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Saccharum officinarum molasses adversely alters reproductive functions in male wistar rats

Eunice Ogunwole[a,b,*], Olufadekemi T. Kunle-Alabi[a], Opeyemi O. Akindele[a], Yinusa Raji[a]

[a] Laboratory for Reproductive Physiology and Developmental Programming, Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria
[b] Department of Physiology, College of Health Sciences, Bingham University, New Karu, Nasarawa, Nigeria

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ABSTRACT

The widely reported anti-androgenic effects of refined sugar led to the exploration of safer alternatives. *Saccharum officinarum* molasses (SOM), a byproduct of sugar processing is gaining popularity as a substitute. This study investigated the effects of SOM and compared them to those of refined sugar on male reproductive functions. Blackstrap® *Saccharum officinarum* molasses were subjected to phytochemical screening and proximate analysis and fractionated to obtain methanol (SOMMF) and aqueous (SOMAqF) fractions. Twelve groups (n = 5) of adult male Wistar rats received distilled water (Control); 0.8, 2.5, 7.9 g/kg SOM; 0.0064 g/kg sugar (Dangote®); 0.0064 g/kg sugar + 7.9 g/kg SOM; 1.0, 3.2, 10.0 g/kg SOMMF and 0.6, 2.0, 6.4 g/kg SOMAqF, respectively. Administrations were done daily by oral gavage for eight weeks. Sperm profile and testicular and epididymal histology were assessed using microscopy. Serum testosterone was quantified using ELISA. Testicular malondialdehyde (MDA) was assayed by spectrophotometry. Data were analyzed using ANOVA at **$p < 0.05$ significance.** Sperm count and viability reduced with 7.9 g/kg SOM, Sugar, 3.2 and 10.0 g/kg SOMMF, 2.0 and 6.4 g/kg SOMAqF. Abnormal sperms increased with 7.9 g/kg SOM, Sugar, 2.0 and 6.4 g/kg SOMAqF. Testosterone level reduced with 6.4 g/kg SOMAqF. Testicular MDA increased with SOM, 3.2 and 10.0 g/kg SOMMF and 6.4 g/kg SOMAqF. Seminiferous tubules and epididymal ducts of 7.9 g/kg SOM, Sugar and SOMAqF-treated rats showed anomalies. *Saccharum officinarum* molasses altered testicular and epididymal integrity *via* lipid peroxidation, thus reducing sperm quality and androgen levels in male Wistar rats.

1. Introduction

Lifestyle and dietary habits are major factors that have been associated with the rise in the occurrence of male reproductive disorders [1]. There is a rising concern on the exposure to and impact of substances with antiandrogenic properties on the health of humans and non-human animals [2,3]. Notable among these anti-androgenic agents are the various sweeteners and ingredients that individuals are exposed to daily in a bid to enhance food color and flavor [4].

Refined sugar, the most commonly used sweetener globally, was previously considered as a non-toxic substance [5,6]. Recently, this non-toxic property of refined sugar has been queried as studies have correlated its consumption with the occurrence of a wide range of diseases [7–9]. Refined sugar has also been strongly associated with low sperm quality and altered testicular steroidogenesis [10,11]. The search for less harmful alternatives has therefore been the focus of much recent research.

One reported substitute is *Saccharum officinarum* molasses (SOM); a natural sweetener obtained as a byproduct during the processing of refined sugar from sugar cane (*Saccharum officinarum*) juice [12]. SOM was the most popular sweetener used until the late 19th century since it was much more affordable than refined sugar at that time. Due to its distinct flavor and high nutritional value, the use of SOM as a refined sugar substitute is on the increase [12,5]. Several positive biological activities have been attributed to SOM [13,14] also, the U.S. Food and Drug Administration (FDA) has considered SOM to be Generally Recognized As Safe (GRAS) [15].

Regarding the effects of SOM on male reproductive physiology, there are a few conflicting reports. [1,2] reported that SOM enhances testicular steroidogenesis. On the other hand, SOM has been reported to

Abbreviations: SOM, Saccharum officinarum molasses; SOMMF, Saccharum officinarum molasses methanol; SOMAqF, Saccharum officinarum molasses aqueous fraction; ELISA, Enzyme-linked immunosorbent assay; MDA, malondialdehyde; ANOVA, analysis of variance

* Corresponding author at: Laboratory for Reproductive Physiology and Developmental Programming, Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria.
E-mail address: eunice@binghamuni.edu.ng (E. Ogunwole).

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cause endocrine disruption [16a,16b and 17]. Since SOM is gaining increasing acceptability as a substitute for refined sugar, this study was therefore designed to compare the effects of refined sugar with both the crude form of *Saccharum officinarum* molasses and its extracts on male reproductive functions in Wistar rats.

2. Materials and methods

Refined sugar (Dangote®, Nigeria) and *Saccharum officinarum* molasses (SOM) (Blackstrap®, Old English Incorporated, USA) were used for the study. Phytochemical screening, proximate analysis and acute oral toxicity of SOM were performed using standard procedures. The methods of [18] and [19] were used in the extraction of SOM to obtain two portions; SOM methanol fraction (SOMMF) and SOM aqueous fractions (SOMAqF) which were subjected to phytochemical screening using standard procedures [20,21].

2.1. Acute oral toxicity test

This was performed using the Limit test procedure following the OECD guideline (2001), to determine the dosage regime for SOM.

