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Full length Research Article

Detrimental Effects of *Saccharum officinarum* Juice on Reproductive Functions of Female Wistar Rats

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Summary: Changing dietary compositions have contributed to the growing epidemic of metabolic diseases with serious impacts on several aspects of health, including reproductive health. *Saccharum officinarum* juice has a natural sweetness that makes the general populace relinquish its use as a sweet course and well-known raw material for the production of refined sugar. Studies have reported adverse effects of this juice on male reproductive functions, but there is a paucity of information on females. This study investigated the effects of fresh *Saccharum officinarum* juice on the reproductive functions of female Wistar rats. A sugarcane press juicer was used to extract *Saccharum officinarum* juice. Twenty female Wistar rats (180-200 g) grouped into four (n = 5) received 1.0 mL/kg/day distilled water (control), and 1.0, 3.2, and 10.0 mL/kg/day of fresh *Saccharum officinarum* juice once daily for 21 days by gavage. Serum follicle-stimulating hormone, luteinizing hormone, and estrogen levels were measured using enzyme-linked immunosorbent assay (ELIZA). The estrous cycle was assessed using the Marcondes principle and histology of the ovary and uterus were assessed by microscopy. Data were analyzed using the Analysis of variance at a significance of $p < 0.05$. *Saccharum officinarum* juice caused an increase in the body weight and serum levels of follicle-stimulating hormone and luteinizing hormone. It altered the estrous cycle by increasing the frequency of occurrence of the proestrus phase but reduced that of the metestrus phase. The juice altered the cytoarchitecture of the ovaries via vacuolations and cysts within the ovarian stroma, while the uterine section showed distorted endometrial lining and glands. *Saccharum officinarum* juice inflamed the ovaries and distorted the estrous cycle and uterine endometrial lining. *Saccharum officinarum* juice consumption may possess deleterious effects on the reproductive functions of female Wistar rats.

Keywords*: Saccharum officinarum juice, Ovary, Uterus, Estrous cycle, Infertility.*

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INTRODUCTION

The inability to conceive by natural means after one year of unprotected sexual intercourse is an issue of global concern (Ombelet *et al*., 2008; WHO, 2018), and it is one of the major causes of unhappy marriages (Inhorn *et al*., 2015; Bakhtiyar *et al.,* 2019; Walker and Tobler, 2022). Though male infertility contributes to some cases of global childlessness, female infertility results in profound social consequences for women regardless of the cause of infertility (Inhorn, 2003; Rutstein and Shah, 2004; Bakhtiyar *et al.,* 2019). The human diet has been related to adverse effects on fertility (Panth *et al.,* 2018: Skoracka *et al.,* 2021). Changing dietary composition has contributed to a growing epidemic of metabolic diseases with serious impacts on several aspects of health, including reproductive health (Norman *et al.,* 2004; Salas-Huetos *et al.,* 2017; Silvestris *et al.,* 2019).

Saccharum officinarum (sugar cane) is commonly consumed as a staple food in some parts of the world, and it is a popular raw material for the production of refined sugar **(**Singh, *et al.,* 2015**).** The natural sweetness of the juice makes it attractive for use as a sweet course. Previous studies showed that *Saccharum officinarum* contains important phytochemicals (such as phenolic compounds, sterols, and policosanols) with antioxidant activity, cholesterol-lowering properties, and other potential health benefits (Singh *et al.,* 2015; Adjatin *et al.,* 2019) that are vital in the treatment of several ailments in numerous parts of the world, such as arthritis, boils, colds, cough, dysentery, fever, sore throat, diuresis (Karthikeyan and Simipillai, 2010; Singh *et al.,* 2015; Ali *et al.,* 2019).

On the other hand, *Saccharum officinarum* contains a high amount of sucrose and some studies have reported harmful consequences of high sucrose intake such as ovarian dysfunction in rats (de Melo *et al.,* 2021). Adekunbi *et al.,* (2016) reported that high sucrose and high salt diet adversely altered sperm functions in Sprague-Dawley rats by reducing testicular weight and testosterone hormone levels. The study also revealed that consumption of *Saccharum officinarum* juice in male rats adversely altered reproductive functions by reducing sperm quality and disrupting testicular architecture (Ogunwole *et al.,* 2020).

