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Full Length Research Paper

Effect of oral intake of sodium benzoate on some haematological parameters of wistar albino rats

IBEKWE, Sixtus Eberechukwu, UWAKWE, Augustine Amadikwa* and MONANU, Michael Okechukwu

Department of Biochemistry, University of Port Harcourt, PMB 5323, Port Harcourt, Nigeria.

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The *in vivo* effects of oral administration of varying concentrations (30, 60 and 120 mg/kg body wt) of sodium benzoate (a commonly used food preservative) on haemoglobin (Hb) concentration, white blood cell (WBC) count, total plasma protein and some plasma electrolytes (Na^+, K^+, Cl^-, and HCO_3^-) levels of wistar albino rats were investigated. The oral intake was done at 48-hourly intervals for 14 days. The rats were fed normal diet *ad libitum* and blood samples for the determinations were obtained at zero, 2-, 6-, and 14-days following intake. The results obtained for Hb (g/l) showed significant ($p<0.05$) decreases in a dose-dependent manner for the three concentrations of sodium benzoate. For WBC, significant ($p<0.05$) decreases were noted for 60 and 120 mg/kg body weight but no significant change occurred with 30 mg/kg body weight of sodium benzoate. The preservative did not significantly affect the total plasma protein concentrations. Significant increases ($p<0.05$) were obtained at the two concentrations of sodium benzoate from the 6^{th} day for Na^+, K^+ while HCO_3^- showed no significant change. No significant change was noted for Cl^- at the various concentrations of the preservative. These findings suggest possible changes in blood chemistry due to the preservative.

Key words: sodium benzoate, haemoglobin, white blood cell, plasma proteins, electrolytes.

INTRODUCTION

The determination of levels of several constituents of blood and plasma of mammals have continued to play valuable role in the assessment of normal functioning of these living organisms as changes from the normal levels have been observed with disease conditions (Cheesebrough, 1991). The assessment of how medicinal plant products affect heamatological parameters of experimental animals such as rats had been performed by several workers (Akpanabiatu et al., 2005; Aboderin and Oyetayo, 2006). In other studies, effects of compounds on the heamatological constituents of experimental animals have been applied in assessing the safe use or otherwise of such compounds in products consumed.

Benzoic acid and sodium benzoate (C_6H_5COONa) are widely applicable as preservatives in a number of products consumed by humans (Chipley, 1983; Baldwin et al., 1995; Ishida, 1996, Villanueva et al., 1994). A number of studies on short- and long-term effects of both compounds had investigated organ disposition. Some reports suggest adverse effects due to both chronic and subchronic intake of sodium benzoate (Fujitani, 1993; Vogt, 1999). Other reports suggest absence of negative effects 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 haemoglobin concentration, total plasma protein and several electrolytes of clinical significance. The findings would further assist in the interpretation of blood chemistry data for individuals who had consumed foods containing the preservatives before

*Corresponding Author's E-Mail: uwadik@yahoo.com.

Table 1. Effects of sodium benzoate on haemoglobin (g/l), white blood cell count (mm^3) and total protein (g/l) of wistar albino rats.

Sodium benzoate (mg/kg body wt)	Na^+ (mmol/l)			
	0-day	2^{nd}-day	6^{th}-day	14^{th}-day
0.0	124.75±0.35	123.99±0.71	124.90±0.84	124.95±0.91
30.0	124.81±0.61	128.00±0.70	134.90±0.84	154.95±0.91*
60.0	124.61±0.55	129.75±0.35	144.75±0.63*	159.90±0.56*
120.0	124.83±0.42	129.95±0.77	149.75±0.35*	175.00±0.70*
K^+ (mmol/l)				
0.0	4.01±0.06	4.05±0.05	4.02±0.08	4.05±0.04
30.0	4.07±0.03	4.15±0.07	4.77±0.11	4.94±0.03*
60.0	4.05±0.02	4.47±0.02	4.87±0.10*	4.99±0.02*
120.0	4.04±0.04	4.50±0.03	4.99±0.08*	96.75±1.22
HCO_3^- (mmol/l)				
0.0	30.00±0.27	98.36±1.45	30.01±0.30	29.99±0.03
30.0	30.18±0.29	99.40±1.28	29.25±0.35	97.51±1.42
60.0	29.99±0.20	99.55±1.53	28.75±0.33	30.00±0.70
120.0	30.00±0.30	99.50±1.14	25.85±0.50	29.75±0.73
Cl^- (mmol/l)				
0.0	98.75±1.55	98.36±1.45	98.80±1.27	99.00±1.30
30.0	98.92±1.02	99.40±1.28	96.75±1.32	97.51±1.42
60.0	99.00±1.31	99.55±1.53	98.75±1.20	97.15±1.49
120.0	98.80±0.99	99.50±1.14	98.05±1.29	4.99±0.07*

Values are mean ± SD for duplicate determinations while those with asterisks are significantly ($p<0.05$) different from control values.

blood-draw.

MATERIALS AND METHODS

Sodium benzoate was from May & Baker Ltd., England; Drabkin's solution was from Ranbaxy Diagnostics, New Delhi, India; while all other reagents were of analytical grades.

Animals

A total of thirty-six (36) male wistar albino rats, average weight of 83.3 g, 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 four (4), and housed in stainless steel cages in a well-ventilated room under 12 h light/dark cycle. The groups were: G1 (control), G2 [30 mg/kg sodium benzoate (SB)], G3 (60 mg/kg SB) and G4 (120 mg/kg SB). The varying concentrations of sodium benzoate were administered orally in 0.5 ml portion at 48 h intervals for the duration of the experiment (14 days). One rat per group was sacrificed at 0, 2, 6 and 14 days in the course of the experiment.