2.2. Experimental animals

Ethical approval was obtained from the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/18/0074). All procedures involving the use of animals were by the EU Directive 2010/63/EU for animal experiments and the study conformed with the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guideline (2010). Sixty (60) male Wistar rats (160–200 g) were obtained from the Central Animal House, University of Ibadan, Ibadan. They had access to rat feed and water *ad libitum* and were acclimatized for two weeks to laboratory conditions before administration commenced.

2.3. Experimental design

The rats were randomly divided into twelve groups (n = 5) which received 1.0 mL/kg distilled water (group 1 - control); 0.8, 2.5 and 7.9 g/kg SOM (groups 2, 3 and 4); 0.0064 g/kg sugar (group 5); 0.0064 g/kg sugar + 0.79 g/kg SOM (group 6);1.0, 3.2, 10.0 g/kg SOMMF (groups 7, 8 and 9) and 0.6, 2.0, 6.4 g/kg SOMAqF (groups 10, 11 and 12) daily *via* oral gavage for eight weeks, respectively. The dosage regime used was the OECD guideline (2001). The body weights of the animals were measured weekly and before sacrifice.

2.4. Blood collection and serum preparation

At the time of sacrifice, fasting blood glucose level was determined using blood from the tail. Thereafter, the rats were bled into plain serum bottles through cardiac puncture. The blood was allowed to clot for at least 45 min after which it was centrifuged at 3000 rpm for 15 min. The supernatant was decanted from the centrifuged blood and stored at −20 °C for assay of hormones using ELISA kits (Fortress diagnostics, UK and Calbiotech Inc. USA). Serum levels of follicle-stimulating hormone, luteinizing hormone and testosterone were assayed.

2.5. Organ collection

Rats were sacrificed under thiopental anesthesia (40 mg/kg, i.p) [22]. They were cut open along the linea alba of the anterior abdominal wall to the thoracic cavity to expose the heart and the organs. The testes, epididymides, seminal vesicle, prostate, liver, and kidney were harvested, freed from adherent tissues and weighed immediately with a digital electronic scale (model EHA501, China). The testes and epididymides were fixed in Bouin's fluid for histological examination.

2.6. Sperm analysis

The left caudal epididymis was collected for sperm analysis. The epididymal fluid was prepared for analysis as described by [23]. Sperm analysis (motility, viability, and morphology) was done as described by [24]. Sperm count was done as described by [25].

2.7. Histological assessment of testes and epididymides

The testes and epididymides were passed through graded concentrations of ethanol after 48 h, sectioned and stained routinely with hematoxylin and eosin for microscopy studies. The slides were cleared in xylene before they were mounted on the microscope and then examined. Photomicrographs of the slides were taken at 100 × magnification.

2.8. Biochemical analysis

Tissue (testes and epididymides) lipid peroxidation was determined by measuring Thiobarbituric Acid Reactive Substances (TBARS/MDA) produced during lipid peroxidation according to the method of [26]. Tissue catalase activity was assessed according to the method of [27]. Assessment of tissue superoxide dismutase (SOD) activity was determined according to the method of [28] and the reduced glutathione level was measured by spectrophotometric assay kit (Oxford Biomedical Research, USA).

2.9. Statistical analysis

Data were expressed as mean ± SEM. The differences in mean were compared by analysis of variance (one-way ANOVA) and $p < 0.05$ was considered statistically significant. Graphpad prism 5 was used for the statistical analysis of data.

3. Results

3.1. Phytochemical constituents, proximate analysis and acute oral toxicity of Saccharum officinarum molasses

The Table 1a shows the chemical constituents present in crude *Saccharum officinarum* molasses (SOM), *Saccharum officinarum* molasses methanol fraction (SOMMF) and *Saccharum officinarum* molasses aqueous fraction (SOMAqF). The proximate analysis shows that crude *Saccharum officinarum* molasses contain 6.2 % ash, moisture content of 16.94 % crude protein 4.38 % and majorly carbohydrate 71.84 % (Table 1b). *Saccharum officinarum* molasses is a non-toxic substance, as the result of the toxicity test did not show any visible and identifiable adverse effects or mortality at the dose of 2 g/kg body weight crude SOM, after the 14th day observatory period. A dosing progression factor

Table 1a
Phytochemical constituents of crude *Saccharum officinarum* molasses, SOMMF and SOMAqF.

Phytochemical	Crude SOM	SOMMF	SOMAqF
Saponin	+ +	+ + + +	+ +
Tannin	–	–	–
Flavonoids	+ +	+ +	+ +
Terpenoid	–	+ +	–
Cardiac glycosides	–	+ + + +	–
Antraquinone	–	–	–
Alkaloids	+	+	–
Reducing sugar	+ + +	+ + + +	–

Key: + present, - absent, + + moderate, + + + high, + + + + very high.
SOM = *Saccharum officinarum* molasses.
SOMMF = *Saccharum officinarum* molasses methanol fraction.
SOMAqF = *Saccharum officinarum* molasses aqueous fraction.

Table 1b
Proximate analysis of *Saccharum officinarum* molasses.

Constituents	Observation
Ash	6.20 %
Moisture	16.94 %
Crude protein	4.38 %
Carbohydrate	71.84 %

Table 1c
Acute oral toxicity effect of *Saccharum officinarum* molasses.

