

Original Research Article

Influence of *Sacoglottis gabonensis* ethanolic extract on the electrolytes of swiss mice administered aspartame

Abstract

Aim: This study aimed at evaluating the influence of *Sacoglottis gabonensis* ethanolic extract on the electrolytes of swiss mice administered aspartame.

Location and Duration of study: The study was carried out in the green house of the Department of Animal and Environmental Biology, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria (Coordinates 4°48'14"N 6°59'12"E). The experiment lasted for Ninety days.

Experimental design: A completely randomized experimental design employing relevant statistical tools for analysis and interpretation.

Methodology: Ninety mice were assigned to six groups (A-F) of fifteen mice each. Group A was the negative control and so they were not given any treatment, but pellet and clean tap water. Group B was the positive control and received 50mg/kg/bw/day of aspartame alone. Group C received 50mg/kg/bw/day of aspartame and 250mg/kg/bw/day of ethanolic extract of *Sacoglottis gabonensis* leaf. Group D received 50mg/kg/bw/day of aspartame and 250mg/kg/bw/day of ethanolic extract of *S. gabonensis* bark. Group E received 50mg/kg/bw/day of aspartame and 250mg/kg/bw/day of a combination of bark and leaf extract. Group F received 50mg/kg/bw/day of aspartame and 500mg/kg/bw/day of a combination of bark and leaf extract. All the groups were exposed to their treatment by oral gavage for 30, 60 and 90days. Feed was withdrawn from the animals 24hours before the termination of experiment. Analysis of serum for electrolytes concentrations followed approved standard procedures.

Result: There was a significant increase in potassium and chloride level across experimental duration compared with the control group. There was significant increase in sodium level in group B across the duration of experiment with the lowest value recorded in group D 30 days. The level of bicarbonate decreased in groups B and C at 30 days, D and F at 60 days and increased in B and C at 90 days. Calcium level increased significantly across experimental duration with the lowest value observed in group F.

conclusion

This study recorded alterations in the electrolyte concentrations in the serum of experimental mice especially group administered aspartame alone compared to the control group. However, these alterations reported in the level of electrolytes may lead to metabolic disorder in individuals exposed to frequent and high intake of aspartame. Therefore, moderate consumption of aspartame is advocated while the consumption of *S. gabonensis* is highly recommended.

Keywords: aspartame, bicarbonate, electrolytes, *Sacoglottis gabonensis*

1. Introduction

The growing concern about health and life quality has encouraged people to exercise, eat healthy food and decrease the consumption of food rich in sugar, salt and fat [1-3]. Subsequently, the use of products

such as artificial sweeteners has increased. Non-nutritive sweetener (NNS) consumption has been historically associated with the increasing prevalence of obesity [4].

Majority of people all over the world consumed food that has one sweetener or the other despite the facts that the foods are termed as sugar free. In recent times, some degenerative diseases, especially diabetes, which was once believed to be adult-lifestyle based disease is now diagnosed in children. This may be as a result of high consumptions of sweetened food, juices, cakes and chocolates that is highly demanded by children [3,4].

Consumers and food manufacturers have long been interested in enhancing flavour while reducing calories intake. Artificial sweeteners have been classified as nutritive and non-nutritive depending on whether they are a source of calories. The nutritive sweeteners include the monosaccharide polyols (e.g., sorbitol, mannitol, and xylitol) and the disaccharide polyols (e.g., maltitol and lactitol) [5]. Artificial sweeteners are many times sweeter than table sugar, smaller amounts are needed to create the same level of sweetness, and which are either not metabolized in the human body or do not significantly contribute to the energy content of foods and beverages. They provide the sweetness of sugar without the calories and produce a low glycaemic response [6].

Aspartame is an artificial (non-nutritive) sweetener used to replace sugar in food and drinks. Nutritive sweeteners are naturally occurring sweeteners such as sucrose and fructose. On the other hand, non-nutritive sweeteners are synthesized in the laboratory such as aspartame, stevia and sucralose. Non-nutritive sweeteners, also referred to as high intensity sweeteners, are typically used in small amounts to reduce the caloric intake while sustaining the desired taste in many food products [7]. The non-nutritive sweeteners, better known as artificial sweeteners, include substances from several different chemical classes that interact with taste receptors and typically exceed the sweetness of sucrose by a factor of 30 to 13,000 times [5]. Aspartame is most stable between pH values of 3 and 5 even with increasing temperature. However, it breaks down and loses its sweetness in normal cooking or baking. Thus its use is limited to table top sweetener in dry foods, soft drinks, and frozen foods like ice cream. Because of its low caloric contribution in producing the same level of sweetness as sucrose (approximately 160-200 times sweeter than sucrose), it is used extensively in 'diet' products [8].

