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

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, serum 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 (Cheesebrough, 1991). 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 (Chipley, 1983; Baldwin *et al.,* 1995; Ishida, 1996, Villanueva *et al.,* 1994). 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 (Fujitani, 1993; Vogt, 1999). Some reports suggest the absence of negative consequence of sodium benzoate intake (Sodemoto and Enomoto,1980; Toth, 1984).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 (Chipley, 1983). For European countries, the limit reported range is from 0.015 to 0.5% (European Commission, 1995). 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

Sodium benzoate was purchased from May & Baker Ltd., England, enzyme kits for AST, ALT and ALP were obtained from Randox laboratory Ltd, San Francisco, U.S.A; while all other reagents were of analytical grade.

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 Pharmacology, 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 Tietz (1976) using sodium tetra-phenyl boron-formulated reagent. Sodium measurement followed the precipitation method described by Henry (1974). Chloride was measured by the titration method described by Ramnik

(1999). For bicarbonate measurements, the method of Ochei and Kolhatkar, (2003) 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 Reitman and Frankel (1957). Alkaline phosphatase (ALP) was measured by end-point colorimetric method of Englehardt (1970).

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 AND DISCUSSION

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.

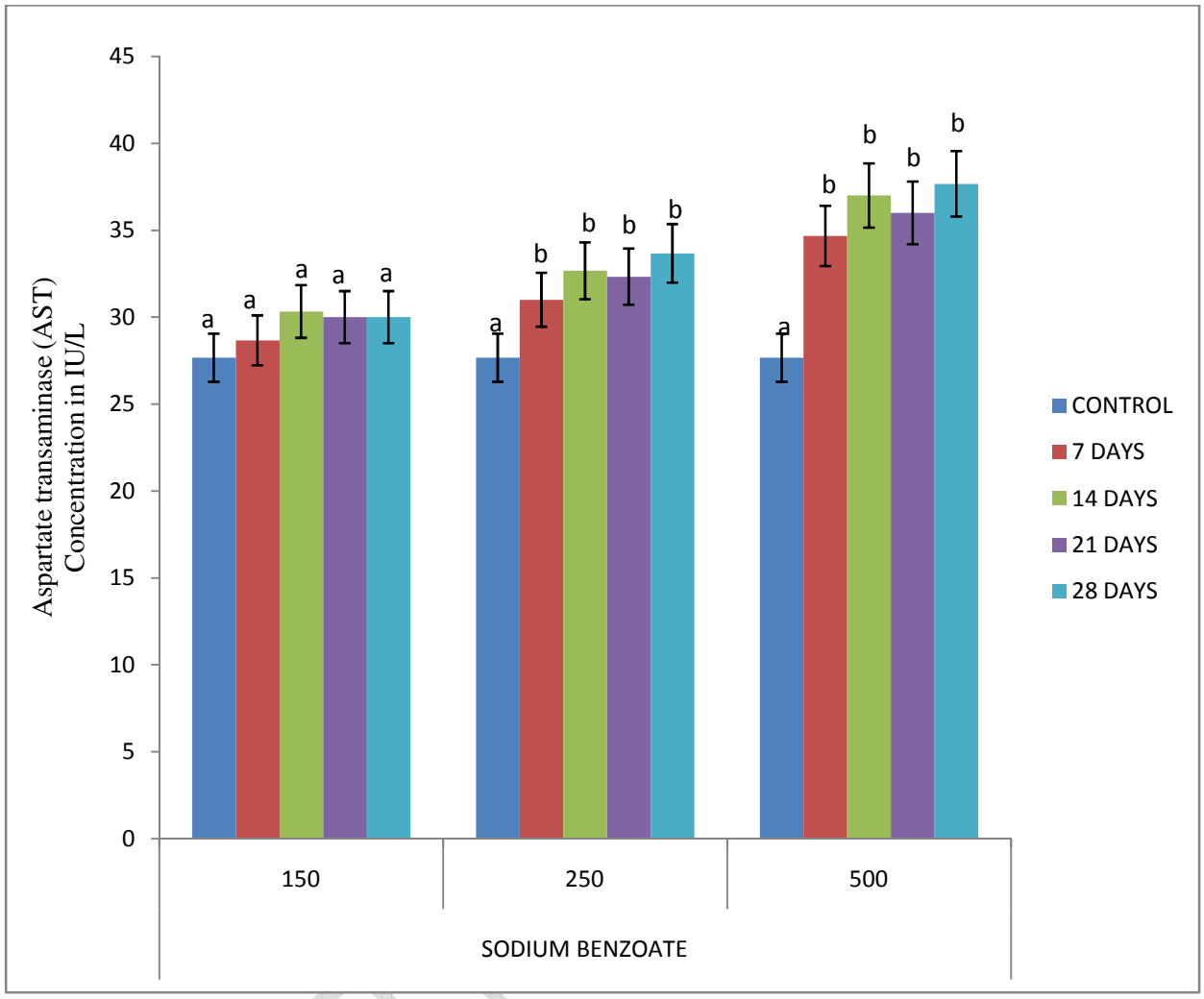


Fig 1: Effects of varying concentrations of sodium benzoate on aspartate transaminase (AST) activity in serum.

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.

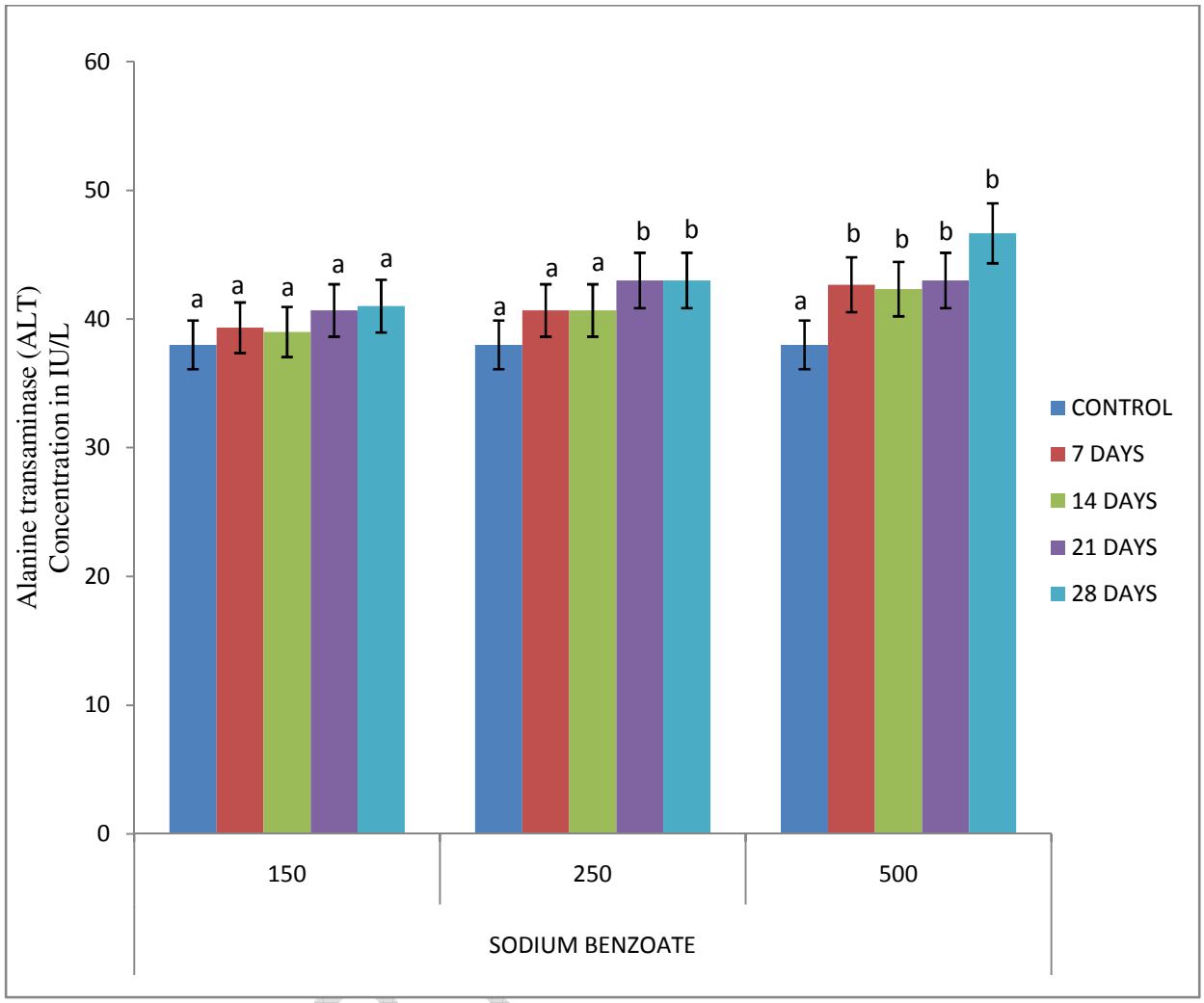


Fig 2: Effects of varying concentrations of sodium benzoate on alanine transaminase (ALT) activity in serum.