No of animal (Male)	Death	Survival
1st	0 %	100 %
2nd	0 %	100 %
3rd	0 %	100 %
4th	0 %	100 %
5th	0 %	100 %

of 3.2 in line with the revised up and down procedure of the OECD was adopted for the test (Table 1c).

3.2. Effect of Saccharum officinarum molasses and sugar on relative organ weights

The weight of the seminal vesicle was significantly decreased ($p < 0.05$) in 0.0064 g/kg sugar as compared with all the treated groups. The weight of the epididymides significantly increased in 0.0064 g/kg sugar + 7.9 g/kg SOM compared with the control. There was a significant increase ($p < 0.05$) in the weight of the liver in 0.6 g/kg SOMAqF compared with the control. (Table 2).

3.3. Effect of Saccharum officinarum molasses and sugar on fasting blood glucose level

There was a significant decrease ($p < 0.05$) in the fasting blood glucose level of rat given 0.0064 g/kg sugar + 7.9 g/kg SOM compared with both SOMMF and SOMAqF, while a significant increase was observed in 10.0 g/kg SOMMF when compared with both the control and SOMAqF (Table 3).

3.4. Effect of Saccharum officinarum molasses and sugar on epididymal sperm characteristics

There was a significant increase ($p < 0.05$) in the sperm count of rats given 2.5 g/kg SOM compared with control and a decrease in sperm count of 6.4 g/kg SOMAqF compared with SOMMF. Sperm viability

Table 3
Effect of sugar and *Saccharum officinarum* molasses on fasting blood glucose level.

Group	Fasting blood glucose level (mmol/L)
Control	101.71 ± 1.37
0.8 g/kg SOM	101.57 ± 3.50
2.5 g/kg SOM	101.43 ± 3.16
7.9 g/kg SOM	103.01 ± 2.65
0.0064 g/kg sugar	104.43 ± 3.17
0.0064 g/kg sugar + 7.9 g/kg SOM	99.86 ± 2.10$^{\#\alpha}$
1.0 g/kg SOMMF	110.20 ± 4.17
3.2 g/kg SOMMF	114.20 ± 3.71
10.0 g/kg SOMMF	120.80 ± 1.79$^{*\alpha}$
0.6 g/kg SOMAqF	114.70 ± 2.20
2.0 g/kg SOMAqF	114.50 ± 3.33
6.4 g/kg SOMAqF	114.50 ± 2.46

Data represent mean ± SEM, n = 5. *$p < 0.05$ compared with control. $^{\#}p < 0.05$ compared with SOMMF. $^{\alpha}p < 0.05$ compared with SOMAqF. SOM = *Saccharum officinarum* molasses SOMMF = *Saccharum officinarum* molasses methanol fraction. SOMAqF = *Saccharum officinarum* molasses aqueous fraction.

significantly decreased ($p < 0.05$) in 7.9 g/kg SOM and 0.0064 g/kg sugar. There were significant decreases ($p < 0.05$) in sperm count and viability of rats that received 3.2 and 10.0 g/kg SOMMF, 0.6, 2.0 and 6.4 g/kg SOMAqF. Sperm motility significantly decreased ($p < 0.05$) in all the SOM and sugar treated groups, in 3.2 and 10.0 g/kg SOMMF treated groups relative to the control and in 10.0 g/kg SOMMF compared with SOMAqF. The abnormal sperm morphology significantly increased ($p < 0.05$) in 7.9 g/kg molasses, 0.0064 g/kg sugar and 6.4 mg/kg sugar + 0.79 g/kg SOM (Table 4). It also increased significantly ($p < 0.05$) in 2.0 and 6.4 g/kg SOMAqF treated rats compared with control and in 6.4 g/kg SOMAqF compared with SOMMF (Table 4).

3.5. Effect of Saccharum officinarum molasses and sugar on serum hormonal profile

Serum testosterone level significantly decreased ($p < 0.05$) in the 6.4 g/kg SOMAqF compared to the control. There were no significant differences in the serum follicle-stimulating hormone, luteinizing hormone and testosterone level of crude SOM treated rats compared to the control. Also, there were no changes in the concentration of the three hormones in SOMMF compared with SOMAqF (Table 5).

3.6. Effect of Saccharum officinarum molasses on lipid peroxidation and antioxidant enzymes

Table 6 show significant increases ($p < 0.05$) in testicular

Table 2
Effect of sugar and *Saccharum officinarum* molasses on relative organ weight(g).

