

**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 ( $\text{Na}^+$ ) 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 ( $\text{Cl}^-$ ) showed significant ( $p \leq 0.05$ ) increase at all administered groups for 250mg/kg and 500mg/kg. Potassium ( $\text{K}^+$ ) was only significantly increased at group 5 for 500mg/kg body wt. while for bicarbonate ( $\text{HCO}_3^-$ ) 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

34 the normal levels have been observed in disease conditions (Cheesebrough, 1991). The effects of  
35 various compounds on biochemical parameters of experimental animals have been applied in  
36 assessing the safe use of compounds in products consumed. Sodium benzoate ( $C_6H_5COONa$ ) is  
37 widely applicable as a preservative in several products consumed by man (Chipley, 1983;  
38 Baldwin *et al.*, 1995; Ishida, 1996, Villanueva *et al.*, 1994). Several studies on the short and long  
39 term effects of sodium benzoate have reported adverse effects due to both chronic and  
40 subchronic intake of sodium benzoate (Fujitani, 1993; Vogt, 1999). Some reports suggest the  
41 absence of negative consequence of sodium benzoate intake (Sodemoto and Enomoto, 1980;  
42 Toth, 1984). The upper limits of benzoate allowable in foods vary with 0.1% reported for United  
43 States of America, while a range of 0.15 to 0.25% had been reported for other countries of the  
44 world (Chipley, 1983). For European countries, the limit reported range is from 0.015 to 0.5%  
45 (European Commission, 1995). There are thus variations in the acceptable limits of these  
46 preservatives in foods. It therefore follows that sodium benzoate could be assimilated widely by  
47 consuming a wide range of food products intentionally preserved with it. The present report  
48 addressed the effects of oral administration of sodium benzoate on serum electrolyte and liver  
49 marker enzymes. The findings of this study would further assist in the interpretation of blood  
50 chemistry data for individuals who consumed foods containing the preservatives.

## 51 **MATERIALS AND METHOD**

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

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56

## 57 **Animals**

58 A total of sixty-six (66) wistar albino rats, with an average weight of 140g were obtained from  
59 the animal house of the Department of Pharmacology, University of Port Harcourt. They were  
60 maintained on normal diet ad libitum, grouped into five (5), and housed in stainless steel cages in  
61 a well ventilated room under 12h light/dark cycle. The sodium benzoate concentrations were  
62 150mg/kg body wt., 250mg/kg body wt and 500mg/kg body weight. The rats were divided into  
63 five groups namely G1 (control group), G2 (7days), G3 (14days), G4 (21days) and G5 (28days).  
64 The varying concentrations of sodium benzoate were administered orally in 1ml portions at 24 h  
65 intervals for the duration of the experiment (7, 14, 21 and 28). At the end of the experimental  
66 duration the rats were sacrificed.

## 67 **Sample collection**

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

## 72 **Determination of plasma electrolytes**

73 Plasma potassium concentrations followed the procedure outlined by Tietz (1976) using sodium  
74 tetra-phenyl boron-formulated reagent. Sodium measurement followed the precipitation method  
75 described by Henry (1974). Chloride was measured by the titration method described by Ramnik

76 (1999). For bicarbonate measurements, the method of Ochei and Kolhatkar, (2003) involving  
77 titration was used.

