Original Research Article

The Effect of Oral Intake of Sodium Benzoate on the Activity of Liver marker enzymes and Electrolyte level of the Wistar Albino Rats

EFEKEMO, Oghenetekevwe, ESSIEN, Eka Bassey, AKANINWOR, Joyce Oronne

Department of Biochemistry, University of Port Harcourt, PMB 5323, Choba, Port Harcourt, Rivers State, Nigeria.

Email address: efekemo_tekevwe@yahoo.com

AUTHORS CONTRIBUTION

The work was carried out in collaboration between all authors. Author Efekemo Oghenetekevwe as the main author designed, analyzed, interpreted and prepared the manuscript, under the supervision of Essien Eka Bassey and Akaninwor Joyce Oronne. All authors read and approved the final manuscript.

ABSTRACT:

The *in vivo* effect of oral administration of varying concentrations (150, 250, 500mg/kg body wt) of sodium benzoate (a known preservative in the food, cosmetic and pharmaceutical industry) on serum liver marker enzyme activity and electrolyte levels of wistar albino rats were investigated. The oral intake was administered at 24 hour intervals

for 7, 14, 21 and 28 days. The groups were labeled; control (group 1), 7days (group 2), 14days (group 3), 21 days (group 4) and 28days (group 5). The rats were fed normal diet *ad libitum* and blood sample for the determination was taken at the end of the duration. For serum electrolytes, the result obtained for sodium benzoate concentrations administered showed significant ($p \leq 0.05$) increase in sodium (Na+) for groups 3, 4 and 5 for 150mg/kg body wt. and group 2, 3, 4 and 5 for 250mg/kg body wt and 500mg/kg body wt. of experimental rats. Chloride (Cl-) showed significant ($p \leq 0.05$) increase at all administered groups for 250mg/kg and 500mg/kg. Potassium (K+) was only significantly increased at group 5 for 500mg/kg body wt. while for bicarbonate (HCO_3) it showed no significant change in all treated groups. Values were all compared to the control. For liver marker enzymes, sodium benzoate significantly increased ($p \leq 0.05$) aspartate transaminase (AST) activity of experimental rats in groups 2, 3, 4 and 5 of 250mg/kg body wt. and 500mg/kg body wt., alanine transaminase (ALT) showed significant increase ($p \leq 0.05$) in group 4 and 5 for 250mg/kg body wt and group 2, 3, 4 and 5 for 500mg/kg body wt., alkaline phosphatase (ALP) showed significant ($p \leq 0.05$) increase in group 2, 3, 4 and 5 for 500mg/kg body wt.These findings suggest possible changes in blood chemistry due to the preservative.

Key words: sodium benzoate; serum; liver marker enzymes; electrolytes.

INTRODUCTION

The investigations of constituents of blood, plasma and serum of mammals have continually played a valuable role in the normal functioning assessment of living organisms. Changes from the normal levels have been observed in disease conditions [1]. The effects of various

compounds on biochemical parameters of experimental animals have been applied in assessing the safe use of compounds in products consumed. Sodium benzoate (C_6H_5COONa) is widely applicable as a preservative in several products consumed by man [2, 3, 4, 5]. Several studies on the short and long term effects of sodium benzoate have reported adverse effects due to both chronic and subchronic intake of sodium benzoate [6, 7]. Some reports suggest the absence of negative consequence of sodium benzoate intake [8, 9].The upper limits of benzoate allowable in foods vary with 0.1% reported for United States of America, while a range of 0.15 to 0.25% had been reported for other countries of the world [2]. For European countries, the limit reported range is from 0.015 to 0.5% [10]. There are thus variations in the acceptable limits of these preservatives in foods. It therefore follows that sodium benzoate could be assimilated widely by consuming a wide range of food products intentionally preserved with it. The present report addressed the effects of oral administration of sodium benzoate on serum electrolyte and liver marker enzymes. The findings of this study would further assist in the interpretation of blood chemistry data for individuals who consumed foods containing the preservatives.

MATERIALS AND METHOD

The experimental analysis was carried out in the Department of Biochemistry Research Laboratory, University of Port Harcourt, Choba, Rivers State, Nigeria. The study duration was for a period of one month, twenty eight days being the longest duration. The animals were purchased from the Department of Biochemistry, Animal House. Sodium benzoate was purchased from May & Baker Ltd., England. AST and ALT Kits were purchased from Randox Laboratory, Ltd. UK, ALP Kit was purchased from TECO Diagnostic Kit, USA; while all other

reagents were of analytical grade. An approval was given by the institutions ethic committee for the commencement of this study.

Animals

A total of sixty-six (66) wistar albino rats, with an average weight of 140g were obtained from the animal house of the Department of Biochemistry, University of Port Harcourt. They were maintained on normal diet ad libitum, grouped into five (5), and housed in stainless steel cages in a well ventilated room under 12h light/dark cycle. The sodium benzoate concentrations were 150mg/kg body wt., 250mg/kg boody wt and 500mg/kg body weight. The rats were divided into five groups namely G1 (control group), G2 (7days), G3 (14days), G4 (21days) and G5 (28days). The varying concentrations of sodium benzoate were administered orally in 1ml portions at 24 h intervals for the duration of the experiment (7, 14, 21 and 28). At the end of the experimental duration the rats were sacrificed.

Sample collection

The rats were anaesthetized with diethyl ether and dissected for blood collection. The blood was collected into lithium heparin bottles and analysis performed within two (2) hrs of collection. Before assays, the blood samples were centrifuged for 5 min using a bench-top centrifuge {MSE-Minor} and the supernatant was then used for the determinations.

Determination of plasma electrolytes

Plasma potassium concentrations followed the procedure outlined by [11] using sodium tetra-phenyl boron-formulated reagent. Sodium measurement followed the precipitation method described by [12]. Chloride was measured by the titration method described by [13]. For bicarbonate measurements, the method of [14] involving titration was used.