Group	Testis	Epididymis	Seminal vesicle	Liver	Kidney
Control	0.53 ± 0.05	0.18 ± 0.14	0.37 ± 0.16	2.67 ± 0.29	0.62 ± 0.05
0.8 g/kg SOM	0.46 ± 0.15	0.11 ± 0.04	0.28 ± 0.18	2.90 ± 0.43	0.64 ± 0.08
2.5 g/kg SOM	0.51 ± 0.08	0.11 ± 0.03	0.39 ± 0.13	3.01 ± 0.36	0.57 ± 0.08
7.9 g/kg SOM	0.52 ± 0.07	0.09 ± 0.03	0.42 ± 0.15	2.83 ± 0.14	0.57 ± 0.03
0.0064 g/kg sugar	0.48 ± 0.04	0.09 ± 0.02	0.09 ± 0.02$^{*\#\alpha}$	2.79 ± 0.45	0.52 ± 0.08
0.0064 g/kg sugar + 7.9 g/kg SOM	0.53 ± 0.06	0.37 ± 0.19*	0.37 ± 0.18	2.63 ± 0.12	0.61 ± 0.04
1.0 g/kg SOMMF	0.52 ± 0.03	0.22 ± 0.02	0.39 ± 0.08	3.01 ± 0.11	0.54 ± 0.01
3.2 g/kg SOMMF	0.48 ± 0.02	0.22 ± 0.01	0.35 ± 0.05	2.63 ± 0.11	0.49 ± 0.02
10.0 g/kg SOMMF	0.50 ± 0.04	0.23 ± 0.02	0.45 ± 0.03	2.81 ± 0.18	0.52 ± 0.02
0.6 g/kg SOMAqF	0.47 ± 0.02	0.25 ± 0.01	0.31 ± 0.03	3.63 ± 0.17*	0.62 ± 0.02
2.0 g/kg SOMAqF	0.60 ± 0.05	0.30 ± 0.03	0.40 ± 0.04	3.23 ± 0.27	0.59 ± 0.01
6.4 g/kg SOMAqF	0.52 ± 0.05	0.22 ± 0.02	0.47 ± 0.04	2.70 ± 0.11	0.54 ± 0.02

Data represent mean ± SEM, n = 5. *$p < 0.05$ compared with control. $^{\#}p < 0.05$ compared with SOMMF. $^{\alpha}p < 0.05$ compared with SOMAqF. SOM = *Saccharum officinarum* molasses SOMMF = *Saccharum officinarum* molasses methanol fraction. SOMAqF = *Saccharum officinarum* molasses aqueous fraction.

Table 4
Effect of crude *Saccharum officinarum* molasses and sugar on epididymal sperm characteristics.

Group	Sperm count (million/ml)	Sperm motility (%)	Sperm viability (%)	Abnormal sperm morphology (%)
Control	49.86 ± 3.46	90.00 ± 1.88	97.14 ± 0.55	10.98 ± 0.09
0.8 g/kg SOM	53.86 ± 3.73	74.29 ± 2.02*	96.29 ± 0.60	11.63 ± 0.09
2.5 g/kg SOM	66.00 ± 3.91*	73.57 ± 1.79*	96.28 ± 0.60	11.38 ± 0.12
7.9 g/kg SOM	60.14 ± 1.29	62.14 ± 4.61*	84.71 ± 4.73*	12.27 ± 0.10*
0.0064 g/kg sugar	57.29 ± 3.83	74.29 ± 2.29*	86.57 ± 4.74*	14.12 ± 0.08*
0.0064 g/kg sugar + 7.9 g/kg SOM	60.14 ± 3.05	60.00 ± 2.18*	92.00 ± 2.50	14.34 ± 0.11*
1.0 g/kg SOMMF	109.30 ± 5.20	90.00 ± 2.60	86.67 ± 1.67	9.50 ± 0.43
3.2 g/kg SOMMF	103.20 ± 9.40*	80.00 ± 3.50	79.17 ± 2.39*	10.33 ± 1.05
10.0 g/kg SOMMF	92.17 ± 10.21*	69.97 ± 4.70*$^{\alpha}$	71.33 ± 5.04*	11.83 ± 0.79
0.6 g/kg SOMAqF	107 ± 7.50*	87.5 ± 2.50	85.83 ± 2.01	9.83 ± 0.70
2.0 g/kg SOMAqF	97.67 ± 5.6 *	86.5 ± 2.75	78.83 ± 2.12*	13.08 ± 1.34*
6.4 g/kg SOMAqF	86.5 ± 3.01 *$^{\#}$	80 ± 2.89	74.17 ± 3.01*	14.17 ± 1.52*$^{\#}$

Data represent mean ± SEM, n = 5. *p < 0.05 compared with control. $^{\#}$ p < 0.05 compared with SOMMF. $^{\alpha}$p < 0.05 compared with SOMAqF. SOMMF = *Saccharum officinarum* molasses methanol fraction. SOMAqF = *Saccharum officinarum* molasses aqueous fraction.

Table 5
Effect of crude *Saccharum officinarum* molasses and sugar on serum follicle-stimulating hormone, luteinizing hormone and testosterone levels.

Group	Follicle-stimulating Hormone (ng/mL)	Luteinizing Hormone (ng/mL)	Testosterone (ng/mL)
Control	0.2825 ± 0.0002	0.5940 ± 0.0008	1.90 ± 0.02
0.8 g/kg SOM	0.2825 ± 0.0003	0.5986 ± 0.0001	1.75 ± 0.05
2.5 g/kg SOM	0.2831 ± 0.0005	0.6000 ± 0.0004	1.91 ± 0.04
7.9 g/kg SOM	0.2827 ± 0.0002	0.5976 ± 0.0005	1.75 ± 0.05
0.0064 g/kg sugar	0.2819 ± 0.0002	0.5977 ± 0.0003	1.74 ± 0.05
0.0064 g/kg sugar + 7.9 g/kg SOM	0.2782 ± 0.0004	0.5979 ± 0.0007	1.76 ± 0.05
1.0 g/kg SOMMF	0.1462 ± 0.0009	0.6700 ± 0.0007	1.72 ± 0.16
3.2 g/kg SOMMF	0.1864 ± 0.0009	0.7300 ± 0.0007	1.94 ± 0.02
10.0 g/kg SOMMF	0.1964 ± 0.0016	0.8510 ± 0.0008	1.78 ± 0.08
0.6 g/kg SOMAqF	0.1456 ± 0.0005	0.6100 ± 0.0008	1.90 ± 0.01
2.0 g/kg SOMAqF	0.1860 ± 0.0006	0.9140 ± 0.0006	1.86 ± 0.04
6.4 g/kg SOMAqF	0.1795 ± 0.0011	0.8240 ± 0.0006	1.66 ± 0.10*