### 78 **Determination of serum liver marker enzymes**

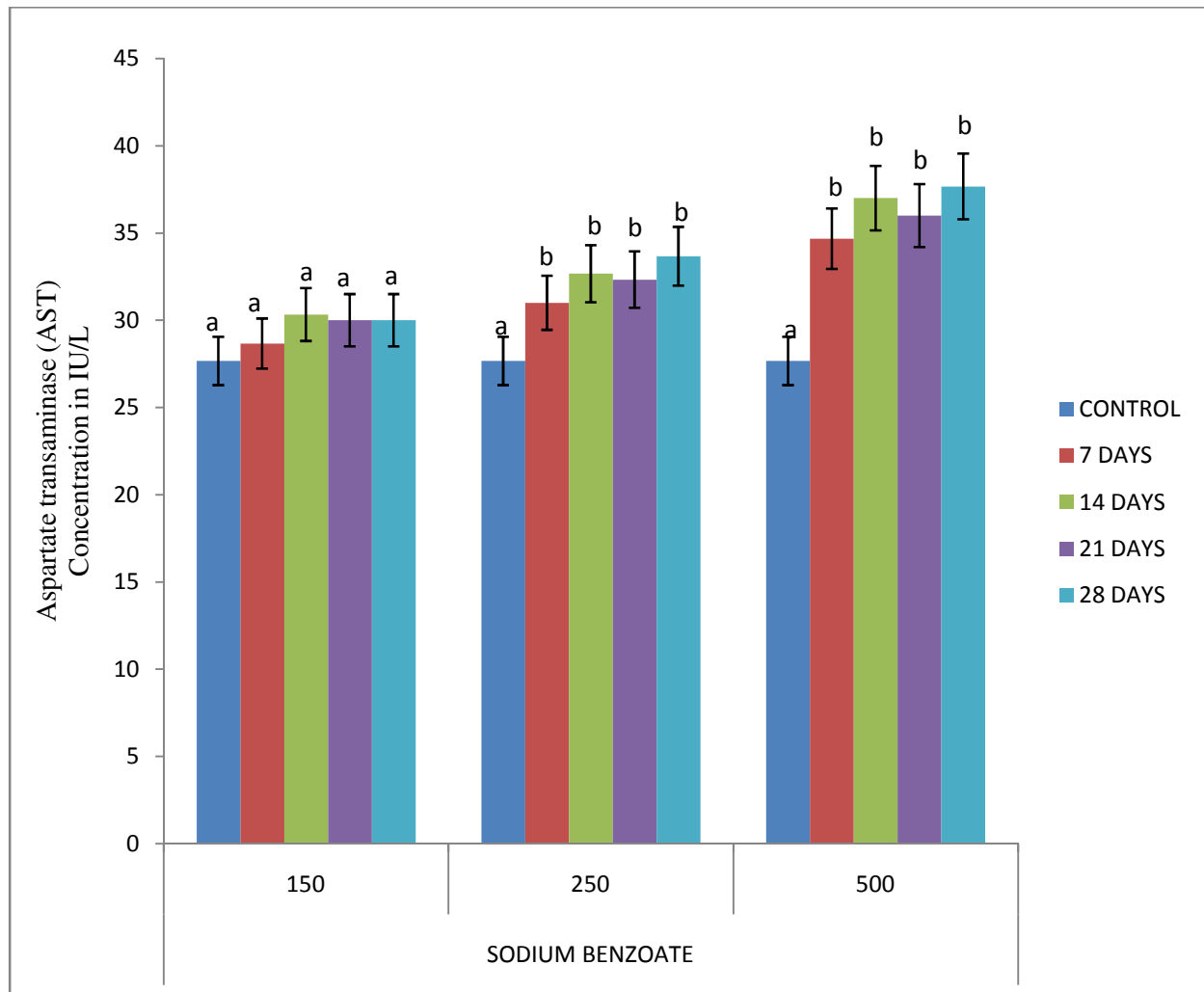
79 Serum liver marker enzymes aspartate transaminase (AST) and alanine transaminase (ALT) were  
80 determined using quantitative method. The activities of ALT and AST were analysed by the end  
81 point colometric method of Reitman and Frankel (1957). Alkaline phosphatase (ALP) was  
82 measured by end-point colorimetric method of Englehardt (1970).