However, there is a scarcity of evidence correlating the reproductive effects of consuming *Saccharum officinarum* juice in females. This study investigated the effects of fresh *Saccharum officinarum* juice on the reproductive functions of female Wistar rats.

MATERIALS AND METHODS

Preparation of *Saccharum officinarum* juice: The *Saccharum officinarum* plant was obtained from Karu, Nasarawa State, Nigeria. It was authenticated at the herbarium of the Department of Botany, University of Ibadan, and assigned the identification number UIH No.: 22613. The cane of *Saccharum officinarum* was stripped off its leaves and stalk. It was cut into small sizes of 1 cm each and the diced sugar cane was crushed using a sugar cane juicer (Vevor, 110LBS/H, China) to obtain the juice. The juice was filtered with a muslin cloth. The filtrate was subjected to phytochemical screening using a standard procedure (Odebiyi and Sofowora 1978; Sofowora, 1993). The rats were administered the fresh juice daily by oral gavage.

Experimental animals and design: All procedures involving the use of animals were according to the EU Directive 2010/63/EU for animal experiments and the study conformed with the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guide-line (2010) and ethical standards of the Department of Physiology, Bingham University for animal experiment. Twenty female Wistar rats (180-200 g) obtained from the National Veterinary and Research Institute, Plateau State were used for this experiment. The rats were kept in Bingham University animal house under standard laboratory conditions and were given feed and water *ad libitum*. The animals were acclimatized for two weeks before the experiment began. They were randomly divided into four groups (n = 5) and received 1.0 mL/kg/day of distilled water (control), 1.0, 3.2, and 10.0 mL/kg/day of SOJ respectively. The dosage regime was in line with the Organization for Economic Co-operation and Development (Ogunwole *et al.*, 2020; OECD, 2022). The administration was done orally once daily for 21 days, body weights were recorded once a week and on the last day of administration using an electronic weighing scale (EK5055, China).

Estrous cycle assessment: This was done pre-treatment and during treatment using the Marcondes principle. As the experiment began, the assessment of the estrous cycle was done for three weeks before treatment. The rats were then administered the fresh juice daily as stated in the design for 21 days, with a concurrent assessment of the estrous cycle between the hours of 7:00-8:00 am every morning as described by (Marcondes *et al.*, 2002). Vaginal-smeared slides were viewed under the microscope and the cell morphology was microscopically assessed using x40 magnification. The result of the estrous cycle study of the pre-treated and treated rats was compared.

Blood collection, serum preparation, and organ harvest: At the end of the third week of administration just before the animal sacrifice, the fasted rats were bled at the tail to get a drop of blood for the determination of the fasting blood glucose level using an automated glucometer (Fine test IGM-00178, UK). Afterward, the rats were sacrificed under thiopental anesthesia (Pereda *et al.*, 2006) and dissected along the linea alba of the anterior abdominal wall to the thoracic cavity to expose the heart and the organs. Blood was obtained through the cardiac puncture into plain serum bottles and allowed to coagulate for at least 45 minutes and then centrifuged at 10,000 rpm for 15 minutes to obtain the supernatant (serum) which was stored at -20 °C. Serum levels of follicle-stimulating hormone, luteinizing hormone, and estradiol were assayed with Enzyme-Linked Immunosorbent Assay (ELISA) kits (Fortress Diagnostics, UK). Furthermore, the ovaries and uteri were harvested, freed from adherent tissues, and weighed immediately with a digital electronic scale (Camry EHA501, China). The ovaries and uteri were fixed in Bouin's fluid for histology.

Histology of the Ovary and Uterus: The ovaries and uteri fixed in Bouin's fluid were processed for microscopy. The tissues were embedded in paraffin and sectioned with a microtome to get the 4–5 μm-thick paraffin sections. The dewaxed sections were stained with hematoxylin and eosin and the slides were viewed under a light microscope at 100× magnification.