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.

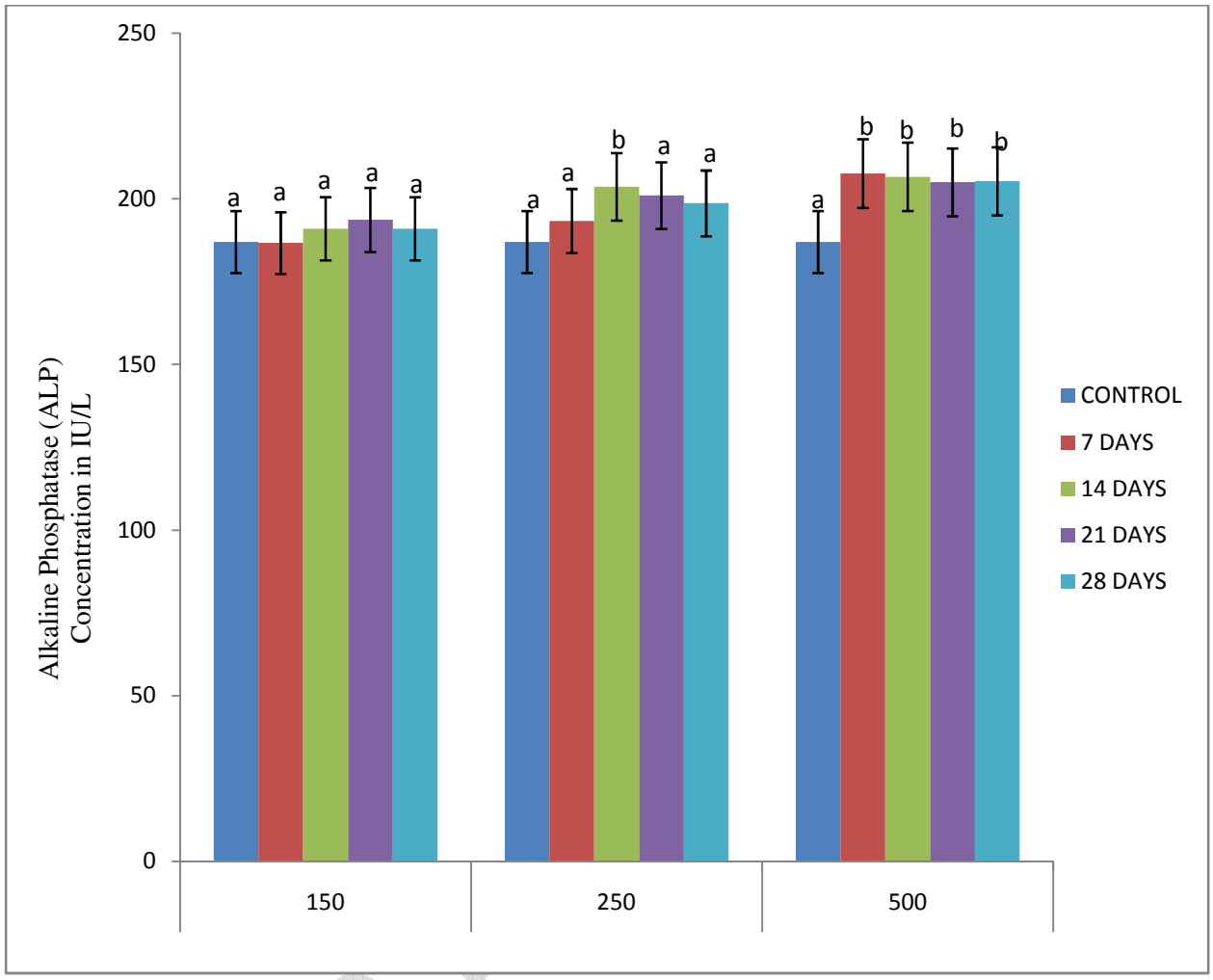


Fig 3: Effects of varying concentrations of sodium benzoate on alkaline phosphatase (ALP) activity in serum.

