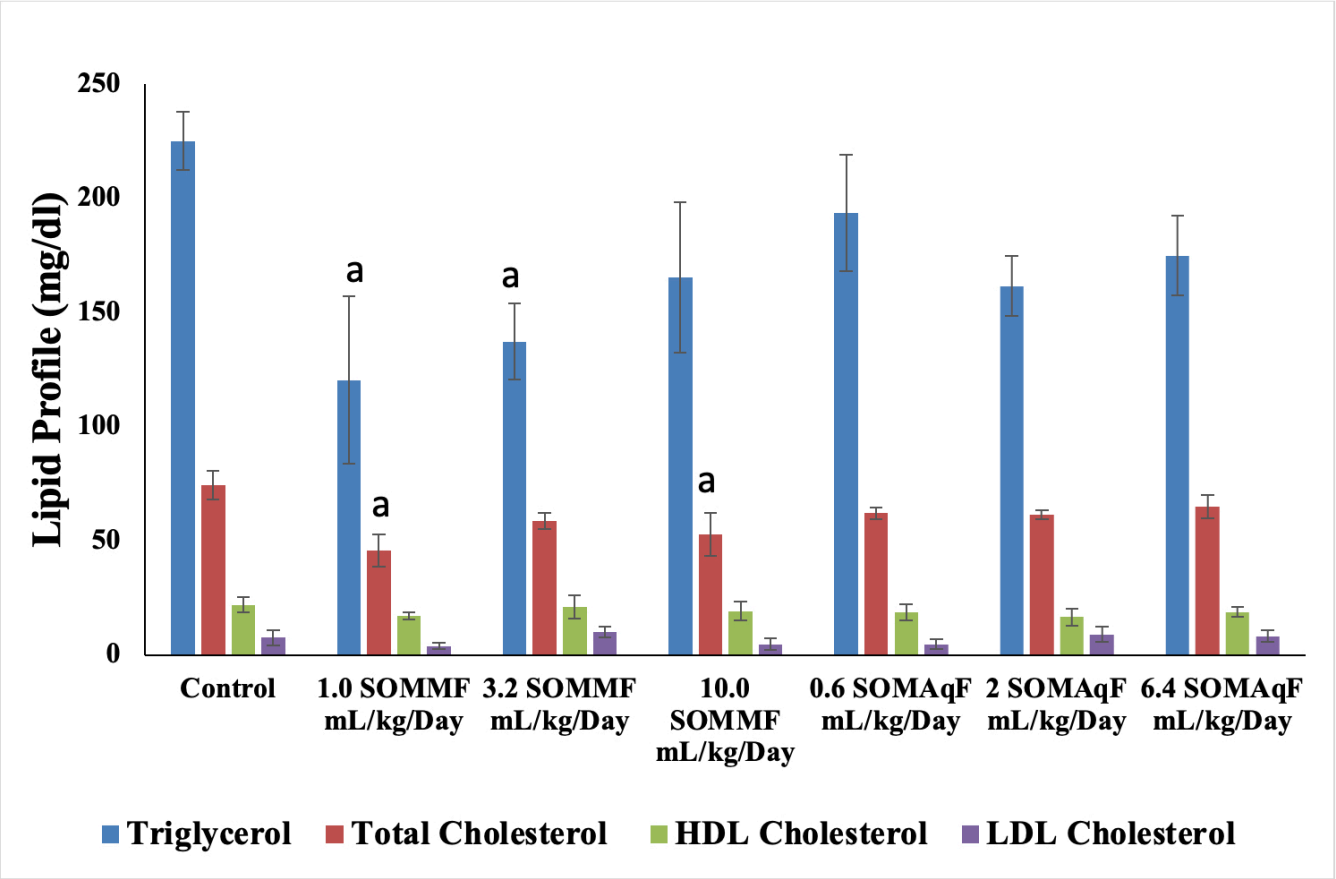

Figure 2. *Effect of Saccharum officinarum molasses on lipid profile level.*

Columns represent mean±SEM, n=5, [a]p<0.05 compared with the control. SOM=Saccharum *officinarum*molasses. SOMMF=Saccharum *officinarum*molasses methanol fraction. SOMAqF=Saccharum *officinarum*molasses aqueous fraction.