Statistical analysis: Data were obtained and evaluated with Graph Pad Prism Statistics software (USA) version 5.0 and presented as mean ± standard error of the mean (mean ± SEM). The level of significance was $p < 0.05$.

RESULTS

Phytochemical screening of *Saccharum officinarum* juice: Phytochemical screening test showed the presence of; cardiac glycoside, terpenoids, saponins, and reducing sugar

Table 1.
Phytochemical screening of *Saccharum officinarum* juice.

TEST	RESULTS
Flavonoids	-
Cardiac glycoside	+ + +
Tannins	-
Anthraquinone	-
Saponins	+
Alkaloids	-
Terpenoids	+
Reducing sugars	+

Key= (+) Present; (-) Absent

Effect of *Saccharum officinarum* juice on fasting blood glucose level.:There were no significant differences in fasting blood glucose levels of all the treated groups as compared to the control.

Effect of *Saccharum officinarum* juice on the percentage change in body weight.: There were no significant changes in the body weight of the rats during pre-treatment. However, significant increases ($p < 0.05$) in body weight were observed in the 1.0 and 10.0 mL/Kg/day *Saccharum officinarum* juice-treated rats respectively relative to the control (Figure 2).

Effect of *Saccharum officinarum* juice on estrous cycle : The result showed that during pre-treatment there were significant increases ($p < 0.05$) in the frequency of occurrence of the proestrus, estrous, and metestrus phases, with a significant decrease ($p < 0.05$) in the frequency of occurrence of the diestrus phase of the estrous cycle as compared to their respective controls. Treatment with 10 mg/Kg/day juice increased the frequency of occurrence of the proestrus phase with a decreased frequency of occurrence of the metestrus phase as compared with their respective controls (Table 3). The number of cycles, as well as the length of the estrous cycle of the treated groups, were not significantly different from that of their respective controls (Table 4).

Effect of *Saccharum officinarum* juice on relative organ weight.
The result shows no significant difference in the weights of the ovary and uterus of the treated groups compared with the control.

Effect of *Saccharum officinarum* juice on serum hormone level: The group treated with 3.2 mL/Kg/day of *Saccharum officinarum* juice showed significant increases ($p < 0.05$) in the serum levels of both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels when compared with the respective control (Figure 3). There was no significant difference in the serum estradiol hormone level of the treated rats relative to the control (Figure 4).

Table 2.
Effect of *Saccharum officinarum* Juice on relative organ weight

	Organ weight (g)	
	Ovary	**Uterus**
Control	0.108±0.01	0.064±0.02
1.0 mL/Kg/day	0.114±0.01	0.068±0.02
3.2 mL/Kg/day	0.114±0.03	0.064±0.02
10.0 mL/Kg/day	0.110±0.00	0.080±0.01

Values represent mean ± standard error of mean, n=5.

Effect of *Saccharum officinarum* juice on the Histology of the Ovary and Uterus: The histology of the ovaries of rats in the treated groups showed vacuolations in the granulosa cell, cysts within the ovarian stroma, and corpus luteum. They also appeared with lymphocyte infiltration, degenerated follicles, and distorted stroma as compared with the ovary of the control group which show normal histology. The uterine sections of the treated groups showed distorted endometrial lining and glands (that were less tubular with distorted cuboidal epithelial cells) as compared with the normal uteri of control.