Sample collection

The rats were anaesthetized with chloroform and dissected for blood collection. The blood was collected into lithium heparin bottles and analysis performed within one (1) h of collection. Before assays, the blood samples were centrifuged for 5 min using a bench-top centrifuge {MSE-Minor} and the supernatant plasma was then used for the determinations.

Haemoglobin determination

Plasma haemoglobin concentrations were determined using the cyanomethaemoglobin method of estimation as described by Ramnik (1999).

Total white blood cell count

This was based on differential lysis of red cells with 2% glacial acetic acid followed by staining with gentian violet as reported by Ramnik (1999).

Total plasma protein

Total plasma protein determination utilized Randox kit formulated for Biuret procedure as described by Ramnik (1999).

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.

Statistical analysis

All data were expressed as mean ± SEM and statistically analyzed with the student's t-test at 95% confidence limit.

Table 2. Effects of sodium benzoate on plasma concentrations of Na^+, K^+, HCO_3^-, and Cl^- (mmol/l) of wistar albino rats.

Sodium benzoate (mg/kg body wt)	Na^+ (mmol/l)			
	0-day	2nd-day	6th-day	14th-day
0.0	124.75±0.35	123.99±0.71	124.90±0.84	124.95±0.91
30.0	124.81±0.61	128.00±0.70	134.90±0.84	154.95±0.91*
60.0	124.61±0.55	129.75±0.35	144.75±0.63*	159.90±0.56*
120.0	124.83±0.42	129.95±0.77	149.75±0.35*	175.00±0.70*
K^+ (mmol/l)				
0.0	4.01±0.06	4.05±0.05	4.02±0.08	4.05±0.04
30.0	4.07±0.03	4.15±0.07	4.77±0.11	4.94±0.03*
60.0	4.05±0.02	4.47±0.02	4.87±0.10*	4.99±0.02*
120.0	4.04±0.04	4.50±0.03	4.99±0.08*	96.75±1.22
HCO_3^- (mmol/l)				
0.0	30.00±0.27	98.36±1.45	30.01±0.30	29.99±0.03
30.0	30.18±0.29	99.40±1.28	29.25±0.35	97.51±1.42
60.0	29.99±0.20	99.55±1.53	28.75±0.33	30.00±0.70
120.0	30.00±0.30	99.50±1.14	25.85±0.50	29.75±0.73
Cl^- (mmol/l)				
0.0	98.75±1.55	98.36±1.45	98.80±1.27	99.00±1.30
30.0	98.92±1.02	99.40±1.28	96.75±1.32	97.51±1.42
60.0	99.00±1.31	99.55±1.53	98.75±1.20	97.15±1.49
120.0	98.80±0.99	99.50±1.14	98.05±1.29	4.99±0.07*

Values are mean ± SD for duplicate determinations while those with asterisks are significantly ($p<0.05$) different from control values.

RESULTS AND DISCUSSION

The results of the effects of different concentrations of orally-administered sodium benzoate on haemoglobin (Hb) and total plasma protein concentrations, and white blood cell counts are shown in Table 1. Haemoglobin concentrations increased significantly ($p<0.05$) from the 6th day at the three concentrations of sodium benzoate tested in this study. For total plasma protein, there was significant ($p<0.05$) increase in white blood cell counts from the 6th day at 60 and 120 mg/kg body weight of sodium benzoate. The total plasma protein levels did not show any significant ($p<0.05$) change.

The results of the plasma levels of Na^+, K^+, Cl^- and HCO_3^-, are shown in Table 2. No significant changes ($p<0.05$) were obtained for Cl^- and HCO_3^-. However, from the 6th day, significant increases were noted for K^+ at 60 and 120 mg/kg body weight of sodium benzoate. For Na^+, significant increases occurred from the 6th day of administering 60 and 120 mg/kg body weight of sodium benzoate.

The significant increases in the levels of some of the blood parameters contrasted with the report by Bedford and Clarke (1972) which indicated no adverse effects of short-term administration of sodium benzoate in blood constituents of cats. Fujitani (1993) reported changes in the serum levels of albumin and total protein in rats. In the present study, total plasma protein did not change significantly at the three concentrations of sodium benzoate tested.

The findings of significant decreases ($p<0.05$) in Hb concentrations from the 6th day at 30, 60 and 120 mg/kg body weight suggests the induction of anaemic condition in the rats (Schalm et al., 1975; Cheeseborough, 1991). The observed significant decrease in WBC levels from the 6th day at 60 and 120 mg/kg body weight of sodium benzoate portrays possible susceptibility to infection as the WBC perform important role in defending against infection (Schalm et al., 1975).

The significant increases ($p<0.05$) in K^+ seemed similar to the study on the effects of *Nauclea latifolia* leaf extract on rat serum electrolytes (Akpanabiatu et al., 2005). However, sodium concentrations significantly ($p<0.05$) increased at 60 and 120 mg/kg body weight from the 6th day of sodium benzoate administration, in contrast with the findings of Akpanabiatu et al. (2005) where no effect was observed for the leaf extract they tested. The K^+ increase was suggested to contribute to the vasodilator effect of the *N. latifolia* leaf extract, and similar suggested effect could be ascribed to sodium benzoate in the present study.

The significant changes obtained in some of the measured parameters following oral administration of sodium benzoate points to need for caution in the interpretation of blood chemistry data of blood samples. Thus, note sh-

ould be taken of possible intakes before blood sample collection.

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