Determination of serum liver marker enzymes

Serum liver marker enzymes aspartate transaminase (AST) and alanine transaminase (ALT) were determined using quantitative method. The activities of ALT and AST were analysed by the end point colometric method of [15]. Alkaline phosphatase (ALP) was measured by end-point colorimetric method of [16].

Statistical analysis

All data were subjected to statistical analysis. The values were reported as mean ± standard error of mean (S.E.M), and analysed by one-way analysis of variance (ANOVA). ANOVA was used to test for differences between treatment groups using statistical package for social sciences (SPSS) version 20. The results were considered significant at P-values of less than 0.05, that is, at 95% confidence level ($P<0.05$).

RESULT

The result of the effects of different concentrations of orally administered sodium benzoate on serum liver marker enzyme activity and serum electrolyte concentrations are shown in figure 1, 2, 3, 4, 5, 6 and 7.

For the aminotranferases, there was significant increase in aspartate transaminase (AST) activity in group 2, 3, 4 and 5 of 250mg/kg body wt and 500mg/kg body wt of sodium benzoate administered groups.. Alanine transaminase (ALT) showed significant ($p \leq 0.05$) increase in activity at grp 4 and 5 for 250mg/kg and all the administered groups for 500mg/kg. Alkaline phosphatase (ALP) showed significant increase in all administered groups at 500mg/kg.

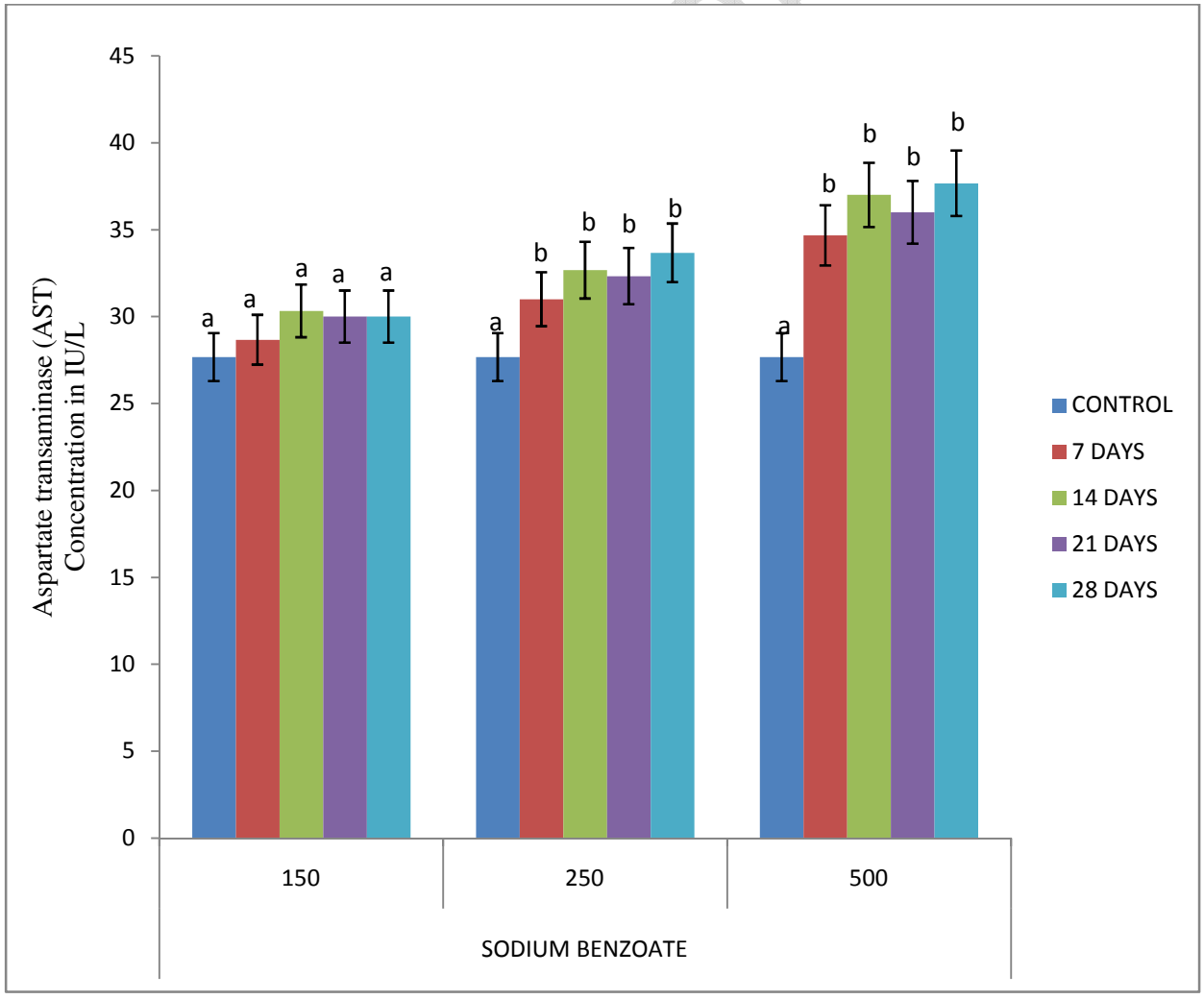


Fig 1: Effects of varying concentrations of sodium benzoate on aspartate transaminase (AST) activity in serum.

Values are means ± Standard Error Mean (SEM). Values with different superscript are statistically significant at ($p \leq 0.05$). Superscript (a,b) compares 7 Days, 14 Days, 21 Days and 28 Days to control.