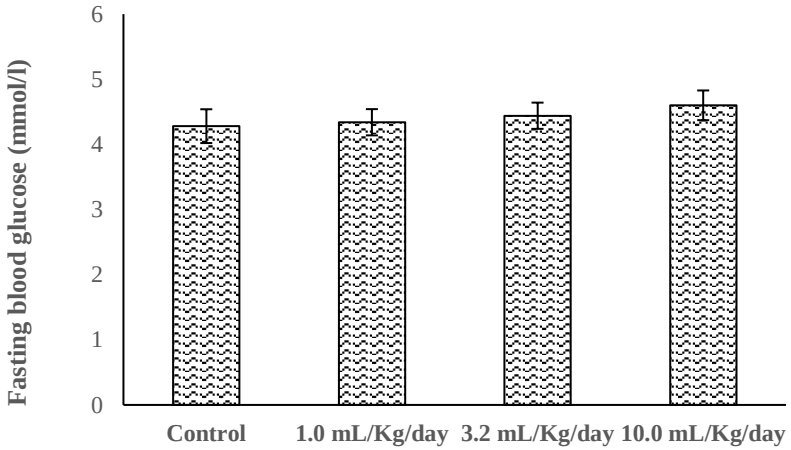

Figure 1.
Effect of *Saccharum officinarum* juice on fasting blood glucose level. Columns represent mean ± standard error of mean, n=5.

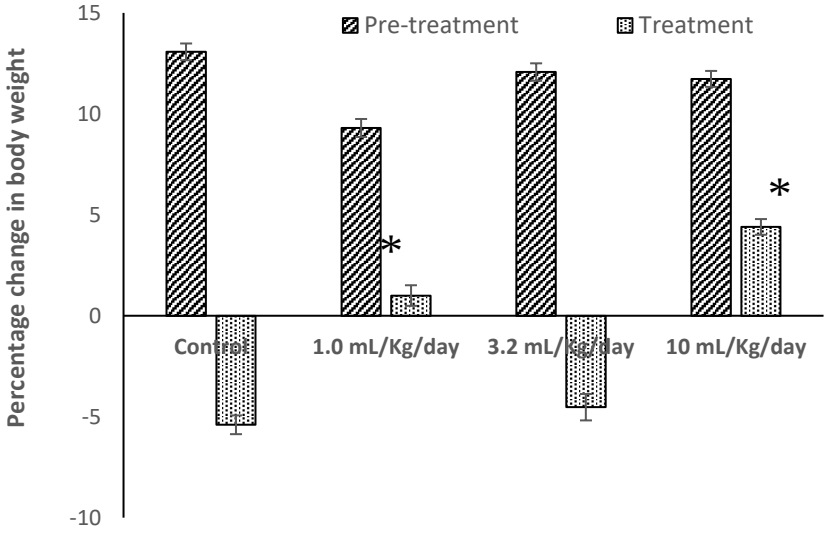

Figure 2.
Effect of *Saccharum officinarum* juice on the percentage change in body weight. Columns represent mean ± standard error of the mean, *$p < 0.05$ compared to control, n=5.

Table 3.
Effect of *Saccharum officinarum* juice on the frequency of occurrence of the phases of the estrous cycle.

	Proestrus		Estrus		Metestrus		Diestrus	
Group	**Pre-treatment**	**Treatment**	**Pre-treatment**	**Treatment**	**Pre-treatment**	**Treatment**	**Pre-treatment**	**Treatment**
Control	20.22 ±4.09	29.22 ±5.65	10.70 ±2.96	13.70 ±2.96	4.49 ±0.23	24.06 ±8.25	65.49 ±4.61	29.62 ±8.56
1.0 mL/Kg/day	20.24 ±2.33	28.58 ±4.00	14.29 ±1.88	6.66 ±1.88	13.1 ±2.32	16.18 ±3.57	52.37 ±1.50	48.58 ±8.85
3.2 mL/Kg/day	28.55 ±5.23	39.00 ±7.53	19.06 ±3.37	10.60 ±3.78	22.63 ±3.5	10.4 ±4.62	29.76 ±4.73	40.0 ±7.09
10.0 mL/Kg/day	41.65 ±5.30*	46.72 ±5.19*	21.45 ±3.19*	15.16 ±3.19	10.41 ±1.03*	12.36 ±5.45*	28.58 ±1.50*	25.76 ±6.25

*Values represent mean ± standard error of mean, n=5, *p < 0.05 compared to control.*

Table 4.
Effect of *Saccharum officinarum* juice on the number and length of the estrous cycle.