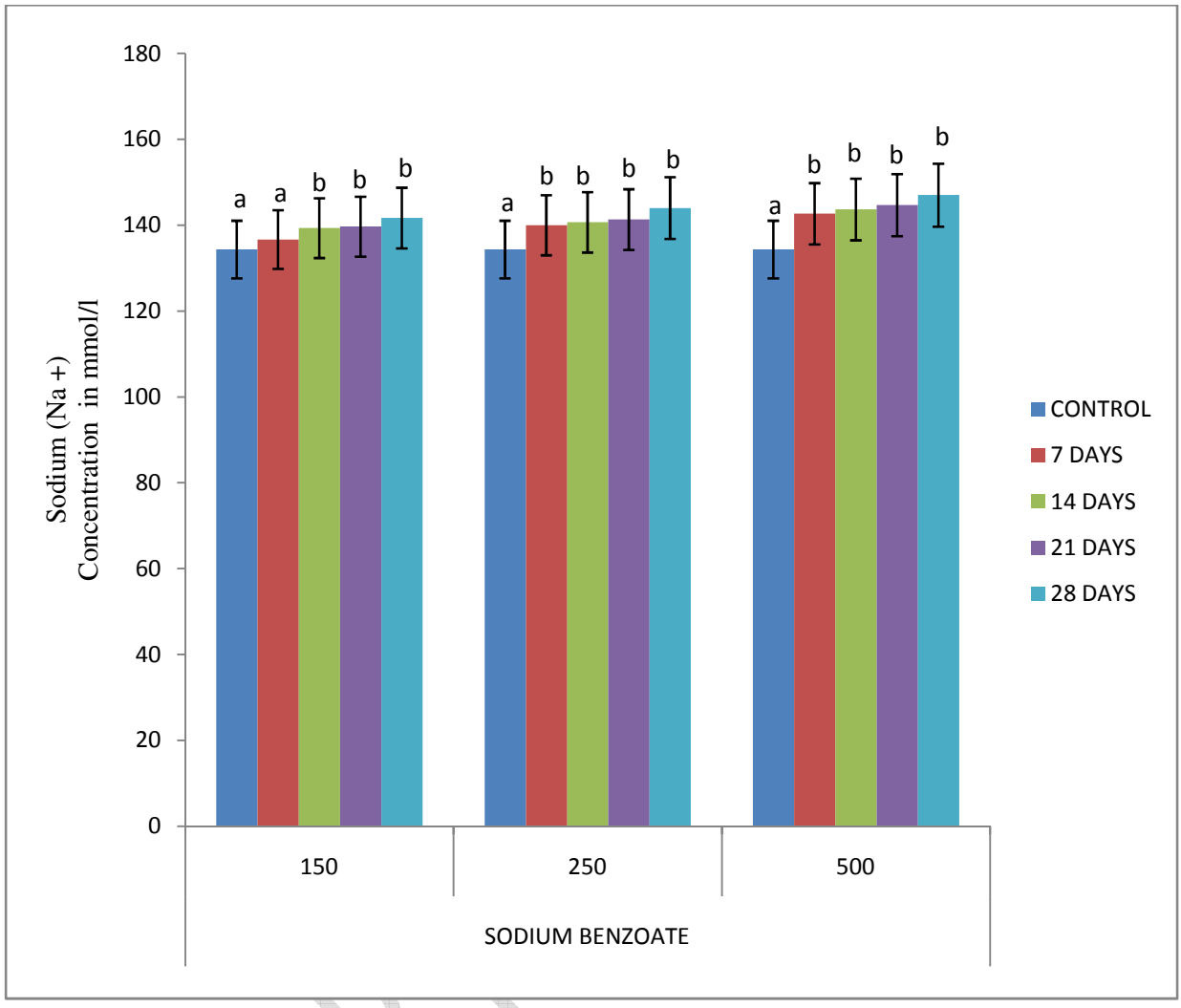
Values are means ± Standard Error Mean (SEM). Values with different superscript are statistically significant at ($p \leq 0.05$). Superscript (a,b) compares 7 Days, 14 Days, 21 Days and 28 Days to control.

