Journal of Research in Applied and Basic Medical Sciences 2023; 9(4): 215-222

Original Article

RABMS Journal of Research in Applied and Basic Medical Sciences

Effects of ethanolic fruit extract of solanum aethiopicum (L.) on saccharin induced hyperlipidemia and sperm abnormalities in male wistar rats

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Abstract

***Background & Aims*:** Saccharin is approximately 300 times sweeter than sucrose, and since its discovery, there has been several controversies regarding its potential toxicity as chronic saccharin consumption negatively influences biochemical parameters. Subsequently, *Solanum aethiopicum* is a good source of bioactive compounds that can be used to treat a variety of ailments including nervous, respiratory, visual, renal, circulatory and fertility issues. The aim of this study was to investigate the effects of ethanolic fruit extract of Solanum Aethiopicum (L.) on saccharin induced hyperlipidemia and sperm abnormalities in male Wistar rats.

***Materials & Methods*:** In this experimental study, 16 Wistar rats were divided into four groups (Groups 1 to 4) comprising of four Wistar Rats per group. Group 1 (control) was given distilled water and feed only, group 2 was administered saccharin (10 mg), group 3 were administered saccharin (10 mg) and 50 mg of extract, while group 4 was administered saccharin (10 mg) and 100 mg of extract. The extract was administered for 21 days, then the rats were sacrificed and blood sample was collected through cardiac puncture for Lipid profile test. Also, semen analysis was conducted to assess sperm count, motility, viability, and morphology. The results were expressed as mean ± SEM. The differences between the four groups were determined using one-way analysis of variance, and results were considered to be significant at $p \leq 0.05$.

***Results*:** The results showed that saccharin adversely affected lipid profile and sperm parameters in Wistar Rats. Also, ethanolic fruit extract of *Solanum aethiopicum* caused a significant dose-dependent increase of lipid profile and sperm parameters in Wistar Rats administered saccharin.

***Conclusion*:** *Solanum aethiopicum* is beneficial for treatment of sperm abnormalities and hyperlipidemia. It is recommended that the fruit be utilized as a less expensive alternative in clinical therapies for treating sperm abnormalities and hyperlipidemia.

***Keywords*:** Artificial Sweeteners, Cholesterol, Hyperlipidemia, Saccharin, *Solanum Aethiopicum*, Sperm

Received 02 April 2023; accepted for publication 28 May 2023

Introduction

Artificial sweeteners have become increasingly popular in recent years as a sugar substitute that provides intense sweetness with little or no energy (1), and given the increasing prevalence of their use as "zero" or "light" sugar alternatives, the consumption of artificial

sweeteners continues to be a hotly debated health issue (2), as intake of high concentrations of artificial sweeteners may increase the risk of metabolic syndrome (3).

Saccharin (also known as E954) is a zero-calorie food additive known for its sweetness and is one of the most famous artificial sweeteners in use since the 1900s, it was accidentally discovered in 1878 by Remsen and Fahlberg while studying the chemistry of cyclic sulfonamides (4). Saccharin is approximately 300 times sweeter than sucrose, and is regarded as one of the most widely used sweeteners in the world. Since its discovery in 1879, discussion has surrounded the propriety of saccharin for human ingestion (5), because chronic saccharin consumption influences biochemical parameters, and reported findings indicate numerous metabolic, neural, and hormonal responses in male and female rats due to the extended use of this sweetener (6).

Fertility in men is primarily determined by the morphology, concentration, and motility of sperms, and the disruption of any of these parameters results in male infertility (7). Male infertility is a public health burden associated with the frequent use of artificial sweeteners (3). Also, one of the risk factors that contribute to and predispose people to cardiovascular diseases is hyperlipidemia (8).

The use of medicinal plants for the management and treatment of human diseases dates back to the dawn of time, and there has been a surge of interest in herbal medicine around the world as a result of its significant contribution to health care delivery and as an alternative to clinical practice (9). These medicinal plants have been shown to be good sources of bioactive compounds (phytochemicals) that can be used to treat a variety of ailments including nervous, respiratory, visual, renal, circulatory, and fertility issues (10).