Group	Number of cycles		Estrous cycle length (days)	
	Pre-treatment	**Treatment**	**Pre-treatment**	**Treatment**
Control	1.2 ±0.2	1.6 ±0.4	16.2 ±0.2	9.70±0.2
1.0 mL/Kg/day	1.2±0.2	1.0±0.0	10.3±0.3	17.2±0.3
3.2 mL/Kg/day	1.6±0.6	1.4±0.2	11.0±0.3	12.1±0.2
10.0 mL/Kg/day	1.8±0.6	1.4±0.2	5.00±0.3	9.60±0.2

Values Columns represent mean ± standard error of mean, n=5

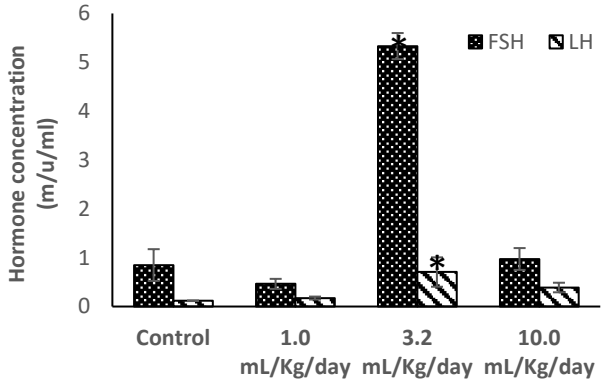

Figure 3.
Effect of *Saccharum officinarum* juice on follicle-stimulating hormone (FSH) and luteinizing hormone (LH) level. Columns represent mean ± standard error of mean, *p < 0.05 compared to control, n=5.

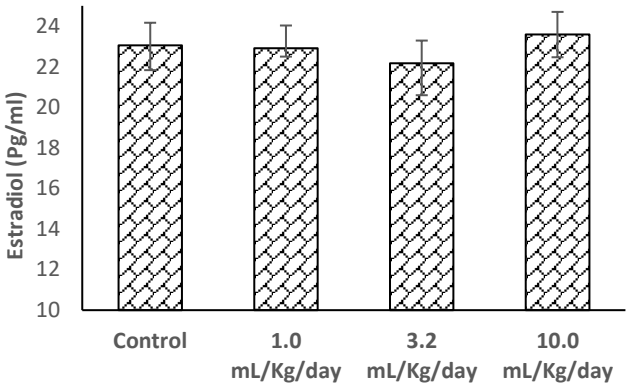

Figure 4.
Effect of *Saccharum officinarum* juice on estradiol level. Columns represent mean ± standard error of mean, n=5.