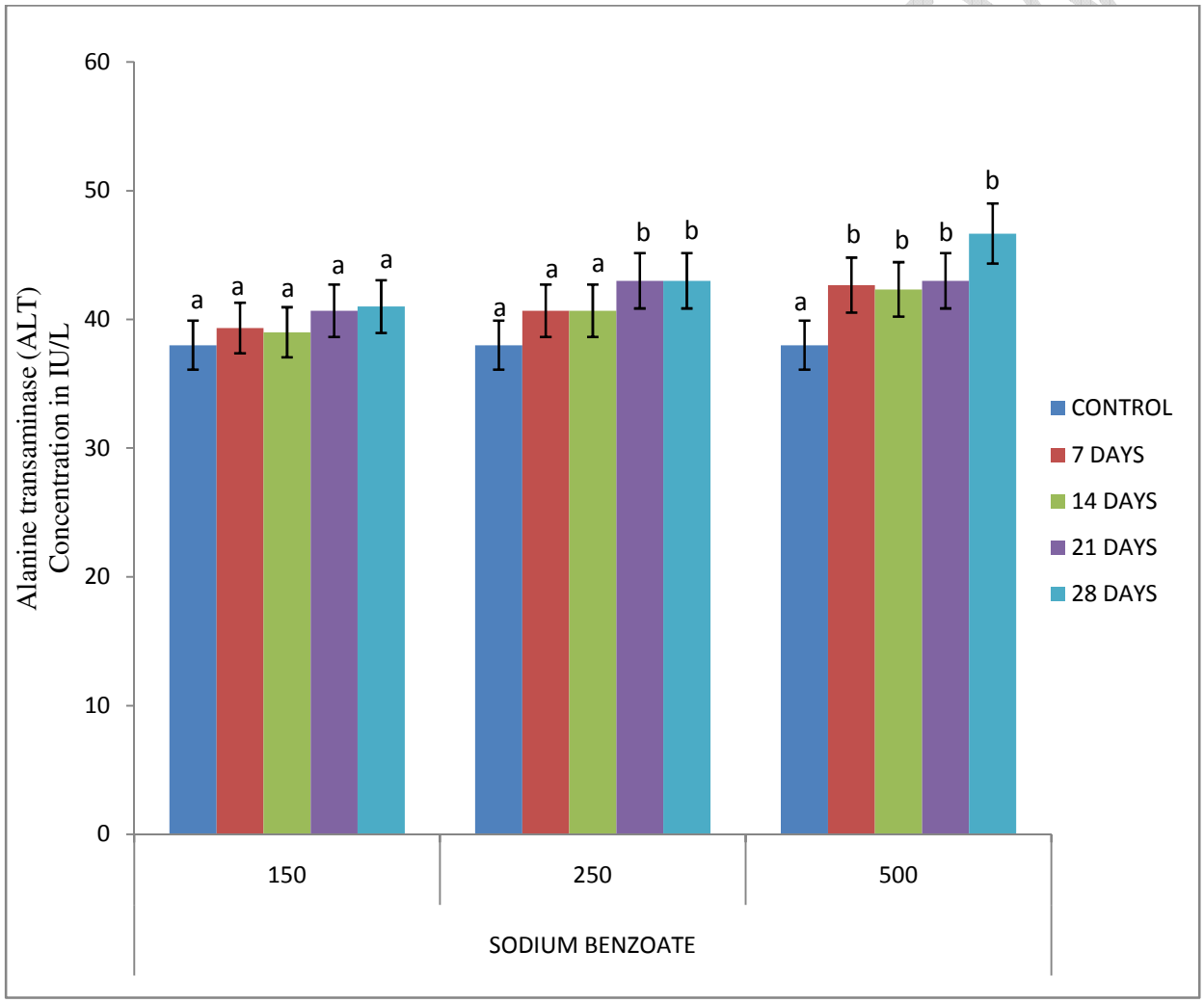


Fig 2: Effects of varying concentrations of sodium benzoate on alanine transaminase (ALT) activity in serum.

Values are means ± Standard Error Mean (SEM). Values with different superscript are statistically significant at ($p \leq 0.05$). Superscript (a,b) compares 7 Days, 14 Days, 21 Days and 28 Days to control.