### 83 **Statistical analysis**

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

### 89 **RESULT AND DISCUSSION**

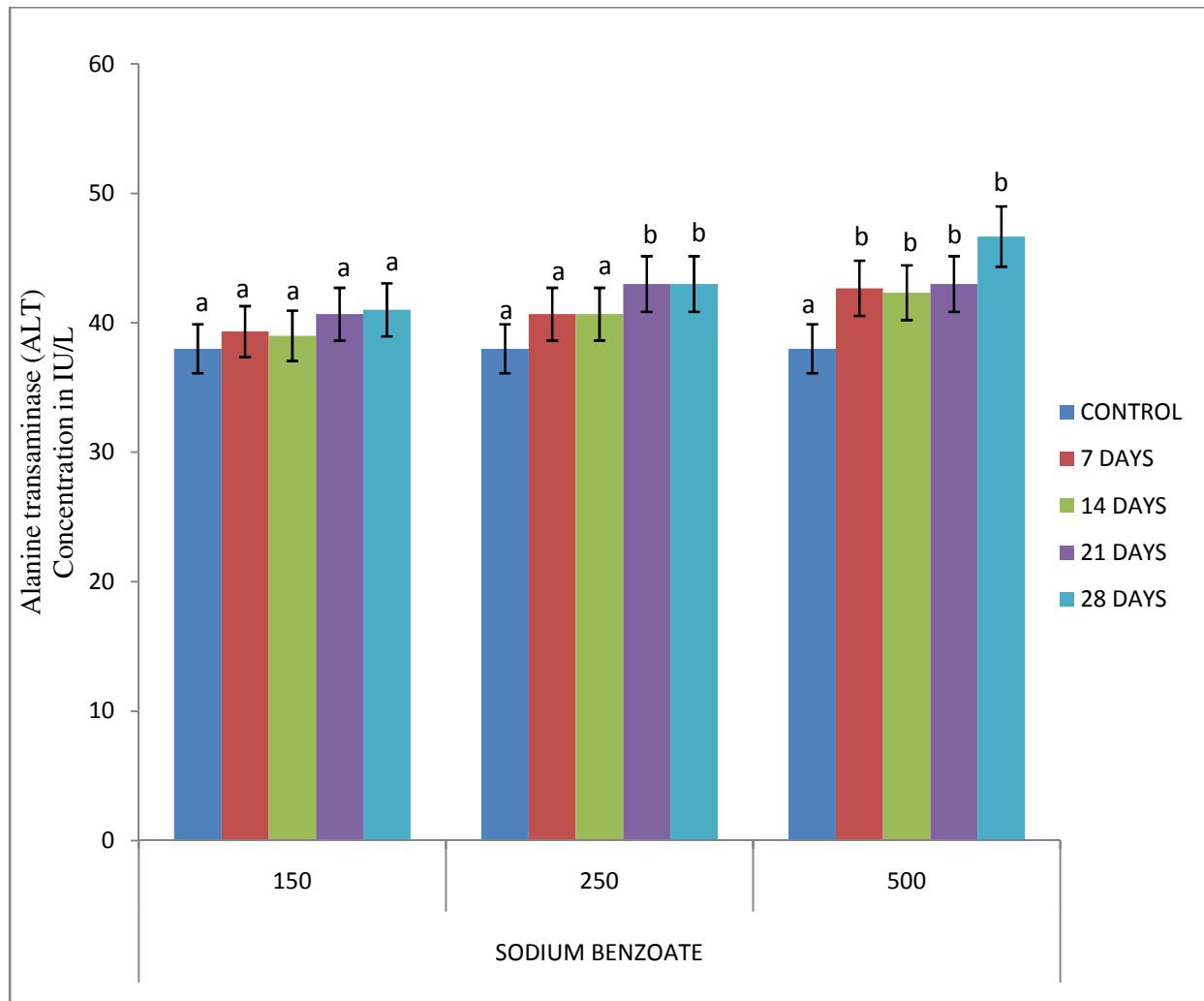
90 The result of the effects of different concentrations of orally administered sodium benzoate on  
91 serum liver marker enzyme activity and serum electrolyte concentrations are shown in figure 1,  
92 2, 3, 4, 5, 6 and 7.



93

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

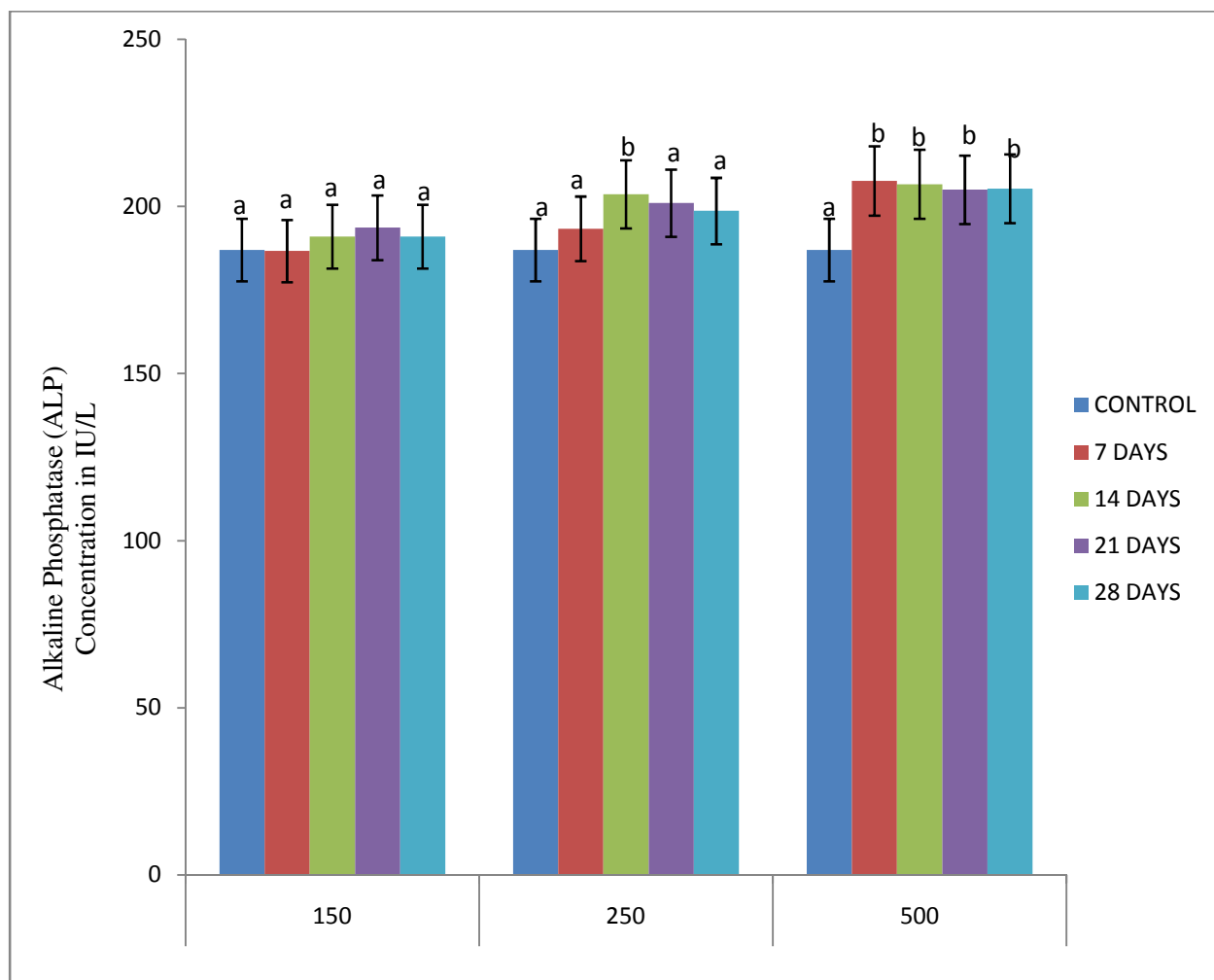
96 Values are means  $\pm$  Standard Error Mean (SEM). Values with different superscript are statistically  
 97 significant at ( $p \leq 0.05$ ). Superscript (a,b) compares 7 Days, 14 Days, 21 Days and 28 Days to control.