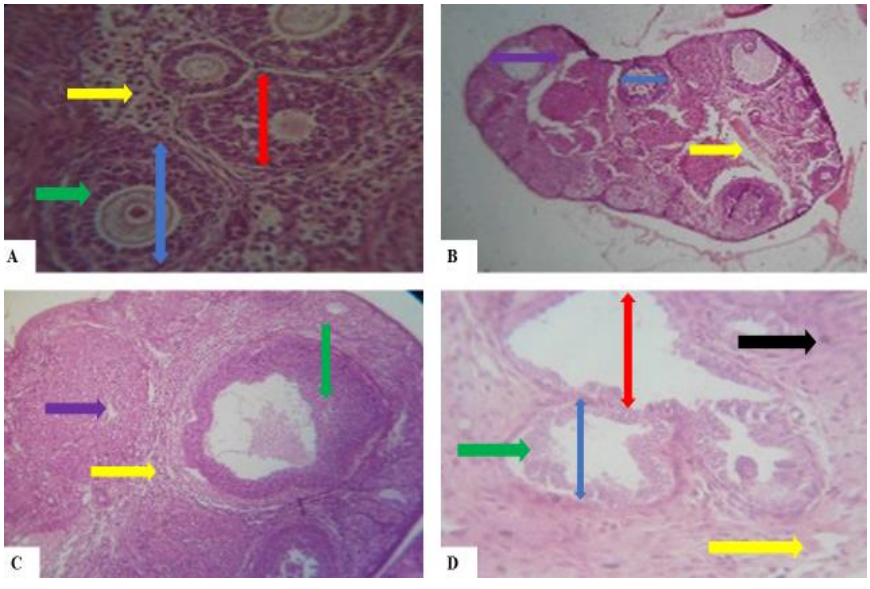

Figure 5a.
Photomicrograph of ovarian sections of control rat and *Saccharum officinarum* juice-treated rats. **A) Control**, appears normal. Note the ovarian primary follicle (red arrow), the secondary follicle (blue arrow), stromal cells (yellow arrow), and well-arranged granulosa cells (green arrow). **B) 1.0 mL/Kg/day of *Saccharum officinarum* juice** Note: vacuolation (purple arrow), cysts within the ovarian stroma (yellow arrow), and degenerated follicles (blue arrow). **C) 3.2 mL/Kg/day *Saccharum officinarum* juice** Note: cysts within the corpus luteum (purple arrow), vacuole in the granulosa cell (green arrow), and distortion of the stroma (yellow arrow). **D) 10 mL/Kg/day *Saccharum officinarum* juice** Note: severe degeneration of the primary follicles (red arrow), secondary follicles (blue arrow), and the granulosa cells (green arrow), lymphocyte infiltration (black arrow), cysts within the secondary follicle (black arrow) and distortion of stroma (yellow arrow). *Stained by H&E. Magnification: x100.*

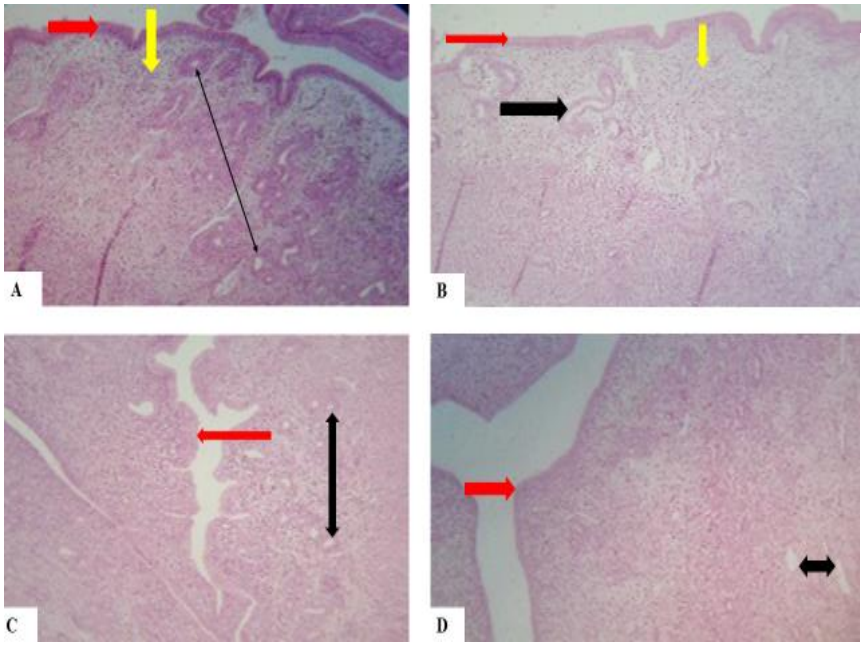

Figure 5b.
Photomicrograph of uterine sections of control rat and *Saccharum officinarum* juice treated rats.
A) Control, appears normal, with endometrium with well-proliferated simple columnar epithelium (red arrow), tubular endometrial glands (black arrow), and lamina propria underlying the endometrial lining (yellow arrow). **B) 1.0 mL/Kg/day of *Saccharum officinarum* juice** Note: endometrial lining (red arrow), distorted endometrial glands (black arrows), and lamina propria underlying the endometrial lining (yellow arrow). **C) 3.2 mL/Kg/day *Saccharum officinarum* juice** Note: a distorted endometrial lining (red arrow), endometrial glands appear less tubular with distorted cuboidal epithelial cells (black arrow). **D) 10.0 mL/Kg/day *Saccharum officinarum* juice** Note: few intact endometrial glands with a distorted base membrane**.**