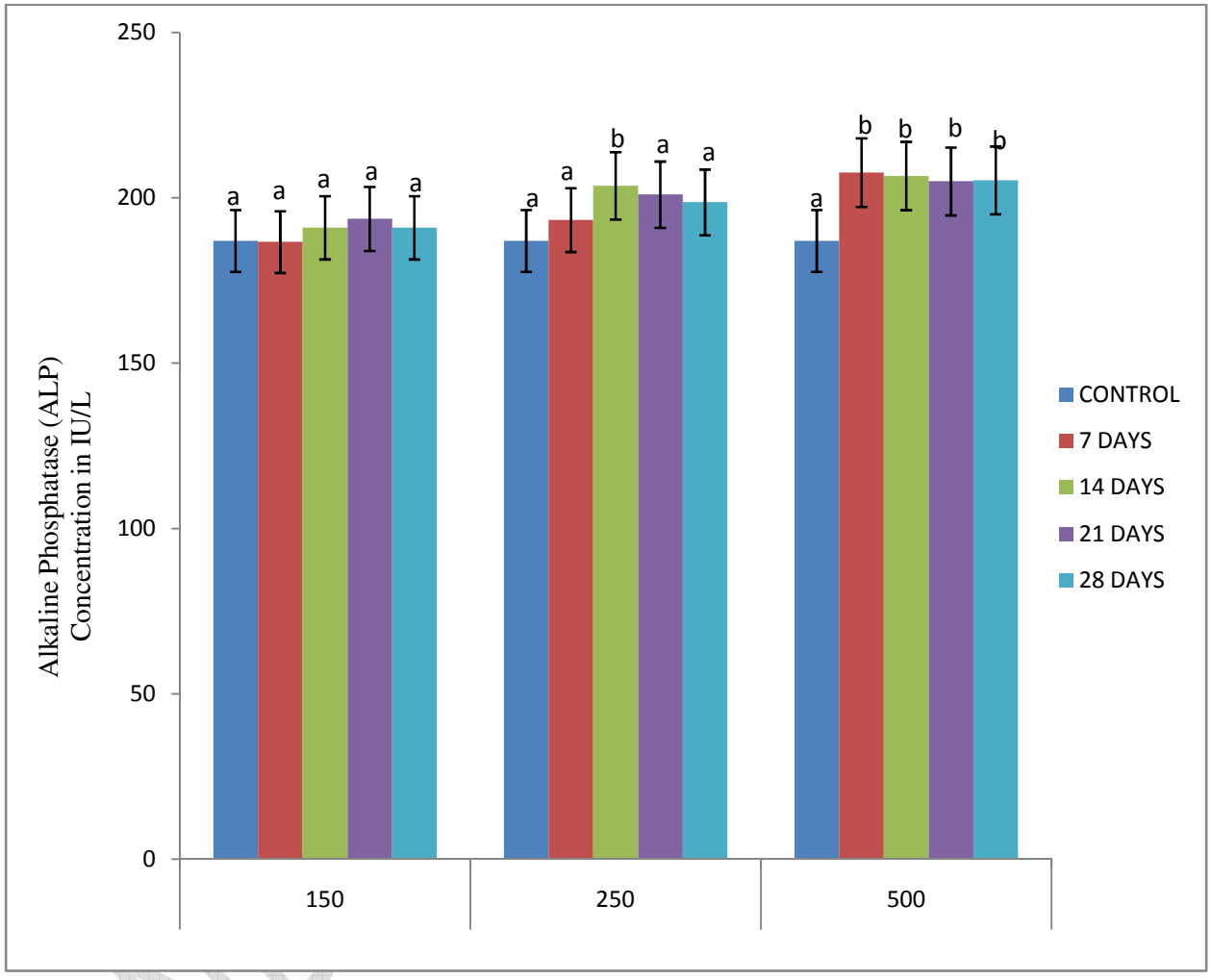
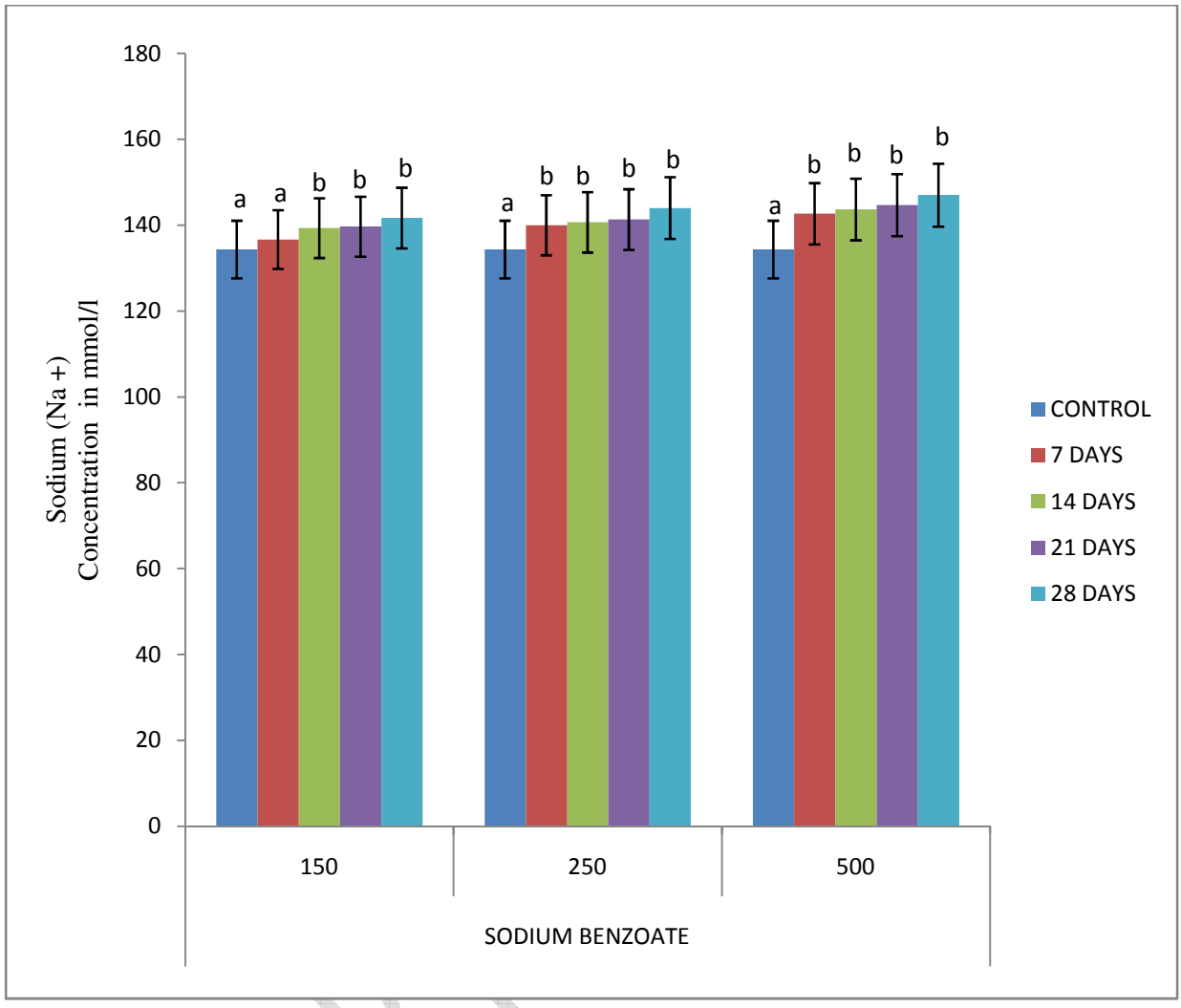


Fig 3: Effects of varying concentrations of sodium benzoate on alkaline phosphatase (ALP) activity in serum.

