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Mitigating Property of Sacoglottis Gabonensis Ethanolic Extract on Spermatogenesis of Swiss Mice Following Chronic Exposure to Aspartame

Aruchi, Wekhe-Emenike; Eme Efioanwan Orlu; Adetutu Olubunmi Obulor

Rivers State University, P.M.B

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ABSTRACT

This study aimed at evaluating the Mitigating property of *Sacoglottis gabonensis e*thanolic extract on Spermatogenesis of Swiss mice following chronic exposure to Aspartame. A total of Ninety mice were assigned to five groups (A-E) of eighteen mice each. Group A was the negative control. Group B was the positive control and received 50mg/kg/bw/day of aspartame. Group C received 50mg/kg/bw/day of aspartame and 250mg/kg/bw/day of ethanolic leaf extract of *Sacoglottis gabonensis.* Group D received 50mg/kg/bw/day of aspartame and 50mg/kg/bw/day of ethanolic bark extract of *S.gabonensis*. Group E received 50mg/kg/bw/day of aspartame and 250mg/kg/bw/day of a combination of bark and leaf extract. All the groups were exposed to the treatment by oral gavage for 30, 60 and 90days. Histological examination of the section of testis of mice at the end of 30, 60, and 90 days of the experimental period revealed normal testicular epithelium with a full complement of spermatogenic elements in group A. In mice exposed to aspartame only (group B), the seminiferous epithelium was devoid of spermatogenic elements, leaving spaces in the lumen. Group C, exposed to aspartame and ethanolic extract of *S. gabonensis* bark showed regeneration of the seminiferous tubule**.** In mice exposed to aspartame and the ethanolic extract of *S. gabonensis* leaf (group D), degeneration of the spermatogenic cells and regeneration of interstitial cells were still observed. Group E showed the regeneration of spermatogenic cells and the elongation of spermatids. Maturing spermatozoa were found in the lumen, and Interstitial cells beginning to regenerate. It was concluded that aspartame is potentially antispermatogenic and, based on its consumption in various forms of juices, drinks, and beverages may be implicated in male secondary infertility. Conversely, S.*gabonensis* has the potential to ameliorate inhibition and impairment of spermatogenesis resulting from chronic exposure to toxicants.

Keywords: Antioxidant, Aspartame, *Sacoglottis gabonensis*, Spermatogenesis

INTRODUCTION

Humans naturally like sweet tastes which boost their appetite. However, there is an increase in the number of people suffering from obesity, diabetes, hypertension, and other heart-related diseases due to the increased consumption of sugary diets. Artificial sweeteners (such as aspartame, saccharine, acesulfame-k) known as non-nutritive sweeteners have sweetening potential very high compared to common sugar (Marinovich *et al.*, 2013). Most diet, beverages, and food products currently in the market contain an artificial sweetener, aspartame. However, controversy surrounds the effects of this non-nutritive artificial sweetener, as it is made up of components such as phenylalanine, aspartic acid, diketopiperazine and methanol, that may have adverse effects on neural functioning, particularly on neurotransmitters, neurons and astrocytes (Humphries *et al.*, 2008; Abu-Taweel, 2016; Abdelmonem, *et al.*, 2019). According to the study of Hozayen *et al.*, 2014 aspartame's mode of action appears to involve the degradation and atrophy of Leydig cells in rats resulting in reduced testosterone synthesis and secretion due to the influence of formaldehyde produced from aspartame. Seif, 2014 reported alterations in the testicular morphology, decreased sperm count, and changes in hormone levels of male Wistar rats exposed to aspartame. Also, Wekhe-Emenike *et al.*, 2022a reported significant alterations in the serum electrolytes of experimental mice administered aspartame compared to the control group Following oral administration to humans and experimental animals, aspartame is completely and rapidly metabolized by

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intestinal *esterases* and *dipeptidases* to aspartic acid, phenylalanine, and methanol, substances normally found in the diet and body (Saleh, 2015). Low-calorie sweeteners are widely used to prevent the increasing rates of obesity and diabetes mellitus in the growing population.