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

malondialdehyde (MDA) level of 3.2 and 10.0 g/kg SOMMF and 6.4 g/kg SOMAqF treated rats compared with control but a decrease in 1.0 g/kg SOMMF compared with SOMAqF. The testicular superoxide dismutase (SOD) activity significantly increased (p < 0.05) in rats that received 3.2 and 10.0 g/kg SOMMF and 0.6 g/kg SOMAqF compared to control as well as in 0.6 g/kg SOMAqF compared with SOMMF. Testicular catalase activity significantly increased (p < 0.05) in 0.6 g/kg SOMAqF relative to control and in 0.6 g/kg SOMAqF compared with SOMMF. No significant differences were observed in the testicular glutathione concentrations of all the treated groups. In the epididymis, there was a significant increase (p < 0.05) in the MDA level and SOD activity of the group that received 10.0 g/kg SOMMF compared to both the control and SOMAqF. Glutathione concentration significant increase (p < 0.05) in 10.0 g/kg SOMMF compared with SOMAqF but not with the control. Catalase activity did not show any significant differences in all treated groups (Table 7).

3.7. Effect of Saccharum officinarum molasses and sugar on testes and epididymides

Testicular section show depletion of germinal cells in group treated with 7.9 g/kg crude SOM. In rats that received 0.0064 g/kg sugar, there were compacted germ cells in seminiferous tubule without lumen (Fig. 1). Testicular sections of 10.0 g/kg SOMMF treated rats show some seminiferous tubules with distorted, degenerated germ cell levels and layers with maturation arrest at the primary level. Some tubules do not have lumen and some presented a wide and empty lumen. Some connective tissues enveloping the tubules appear thickened and rats given SOMAqF show interstitial Leydig cells with hyperplasia and congested interstitial spaces (Fig. 1). Infiltration of inflammatory cells of the epididymides were observed in 0.0064 g/kg sugar + 7.9 g/kg SOM) (Fig. 2). In the 10.0 g/kg SOMMF, the epididymal sections show few ducts without spermatozoal in their lumen, interstitial spaces with aggregate of inflammatory cell and in 0.0064 g/kg SOMAqF there were a few interstitial tissues appearing fibrotic (Fig. 2).

4. Discussion

There was no lethality observed in rats even at a limit dose of 2000 mg/kg weight, which implies a wide safety margin for *Saccharum officinarum* molasses when ingested orally. The use of *Saccharum officinarum* molasses as a sweetener, energy source and possibility of abuse propelled this test (OECD 425, 2001). *Saccharum officinarum* molasses consists of an array of phytochemicals. [29] reported that flavonoids are the main source of colorants that are essentially considered impurities during the processing of sugar from sugarcane. They are known antioxidants useful in the treatment of various pathologies [30] and promote male reproductive functions [31,32]. Saponins possess ahypolipidemic effect useful in treating cardiovascular diseases [33], it also has anti-inflammatory and anti-pyretic activity [34]. Cardiac glycosides and terpenoids are useful in the prevention and therapy of several

Table 6
Effect of *Saccharum officinarum* molasses on lipid peroxidation and antioxidant enzymes of the testes.

Group	Control	1.0 g/kg SOMMF	3.2 g/kg SOMMF	10.0 g/kg SOMMF	0.6 g/kg SOMAqF	2.0 g/kg SOMAqF	6.4 g/kg SOMAqF
MDA (U/mg)	1.7 ± 0.24	1.4 ± 0.01$^{\alpha}$	2.4 ± 0.18*	2.6 ± 0.13*	2.2 ± 0.20	2.1 ± 0.26	2.6 ± 0.44*
SOD (U/mg)	174.8 ± 23.72	214.7 ± 49.21	329.4 ± 54.64*	290.2 ± 34.93	3299.0 ± 2.84*$^{\#}$	270.6 ± 34.36	260.5 ± 34.02
CATALASE (IU/L)	698.2 ± 62.94	539.3 ± 44.62	676.0 ± 65.25	771.5 ± 158.60	1101.0 ± 87.77*$^{\#}$	792.7 ± 63.14	833.9 ± 111.0
GSH (uM/mg)	2.9 ± 0.31	2.9 ± 0.27	3.2 ± 0.43	2.7 ± 0.29	4.4 ± 0.95	3.28 ± 0.86	3.94 ± 0.42

Data represent mean ± SEM, n = 5. *p < 0.05 compared with control. $^{\#}$ p < 0.05 compared with SOMMF. $^{\alpha}$p < 0.05 compared with SOMAqF. SOMMF = *Saccharum officinarum* molasses methanol fraction. SOMAqF = *Saccharum officinarum* molasses aqueous fraction.

Table 7
Effect of *Saccharum officinarum* molasses extracts on lipid peroxidation and antioxidant enzymes of the Epididymides.