Values are means ± Standard Error Mean (SEM). Values with different superscript are statistically significant at ($p \leq 0.05$). Superscript (a,b) compares 7 Days, 14 Days, 21 Days and 28 Days to control.

For the electrolytes sodium, potassium, chloride and bicarbonate had varying activity as shown in fig 4, 5, 6, 7. Sodium benzoate significantly ($p \leq 0.05$) increased the levels of sodium at grp 3, 4 and 5 for 150mg/kgb.w and all administered groups for 250mg/kgb.w and 500mg/kgb.w. It significantly ($p \leq 0.05$) increased potassium only at grp 5 for 500mg/kgb.w. Bicarbonate had no signicant difference in all the treated groups and chloride was significantly ($p \leq 0.05$) increased in all treated groups for 250mg/kgb.w and 500mg/kgb.w and no significant difference in 150mg/kgb.w. The levels of sodium, potassium bicarbonate and chloride in test groups where all compared to the control group.



144

145 Fig 4: Effects of varying concentrations of sodium benzoate on sodium (Na^+) levels in serum.

146 Values are means ± Standard Error Mean (SEM). Values with different superscript are
147 statistically significant at ($p \leq 0.05$). Superscript (a,b) compares 7 Days, 14 Days, 21 Days and 28
148 Days to control.

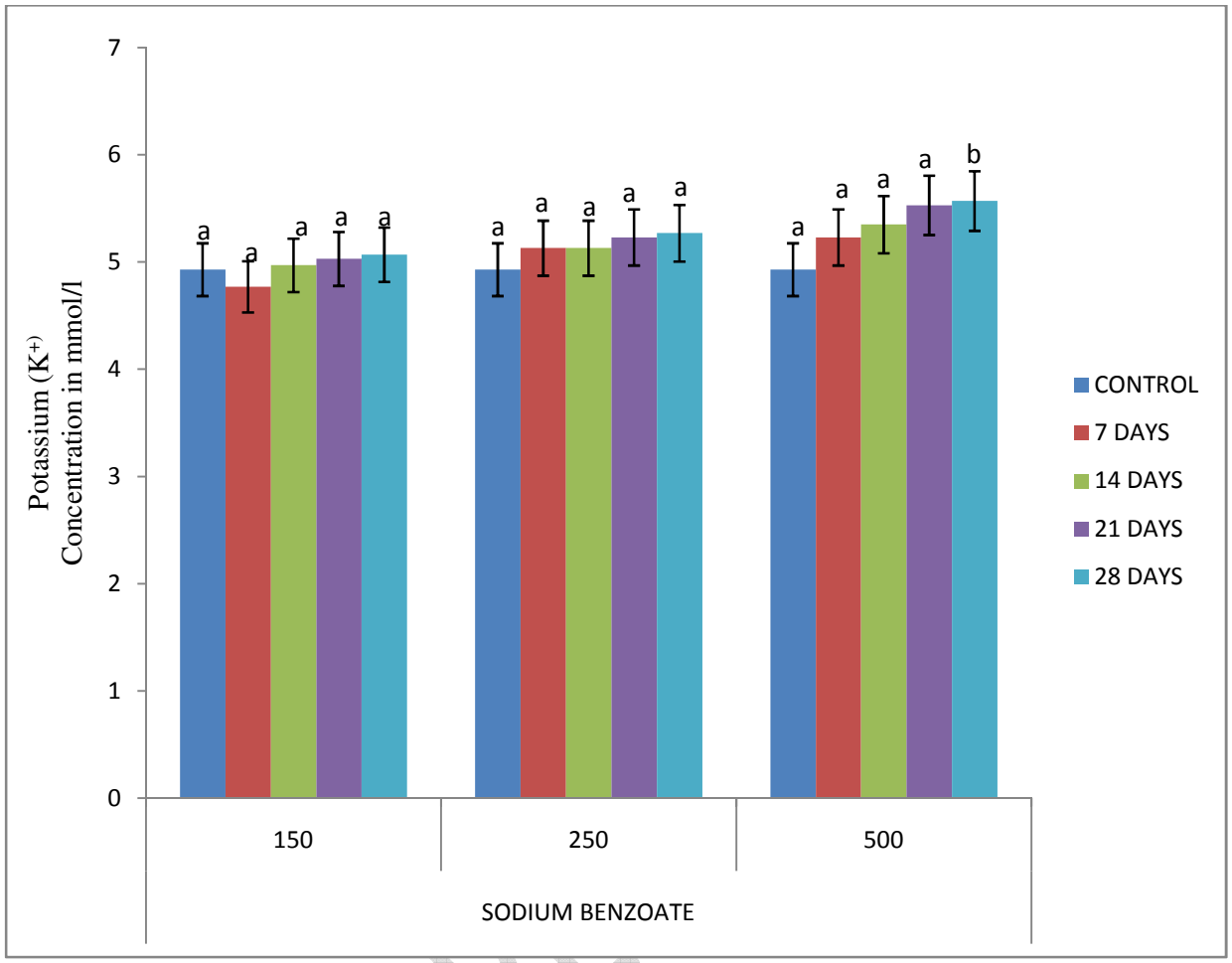


Fig 5: Effects of varying concentrations of sodium benzoate on potassium (K^+) levels in serum.

