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Potassium bromate content of selected bread samples in Ilorin, Central Nigeria and its effect on some enzymes of rat liver and kidney

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ABSTRACT

Bread samples from five different locations (Gaa-Akanbi, Saw-Mill, Oloje, Fate-Basin and Zango) in Ilorin metropolis, Central Nigeria were analyzed for their potassium bromate content before they were employed as a source of carbohydrate in the formulation of diet for albino rats. A total of sixty (60) albino rats (*Rattus norvegicus*) were grouped into six (6) of ten (10) rats each. The rats in the first group served as control and they were placed on diet formulated with bromate-free bread. Animals in Groups 2–6 were placed on diet formulated with bread samples obtained from the five different locations in Ilorin metropolis. At the expiration of thirty (30) days feeding period, the animals were sacrificed and blood samples, liver and kidney tissues were collected for the assay of ALP, AST and ALT activities. The results showed a significant reduction ($p < 0.05$) in the activities of these enzymes in the tissues when compared with the control. However, a significant increase ($p < 0.05$) was observed in the activities of the selected serum enzymes. Overall, the data indicate adverse effects on the liver and kidney of rats fed on diet containing potassium bromate.

1. Introduction

Bread is an important staple food of many countries of the world especially the African countries and South East part of Asia (Pomeranz, 1987; Owens, 1997). In fact, statistical analysis in Nigeria showed that bread is one of the most consumed food type with predominant consumption among the poor (Maziya-Dixon et al., 2004). It is a carbohydrate source made from flour and yeast with the flour fortified with potassium bromate for aesthetic purposes (Vadlamani and Seib, 1999).

Potassium bromate is a flour improver that acts as a maturing agent (Vadlamani and Seib, 1999). It has been in use as a food additive for the past 80 years. It acts principally in the late dough stage giving strength to the dough during the late proofing and early baking (Vadlamani and Seib, 1999). In laymen's term, potassium bromate prevents dough from falling. This property has been manipulated by many Nigerian bakers in profit making. Over time, it has been discovered that potassium bromate is toxic and is a possible carcinogen in man (Kurokawa et al., 1986). This led to the proposal for its ban in the United States. Potassium bromate has been banned in several countries including the United Kingdom in 1990, Nigeria in 1993 and Canada in 1994. However, since the ban, not too many Nigerian bakers have complied and stopped its use even as the National Agency for Food and Drug Administration and Control (NAFDAC) has not relented in its enlightenment and enforcement efforts.

Toxicological studies have convincingly shown that potassium bromate affects the nutritional quality of bread as the main vitamins available in bread are degraded (Sai et al., 1992). Carcinogenic and mutagenic effects of potassium bromate have been reported in experimental animals (Kurokawa et al., 1987). Lethal oral doses of bromate in humans have been estimated to be between 154 and 385 mg/kg body weight while serious poisoning results at doses of 46–92 mg/kg body weight (Mark, 1988). Oral doses of 185–385 mg/kg body weight results in irreversible toxic effects like renal failure and deafness in humans while lower doses are associated with vomiting, diarrhea, nausea and abdominal pain (Mark, 1988).

Potassium bromate is extremely irritating and injurious to tissues especially those of the central nervous system and kidneys. The pathologic findings include kidney damage and haemolysis (Robert and William, 1996). Bromate was first found to cause tumour in rats in 1982. Subsequent studies on rats and mice confirmed that it causes tumour of the kidney, thyroid and other organs (CSPI, 1999). It is known that potassium bromate induces oxidative stress in tissues (Sai et al., 1991; Watanabe et al., 1992; Parsons and Chipman, 1992, 2000). Indeed, oxidative damage appears to be the basis of bromate-induced carcinogenesis (Chipman et al., 2006). Several cases of accidental poisoning in children resulting from ingestion of bromate solution and sugar contaminated with bromate were reported as the source of an outbreak of mild poisoning in New Zealand (Paul, 1966).

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The present study attempts to assess the potassium bromate content in selected bread samples consumed in Ilorin, Central Nigeria with a view to finding out the effect of their consumption on some enzymes of rat liver and kidney.

2. Materials and methods

2.1. Detection of potassium bromate in bread samples and formulation of diet

Bread samples were obtained from five different locations within Ilorin namely Gaa-Akanbi, Saw-Mill, Oloje, Fate-Basin and Zango areas. The bread samples were then analyzed qualitatively to detect the presence of potassium bromate in them according to the method described by Armstrong (1994) after which they were employed as a source of carbohydrate to formulate diet for albino rats. The diet for each group was formulated by mixing known quantities of sources of each food class (Table 1). The food items were mixed together and manually made into pellets to feed albino rats.

2.2. Experimental rats and treatments

Sixty (60) albino rats (*Rattus norvegicus*) with an average weight of 50.20 ± 4.24 g were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria where they received humane care in line with the Departmental Ethical Committee on the Care and Use of Experimental Animals. The animals were grouped into six (6) with each group containing ten (10) rats as follows:

Group 1: Rats fed on diet formulated with bread sample without potassium bromate (control).
Group 2: Rats fed on diet formulated with bread sample obtained from Gaa-Akanbi (GAB).
Group 3: Rats fed on diet formulated with bread sample obtained from Saw-Mill (SMB).
Group 4: Rats fed on diet formulated with bread sample obtained from Oloje (OLB).
Group 5: Rats fed on diet formulated with bread sample obtained from Fate-Basin (FBB).
Group 6: Rats fed on diet formulated with bread sample obtained from Zango (ZGB).