For the 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. The elevation of aminotransferase activity in serum may be due to tissue damage particularly in liver

and heart, and increased permeability of cell membrane. (Amin *et al.,* 2010). 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 (Moss and Rosalki, 1996). 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 (Wang and Srivastava, 2002). 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 (Abdel-Rahim *et al.,*1989). Alkaline phosphatase occurs in the canalicular and sinusoidal membranes of the liver, thus damage to the liver will result in elevated serum ALP activity (McComb *et al.,* 1979). 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 (McComb *et al.,* 1979). 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 (Amin *et al.,* 2010). 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 (Kubota & Ishizaki 1991). 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 (Al-Mamary *et al.,* 2002). Liver enzymes levels are usually raised in acute hepatoxicity, but tend to decrease with prolonged intoxication due to damage to the liver (Obi *et al.,* 2004). According to Ranjna *et al.,* 1999, 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 (Woss and Hendersson, 1996). 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.

The electrolytes sodium, potassium, chloride and bicarbonate had varying activity as shown in fig 4, 5, 6, 7.



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

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

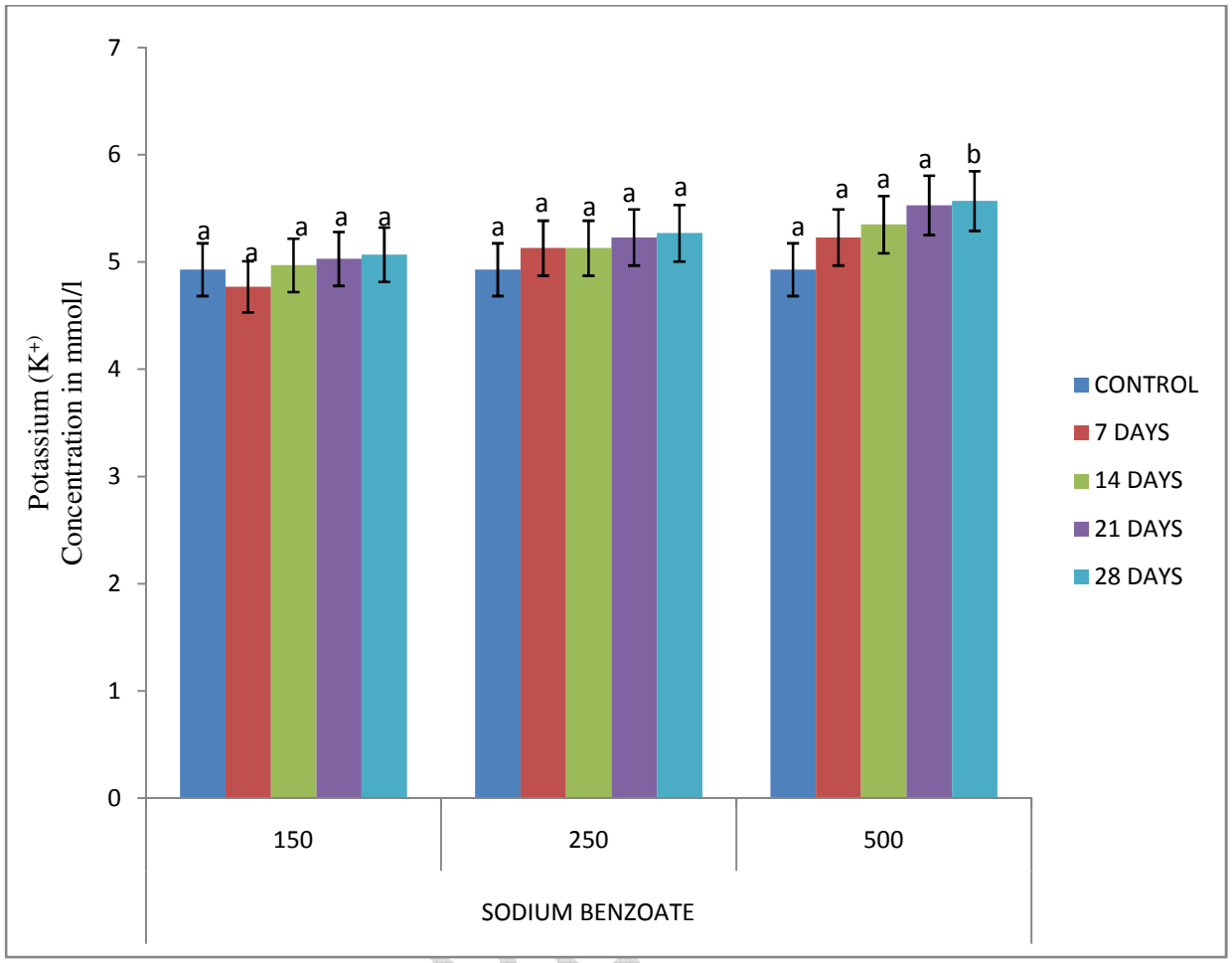

Fig 5: Effects of varying concentrations of sodium benzoate on potassium (K^+) levels in serum.