Values are means ± Standard Error Mean (SEM). Values with different superscript are statistically significant at ($p \leq 0.05$). Superscript (a,b) compares 7 Days, 14 Days, 21 Days and 28 Days to control.

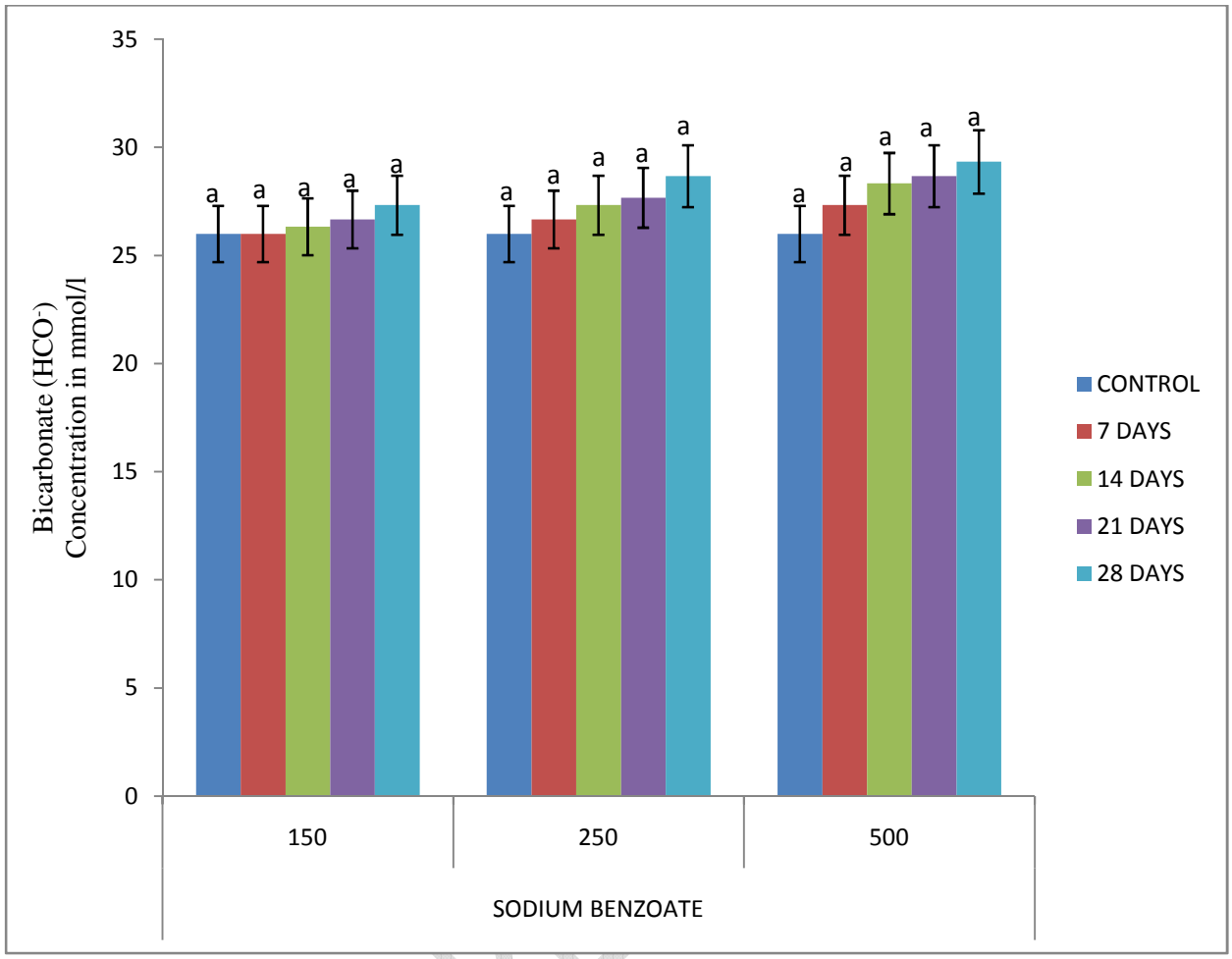


Fig 6: Effects of varying concentrations of sodium benzoate on bicarbonate (HCO^-) levels in serum.

Values are means ± Standard Error Mean (SEM). Values with different superscript are statistically significant at ($p \leq 0.05$). Superscript (a,b) compares 7 Days, 14 Days, 21 Days and 28 Days to control.