The feeding lasted for a period of 30 days and was preceded by 5 days acclimatization period.

2.3. Collection of blood sample and isolation of liver and kidney

At the expiration of 30 days, the animals were sacrificed by ether anaesthetization and blood samples were collected by cutting the jugular vein with a sharp sterile blade. The blood sample collected (5 ml) was spinned using a centrifuge at 4000 rpm for 35 min and the serum was collected using a Pasteur's pipette for enzyme assay. The rats were thereafter dissected and the liver and kidney were excised into a beaker containing ice-cold 0.25 M sucrose solution. Known weights of the liver and kidney were cut, chopped into small pieces and then homogenized using pre-cooled pestle and mortar in a bowl of ice chips. The homogenized tissue was diluted with 0.25 M sucrose solution to obtain a 1 in 5 dilution for enzyme assay.

Table 1
Composition of the formulated diet.

Feed components	Quantity (g)
Bread[a]	516
Soy bean	250
Soy bean oil	40
Cellulose	100
Methionine	50
Mineral/vitamin mixture[b]	44
Total	1000

[a] Bread samples are from different locations within Ilorin metropolis while the control diet contains bromate-free bread.
[b] Vitamin A, 15,000,000 IU; vitamin D, 32,000 IU; vitamin E, 12,000 IU; vitamin K, 2 IU; thiamine, 1.5 g; riboflavin, 25 g; pyridoxine, 5 g; folic acid, 0.5 g. For the mineral mixture, manganese 75 g, zinc 45 g, iron 20 g, copper 5 g, iodine 1 g and selenium 100 mg.

2.4. Enzyme assay

Total protein in the liver was determined according to the method of Henry et al. (1974). Alkaline phosphatase (ALP) activity was determined using the method of Wright et al. (1972). Activities of aspartate transaminase (AST) and alanine transaminase (ALT) were determined based on the method described by Schmidt and Schmidt (1963).

2.5. Statistical analysis

All data were analyzed statistically using Analysis of Variance (ANOVA) by employing the method of Steel and Torrie (1960). Significant difference between the treatment means was determined at 5% confidence level using Duncan's Multiple Range Test (Duncan, 1955).

3. Results

The specific activity of alkaline phosphatase (ALP) in the liver, kidney and serum of rats fed on diet formulated with bread samples containing potassium bromate is as presented in Table 2. The data revealed a significant reduction ($p < 0.05$) in the activity of ALP in the liver and kidney when compared with the control. It was also observed that the activity of ALP in the liver and kidney of rats in Groups 2–6 are not significantly different ($p > 0.05$).

The specific activities of aspartate transaminase (AST) and alanine transaminase (ALT) in the liver and serum of rats fed on diet formulated with bread samples containing potassium bromate is as presented in Tables 3 and 4. Compared with the control, a significant reduction ($p < 0.05$) in the activities of the two enzymes was observed in the liver with a corresponding significant increase ($p < 0.05$) in activities of the enzymes in the serum when compared with the control. In most cases, the data obtained for GAB group are not significantly different ($p > 0.05$) from the control.

4. Discussion

The significant increase ($p < 0.05$) in ALP activity observed in the serum of rats fed bromate-containing diet compared with the control may be attributable to loss of membrane components due to a possible reaction between potassium bromate in the bread samples and the membranes of liver and kidney cells, causing leakage of the enzyme into the serum. This observation was supported by Fleischer and Schwartz (1971) who reported that any damage done to the cell membrane may lead to leakage of ALP, which is a marker enzyme in the plasma membrane, into extracellular fluid.

Increased activities of serum enzymes have been reported in conditions of tissue damage (Hanley et al., 1986). Normally, enzymes will not always be found in the serum except there is damage to one or more organs of the body. Therefore, enzymes from diseased organs may become manifested in the serum resulting in increased activity since they must have leaked from the diseased organ. The increased activity of the serum enzyme is often

Table 2
Specific activity of alkaline phosphatase in selected tissues of rat fed on diet formulated with potassium bromate-containing bread samples produced in Ilorin metropolis.

Specific activity (IU/mg protein)			
Groups	Liver	Kidney	Serum
Control	4.05 ± 0.55[a]	100.05 ± 2.63[a]	1.98 ± 0.05[a]
GAB	4.06 ± 0.35[a]	89.58 ± 1.82[b]	2.01 ± 0.15[a]
SMB	3.75 ± 0.25[b]	82.75 ± 1.95[c]	6.85 ± 0.85[b]
OLB	3.73 ± 0.26[b]	81.77 ± 1.86[c]	6.96 ± 0.86[b]
FBB	3.74 ± 0.23[b]	81.75 ± 1.88[c]	6.89 ± 0.89[b]
ZGB	3.73 ± 0.25[b]	80.95 ± 2.57[c]	6.88 ± 0.87[b]

a, b and c values along the same column with different superscripts are significantly different ($p < 0.05$). Each value is a mean of 10 determinations ± SEM.