Bitter bark cherry tree *(Sacoglottisgabonensis)* is a tree found in the tropical rainforest region of Africa and America. It belongs to the family Humiriaceae. In certain rural communities of Nigeria, the stem bark is commonly used as an additive to palm wine, a local alcoholic brew which is an exudate from the phloem of *Raphia* species especially *Raphia vinifera* (P.Beauv) and palm trees (*Elaeis guineensis* Jacq). Palm wine is a generic name for a group of alcoholic beverages obtained by fermentation from the sap of palm trees and *Raphia* trees. Freshly harvested unfermented sap is a clear colourless liquid or suspension with a sweet sugary taste and low alcohol content. Following fermentation from its yeast content, it becomes milkier and increasingly less sugary with increased alcohol content which result in intoxication depending on the volume consumed. The stem bark extract of *S. gabonensis*is normally added when the palm wine is fresh as it is believed to prolong the shelf life and reduce foaming and effervescence. It imparts a bitter taste to the sugary palm wine, thereby making the beverage more acceptable with the amber colour. It has been traditionally used to treat various ailments and may help reduce cellular damage caused by toxins. Wekhe-Emenike *et al.*, 2022b reported a significant decrease in liver injury biomarkers, increase in oxidative stress biomarkers, and number of hepatocytes in liver epithelium of animals coadminiatered *S.gabonensis.* also, Wekhe-Emenike *et al.*, 2024 reported no alteration in the gestation length and behavioural changes during gravid period in experimental animals coadminiatered *S.gabonensis*.

Therefore this study is designed to investigate mitigating properties of *S. gabonensis* in spermatogenesis of mice exposed to aspartame.

MATERIALS AND METHODS

Study location

The study was carried out in the green house of the Department of Animal and Environmental Biology, Rivers State University, NkpoluOroworukwo, Port Harcourt, Nigeria (Coordinates 4º48'14"N6º59'12"E).

Study duration: The experiment was conducted from January to April, 2021.

Sources and Preparation of Plant Material

The bark and leaves of *Sacoglottis gabonensis* were harvested in Etche Local Government area of Rivers State, Nigeria and allowed to dry under room temperature (18ºc-27ºc).The dried samples were blended into a fine powder and stored for use. 50g of the fine powder of *S.gabonensis* was dissolved in 200ml of ethanol. The mixture was allowed to stay for fourteen days before they were administered to the experimental animals

Animal care and management

A total of Ninety (90) adult male mice (mean weight 18.57±3.35g) were used for the study. The mice were housed in a rubber case under standard conditions and acclimatized for two weeks. All animals were fed with standard rodent pellets and cool, clean water *ad libitum.* All experiments were conducted according to the institutional animal care protocol at the Rivers State University, Nigeria, and followed approved guidelines for the ethical treatment of the experimental animals.

Experimental design

Ninety mice were assigned to five groups (A-E) of eighteen mice each. Group A was the negative control, and they were not given any treatment, but only given pellet and clean tap water. Group B was the positive control and received 50mg/kg/bw/day of aspartame. Group C received 50mg/kg/bw/day of aspartame and 250mg/kg/bw/day of ethanolic leaf extract of *Saboglottis gabonensis.* Group D received 50mg/kg/bw/day of aspartame and 250mg/kg/bw/day of ethanolic bark extract of *S.gabonensis*. Group E received 50mg/kg/bw/day

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of aspartame and 250mg/kg/bw/day of a combination of bark and leaf extract. All the groups were exposed to the treatment by oral gavage for 30, 60, and 90days. Feed was withdrawn from the animals 24 hours before the termination of the experiment.

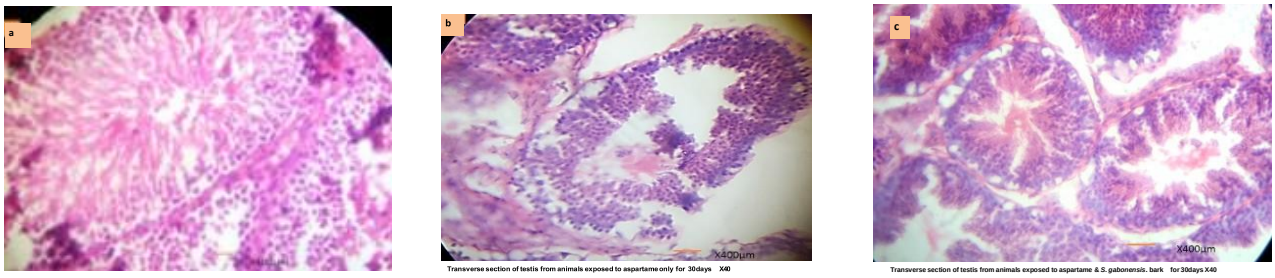
Histopathological analysis of the testis

Immediately after dissection of each animal, 0.5g of testis was fixed in 10% neutral formalin and sectioned with a digital rotatory microtome at 5µm. Histological sections mounted on slides were stained with Haematoxylin and counter-stained with Eosin (H&E). Photomicrographs were generated with a digital microscope Biosphere Miller B with an image processor DN2–microscopy image processing software at x40 magnification.