Effect of S. officinarum molasses on some serum electrolytes levels

Serum level of zinc ion was significantly decreased (p<0.05) in all SOMAqF treated rats when compared with the control and SOMMF groups. Serum sodium ion level was significantly decreased (p<0.05) in 1.0 mg/kg/day SOMMF, 0.6 and 2.0 mg/kg/day SOMAqF treated rats compared to control and the 10.0 mL/kg/day SOMMF group. There were significant increases (p<0.05) in the serum levels of potassium and iron in 0.6 and 2.0 mg/kg/day SOMAqF treated rats in comparison with the control and all treated groups, respectively. Calcium and inorganic phosphate did not show significant differences in serum concentrations (Table 2).

		SOMMF (mL/kg/Day)			SOMAqF (mL/kg/Day)		
Group	Control	1.0	3.2	10.0	0.6	2.0	6.4
Zinc (Mmol/L)	25.37±5.803	26.35±5.947	23.54±3.114	25.32±3.11	22.0±3.346 [ab]	20.74±6.677[ab]	16.58±2.014[ab]
Sodium (Mmol/L)	108.3±5.65	73.58±11.08[ac]	110.8±15.25	124.9±17.73	48.15±4.65[ac]	53.41±10.36[ac]	106.7±3.874
Iron (Mmol/L)	41.13±2.828	42.52±5.088	46.8±3.509	54.06±6.058	51.68±6.57	64.51±10.99[a]	42.43±8.935

Potassium (Mmol/L)	3.966±0.253	3.209±0.538	3.302±0.490	3.854±0.259	5.246±0.865[a]	3.397±0.244	3.328±0.191
Calcium (Mmol/L)	8.191±0.343	6.932±0.241	8.866±0.878	8.773±0.691	8.165±0.516	8.302±0.707	7.992±0.307
Inorganic Phosphate (Mmol/L)	2.581±0.195	2.38±0.069	2.777±0.254	2.988±0.333	2.369±0.454	2.051±0.082	2.158±0.097

Table 2. *Effect of Saccharum officinarum molasses on serum electrolytes levels.*

Data are presented as mean±SEM. n=5. [a]p<0.05 compared with the control. [b]p<0.05 compared with SOMMF.[c]p<0.05 compared with 10.0 mL/kg/day SOMMF. SOMMF=Saccharum *officinarum*molasses methanol fraction. SOMAqF = Saccharum *officinarum*molasses aqueous fraction.

Effect of S. officinarum molasses on lipid peroxidation and antioxidant enzymes of the liver.

The superoxide dismutase (SOD) activities of the lowest doses of SOMMF and SOMAqF treated rats significantly decreased (p<0.05) compared to the control and other treated groups. Catalase activity of 0.6 g/kg/day SOMAqF treated rats significantly increased (p<0.05) when compared to the control and SOMMF treated groups. There were no significant differences in the malondialdehyde (MDA) level and glutathione concentration (Table 3).

		SOMMF (mL/kg/Day)			SOMAqF (mL/kg/Day)		
Group	Control	1.0	3.2	10.0	0.6	2.0	6.4
MDA (U/mg)	4.572±0.52	3.131±0.28	3.878±0.79	4.92±0.87	2.752±0.52	2.288±0.41	3.75±0.88
SOD (U/mg)	560.7±147.8	2 20.7±53.22[abc]	430±103.2	345.9±148.4	2 65.6±60.35[abc]	288.7±64.81	609.8±82.1
Catalase (IU/L)	698.2±62.94	539.3±44.62	676±65.25	771.5±158.6	1101±87.77[a#]	792.7±63.14	833.9±111
GSH (uM /mg)	4.715±0.60	4.352±0.28	4.783±0.20	4.197±0.33	3.607±0.94	3.589±0.4	5.452±0.41

Table 3. *Effect of Saccharum officinarum molasses on lipid peroxidation and antioxidant enzymes of the liver.*

Data are presented as mean±SEM, n=5, [a]p<0.05 compared with the control. [b]p<0.05 compared with 3.2 mL/kg/day SOMMF, [c]p<0.05 compared with 6.4 mL/kg/day SOMMF, [#]p<0.05 compared with SOMMF. SOM= Saccharum *officinarum*molasses. SOMMF=Saccharum *officinarum*molasses methanol fraction. SOMAqF = Saccharum *officinarum*molasses aqueous fraction.

