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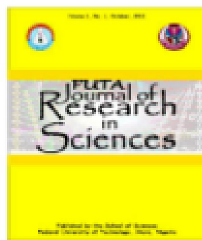

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PROTECTIVE ACTIVITY OF ACETYLSALICYLIC ACID ON SELECTED TISSUES OF ALBINO RATS TREATED WITH POTASSIUM BROMATE.

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ABSTRACT

The protective role of acetylsalicylic acid on potassium bromate-induced toxicity in rat tissues was investigated. Twenty male albino rats of average weight, 200±4.5g were grouped randomly into four groups of five rats per group. Group 1 received distilled water only and served as the control. Group 2 received 1 ml solution containing 20mg/kg body weight of potassium bromate ($KBrO_3$). Group 3 received 1ml solution containing 20mg/kg body weight of acetylsalicylic acid (ASA). And group 4 received 1 ml solution of 20 mg/kg body weight of $KBrO_3$ and ASA concurrently. Distilled water and the solution of chemical substances were administered to rats orally for 21 days. Physical observations of the rats administered with potassium bromate include rapid breathing, impaired locomotion and diarrhoea. Enzymes assay revealed significant reductions ($P<0.05$) in activities of major marker enzymes-alkaline phosphatase, (ALP); Lactate dehydrogenases, (LDH) and glutamate dehydrogenase, (GDH) in the tissues followed by corresponding significance increases ($P<0.05$) in activities of these enzymes in the serum upon administration of solution of $KBrO_3$. Acid phosphatase activity was not significantly ($P>0.05$) affected. When ASA alone was administered and enzyme activities were compared with both the control and the $KBrO_3$ group, it was discovered that activity values in the tissues and serum tend towards the control as well as being significantly higher ($P<0.05$) than the values obtained when $KBrO_3$was administered. However, concurrent administration of $KBrO_3$ and ASA solutions to the rats, nullified the effects of $KBrO_3$ on these enzymes activities rather the activity values were elevated with no significant difference ($P>0.05$) compared to the control but significantly higher ($P<0.05$) when compared to the administration of $KBrO_3$ alone. The observed trends in enzymes activities following administration of $KBrO_3$ suggested damage to the plasma membrane of cells. The trend however changed when solutions of $KBrO_3$ and ASA were administered concurrently indicating a probable ability of ASA to prevent the disruption of the ordered membrane structure of the cells. The dose of acetylsalicylic acid that was employed in this work was able to prevent enzyme leakage through the membrane of the cells and it is the likely attribute of acetylsalicylic acid as a membrane stabilizer.

Keywords: Potassium bromate, acetylsalicylic acid, liver, kidney

INTRODUCTION

Chemical additives in food are either intentionally or unintentionally added to food in order to enhance its quality (Magnus, 1982; Akanji, 2002; Shubhangini, 2007). Additives have been in use long before food technology was developed as an application of science (Potter and Hotchkiss, 1998). Their inclusion in the food processing protocol became necessary in the quest to preserve excesses, since food production surpasses rate of consumption (Akanji, 2002).The interaction of these compounds with cellular components necessitated investigation into their effects at the molecular level. Even though they add values to food, some of the additives could become toxic or deleterious to health when ingested (Achukwu *et al.,* 2009). The toxic implication of these compounds at cellular level should undoubtedly arouse the interest of a biochemist. Therefore, whatever is the aim of a food technologist, it is imperative to consider whether the food additives interfere with the quality of the commodity, hence, the

health of the consumers (Magnus, 1982; Achukwu *et al.,* 2009).

Enzyme bioassay is a rapid and convenient means to obtain information on the mode of interaction of these chemical compounds with cells/tissues before their structural effects using conventional histological techniques.

Potassium bromate ($KBrO_3$), a white crystalline salt with molecular mass of 167g, has been used successfully as flour improver in bakeries (WHO, 1996; Laba, 2003; Uwumarongie *et al.,* 2007) for a number of years where it enables production of high quality loaves even from low protein wheat (Kurokawa, 1990; Cavanaugh, 2002). Despite these advantages, $KBrO_3$ was reported to have harmful effect on the nutritional qualities of bread (Laba, 2003).

Results from mechanistic studies also reported that $KBrO_3$ have caused renal toxicity through formation of reactive intermediates which could cause lipid peroxidation and DNA damage in the kidney (Kasai *et al.,* 1987; Adekoya *et al.,* 2011). Also in a separate study using rats, Akanji *et al.* (2008) investigated the effect of chronic administration of $KBrO_3$ on some 'marker' enzymes of rat cellular system. The results of the study showed that exposure of rats to $KBrO_3$ led to labialization of the cell plasma membrane and hence loss of both membrane and cytosolic enzymes.