Group	Control	1.0 g/kg SOMMF	3.2 g/kg SOMMF	10.0 g/kg SOMMF	0.6 g/kg SOMAqF	2.0 g/kg SOMAqF	6.4 g/kg SOMAqF
MDA (U/mg)	15.7 ± 6.89	13.3 ± 3.05	19.9 ± 8.16	43.0 ± 5.20*$^{\alpha}$	13.2 ± 2.36	23.9 ± 3.94	22.9 ± 2.0
SOD (U/mg)	1620 ± 379.2	2350 ± 550.5	3513 ± 1089	5383 ± 1479*$^{\alpha}$	1729 ± 361.8	1989 ± 594.1	2317 ± 912.1
CATALASE (IU/L)	3669 ± 898.3	3338 ± 771.8	6558 ± 1716	6446 ± 1707	2144 ± 467.6	3970 ± 1721	3553 ± 1070
GSH (uM/mg)	9.1 ± 1.97	7.1 ± 1.99	13.4 ± 6.31	15.8 ± 4.17$^{\alpha}$	5.6 ± 2.12	9.2 ± 1.49	7.7 ± 1.97

Data represent mean ± SEM, n = 5. *p < 0.05 compared with control. $^{\alpha}$p < 0.05 compared with SOMAqF. SOMMF = *Saccharum officinarum* molasses methanol fraction. SOMAqF = *Saccharum officinarum* molasses aqueous fraction.

diseases, including cancer [35]. Reducing sugars contain a variety of sugars, including sucrose, glucose, and fructose [36]. The result of the proximate analysis supports the findings of [37], that molasse is a nutritive substance composed of either monosaccharides or disaccharides responsible for its sweetness and function as the body's primary source of fuel. The high moisture content observed may be responsible for its consistency and fluidity which possibly improves its shelf life, preserve and inhibits the growth of bacteria, molds, and yeast, in line with reports of [38]. The increased fasting blood glucose level and organ weights corroborate the findings of [39] that sugar-sweetened beverages predispose to insulin resistance as they may contain a variety of sugars, such as sucrose, glucose, and fructose.

The hypothalamus, anterior pituitary, and testes are three components of the male reproductive system known as the hypothalamic-pituitary-gonadal (HPG) axis that forms a finely tuned system which is controlled through a classic negative feedback mechanism. As testosterone level in the blood rises, the anterior pituitary becomes less responsive to stimulation by gonadotropin-releasing hormone (GnRH), resulting in reduced luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion. The crude *Saccharum officinarum* molasses **(**SOM) and refined sugar caused no change in the serum hormone levels. Also, *Saccharum officinarum* molasses methanol fraction (SOMMF) did not alter serum levels of FSH and LH, but *Saccharum officinarum* molasses aqueous fraction (SOMAqF) was able to cause a significant decrease in serum testosterone level in the group that received the highest dose. This infers that the action of SOM may not be through the gonadotropins but may be directly on the testes. Testosterone is the primary androgen responsible for the development, growth, maintenance of male reproductive functions and sexual characteristics [40,41]. The decreased serum testosterone level by the SOMAqF is an indication that SOM constitutes substances that may alter the reproductive functions.

The crude SOM at its highest dose and refined sugar significantly increased the sperm count and percentage of the aberrant spermatozoa but decreased sperm motility and viability. The sperm count, viability and motility significantly decreased as the dose of SOMMF administered was increased. Concurrently, reduced sperm count and viability with increased percentage aberrant spermatozoa were noted as the concentration of SOMAqF was increased. These suggest possible alterations during spermiation as it was released from the protective Sertoli cells into the lumen of the seminiferous tubule [42]. It is implicit that *Saccharum officinarum* molasses may be a potential causative factor of male infertility in the long run.