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.

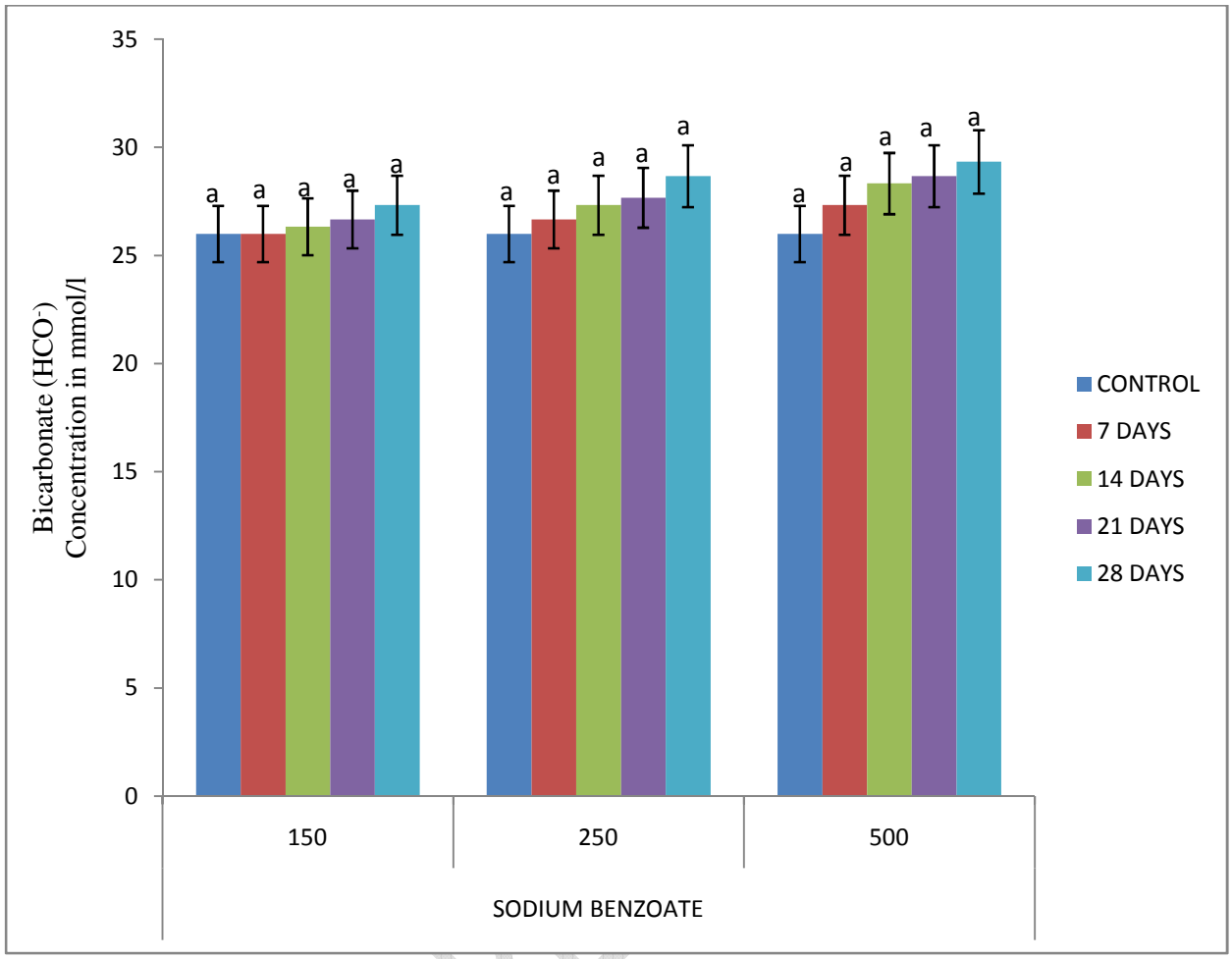

Fig 6: Effects of varying concentrations of sodium benzoate on bicarbonate (HCO^-) levels in serum.

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.