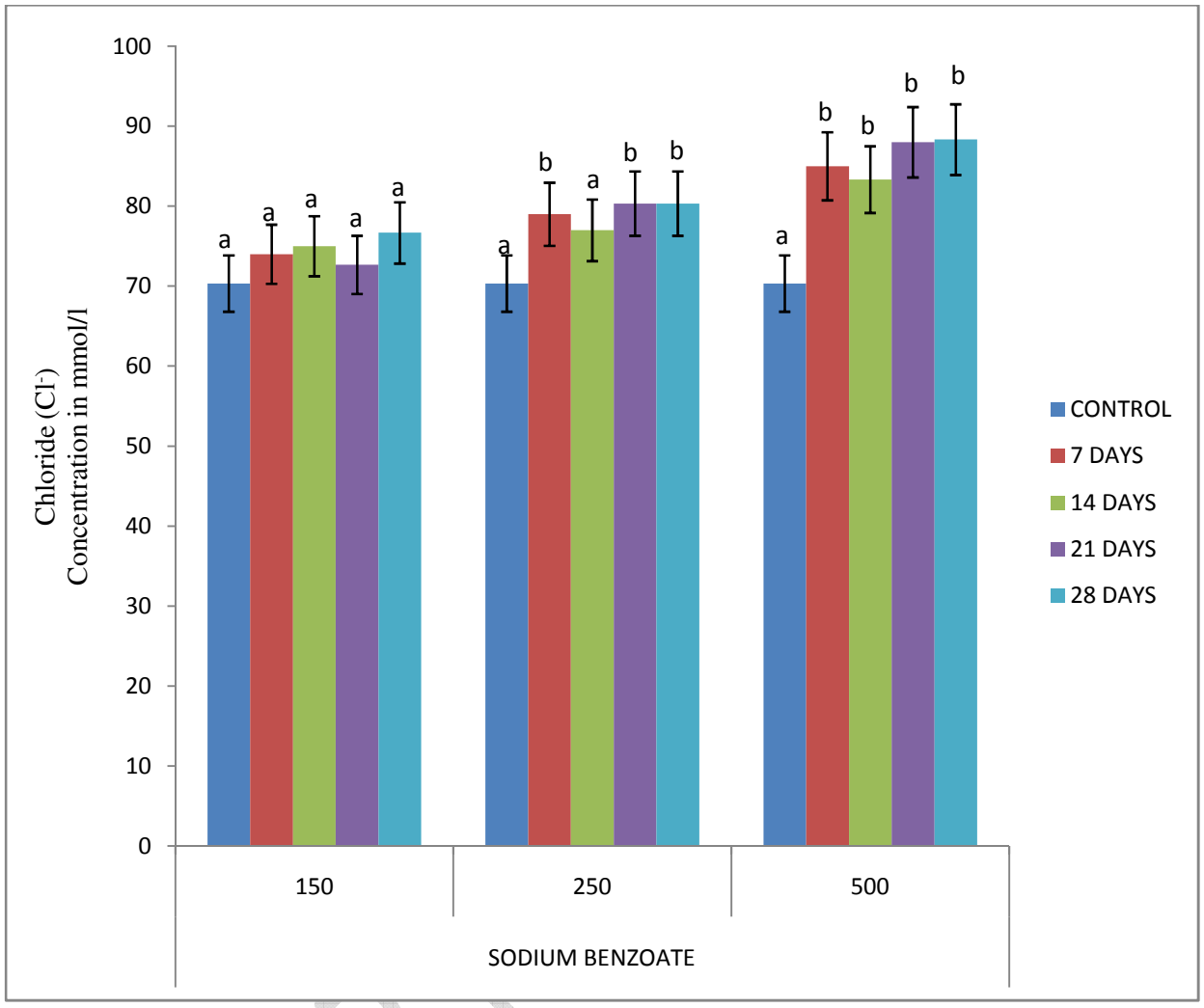


Fig 7: Effects of varying concentrations of sodium benzoate on chloride (Cl^-) levels in serum.

Values are means ± Standard Error Mean (SEM). Values with different superscript are statistically significant at ($p \leq 0.05$). Superscript (a,b) compares 7 Days, 14 Days, 21 Days and 28 Days to control.

DISCUSSION

The elevation of aminotransferase activity in serum may be due to tissue damage particularly in liver and heart, and increased permeability of cell membrane [17]. The study revealed that rats

that consumed sodium benzoate exhibited a significant ($p \leq 0.05$) increase in serum ALT, AST, and ALP activities when compared to control rats. Sodium benzoate caused derangement of liver function as revealed by significant elevation of serum ALT and AST as well as significant reduction of these enzymes in the liver. Determination of AST, ALT and ALP in the serum is largely used in the assessment of liver damage [18]. Membrane damage to the liver releases the enzymes into circulation and hence can be measured in the serum. Previous studies showed that sodium benzoate showed a significant ($p \leq 0.05$) increase in serum AST, ALT, and ALP activities and these results were attributed to hepatocellular damage which was caused by the toxic effect of sodium benzoate. It was indicated by vacuolation, swelling and necrosis of the liver cells [19]. Increase in both serum AST and ALT of rats was attributed to the changes in liver function and hepatocellular impairment which subsequently caused the release of greater than normal levels of intracellular enzymes into the blood [20]. Alkaline phosphatase occurs in the canalicular and sinusoidal membranes of the liver, thus damage to the liver will result in elevated serum ALP activity [21]. Cholestatic liver disease is characterized by an increased level of ALP. The trend of ALP significantly increase gave an indicator that the hepatic capacity of the liver is affected by sodium benzoate [21]. Also, the significant elevation of serum aminotransferases may be attributed to the fact that under pathological conditions, the parenchymal cells of hepatic lobules fail to carry out vital functions, which usually results in disturbed or imbalanced intermediatory metabolism. As a result of cellular damage, several enzymes like ALT, AST and ALP leach out into the serum and hence their level indicate the type and extent of damage inflicted [17]. Sodium benzoate caused derangement of liver function as revealed by significant elevation of serum ALT, AST and ALP. In blood plasma, sodium benzoate has a binding affinity for plasma proteins where it is carried out to different tissues. In the liver, it is metabolized by conjugation

with glycine, resulting in the formation of hippuric acid [22]. Alkaline phosphatase is present on cell surfaces in most human tissues, especially those of the intestine, liver, bones, spleen and kidneys. The specific location of the enzyme within sinusoidal and bile canalicular membranes could account for its serum elevation in the current study in response to sodium benzoate administration. The ALT enzyme is a strong positive indicator of insulin resistance, diabetes mellitus and obesity which are risk factors for coronary heart disease and is also a sensitive marker of liver damage [23]. Liver enzymes levels are usually raised in acute hepatoxicity, but tend to decrease with prolonged intoxication due to damage to the liver [24]. According to Ranjna *et al.,* [25], both AST and ALT enzymes are excellent markers of liver damage caused by exposure of liver to toxic substances. However, ALT is more specific liver enzyme for diagnostic use when the integrity of the hepatocellular membrane is compromised [23]. The increased transaminase levels of test rats against the control as observed in the present study could be linked to consumption of sodium benzoate. It was mentioned that the release of abnormally high levels of specific tissue enzymes into blood stream is dependent on both the degree and the type of damage exerted by the toxic compound administration. For the electrolytes, the observe increase in the groups could be an effect on their pumps which can be linked to sodium benzoate administration. This may interfere with these electrolytes in several metabolic pathways leading to increase in their levels in the serum [26].