Effect of S. officinarum molasses on histology of liver

The liver section of the 1.0 mL/kg/day SOMMF group shows mildly dilated sinusoids and infiltration of inflammatory cells. The liver sections of the SOMAqF groups show portal tracts with lymphocytes aggregating and mildly dilated sinusoids, as well as mild periportal infiltration of inflammatory cells in liver parenchyma (Figure 5).

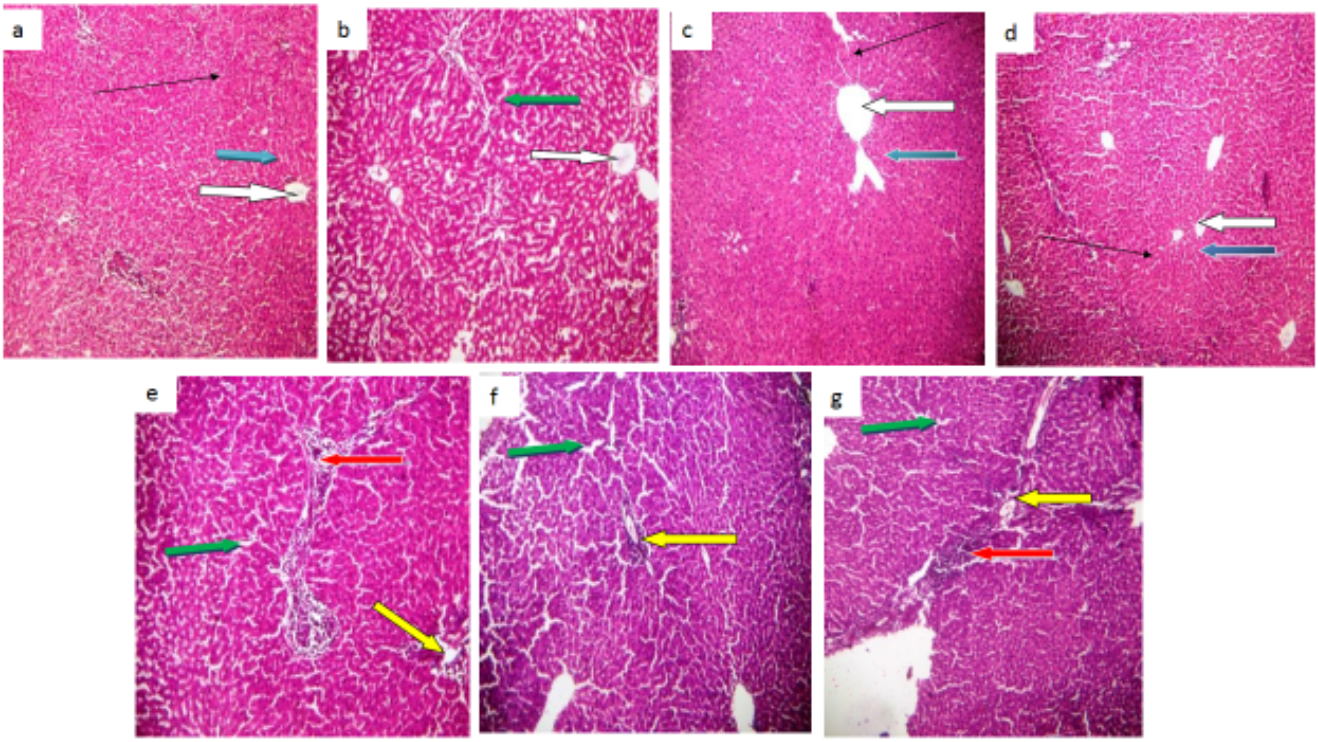

Figure 3. *Photomicrograph of liver sections of control rat and Saccharum officinarum molasses treated rats. a- control, b, c and d (1.0.3.2 and 10.0 g/kg SOMMF, respectively), e, f and g (0.6, 2.0 and 6.4 g/kg SOMAqF, respectively). Note the central venules (white arrows), normal sinusoids (blue arrows), hepatocytes (slender arrows). Mild perivascular and periportal infiltration of inflammatory cells (yellow arrows). Mildly dilated sinusoids with infiltration of inflammatory cells (green arrows). Lymphocyte aggregation (red arrows). Stained by H&E, ×100 magnification.*