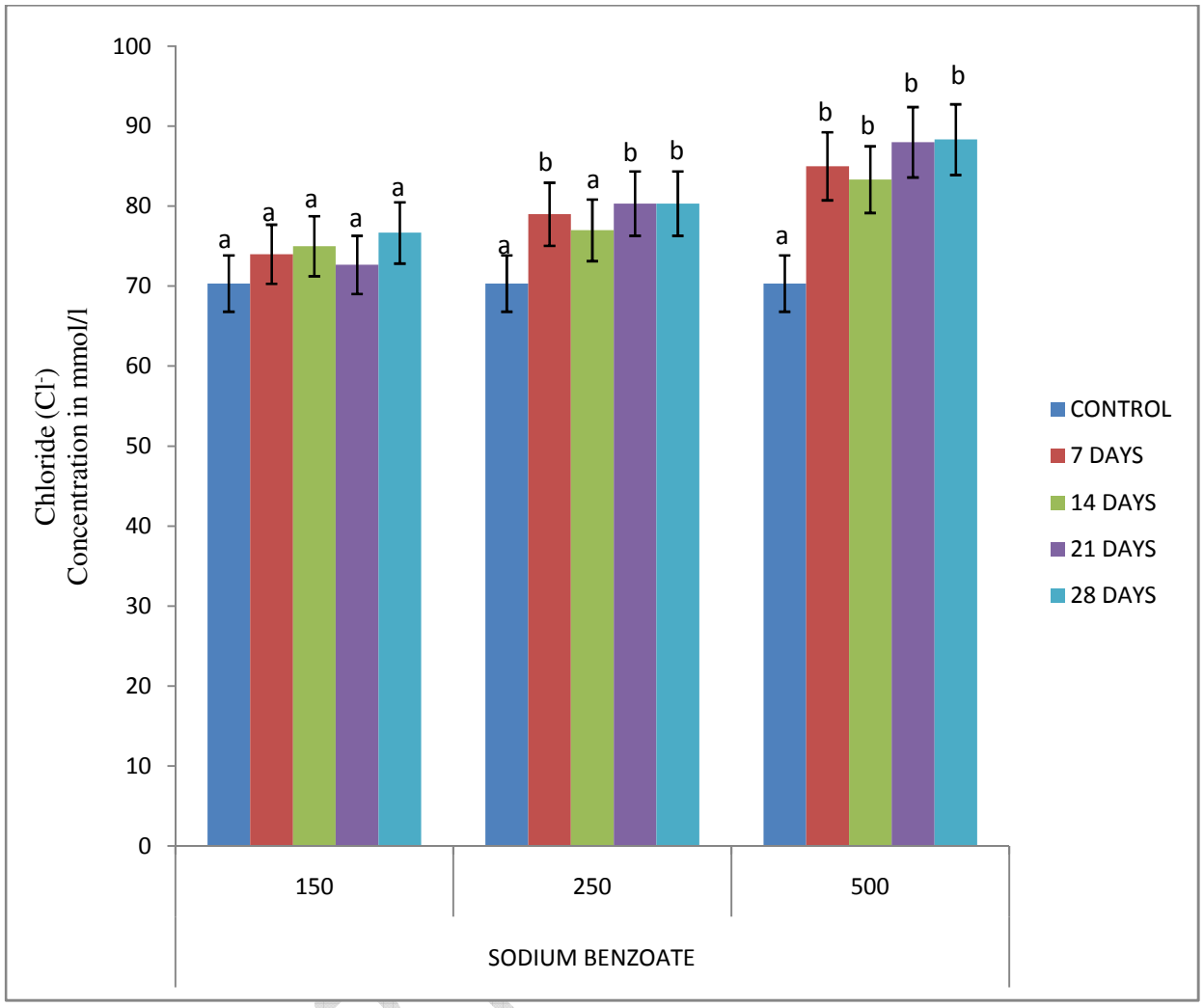

Fig 7: Effects of varying concentrations of sodium benzoate on chloride (Cl^-) levels in serum.

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.

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. The observe increase in the group 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 (El-Sheikh and Khalil, 2011). The significant changes obtained in some of the measured parameters following oral administration of sodium benzoate points to the need for caution in the interpretation of blood chemistry data of blood samples.

REFERENCES

Abdel- Rahim, E. A., Ashoush, Y. A., Afify, A. S. and Hewedi, F. (1989). Effect of some synthetic food additives on blood haemoglobin and liver function of rats. *Menofia Journal of Agricultural Research*, **14**(1): 557.