98

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

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



103  
 104 Fig 3: Effects of varying concentrations of sodium benzoate on alkaline phosphatase (ALP) activity in  
 105 serum.

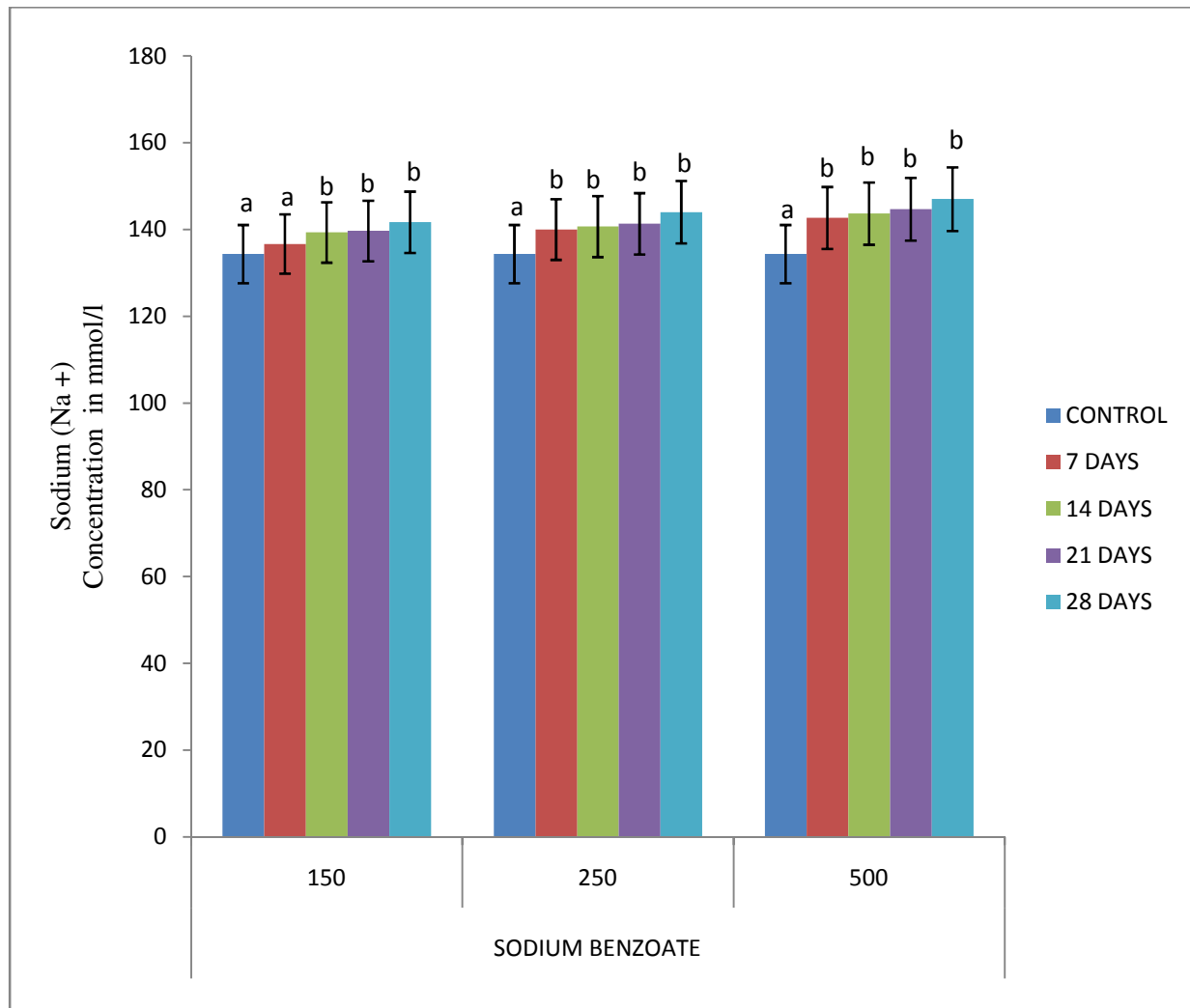
106 Values are means  $\pm$  Standard Error Mean (SEM). Values with different superscript are statistically  
 107 significant at ( $p \leq 0.05$ ). Superscript (a,b) compares 7 Days, 14 Days, 21 Days and 28 Days to control.