CONCLUSION AND RECOMMENDATION

The main aim of this research was to determine the toxicology effect of the oral administration of sodium benzoate. From the result obtained from the experimental duration it can be observed that sodium benzoate at the varying concentrations, significantly raised serum liver marker

enzyme activity and electrolyte levels. The significant changes obtained in some of the measured parameters following oral administration of sodium benzoate points to the need for caution on usage and in the interpretation of blood chemistry data of blood samples, especially for samples drawn from individuals who may have consumed sodium benzoate containing foods before sample collection..

Further studies can be carried to determine its possible toxicology effect on longer periods of administration.

COMPETING INTERESTS

Authors have declared no competing interest exist.

ACKNOWLEDGEMENT

I want to acknowledge the contribution of Dr. D. E. Peters and Mr. Aleme B to the success of this research work.

REFERENCES

1. Cheesbrough M. Medical laboratory manual for tropical countries. 2nd edition. Tropical Health Technology and Butterworth Scientific Limited. 1991;(1):494-526.
2. Chipley JR. Sodium benzoate and benzoic acid. In: Branen AL, Davidson PM, eds. Antimicrobials in foods. New York, M. Decker. 1983;11-35.
3. Baldwin EA, Nisperos-Carriedo MO, Baker RA. Use of edible coatings to preserve quality of lightly (and slightly) processed products. Critical Review in Food Science and Nutrition. 1995;35(6):509-524.

4. Ishida H. Levels of preservatives in toothpastes and possibility of their intake during brushing of teeth. Shokuhin Eiseigaku Zasshi. Journal of the Food Hygienic Society Japan. 1996;37:234-239.
5. Villanueva MBG, Jonai H, Kanno S. Takeuchi Y. Dietary sources and background levels of hippuric acid in urine: Comparison of Philippine and Japanese levels. Industrial Health. 1994;32: 239-249.
6. Fujitani T. Short-term effect of sodium benzoate in F344 rats and B6C3F1 mice. Toxicology Letters. 1993;69:171-179.
7. Vogt T, Landthaler M, Stolz W. Sodium benzoate-induced acute leukocytoclastic vasculitis with unusual clinical appearance. Archives of Dermatology. 1999;135:726-727.
8. Sodemoto Y. Enomoto M. Report of carcinogenesis bioassay of Sodium benzoate in rats: absence of carcinogenicity of sodium benzoate in rats. Journal of Environmental Pathology, Toxicology and Oncology. 1980;4:87-95.
9. Toth B. Lack of tumorigenicity of sodium benzoate in mice. Fundamental Applied Toxicology, 1984;4:494-496.
10. European Commission. European Union Directive 95/2/CE from 20.02.1995 on food additives, colourants and sweeteners. European Commission; 1995
11. Tietz NW. Fundamentals of Clinical Chemistry, 2nd edition, Saunders, Philadelphia. 1976;876.
12. Henry RJ. Clinical Chemistry. Harper & Row, New York, 2nd edition. 1974;643.
13. Ramnik S. Medical Laboratory Technology: methods and Interpretations, 5th edition. Jayee Brothers, New Delhi. 1999;279.

14. Ochei J, Kolhatkar A. Medical Laboratory Science: theory and practice. Tata McGraw-Hill Pub. Co. Ltd, New Delhi, India. 2003;200.
15. Reitman A, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology. 1957;28(1):56 - 63.
16. Englehardt VA. Measurement of Alkaline phosphate. Aerztl Labour. 1970;16: 42.
17. Amin KA, Abdel Hameid H, AbdElsttar AH. Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats. Food and Chemical Toxicology. 2010;(48):2994–2999.
18. Moss DW, Rosalki SB. Enzyme tests in diagnosis. Edward Arnold. London. 1996;187-196
19. Wang W, Srivastava S. "Serological markers". In Breslow, Lester. Encyclopedia of Public Health. New York. Macmillan, USA.2002;1088-1090.
20. Abdel-Rahim EA, Ashoush YA, Afify AS, Hewedi F. Effect of some synthetic food additives on blood haemoglobin and liver function of rats. Menofia Journal of Agricultural Research. 1989;14(1):557.
21. McComb RB, Bowers GN, Posen S. Alkaline phosphatase. In: Biological Chemistry (3rd ed.). B. Milner (ed.). 1979;465 - 468
22. Kubota K, Ishizaki T. Dose-dependent pharmacokinetics of benzoic acid following oral administration of sodium benzoate to humans. European Journal of Clinical Pharmacology. 1991;41:363-368.

23. Al-Mamary M, Al-Habori M, Al-Aghbari AM, Baker MM. Investigation into the Toxicological Effects of Catha Edulis Leaves: A Short-Term Study in Animals. Phytoetherapy Research. 2002;(16):127-132.
24. Obi E, Orisakwe OE, Asomugha LA, Udemezue OO. The hepatotoxic effect of halofantrine in guinea pigs. Indian Journal of Pharmaceutical Sciences. 2004;36(5):303-305.
25. Ranjna C. Practical Clinical Biochemistry Methods and Interpretation, 2nd edition; 1999.
26. El-Sheikh NM. Khalil FA. L-Arginine and L-glutamine as immunonutrients and modulating agents for oxidative stress and toxicity induced by sodium nitrite in rats. Food and Chemical Toxicology. 2011;12(49):758–762.