We have therefore, in the present study, employed the use of acetylsalicylic acid (ASA) a known membrane stabilizer (Ngaha and Akanji, 1982) to explore its membrane protective potential as remedy for the bromate-induced cellular toxicity.

Acetylsalicylic acid, (ASA) is a non-steroidal anti-inflammatory agent (Vane, 2003; Vane and Bonting, 2003; Holmes *et al.,* 2010) and certain anti-inflammatory drugs have been reported to be potent membrane stabilizers (Ignarro, 1971; Hudson *et al.,* 2008). ASA has equally been reported to stabilize rat liver lysosomes *in vitro* (Miller and Smith, 1966) just as it stabilizes rat kidney lysosomal membrane (Ngaha and Akanji, 1982). The stabilizing role of ASA was also demonstrated in a study using sodium metabisulphite, a food preservative, to induce cellular damage in rat liver and kidney tissues and then treated with ASA (Olajide *et al.,* 2005, 2009).

The potency of ASA as a membrane stabilizer in the presence of $KBrO_3$was investigated in this study. This is because $KBrO_3$ is a powerful oxidizer whose metabolism produces reactive oxygen species (ROS) that is capable of oxidizing polyunsaturated membrane lipids as well as reducing antioxidant enzymes (Farombi *et al.,* 2002).

MATERIALS AND METHODS

Materials

Twenty male albino rats (*Rattus norvegicus*) of Wistar strain with average weight of 200 ± 4.45g were obtained from the Animal House of Kogi State University, Anyigba, Kogi State, Nigeria. Assay kits for LDH, GDH, ALP and ALT were obtained from LabkitsChemelex, S.A Pol. Canovelles-Barcelona, Spain.

Potassium bromate was obtained from Labtech Chemicals Nigeria Limited, Lagos, Nigeria. Acetysalicyclic acid used is a product of Tega Laboratories, Chelsea, London. All other reagents used in this work were of analytical grade and were prepared in glass distilled water and stored in reagent bottles until required for use.

Animals

The animals were all housed in metabolic cages and were kept under standard experimental conditions (12 hours light/12 hours dark, 26 ± 4^oC and 45-50% humidity). They were allowed to acclimatize to the environment for two weeks with free access to normal rat diet cubes produced by Vital Feed, Nigeria and water *ad libitum.*

Bioassay

The animals were then grouped randomly into four (4) with each group containing five (5) rats. Both potassium bromate and acetylsalicylic acid were dissolved in sterile distilled water to obtain the required concentration. They were then administered orally to rats in such a way that a rat received a maximum of 1.0ml of the dissolved compound.

Group 1 - Rats that were administered orally with 1 ml of distilled water and represents the control group.

Group 2 - Rats that were administered with 20mg/kg body weight of potassium bromate.

Group 3 - Rats that were administered with 20mg/kg body weight acetylsalicylic acid.

Group 4 - Rats that were administered with 20 mg/kg body weight of potassium bromate and 20mg/kg body weight acetylsalicylic acid concurrently.

The animals were given daily oral dose for 21 days. The rats from each group were sacrificed 24 hours after the twenty-one days of administration.

Animal sacrifice, preparation of serum and tissue homogenates

After the administration of appropriate doses and completion of the experiment, rats were placed under ether anaesthesia. The fur and skin on the neck area were cleared to expose the jugular veins. The veins were slightly displaced to avoid contamination with interstitial fluids (Yakubu *et al.,* 2005) and were cut sharply with clean sterile blades while the rats were held head downward. Blood was allowed to drift into clean sterile glass tubes and left on the bench for 30 minutes (Akanji and Ngaha, 1989). The blood collected was centrifuged at 4000 rpm for 30 minutes (Yakubu *et al.,* 2005; Akanji *et al.,* 2008) using Heraus-Christ GMBH Osterode refrigerated centrifuge and the sera was also collected by aspiration into clean, dry sample bottles using Pasteur pipette. This was kept frozen overnight and used for enzyme assay (usually not longer than 48 hours). The rats thereafter were quickly dissected; the liver and kidney were excised into beakers containing ice-cold 0.25M sucrose solution. The kidney was de-capsulated and known weight of the organs were cut, chopped into small pieces and then homogenized in 0.25M sucrose solution (1:5w/v) using Tissues Tear or Homogenizer Model 985370-375. The homogenates were stored frozen for 24 hours before being used for enzymes analyses (Akanji *et al.,* 2008).