108  
 109 For the aminotranferases, there was significant increase in aspartate transaminase (AST) activity  
 110 in group 2, 3, 4 and 5 of 250mg/kg body wt and 500mg/kg body wt of sodium benzoate  
 111 administered groups.. Alanine transaminase (ALT) showed significant ( $p \leq 0.05$ ) increase in  
 112 activity at grp 4 and 5 for 250mg/kg and all the administered groups for 500mg/kg. Alkaline  
 113 phosphatase (ALP) showed significant increase in all administered groups at 500mg/kg. The  
 114 elevation of aminotransferase activity in serum may be due to tissue damage particularly in liver

115 and heart, and increased permeability of cell membrane. (Amin *et al.*, 2010). The study revealed  
116 that rats that consumed sodium benzoate exhibited a significant ( $p \leq 0.05$ ) increase in serum ALT,  
117 AST, and ALP activities when compared to control rats. Sodium benzoate caused derangement  
118 of liver function as revealed by significant elevation of serum ALT and AST as well as  
119 significant reduction of these enzymes in the liver. Determination of AST, ALT and ALP in the  
120 serum is largely used in the assessment of liver damage (Moss and Rosalki, 1996). Membrane  
121 damage to the liver releases the enzymes into circulation and hence can be measured in the  
122 serum. Previous studies showed that sodium benzoate showed a significant ( $p \leq 0.05$ ) increase in  
123 serum AST, ALT, and ALP activities and these results were attributed to hepatocellular damage  
124 which was caused by the toxic effect of sodium benzoate. It was indicated by vacuolation,  
125 swelling and necrosis of the liver cells (Wang and Srivastava, 2002). Increase in both serum AST  
126 and ALT of rats was attributed to the changes in liver function and hepatocellular impairment  
127 which subsequently caused the release of greater than normal levels of intracellular enzymes into  
128 the blood (Abdel-Rahim *et al.*, 1989). Alkaline phosphatase occurs in the canalicular and  
129 sinusoidal membranes of the liver, thus damage to the liver will result in elevated serum ALP  
130 activity (McComb *et al.*, 1979). Cholestatic liver disease is characterized by an increased level of  
131 ALP. The trend of ALP significantly increase gave an indicator that the hepatic capacity of the  
132 liver is affected by sodium benzoate (McComb *et al.*, 1979). Also, the significant elevation of  
133 serum aminotransferases may be attributed to the fact that under pathological conditions, the  
134 parenchymal cells of hepatic lobules fail to carry out vital functions, which usually results in  
135 disturbed or imbalanced intermediary metabolism. As a result of cellular damage, several  
136 enzymes like ALT, AST and ALP leach out into the serum and hence their level indicate the type  
137 and extent of damage inflicted (Amin *et al.*, 2010). Sodium benzoate caused derangement of

138 liver function as revealed by significant elevation of serum ALT, AST and ALP. In blood  
139 plasma, sodium benzoate has a binding affinity for plasma proteins where it is carried out to  
140 different tissues. In the liver, it is metabolized by conjugation with glycine, resulting in the  
141 formation of hippuric acid (Kubota & Ishizaki 1991). Alkaline phosphatase is present on cell  
142 surfaces in most human tissues, especially those of the intestine, liver, bones, spleen and kidneys.  
143 The specific location of the enzyme within sinusoidal and bile canalicular membranes could  
144 account for its serum elevation in the current study in response to sodium benzoate  
145 administration. The ALT enzyme is a strong positive indicator of insulin resistance, diabetes  
146 mellitus and obesity which are risk factors for coronary heart disease and is also a sensitive  
147 marker of liver damage (Al-Mamary *et al.*, 2002). Liver enzymes levels are usually raised in  
148 acute hepatotoxicity, but tend to decrease with prolonged intoxication due to damage to the liver  
149 (Obi *et al.*, 2004). According to Ranjna *et al.*, 1999, both AST and ALT enzymes are excellent  
150 markers of liver damage caused by exposure of liver to toxic substances. However, ALT is more  
151 specific liver enzyme for diagnostic use when the integrity of the hepatocellular membrane is  
152 compromised (Woss and Hendersson, 1996). The increased transaminase levels of test rats  
153 against the control as observed in the present study could be linked to consumption of sodium  
154 benzoate. It was mentioned that the release of abnormally high levels of specific tissue enzymes  
155 into blood stream is dependent on both the degree and the type of damage exerted by the toxic  
156 compound administration.