Solanum aethiopicum is a popular traditional plant cultivated mainly for its leaves and fruits in tropical Africa and together with okra, onion, pepper, and tomato, is one of the five most important vegetables in Central and West Africa. It also is cultivated in the Caribbean, Brazil and southern Italy (11). *Solanum aethiopicum* was initially known as *Solanum anguivi* or *Solanum gilo* (12), and other African species like *Solanum macrocarpon* L. and *Solanum melongena* L. are close relatives of *S. aethiopicum* (13). The plant has a chromosome number of 24, and is a member of the Solanaceae family. It is also known as African eggplant, bitter tomato, garden eggs, and scarlet eggplant (11, 13). Among the popular Nigerian tribes, it goes by the name *Afufa* in Igbo, *Gauta* in Hausa, and *Igbagba* in Yoruba (12). The fruits of *S. aethiopicum* can be consumed raw, steamed, boiled, or combined with other vegetables to make stews, and has been established that almost all its parts are useful for industrial, cosmetics, nutritional, and medicinal uses (14). It has been demonstrated the potential use of it to treat a number of chronic illnesses (15).

Materials & Methods

Plant material:

Fresh fruits of *S. aethiopicum* were purchased from Biu central market in Biu Local Government Area of Borno State, North-East Nigeria in August 2022. The fresh fruits were identified and authenticated in the Biology Department, Faculty of Natural and Applied Sciences of Nigerian Army University Biu, Borno, Nigeria by two Botanists: Dr. B.C. Essien and Mr. S.M. Kabiru.

Fruit Extract Preparation:

The fruits were carefully selected and thoroughly washed in distilled water to remove unwanted particles and dirt. The stalks were removed and the edible portion of the fruits were air dried for 12 days. The air-dried fruits were milled with an automatic electrical blender (QBL-18L40) to a powdered form. 50 g of the powdered fruit was weighed using an electronic weighing balance (Ohaus PX84) and soaked in ethanol for 24h. The mixture was subjected to filtration using a filter paper, and the solvent was evaporated using a rotary evaporator (RE-52A) (12).

Experimental Male Wistar Rats:

Sixteen Male Wistar Rats with an average weight of 150 g and 8 weeks' old purchased from the National Veterinary Research Institute Vom, Plateau State Nigeria were used for the study. The male Wistar Rats

were acclimatized for 14 days before the experimental work, and were given free access to distilled water and fed with standard finishers feed (Top Feeds, Nigeria) bought from the local market.

Condition of Experimental Male Wistar Rats:

The male Wistar Rats were housed in a twelve-hour light and twelve-hour dark cycle (lights from 08:00 to 20:00Hrs) in clean cages in an air-conditioned room under standard environmental conditions (23–25°C) throughout the experimental period. Furthermore, the animal experimental models used conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (16).

Experimental Design and Animal Grouping:

The sixteen male Wistar Rats were randomly divided into four equal groups (Groups 1 to 4) comprising of four male Wistar Rats per group and subsequently treated as follows:

S/N	Group Name	Substance Administered
1	Group 1 (Control) 1A, 1B, 1C, 1D	Distilled water and feed only
2	Group 2 2A, 2B, 2C, 2D	10 mg of Saccharin with distilled water and feed
3	Group 3 3A, 3B, 3C, 3D	10 mg of Saccharin + 50 mg of *Solanum aethiopicum* extract with distilled water and feed
4	Group 4 4A, 4B, 4C, 4D	10 mg of Saccharin + 100 mg/kg BW *Solanum aethiopicum* extract with distilled water and feed

Euthanization of Experimental Male Wistar Rats and Sample Collection:

At the expiration of 21 days, the experimental male Wistar Rats were euthanized using chloroform anesthesia, then blood samples were collected through cardiac puncture for Lipid profile test, and the blood was stored in sample bottles for further analysis. The abdominal cavity was opened up through a midline abdominal incision to expose the reproductive organs. The testis was detached and cleared free of the surrounding tissue. The cauda epididymis of one testis was removed and transferred to a sterilized Petri dish containing 2 mL warm normal saline at 37°C, and then macerated by sterilized scissor to obtain a suspension of the epididymal content used to estimate the sperm concentration, motility, morphology, and viability (17).

Evaluation of Parameters:

Lipid parameters:

The samples were analyzed using a COBAS C111 Chemistry analyzer. The parameters analyzed included Total Cholesterol (TC), Triglycerides, High-Density Lipoproteins (HDL) and low-Density Lipoproteins (LDL).

Semen Concentration:

Semen concentration was determined as described previously by Hosseini et al. (18) with minor modifications. Sperm suspension was diluted in 3% normal saline, and then a drop of the sperm suspension was gently transferred to a glass slide using a micropipette. After five minutes using a magnification of 40x, spermatozoa with head, middle, and tail pieces were counted. Epididymal sperm cell counts were expressed as million/ml of suspension.