After oral administration, ASP is metabolized into three components, two amino acids (50% phenylalanine - Phe and 40% aspartic acid - aspartate, Asp) and 10% methanol (MeOH). Aspartame produces effects through its metabolites the concentration of which increases in the blood [9].

Activity measurements of serum electrolytes (Na, K, Ca^{2+}) are a valuable tool in clinical diagnosis. The measurement of enzymes' activities in tissues and body fluids can be used to estimate the degree of toxicity of a chemical compound on an organ/tissues [10, 11].

Bitter bark cherry tree (*Sacoglottis gabonensis*) is a tropical rainforest tree found in the tropical rainforest region of Africa and America. It belongs to the family *Humiriaceae*. Is rich in compounds such as phenols, tannins, oxalate, saponins, flavonoids alkaloids and phytate which contains antioxidants, anti-inflammatory, antibacterial, suppresses lipid peroxidation and contain anticancer properties. In certain rural communities of Nigeria especially Abia, Akwa- Ibom, Rivers, Delta, Edo and Imo State, the stem bark of this tree is commonly used as an additive to palm wine, a local alcoholic brew which is an exudates from the phloem of *Raphia species* especially *Raphia vinifera* and palm trees (*Elaeis guinensis*). Traditionally in central African countries, Cameroon and Congo in particular, the stem bark of *Sacoglottis gabonensis* are commonly taken to treat fever, diarrhoea, gonorrhoea and abdominal pain, and they are used to treat hypertension and diabetes sometimes[12,13]. The Kola pygmies and Mvae people of Cameroon use a decoction of the crushed bark mixed with leaves of *Dioscorea minutiflora* as a rectal enema to treat acute abdominal pain [13]. A decoction of the stem bark is used to cure difficult cases of dermatitis in Congo [13]. A bark decoction is used to treat stomach-ache and it is also used as a spice in food to induce heat in nursing and pregnant mothers in Sierra Leone [14].

Clinical studies have shown that the administration of ASP could be responsible for neurological and behavioral disturbances in sensitive individuals [15, 16]. In addition, the chronic use of Aspartame might contribute to hypersensitivity reactions and atherosclerosis [1, 15, 17].

Ali *et al*[18] reported subchronic intake of aspartame (240mg/kg orally) and cola soft drink (free access) in thirty rats for two months significantly altered serum electrolytes (increased Ca and Na, and depleted Cu, Fe, Zn and K levels).

Andullu and Vardacharvulu[19] Reported a significant ($P < 0.05$) reduction in the level of potassium, Zinc, sodium and Calcium in ethanol exposed groups compared to the control. Moreover, *P. guineense* and *G. latifolium* extracts normalize the calcium level in a dose dependent fashion.

Therefore this study aimed at assessment of the impact of aspartame coadministered *Saccoglottis garbonnensis* on electrolytes in male Swiss Mice.

2. MATERIALS AND METHODS

2.1 Experimental Location

The study was carried out in the green house of the Department of Animal and Environmental Biology, Rivers State University, Nkpolu Oroworukwo, Port Harcourt, Nigeria (Coordinates $4^{\circ}48'14''N$ $6^{\circ}59'12''E$). The experiment was conducted from January to April, 2021.

2.2 Plant Extraction

Plant samples (bark and leaf) were collected, washed and allowed to dry under room temperature. They were homogenised into powdery form by using an electric blender. Ethanoic extract of both samples were prepared.

2.3 Experimental Animal and Management

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

2.4 Experimental Design and Procedure:

Ninety mice were assigned to six groups (A-F) of fifteen mice each. Group A was the negative control and so they were not given any treatment, but 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 extract of *Saboglottis gabonensis* leaf. Group D received 50mg/kg/bw/day of aspartame and 250mg/kg/bw/day of ethanolic extract of *S. gabonensis* bark. Group E received

50mg/kg/bw/day of aspartame and 250mg/kg/bw/day of a combination of bark and leaf extract. Group F received 50mg/kg/bw/day of aspartame and 500mg/kg/bw/day of a combination of bark and leaf extract. All the groups were exposed to their treatment by oral gavage for 30, 60 and 90days. Feed was withdrawn from the animals 24hours before the termination of experiment.