The presence of highly unsaturated fatty acids and increased

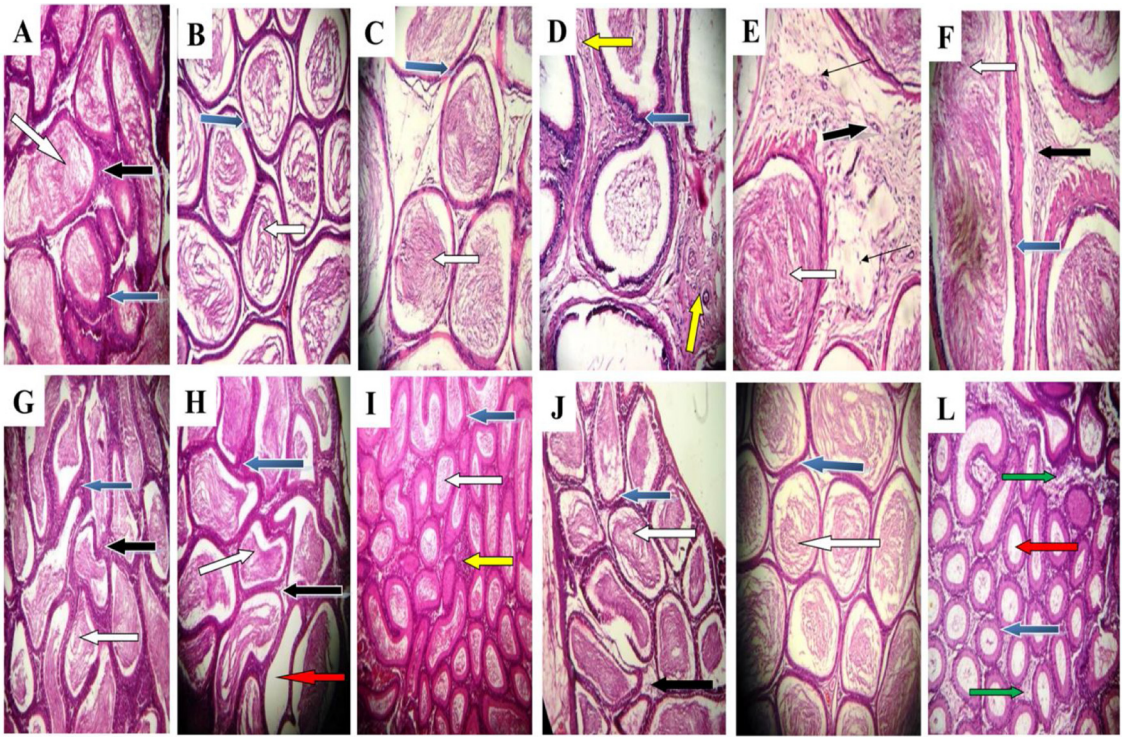

Fig. 1. Photomicrograph of testicular sections of treated rats. A (Control), B, C and D (0.8, 2.5 and 7.9 g/kg SOM respectively), E (0.0064 g/kg sugar), F (0.0064 g/kg sugar + 7.9 g/kg SOM), G, H and I (1.0,3.2 and 10.0 g/kg SOMMF respectively), J, K and L (0.6, 2.0 and 6.4 g/kg SOMAqF, respectively). Note the normal seminiferous tubules (white arrows), Interstitium (grey arrows), Interstitial congestion (blue arrows), depleted germinal cells (yellow arrows), compacted germ cells and absence of lumen in seminiferous tubule (black arrows), degenerated sertoli and germ cell layers - maturation arrest (spanning arrows), thickened connective tissues (red arrows), hyperplasia (green arrows). Stained by H&E. Magnification: X100.

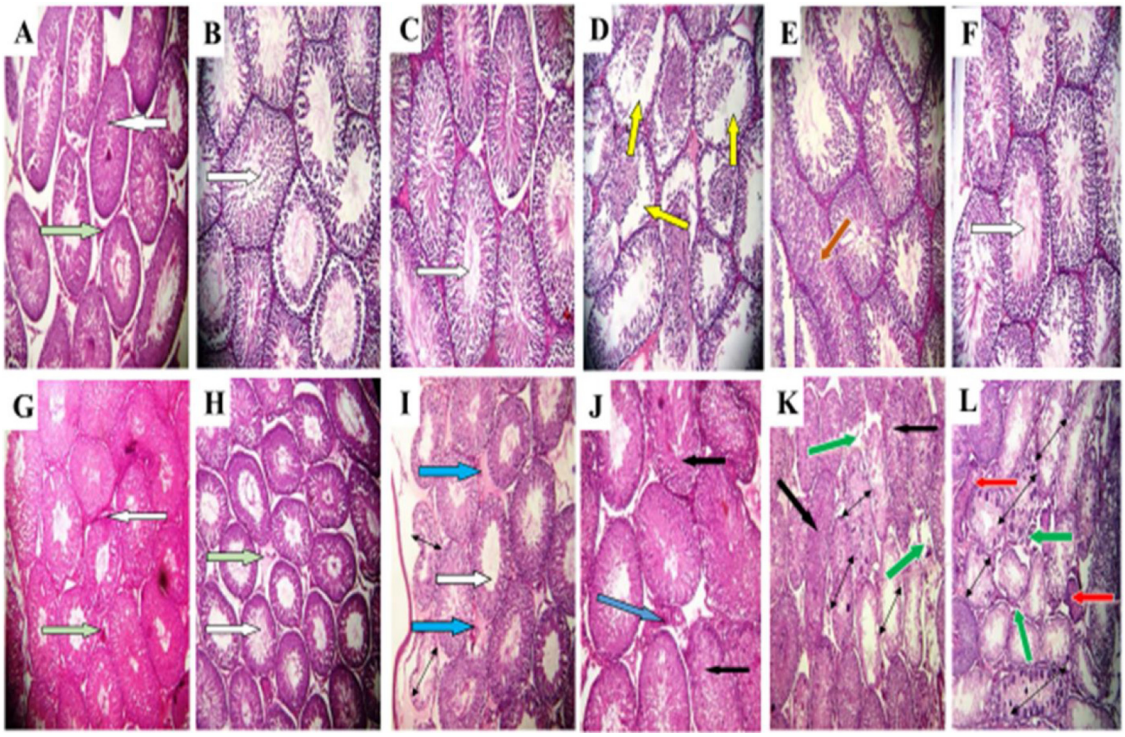

Fig. 2. Photomicrograph of epididymal sections of treated rats. A (Control), B, C and D (0.8, 2.5 and 7.9 g/kg SOM respectively), E (0.0064 g/kg sugar), F (0.0064 g/kg sugar + 7.9 g/kg SOM), G, H and I (1.0,3.2 and 10.0 g/kg SOMMF respectively), J, K and L (0.6, 2.0 and 6.4 g/kg SOMAqF respectively). Note the smooth muscle and epithelial layers (blue arrows), spermatozoa stored within the lumen (white arrows), normal interstitial spaces (black arrows), and infiltration of inflammatory cells in interstitium (yellow arrows), empty ducts (red arrows), fibrosis (green arrows). Stained by H&E. Magnification: X100.