RESULTS

Histological analysis of the Testis of Mice exposed to aspartame and *Sacoglottis gabonensis* for 30 days.

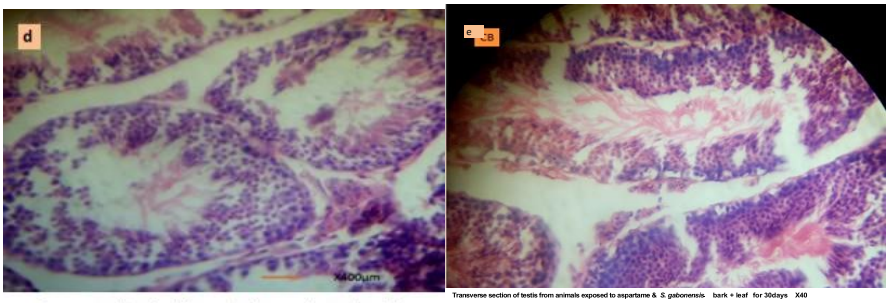
The result for the histological examination of hematoxylin and eosin sections of the testes of experimental mice exposed to aspartame and *S. gabonensis*,30 days examined at X400 magnification, is presented in Fig. 3a-3f. Figure 3a was the negative control group shows seminiferous epithelium of the testis of mice with visible spermatogonia undergoing active mitotic division in the basal compartment, premeiotic, primary and secondary spermatocytes, as well as, as round and elongating spermatids occupied the adluminal compartment and maturing spermatozoa awaiting spermiiation into the lumen. Fig. 3b was the positive control and received aspartame alone showed degeneration of spermatogenic elements, loss of interstitial cells of the Leydig, inhibition of elongation and maturation of spermatozoa. Fig. 3c-3f showed the mouse testis exposed to aspartame and the extracts of *S. gabonensis*. There was visible regeneration of the spermatogenic element in the seminiferous epithelium, traces of degeneration of some spermatogenic cells still observed, interstitial cells regeneration, elongating spermatids, few maturing spermatozoa found in the lumen.



Transverse section of testis from animals exposed to aspartame only for 30days X40

Transverse section of testis from animals exposed to aspartame & *S. gabonensis*. bark for 30days X40

Transverse section of testis from control animals for 30days X40



Transverse section of testis from animals exposed to aspartame & *S. gabonensis*. leaf for 30days X40

Transverse section of testis from animals exposed to aspartame & *S. gabonensis*. bark + leaf for 30days X40

Fig 3a-3e: Micrograph of Mice Testes exposed to aspartame and *Sacoglottis gabonensis f*or 30 days @ X40.

Histological analysis of the Testis of Mice exposed to Aspartame and *Sacoglottis gabonensis* for 60 Days.

The result for the histopathological examination of hematoxylin and eosin section of the testis of mice exposed to aspartame and *S.gabonensis* for 60 days examined at X40 magnification is presented in Figure 3f-3j. Figure 3f showed the mouse testis for negative control with normal architecture of testicular epithelium filled with spermatogenic elements. Lumen filled with fully formed elongated spermatozoa. Interstitial space filled with

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interstitial cells of Leydig. Figure 3g showed the mouse testis exposed to aspartame alone (positive control). Degeneration of spermatogenic elements is pronounced in the seminiferous epithelium, loss of the interstitial cells of the Leydig, detached basal membrane, and reduction of mitotic cells in the basal compartment Fig 3h-3j shows Seminiferous epithelium of the mouse testis exposed to aspartame and extracts of *S.gabonensis*. Regeneration of spermatogenic elements was observed, and the Lumen gradually filled with maturing spermatozoa. The seminiferous epithelium is recovering, All the spermatogenic elements are regenerated in the epithelium of animals in group h that received aspartame and *S. gabonensis*(fig 3h). The group that received aspartame and leaf extract of *S. gabonensis* showed regenerating seminiferous epithelium with sparse spermatogenic elements, empty lumen, and scarce maturing spermatocytes (fig 3i) A normal spermatogenic elements, regenerating lumen, visible primary and secondary spermatocytes was observed in fig 3j which received aspartame and the combination of bark + leaf extract of *S. gabonensis*.