Analysis of blood electrolytes: Twenty four hours after the last dose administration, the animals were anaesthetized with chloroform vapour [20]. Serum was used for electrolyte analysis. The estimation of calcium, potassium and sodium were carried out according to the methods described by Tietz, while bicarbonate was determined by Pause et al., method and chloride by Levinson method. The serum was analyzed for electrolytes using Audicom full auto electrolyte analyzer [21].

Statistical analysis: Data management and statistical analysis was conducted using Statistical Analyses System SAS 9.4 (SAS Institute, Cary, North Carolina, USA). Graphical representations and data visualizations were carried out using the JMP statistical discovery™ software version 14.3 (SAS Institute, Cary, NC, USA).

3.0 Results

3.1 Effect of aspartame and *Sacoglottis gabonensis* on serum electrolytes for 30,60 and 90 days.

The effect of coadministration of aspartame and *S. gabonensis* for 30 days is presented in Fig 1. The concentration of calcium was at the highest given a value of 2.95 ± 0.21 in group F coadministered 750mg/kg/bw of *S. gabonensis*. While Ca concentration non-significantly decreased in all the other groups compared with group A. The level of Potassium showed significance ($P < 0.0001$) decrease across the groups. The highest value was recorded in group B administered aspartame only with 5.10 ± 0.17 mmol/l. The value decreased significantly ($P < 0.0001$). To 2.67 ± 0.24 mmol/l in group D co-administered aspartame and bark extract of *S. gabonensis* and 3.30 ± 0.06 mmol/l in group C co-administered aspartame and leaf extract of *S. gabonensis*.

There was a no significance difference in sodium ions (Na mmol/l) concentration in the groups. The highest value recorded in group F, with 2.95 ± 0.21 decreased in other groups ranging from 2.39 ± 0.08 in group A to 2.01 ± 0.02 in group E.

The chloride level observed in this study decreased significantly from 94.00 ± 2.89 in group D coadministered bark extract of *S.gabonensis* compared to those from other groups. Moreover, group B which received aspartame only had the lowest value of 78.67 ± 3.48 while other groups had values close to the group A (control). The concentration of HCO_3 showed no significant difference among groups compared to group A.

Result for the comparisons of electrolytes for 60 days is presented in Fig 2. There is significant difference in electrolytes across treatment group except for HCO_3 . The lowest concentration of Potassium was observed in group C with 3.30 ± 0.06 while group D and B had the highest concentrations of HCO_3 with 6.20 ± 0.06 and 5.47 ± 0.15 respectively. Other values recorded for Sodium, chloride and potassium followed the same decreased trend

The results for the comparisons of electrolytes in Swiss mice after treatment with aspartame and *Sacoglottis gabonensis* for 90days are presented in Fig 3. Group B showed a significant increase in all Parameters considered when compared with group A. The highest value in all parameters were seen in group B while other groups recorded values not significantly different from group A.

The results of combined monthly influence of treatment with aspartame and *Sacoglottis gabonensis* on electrolytes in Swiss mice is presented in Fig 4. There is significant increase in potassium level across experimental duration. The highest value was observed in group D at 60 days, while the lowest value was observed in group D at 30 days. However, for sodium concentration, the highest value was observed in group C at 90 days, while the lowest value was seen in group D at 30 days. There was a significant increase in chloride concentration across treatment duration. The highest chloride concentration was also observed in group D at 30 days, while the lowest value was observed at 90 days in group E. The level of bicarbonate increased across the experimental groups and duration with the lowest concentration

recorded at 30 days in group B administered aspartame alone. Calcium level increased significantly across experimental duration with the lowest value observed in group F.

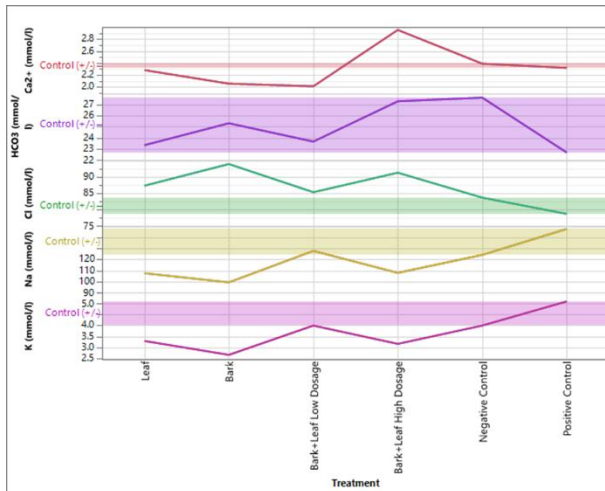


Fig 1: Electrolytes Levels in Swiss Mice after Treatment with Aspartame and *Sacoglottis gabonensis* for 30 Days