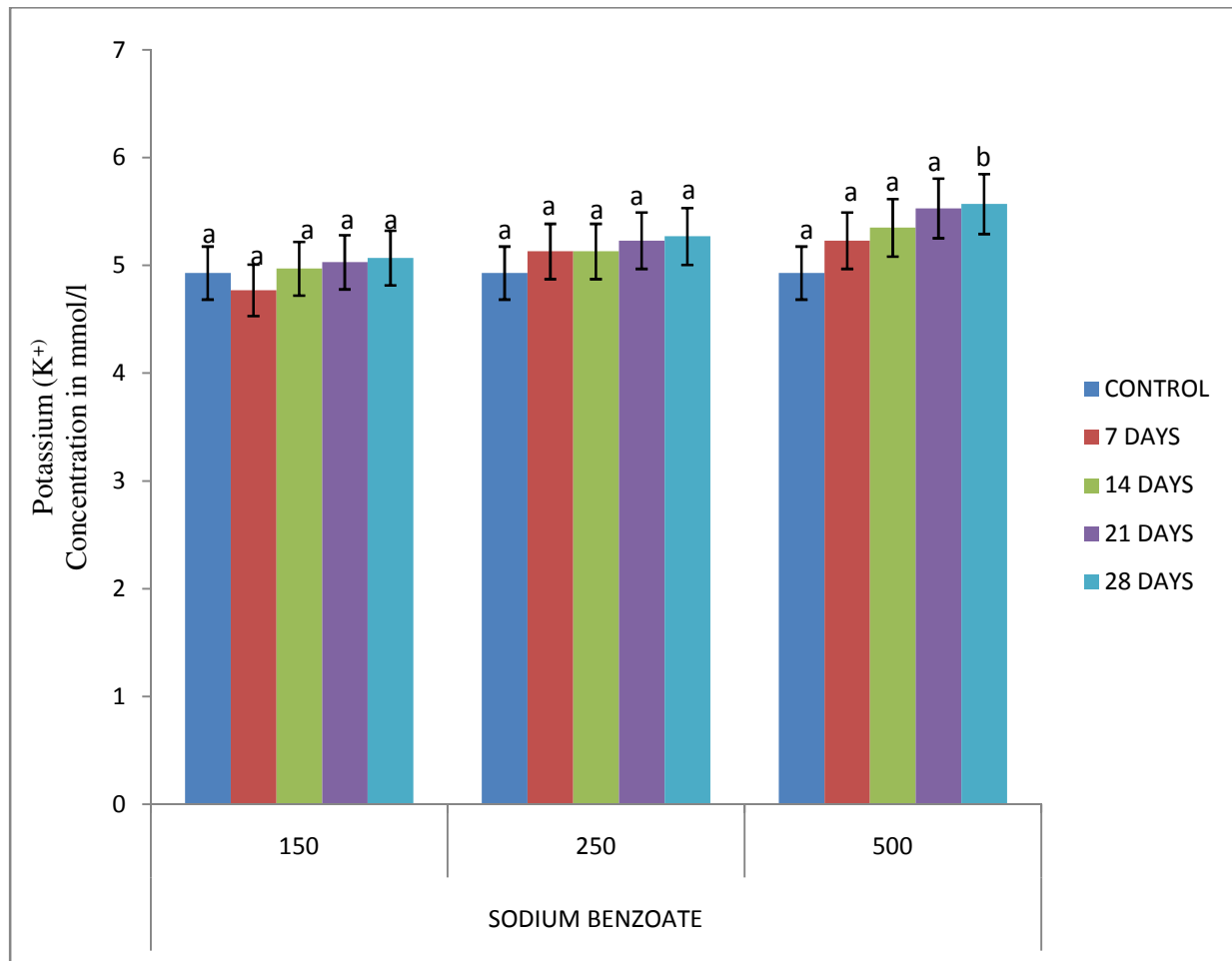
157 The electrolytes sodium, potassium, chloride and bicarbonate had varying activity as shown in  
158 fig 4, 5, 6, 7.