Al-Mamary, M., Al-Habori, M., Al-Aghbari, A. M. and Baker, M. M. (2002). Investigation into the Toxicological Effects of Catha Edulis Leaves: A Short-Term Study in Animals. *Phytoetherapy Research*, (16): 127-132.

Amin, K.A., Abdel Hameid, H. and AbdElsttar, A. H. (2010). 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*, (48): 2994–2999.

Baldwin, E. A., Nisperos-Carriedo, M. O., Baker, R. A. (1995). Use of edible coatings to preserve quality of lightly (and slightly) processed products. *Critical Review in Food Science and Nutrition*, **35**(6): 509-524.

Cheesbrough, M. (1991). Medical laboratory manual for tropical countries. 2nd edition. *Tropical Health Technology and Butterworth Scientific Limited*. Vol. 1, pp: 494-526.

208 Chipley, J. R. (1983). Sodium benzoate and benzoic acid. In: Branen AL,
209 Davidson PM, eds. *Antimicrobials in foods*. New York, M. Decker, 11-35.

210 El-Sheikh, N. M. and Khalil, F. A. (2011). L-Arginine and L-glutamine as
211 immunonutrients and modulating agents for oxidative stress and toxicity
212 induced by sodium nitrite in rats. *Food and Chemical Toxicology*,
213 **12**(49):758–762.

214 European Commission (1995). European Union Directive 95/2/CE from
215 20.02.1995 on food additives, colourants and sweeteners. European
216 Commission.

217 Fujitani, T. (1993). Short-term effect of sodium benzoate in F344 rats and B6C3F1
218 mice. *Toxicology Letters*, **69**: 171-179.

219 Henry, R. J. (1974). Clinical Chemistry. Harper & Row, New York, 2nd edition,
220 643.

221 Ishida, H. (1996). Levels of preservatives in toothpastes and possibility of their
222 intake during brushing of teeth. Shokuhin Eiseigaku Zasshi. *Journal of the*
223 *Food Hygienic Society Japan,* **37**: 234-239.

224 Kubota, K., Ishizaki, T. (1991). Dose-dependent pharmacokinetics of benzoic acid
225 following oral administration of sodium benzoate to humans. European
226 Journal of Clinical Pharmacology, **41**: 363-368.

227 McComb, R. B., Bowers, G. N. (Jr.), and Posen, S. (1979). Alkaline phosphatase.
228 In: *Biological Chemistry* (3rd ed.). B. Milner (ed.), 465 - 468

229 Moss, D. W., Rosalki, S. B. (1996). Enzyme tests in diagnosis. Edward Arnold.
230 London, 187-196

Obi, E., Orisakwe, O. E., Asomugha, L. A and Udemezue, O.O (2004). The hepatotoxic effect of halofantrine in guinea pigs. *Indian Journal of Pharmaceutical Sciences,* **36**(5):303-305.

Ochei, J. and Kolhatkar, A. (2003). Medical Laboratory Science: theory and practice. Tata McGraw-Hill Pub. Co. Ltd, New Delhi, India, 200.

Ramnik, S. (1999). Medical Laboratory Technology: methods and Interpretations, 5th edition. Jayee Brothers, New Delhi, 279.

Ranjna, C. (1999). Practical Clinical BiochemistryMethods and Interpretation, 2nd edition.

Sodemoto, Y. and Enomoto, M. (1980). Report of carcinogenesis bioassay of Sodium benzoate in rats: absence of carcinogenicity of sodium benzoate in rats. *Journal of Environmental Pathology, Toxicology and Oncology,* **4**: 87-95.

Tietz, N. W. (1976). Fundamentals of Clinical Chemistry, 2nd edition, Saunders, Philadelphia, 876.

Toth, B. (1984). Lack of tumorigenicity of sodium benzoate in mice. *Fundamental Applied Toxicology*, **4**: 494-496.

Villanueva, M. B. G., Jonai, H., Kanno, S. and Takeuchi, Y. (1994). Dietary sources and background levels of hippuric acid in urine: Comparison of Philippine and Japanese levels. *Industrial Health*, **32**: 239-249.

Vogt, T., Landthaler, M. and Stolz, W. (1999). Sodium benzoate-induced acute leukocytoclastic vasculitis with unusual clinical appearance. *Archives of Dermatology*, **135**: 726-727.

254 Wang, W. and Srivastava, S. (2002). "Serological markers". In Breslow, Lester.
255 Encyclopedia of Public Health. New York. Macmillan, USA, 1088-1090.

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