Semen Morphology:

Semen morphology was determined as described previously by Abdullahi et al. (19). Sperm smears were prepared by placing a drop of minced sample of caudal epididymis and one drop of eosin stain on a clean slide. The smears were allowed to air dry and then examined using high power (100x) microscope oil immersion objective. Sperm cells from different fields were examined and the number of normal and abnormal sperm cell forms were estimated in percentage. In this study, a spermatozoon was considered abnormal morphologically if it had one or more of the following

features: rudimentary tail, round head, and detached head, and was expressed as a percentage of morphologically normal sperm.

Semen Motility:

Semen motility was determined as described previously by Kolawole et al. (20) with minor modifications. One drop of the semen suspension was placed on a glass slide and the number of motile and non-motile spermatocytes was counted in four random fields. The number of motile spermatocytes was then expressed as a percentage of the total number of the counted spermatocytes. Motility was expressed as percentage of progressive (rapid and slow), non-progressive, immotile spermatozoa.

Semen Viability:

Semen viability was estimated using the improved one-step eosin staining technique according to Abdullahi et al. (19). A fraction of each suspension of the sperm samples was mixed with an equal volume of eosin stain and prepared on glass slides. The slides were examined under the microscope for percentage viability. Normal live sperm cells (viable) exuded the eosin, while dead sperm cells took up the stain.

Statistical Analysis:

The results were expressed as mean ± SEM (standard error of means). The differences between the four groups were determined using one-way analysis of variance, and results were considered to be significant at $p \leq 0.05$.

Results

Table 1 shows the results of semen parameters for four groups of men. The semen parameters tested are morphology (the shape of the sperm), viability (the percentage of sperm that are alive), concentration (the number of sperm per milliliter of semen), and motility (the percentage of sperm that are moving).

Table 1. Semen parameters results

	Morphology (%)	Viability (%)	Concentration (million/ml)	Motility (%)
Group 1 (Control)	93.25	73.5	53.5	85
Group 2	90.5	51.5	33.75	54.5
Group 3	92.25	61.25	41.25	68.5
Group 4	92.75	69.50	47.75	83.25

Table 2 shows the results of lipid profile tests for four groups of people. The lipid profile tests measure the levels of triglycerides, LDL cholesterol, HDL cholesterol, and total cholesterol in the blood.

Table 2. Lipid profile results

	Triglyceride (mg/dl)	Low-density lipoprotein (mg/dl)	High density lipoprotein (mg/dl)	Total cholesterol (mg/dl)
Group 1 (Control)	152.67	96.1	23.5	150
Group 2	158	47	20.17	99
Group 3	121	87	23	133
Group 4	140.33	69	30	171.67

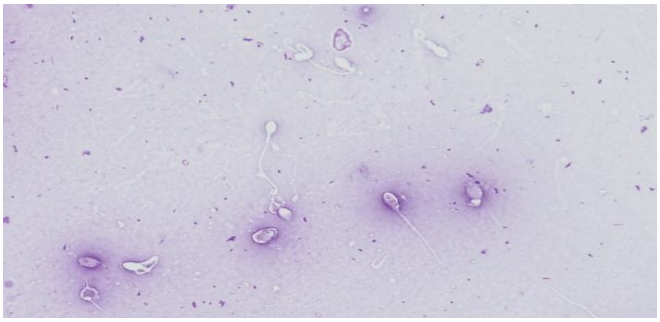

Fig. 1. Sperm cell without tail

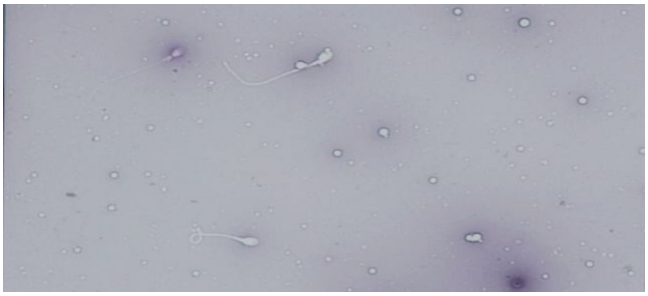

Fig. 2. Sperm cell with coiled tail

Discussion

Effects on Lipid profile:

The hyperlipidemia seen in this study following saccharin administration may be due to the enhancement of reactive oxygen species (ROSs) production in the form of oxidative stress, as a positive relationship between oxidative stress and saccharin consumption has been established in a study by Azeez et al. (21), which deduced that the lowest dose of saccharin can lead to a release of reactive oxygen species which could be overcome by the body's ability to produce antioxidants such as catalase as a result of cellular protection against reactive oxygen species. Furthermore, it may be caused by free radical's interaction with cellular membranes or may be attributed to reduce total cholesterol synthesis by the suppression of in vivo liver enzymatic activity of Acetyl-CoA synthetase, citrate lyase, and mitochondrial citrate exchange leading to a reduction of available cytoplasmic Acetyl-CoA, which is required for the synthesis of cholesterol and fatty acids (22).