159

160 Fig 4: Effects of varying concentrations of sodium benzoate on sodium (Na<sup>+</sup>) levels in serum.

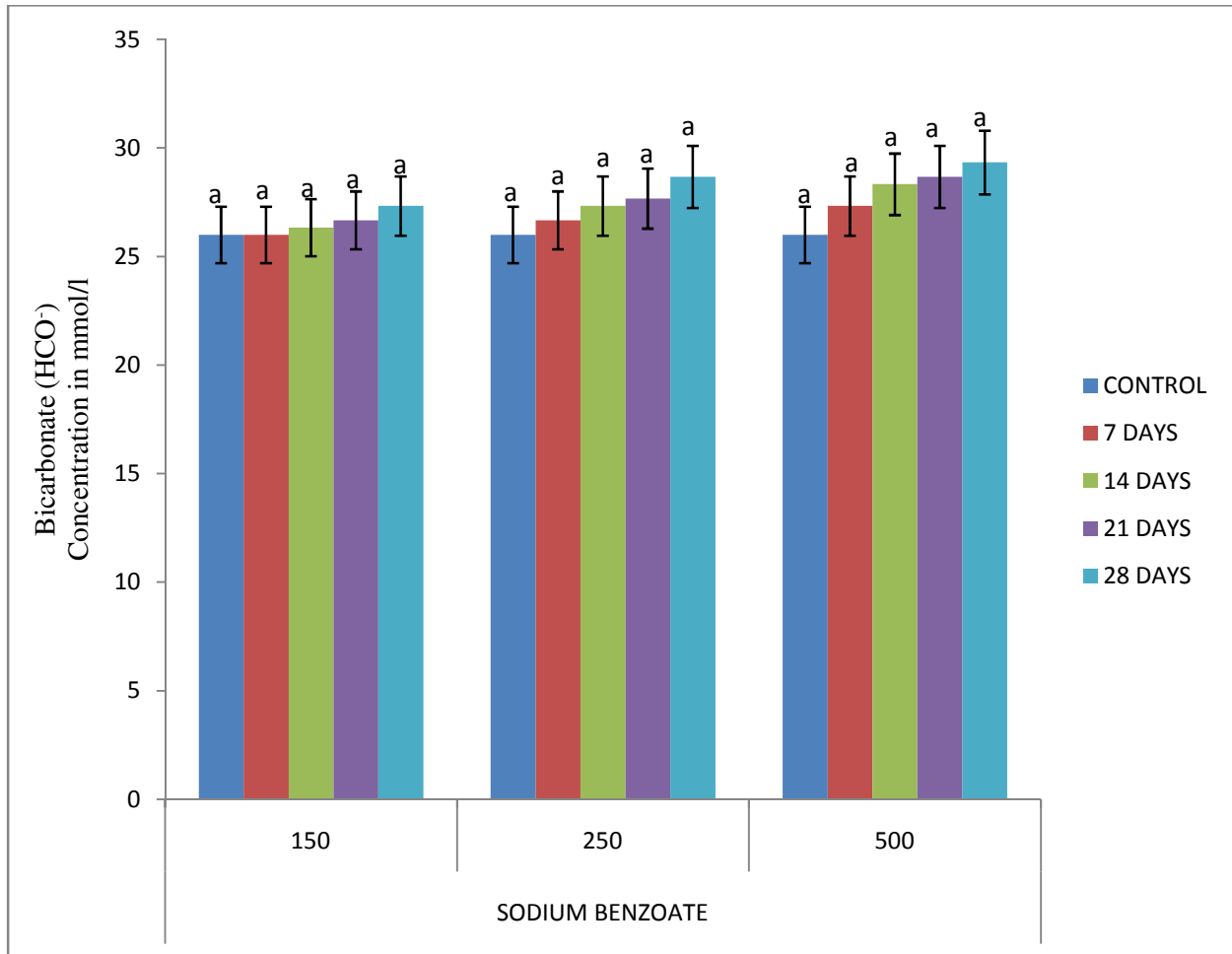
161 Values are means ± Standard Error Mean (SEM). Values with different superscript are statistically  
 162 significant at (p<0.05). Superscript (a,b) compares 7 Days, 14 Days, 21 Days and 28 Days to control.