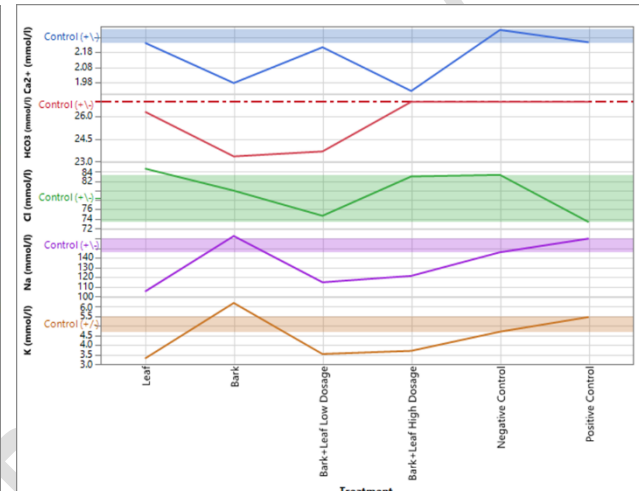


Fig2: Electrolytes Levels in Swiss Mice after Treatment with Aspartame and *Sacoglottis gabonensis* for 60 Days.

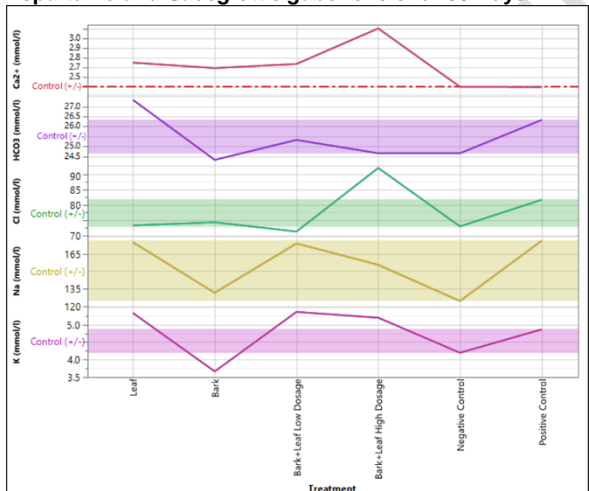


Fig 3: Electrolytes Levels in Swiss Mice after Treatment with Aspartame and *Sacoglottis gabonensis* for 90 Days.

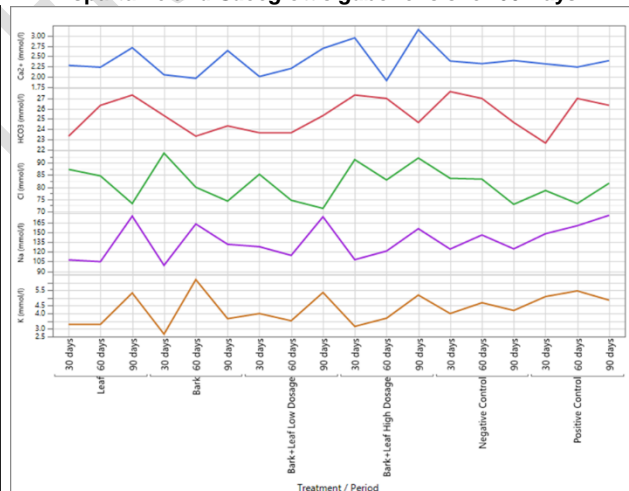


fig 4: Comparisons of electrolytes in Swiss mice after with treatment with aspartame and