generation of free radicals as potential reactive oxygen species (ROS) generating systems in the testes make it vulnerable to oxidative stress [43–45]. The SOMMF and SOMAqF significantly increased testicular lipid peroxidation, this may be due to increased ROS generation [46]. reported that increased ROS generation causes lipid peroxidation and production of a cytotoxic substance such as MDA which triggers deleterious events that can affect sperm chromatin integrity and cause a high frequency of DNA strand breaks [47]. To overcome oxidative stress, the testes consist of an elaborate array of antioxidant enzymes and free radical scavengers which ensures that its spermatogenic and steroidogenic functions are not impacted by oxidative stress as peroxidative damage is currently regarded as the single most important cause of impaired testicular functions [44,48]. Although SOMMF and SOMAqF caused a significant increase in the activity of testicular superoxide dismutase (SOD), SOMAqF further increased catalase activities, both of them did not change the concentration of glutathione within the testes [49]. reported that overexposure to environmental toxicants can impair the pro-oxidant/antioxidant balance in the testes and thereby hamper testicular function. The result revealed the possibility of an imbalance between the pro-oxidant and antioxidant systems that may have disrupted the oxidant status despite the increases in activities of testicular antioxidants. It is plausible that the presence of SOM increased the production of ROS within testicular cells in agreement with [50] that whenever the production of ROS exceeds or overwhelms the scavenging capacity of antioxidants, it results in oxidative stress. Also, the treatment was done for eight weeks, perhaps this probably leads to overexposure of the animals to both SOMMF and SOMAqF as well resulting in an imbalance in the pro-oxidant/antioxidant of the testes.

Within the epididymis, SOMMF caused significant increases in both MDA and SOD of the same group but showed no significant changes in catalase activity and glutathione concentration. The presence of MDA, a known stable by-product of lipid peroxidation has been used to quantify lipid peroxidation and it is a well-known mechanism of cellular injury in animals [51]. The lipid peroxidation may have caused the anomalies in the sperm parameters observed following the report of [52] that increase MDA causes an increase in abnormal semen parameters in humans. Superoxide dismutase has been shown to act by catalyzing the conversion of superoxide to form oxygen and hydrogen peroxide [53]. Although SOD activity was increased, it was probably overwhelmed by the quantity and rate of MDA liberated.

Sertoli cells are components of the seminiferous epithelium with a pivotal role in testicular homeostasis. They help to make up the blood testes barrier, support and synchronize germ cells as they differentiate and mature [54,55]. Sertoli cells have been reported to be the most resistant cells in the testis [56,57]. The sugar consumption caused compaction of germ cells and the absence of lumen in some tubules in line with the previous reports on cytotoxicity of sugar and its adverse effects on spermatogenesis [10]. The crude SOM caused the loss of some germ cells. This probably may have disrupted the cell cycle or cell division processes during spermatogenesis following [58] who stated that depletion of germ cells can alter the expression of Sertoli cell genes and secretion of specific Sertoli cell proteins. The detachment of these developing germ cells from the seminiferous epithelium may lead to their premature release into the tubular lumen leading to infertility [59].

[60] stated that alterations in Sertoli cells cause severe damage to spermatogenesis. The abnormal sperm cells observed may be as a result of the toxicant effect of SOM intake in support of [54] who noted that when Sertoli cells are subjected to a toxicant, they manifest a variety of changes in their morphology which may cause massive death of germ cells that they were meant to sustain. The previous reports had shown that as germ cells are being nursed by the Sertoli cells, they concurrently play a feedback role in regulating the actions of the Sertoli cells [58]. The SOMMF and SOMAqF caused degeneration of germ cells and their layers, this may be the reason for the abnormal Sertoli cells that were formed, corroborating [61] that death of germ cells can lead to functional and morphological alterations of Sertoli cells. It is also plausible that the alterations caused to the Sertoli cells may be the secondary effects of the germ cell [57]. The lack of spermatogonia,

maturation arrest at the primary level with the wide and empty lumen in some of the tubule corroborates [62] that arrest of spermatogenesis may be the reason for the wide and empty lumen as matured elongating spermatids may not be released into the tubular lumen [62]. Thus, SOM may be considered as a potential Sertoli cell toxicant, altering normal testicular functions.

The SOMMF caused infiltration and aggregation of inflammatory cells in the interstitial spaces of the epididymis. Inflammatory infiltrates are commonly observed in the interstitium of animals, it may have been driven by an imbalance in the dynamic equilibrium between immune tolerance and toxicant mediated activation of inflammation in the epididymis that was posed by the biological effects of the SOM administered [49]. The SOMMF and SOMAqF caused empty epididymal ducts, the SOMAqF further caused fibrosis of the interstitial tissues. The epididymis depends on androgen to maintain the outflow of mature spermatids as it supports maturation into fertile spermatozoa [63]. Testosterone deprivation causes a reduction in the number of qualitative normal sperm entering the epididymis. Also, various activities take place within specific portions of the epididymis and this includes protein synthesis, resorption of fluid, secretion and hormonal modulation [41]. The disruption of these processes may result in infertility.

5. Conclusion

The refined sugar and *Saccharum officinarum* molasses adversely altered sperm quality and disrupted the normal architecture of the testes and epididymides. *Saccharum officinarum* molasses fractions further caused reduced testosterone levels and lipid peroxidation. Hence, *Saccharum officinarum* molasses also possess anti-gonadal properties and therefore is not a suitable substitute for refined sugar with regards to its effects on male reproductive functions.

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Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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