163

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

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

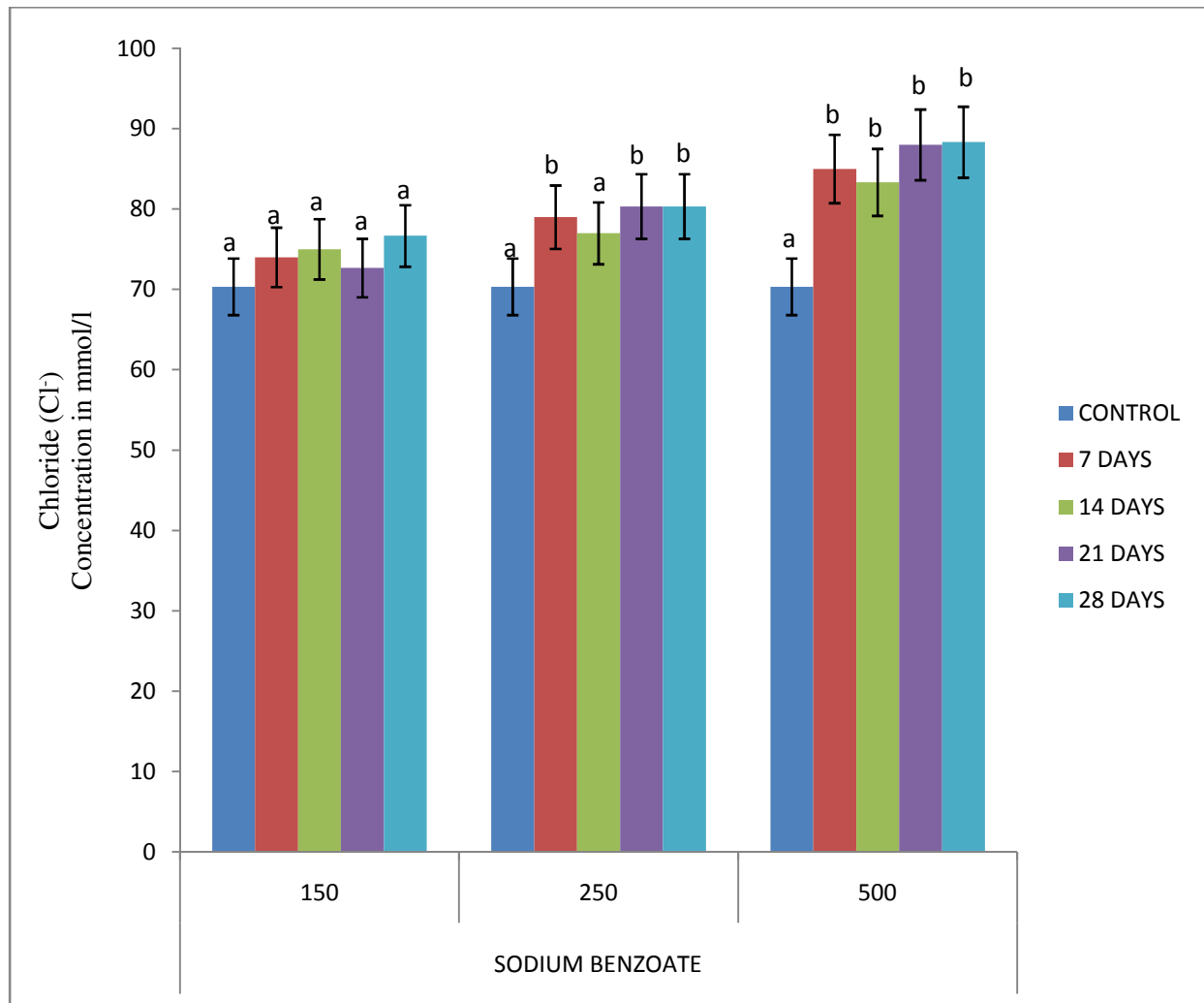


167

168 Fig 6: Effects of varying concentrations of sodium benzoate on bicarbonate (HCO<sup>3-</sup>) levels in serum.

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

171



172

173 Fig 7: Effects of varying concentrations of sodium benzoate on chloride (Cl<sup>-</sup>) levels in serum.

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

176

177 Sodium benzoate significantly (p≤0.05) increased the levels of sodium at grp 3, 4 and 5 for

178 150mg/kgb.w and all administered groups for 250mg/kgb.w and 500mg/kgb.w. It significantly

179 (p≤0.05) increased potassium only at grp 5 for 500mg/kgb.w. Bicarbonate had no significant

180 difference in all the treated groups and chloride was significantly (p≤0.05) increased in all

181 treated groups for 250mg/kgb.w and 500mg/kgb.w and no significant difference in

182 150mg/kgb.w. The levels of sodium, potassium bicarbonate and chloride in test groups where all

183 compared to the control group. The observe increase in the group could be an effect on their  
184 pumps which can be linked to sodium benzoate administration. This may interfere with these  
185 electrolytes in several metabolic pathways leading to increase in their levels in the serum (El-  
186 Sheikh and Khalil, 2011). The significant changes obtained in some of the measured parameters  
187 following oral administration of sodium benzoate points to the need for caution in the  
188 interpretation of blood chemistry data of blood samples.

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UNDER PEER REVIEW