Discussion

The widespread use of S. officinarum molasses sweetener may cause concerns for possible adverse health effects, but this study reveals that it has a wide safety margin as there was no lethality even at a dose of 2000-mg/kg weight. Hematological components are valuable in monitoring general health status and changes in the blood due to toxicity.[22,23]*Saccharumofficinarum*molasses caused significant decreases in hemoglobin (Hb) concentration, red blood cell (RBC) count, and hematocrit/packed cell volume (PCV). Hemoglobin (Hb) is the iron-containing metalloprotein that transports oxygen to tissues for the breakdown of food substances for the release of energy used by the body and transport of carbon dioxide out of the body.[24]Red blood cell carries hemoglobin that combines with oxygen to form oxyhemoglobin during respiration.[25,26]The PCV is the percentage of RBC in blood and is also known as the hematocrit.[27]Thus, the significant reduction in hemoglobin, MCHC, RBC count, and PCV reveals that SOM may adversely alter the amount of oxygen that would be carried to the tissues and probably predispose to hypoxia.[28,29]The PCV, hemoglobin and mean corpuscular hemoglobin are major indices for evaluating bone marrow capacity to produce red blood cells, circulatory erythrocytes, and diagnosis of anemia.[29,30]The findings of this study support an earlier report that blood toxicity is usually accompanied by significant decreases in the values of RBC, HB, PCV due to possible suppression of erythropoietic processes or hemolysis of the available RBC.[31]This may also result in an anemic condition in line with the study of[32] who noted that S. officinarum peel extract caused a decline in some of these blood indices.

Studies have shown that the amount, type, and composition of lipid sources in the diet are determining factors of the serum lipid profile.[33,34]Lipids have effects on cardiovascular functions

and a change of diet is the first strategy applied to prevent and treat cardiovascular diseases.[35]Saccharum officinarum molasses is incorporated as a sweetener in both animal and human diets, this study shows that it caused a significant reduction in serum triglycerol and total cholesterol levels. It had been earlier stated that a 1 % decrease in serum cholesterol results in a 3 % decrease in risk of congestive heart disease.[36]SOM may be regarded as safe since it does not pose a possibility of an adverse effect on the heart as implicated by the decrease in the lipid profile.

The electrolytes in plasma contribute to the osmotic balance which controls the movement of water between cells and their environment.[37]Potassium is the major intracellular cation, it establishes the resting membrane potential in neurons and muscle fibers after membrane depolarization and action potentials.[38]Along with sodium, it regulates water balance and the acid-base balance in the blood and tissues. The observed inadequate changes in potassium and sodium ions levels, implied that prolonged consumption of higher doses of Saccharum *officinarum*molasses may cause hyponatremia or hyperkalemia and its related health effects. Serum iron concentration is a measure of the circulating iron (Fe^{3+})bound to transferrin, and only 0.1% of total body iron is bound to transferrin at any one time.[39]The slightly increased serum iron level by SOMMF is an indication that Saccharum *officinarum*molasses may help improve iron level due to its usefulness in the synthesis of oxygen transport proteins (hemoglobin and myoglobin), and for the formation of heme enzymes and other iron-containing enzymes involved in electron transfer and oxidation-reductions.[40,41]

The ability of S. officinarum molasses to increase catalase activity is an indication that it possibly possesses some amount of antioxidant properties. Catalase is one of the main enzymes that act as an oxidant scavenger, via the degradation of hydrogen peroxide to oxygen and water.[42] The perivascular infiltration and lymphocytes aggregation caused by SOMAqF may be from inflammation of either the neutrophils, eosinophils, lymphocytes plasmacytes, macrophages,or mast cells that infiltrate around the blood vessels. The portal tract's inflammation probably resulted from deposits of blood clots or bleeding in the blood vessels.[43,44]The inflammatory cells in the hepatic tissue suggest that SOM may contain constituents that can interact with proteins and enzymes of the hepatic interstitial tissue interfering with the antioxidant defense mechanism to generate reactive oxygen species that may, in turn, imitate an inflammatory response.[45]Plausibly the observed mild dilation of the sinusoid was by peri-sinusoidal fibrosis nodular regenerative hyperplasia or hepatoportal sclerosis which has been related to xenobiotics.[46,47]

Conclusion

Saccharum officinarum molasses increased serum levels of iron, potassium, and liver catalase, but adversely disrupted the lipid profile, hematological and hepatic functions of male Wistar rats. Saccharum officinarum molasses possess harmful effects on health status.

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