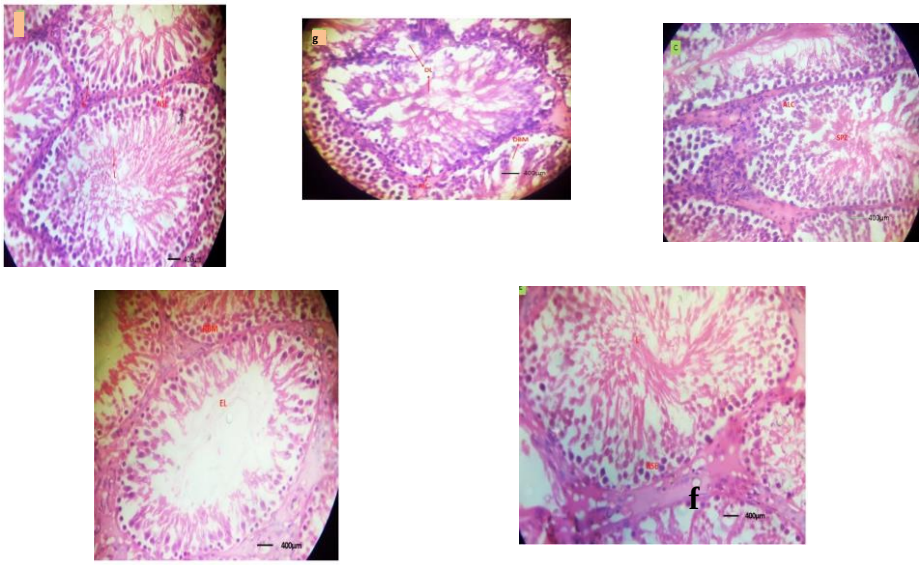
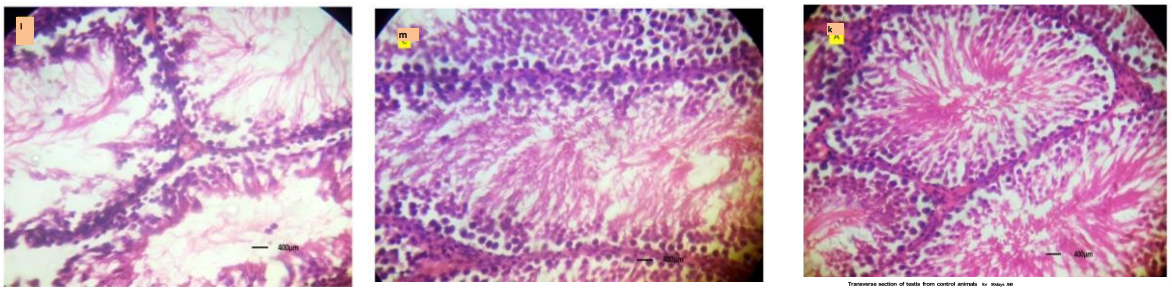


Fig 3f-3j: Micrograph of Mice Testes exposed to aspartame and *Sacoglottis gabonensis* for 60 days period @ X40

Histological analysis of the Testis of Mice exposed to aspartame and *Sacoglottis gabonensis* for 90 Days.

The result of the histopathological examination of hematoxylin and eosin section of the testis of mice exposed to aspartame and *Sacoglottisgabonensis*90 days, examined at X40 magnification is presented in fig 3k-3o. Figure 3k showed the mouse testis for the negative control. Normal architecture of the seminiferous tubules showing full spermatogenic elements. Figure 3l showed mouse testis exposed to aspartame alone. Massive degeneration of the seminiferous epithelium, complete loss of spermatogenic elements, empty lumen, no primary and secondary spermatocytes, loss of the interstitial cells of Leydig, mitotic spermatogonia observed lining the basal membrane. Figures 3m-3o showed the mouse testis exposed to aspartame and *Sacoglottis gabonensis*. Lumen filled with maturing spermatocytes, regeneration of the interstitial cells of the Leydig, and maturing spermatocytes are seen.