4.DISCUSSION

Electrolytes are biomolecules that have a natural positive or negative electrical charge, they help the body to regulate chemical reactions, maintain the balance between fluids inside and outside the cell. The

results for electrolytes levels in Swiss mice after 30 days following treatment with *Sacoglottis gabonensis* showed a significant difference in calcium ion Ca^{2+} (cation). Group C, E, A and B administered the leaf, bark + leaf low dose extracts of *S. gabonensis*, negative control and positive control groups all have increased calcium ion concentrations while the group D and F administered the bark, bark + leaf high dose extracts of *S. gabonensis* have decreased calcium (Ca^{2+}) ion concentrations. Calcium (Ca^{2+}) contributes to the hardness of skeletal bones in the body which serves as a mineral reserve for calcium and its salts for the rest of the tissues, also teeth have high deposits of calcium within them. The administration of aspartame alone and aspartame and the leaf, bark + leaf low dose, conferred high calcium deposits in the body of the experimental animals, these findings is in line with [7] who reported increase in serum electrolytes of calcium and sodium ions in wistar rats exposed to soft drinks and menthol candy for forty two days. Group D and F administered bark, bark + leaf high dose extract of *S. gabonensis* had low calcium deposits. This implies that the bark extract of *S. gabonensis* reduced the calcium content in the experimental animal group co-administered with aspartame.

There is significant difference in bicarbonate (HCO_3^-) across the experimental groups. Groups A, B and F that is the negative control, positive control, bark + leaf high dose and the group that received the leaf extracts of *S. gabonensis* had increased bicarbonate (HCO_3^-) concentrations, while the groups D and E that received the bark + leaf low dose and bark of *S. gabonensis* have decreased bicarbonate concentrations. Bicarbonate is the second most abundant anion in the blood, its main function is to maintain a balance acid to base ratio by being a part of the buffer systems. When the bicarbonate levels are higher or lower than, it suggests that the body is having trouble maintaining its acid- base balance or that their electrolyte balance has upset, perhaps by loss or retention of fluid [22, 23]. This result implies that groups A, B were unable to maintain stable blood bicarbonate concentrations which will definitely results to a shift in acid to base ratio in the body of experimental mice.

There is significant difference in chloride ion (Cl^-) ion. There is significant increase across the treatment groups compared with the control group. Chloride ion is the predominant extracellular anion, it maintains the osmotic pressure gradient between the intracellular fluid (ICF) and the extracellular fluid (ECF), and it also maintains proper hydration in the body. Group B had significant decreased concentrations of chloride ion (Cl^-) in the serum of experimental mice. This result implies that aspartame reduced the chloride ion (Cl^-)

) concentrations which will result in a shift in osmotic gradient between the intracellular fluid (ICF) and the extracellular fluid (ECF).

There is significant difference in sodium ion (Na^+) concentration in the experimental animals groups. The positive, negative control groups and the group that received bark extracts of *S. gabonensis* experienced increased sodium ion concentrations, while the bark + leaf (750mg/kg/bw) experienced decreased sodium ion concentrations. Sodium ion (Na^+) is the major cation of the extracellular fluid that is responsible for osmotic pressure gradients that exist between the interior of cells and their surrounding environment. This result means that the experimental animals in the group that received the bark extract of *S. gabonensis*, negative and the positive control groups have a stable osmotic pressure gradient, while the experimental animals in groups that received the leaf extract, bark + leaf (250 and 750mg/kg/bw) groups experienced shift in osmotic pressure gradients. This implies that neither aspartame nor the bark extract of *S. gabonensis* had a negative impact on the experimental mice as the osmotic gradient controlled by sodium ion (Na^+) remain stable in those groups that received aspartame and the bark extract of *S. gabonensis*.

There is significant difference in the result of potassium ion (K^+) concentrations in the experimental animals. In all the experimental groups studied, the potassium ion (K^+) is within the normal range which is 3.50-5.00 (mmol/l). The group that received the bark extract of *S. gabonensis* have the highest concentration of blood potassium ion (K^+) concentrations followed by the group that received aspartame and then the negative control group, while the other experimental groups have lower potassium ion (K^+) concentrations. Potassium is one of the major intracellular cation, whose function is to open up resting membrane cells of the neurons and muscles fibres after membrane depolarisation. Neither aspartame nor the extracts of *S. gabonensis* have negative impact on the experimental animals as the potassium ion (K^+) concentrations remained within the normal range.

Conclusion

Electrolytes fluctuations and alterations are implications in liver and kidney damage. This study recorded alterations in the electrolyte concentrations in the serum of experimental mice especially those administered aspartame alone compared to the control group. However, these alterations may lead to

metabolic disorder in individuals exposed to frequent and high intake of aspartame. Therefore, moderate consumption of aspartame is advocated while the consumption of *S. gabonensis* is highly recommended.

Ethical Approval

Animal Ethic committee approval has been taken to carry out this study.

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