DISCUSSION

The phytochemicals present in *Saccharum officinarum* juice include cardiac glycoside, terpenoids, saponins, and reducing sugar in line with previous findings that it contains phenolic compounds with great biological activities (De-Armas *et al.*, 1999; Ashade *et al.*, 2014; Ogunwole *et al.*, 2020). The observed inviolate fasting blood glucose level of the treated rats implied that the glycemic index of *Saccharum officinarum* juice was probably adequate to maintain a normal fasting blood glucose level of the female rats during the short period of 21 days juice administration in contrast with Ogunwole *et al.* (2020) who reported increases in fasting blood glucose levels after eight weeks of administration of *Saccharum officinarum* juice to male Wistar rats.

Another factor could be the gender of the rats since hormones in females particularly estrogen has been reported to affect glucose homeostasis (Kumar *et al.*, 2011; Mauvais-Jarvis, 2018). This study showed an increased percentage change in body weight in the *Saccharum officinarum* juice-treated rats, which may be a result of energy gained from the nutrients in the juice and the feed given, supporting Swinburn *et al.* (2004) who noted that weight is gained generally from food consumption. On the other hand, the study disagrees with the findings of Flavel *et al.* (2021) who revealed that polyphenol-rich sugar cane extract reduced body weight in mice fed with high fat, and high carbohydrate diet, and Ogunwole *et al.* (2020) who noted reduced body weights after consumption of *Saccharum officinarum* juice for a longer period in male Wistar rats.

The estrous cycle of rats consists of four phases, usually identified according to cell types observed in vaginal smears. These phases are the Diestrus (resting stage - last for 57 hours), Proestrus (preovulatory stage - last for 12 hours), Estrus (ovulatory stage - last for 12 hours), and Metestrus (degenerative stage - last for 21 hours), (Hebel and Stromberg, 1986). The result showed an increased frequency of occurrence of the proestrus phase and reduced occurrence of the metestrus phase implying that *Saccharum officinarum* juice had an impact on the estrous cycle of the rats. During a normal proestrus phase in rats, there is an increase in follicle-stimulating hormone and luteinizing hormone levels that cause the ovarian follicles to grow faster and a corresponding increase in estradiol level (Hebel and Stromberg, 1986; Maeda *et al.*, 2000). However, in this present study, though the juice administered caused the proestrus phase to appear more frequently and increased the follicle-stimulating hormone and luteinizing hormone levels, it did not alter the estradiol level, indicating an abnormal proestrus phase that was probably either longer or shorter in period and did not lead to the estrus phase. This may be the reason for the metestrus phase that also occurred less frequently as well, resulting in a disrupted estrous cycle. The histology showed alterations in the cytoarchitecture of the ovary and uterus. The observed ovarian lymphocyte infiltration may be from inflammatory reactions that probably occurred in response to the pre-ovulatory gonadotropin stage, preceding ovulation (Oliver *et al.*, 2010). The follicular cells appeared degenerated with distorted granulosa cells and may have resulted from granulosa cell apoptosis due to the presence of Fas antigen, a cell surface receptor protein that is expressed on granulosa cells that mediate signals which induces apoptosis, a vital role in follicular atresia (Sakamaki *et al.*, 1997). The reported vacuolations within the corpus luteum and stroma may be a result of pregnancy failure (Oliver *et al.*, 2023). The noted vacuolations in the endometrial lining and distorted endometrial glands may be caused by atrophy of the uterus. Atrophy is a common age-related change that can be induced by agents that cause ovarian damage. It affects the endometrium and myometrium resulting in fewer endometrial glands and the collapse of the stroma (Davis *et al.*, 1999).

In conclusion, *Saccharum officinarum* juice inflamed the ovaries, distorted the estrous cycle, and caused vacuolations of the uterine tissues. *Saccharum officinarum* juice consumption may possess deleterious effects on the reproductive functions of female Wistar rats.

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