Transverse section of testis from animals exposed to aspartame only for 90days

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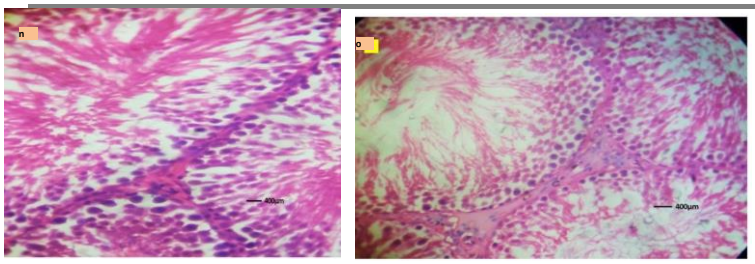

Fig 3k-3o: Micrograph of Mice Testes exposed to aspartame and *Sacoglottis gabonensis* for 90 days @ X40.

DISCUSSION.

Spermatogenesis, the biological process of producing male germ cells or spermatozoa, is a critical function, and recent research indicates a rise in male infertility. This rise in male secondary infertility has been linked to environmental toxins such as heavy metals, pesticides, food additives, and preservatives. The histopathological micrograph of the negative control group showed epithelium of the testis of mice with visible mitotic spermatogonia in the basal compartment, premeiotic primary, secondary elongated spermatocytes and maturing spermatozoa in the adluminal compartment awaiting spermiation into the lumen. The positive control group showed degeneration of spermatogenic elements, loss of interstitial cells of Leydig, detached basal membrane, inhibition of mitotic process of spermatogonia, loss of primary and secondary spermatocytes ; inhibition of elongation and maturation of spermatozoa. The observed adverse effect on the positive control group is indicative that aspartame may contain antispermatogenic components, as observed in the experimental mice. These findings validates Al-Qudsi, & Al-Dossary,(2022) who reported disruption of spermatogenic process observed by increased number of deformed seminiferous tubules, shrinkage in their size and disappearance of the basal lamina in mice exposed to 50mg/kg/day of commercial artificial sweeteners. In this investigation Mice treated with aspartame exhibited dose-dependent adverse effects including vacuolation, exfoliated germ cell in seminiferous tubules and loss of Leydig cells in the interstitial space. El -Alfy, *et al.*, (2023) also reported that aspartame induced testicular toxicity in rat at the dose 100mg/kg when administered three times a week for a period of 12 weeks.

The experimental groups that received aspartame and the extracts of *S. gabonensis* showed visible regeneration of the spermatogenic elements in the seminiferous epithelium, regeneration of interstitial cells of the Leydig, primary and secondary spermatocytes, elongation of round and maturing and matured spermatozoa as well as spermiation as observed in the lumen filled with spermatozoa.It is therefore, deduced that *S. gabonensis* reversed gonadotoxic effects of aspartame on the experimental mice further proving its antioxidative potentials derived from its phytochemical components (Tchouya, *et al.*, 2016).

This result is in line with Titus *et al.*, (2018) who reported regeneration of spermatogenic elements in mice coadministered *Citrullus lanatus* and *Annona muricata*, following exposure to Lambda cyhalothrin, Obulor & Orlu (2019) who reported protective role of Lycopene on spermatogenesis in Sprague-Dawley rat following exposure to Cypermethrin and Obulor *et al.*, (2022) also reported the prevention of insecticides induced reproductive toxicity in rat by different local spices. Moreover, previous reports on coadministration of aspartame and *S. gabonensis* show significant decrease in liver injury biomarkers, increase in oxidative stress biomarkers, and number of hepatocytes in liver epithelium of animals coadminiatered *S.gabonensis* (Wekhe-Emenike *et al.*, 2022a), but no alteration in the gestation length and behavioural changes during gravid period in experimental animals coadminiatered *S.gabonensis* (Wekhe-Emenike *et al.*, 2024).

CONCLUSION

The histological examination of the testis of experimental mice exposed to aspartame alone indicated that aspartame is potentially antispermatogenic and based on its consumption in various forms of juices, drinks, and beverages, may be implicated in male secondary infertility. Conversely, S.*gabonensis* has the potential to ameliorate inhibition and impairment of spermatogenesis resulting from chronic exposure to aspartame.

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