The reversal of hyperlipidemia as seen in this study after administration of *S. aethiopicum* fruit extract could be ascribed to the importance of Solanum aethiopicum as a potent antioxidant and free radical scavenger (23), this is in line with the work of Adewoyin et al. (24) which established that potent antioxidants ameliorate increased free radicals generated by both natural and experimental stress, thereby causing a substantial elevation in lipid profile.

Effects on sperm parameters:

The decline of semen parameters following saccharin administration as seen in the present study suggests that saccharin might prevent spermatogenesis by crossing through the blood-testis barrier and entering the germinal cells (25), leading to the enhancement of reactive oxygen species production in the form of oxidative stress as spermatozoa are highly susceptible to damage by excessive concentrations of reactive oxygen species due to the high content of polyunsaturated fatty acids within their plasma membrane which leads to lipid peroxidation that destroys the structure of lipid matrix in the membranes of spermatozoa, and loss of motility and impairment of spermatogenesis (26). Consequently, the decline of semen parameters could also be ascribed to

the establishment of a positive relationship between oxidative stress and saccharin consumption in a study by Azeez et al. (21), which deduced that the lowest dose of saccharin can lead to a release of reactive oxygen species.

On the other hand, the statistically significant increase of sperm parameters in the treatment groups (group 3 and group 4) in relation to the saccharin group (group 2) indicates the fruit extract demonstrated the ability to ameliorate the damage done on the sperm parameters by saccharin in a dose-dependent manner; this could be ascribed to the importance of *S. aethiopicum* as a potent antioxidant and free radical scavenger (23). This is in line with the work of Adewoyin et al. (24), which established that potent antioxidants ameliorate increased free radicals generated by both natural and experimental stress, and thereby causing a substantial elevation in spermatogenic activity by increasing the production of testosterone from the Leydig cells. Apart from the ability to scavenge reactive oxygen species generated by saccharin, the results of this study also indicate that the fruit extract of *S. aethiopicum* may have an effect on the mitochondria of the spermatozoa, where energy is being synthesized in the form of adenosine triphosphate (ATP) and increases sperm motility (27).

This result also is in line with finding of the research done by (9) on Male Wistar Rats which showed that the continuous consumption of *S. aethiopicum* (garden egg) may increase the concentration of reproduction hormones and cause an appreciable improvement in sperm parameters in male Wistar Rats and therefore capable of boosting their reproductive potential. Furthermore, phytochemical screening of *S. aethiopicum* has revealed high values of vitamins A, C, D, E (28), and vitamin E supplementation has been found to increase fertilization rates, possibly by improving membrane integrity, reducing oxidative damage and lipid peroxidation potential (29).

Conclusion

The research found that saccharin consumption adversely affected lipid profile and sperm parameters in male Wistar rats. Also that the ethanolic fruit extract of *S. aethiopicum* fruit caused a significant dose-dependent increase in Lipid profile and sperm parameters of male Wistar rats administered saccharin. Hence, the findings of this study suggest that *S. aethiopicum* fruit has pro-fertility properties that may benefit those who consume it, particularly those who consume saccharin on a regular basis. This indicates that *S. aethiopicum* fruit may be beneficial in the treatment of hyperlipidemia and sperm abnormalities thus boosting reproductive potential. It is therefore recommended that study focus should be placed on isolating and purifying the compounds in S. aethiopicum fruit which has been found to be effective in treating hyperlipidemia and sperm anomalies as seen from this study. Additional research to determine the precise mechanism of action leading to substantial elevation in spermatogenic activity and improvement in lipid profile by S. aethiopicum fruit should be conducted and the effects of ethanolic fruit extract of S. aethiopicum fruit on lipid profile and sperm parameters be studied in the long term to see further relationship or probable variance with this study.

Acknowledgments

The authors thank the patients for participating in the study.

Conflict of interest

The authors have no conflict of interest in this study.

Funding

No funding was required for the study.

Ethical Statement

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals. The study protocol was approved by the Ethics Committee of Nigerian Army University . All animals were purchased from the National Veterinary Research Institute Vom, Plateau State Nigeria.

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