Enzyme Activity Measurement

Lactate dehydrogenase (EC 1.1.1.27) and glutamate dehydrogenase (EC 1.4.1.2) activities were determined according to the methods described by (Pesce, 1984 and Schmidt *et al.,* 1965) respectively.

While alkaline phosphatase (EC 3.1.3.1) and acid phosphatase (EC3.1.3.2) activities were determined according to the method described by (Wenger *et al.,* 1984) and Abbort *et al.,* 1984) respectively. All measurements were carried out using UV/Visible spectrophotometer (Agilent 8453 UV-visible).

Statistical Analysis

The results were expressed as mean of 5 replicates $\pm$ standard deviation (SD) and results were analysed using Graph pad instat (Data set 1. SD) Software.

Duncan Multiple Range Test (DMRT) was conducted for the pair-wise mean comparisons, to determine the significant difference at 95% level of confidence. Values were considered statistically significant at $P < 0.05$, and are denoted by different alphabets (Mahajan, 1997). That is, mean value bearing the same alphabet with control are not statistically different from the control and other test groups for each parameter are statistically significant ($p<0.05$).

RESULTS

The effects of oral administration of 20mg/kg body weight of $KBrO_3$, ASA and their combination on the activities of ALP, ACP, LDH and GDH of rat liver, kidney and serum are presented in Table 1-4 respectively. There was a significant decrease ($P<0.05$) in activity of ALP by about 50% in liver and about 20% in the kidney compared with the control when potassium bromate was administered. These reductions were accompanied by corresponding significant increase ($P<0.05$) in serum (Table 1).

When ASA alone was administered, ALP activity value was significantly higher than when bromate was administered with the value being comparable with the control. Serum value of ALP was also close to the control (Table 1). However, when both potassium bromate and ASA were administered concurrently, there were no significant differences ($p<0.05$) in the activity of ALP in both tissues (liver and kidney) as compared

with the control. However, it was significantly higher than when $KBrO_3$ alone was administered.

The serum ALP activity value was comparable with the control value. The activities of ACP in both the liver and kidney were higher than the control when $KBrO_3$ was given. The value of activity of ACP in serum does not however show corresponding significance difference (P>0.05) (Table 2). The administration of ASA and combination of $KBrO_3$ and ASA concurrently did not produce any appreciable change in activity as compared with the control as well as with the $KBrO_3$ group (Table 2). The serum activities of ACP in all the groups did not follow any trends (Table 2).

Following the administration of $KBrO_3$ alone, the LDH activities was significantly reduced (P <0.05) in both the kidney and the liver by 33% and 60% of the control value respectively. The activity of the enzyme showed a concomitant increase in the serum (Table 3).

The concurrent administration of $KBrO_3$ and ASA produced activity that was not significantly different (P > 0.05) from the control but significantly higher (P<0.05) than when $KBrO_3$ alone was given. The serum activity value of the enzyme was also brought close to the control from its initial value obtained following administration of $KBrO_3$ alone (Table 3).

Table 1: Effect of chronic administration of 20mg/kg body weight each of potassium bromate, acetylsalicylic acid and combination of potassium bromate and acetylsalicylic acid on alkaline phosphatase (ALP) activities of liver, kidney and serum of rats.

Organs → / Groups↓	Liver ALP (U/l)	Kidney ALP (U/l)	Serum ALP (U/l)
Control	3.80 ± 0.04^{a}	16.35 ± 0.23^{a}	0.18 ± 0.02^{a}
$KBrO_3$	2.05 ± 0.12^{b}	11.64 ± 0.06^{c}	0.32 ± 0.03^{b}
ASA	3.75 ± 0.04^{a}	15.85 ± 0.15^{b}	0.20 ± 0.04^{a}
$KBrO_3$ + ASA	3.69 ± 0.03^{a}	15.48 ± 0.19^{b}	0.21 ± 0.06^{a}

Values are presented as mean ± S.D, n=5. Values with superscript a, b and c different from their controls are significantly different (p<0.05). ASA= acetylsalicylic acid, $KBrO_3$ = Potassium bromate.

Table 2: **Effect of chronic administration of 20mg/kg body weight each of potassium bromate, acetylsalicylic acid and combination of potassium bromate and acetylsalicylic acid on acid phosphatase (ACP) activities of liver, kidney and serum of rats.**

Organs → / Groups↓	Liver ACP (U/l)	Kidney ACP (U/l)	Serum ACP (U/l)
Control	19.50 ± 0.04^{a}	28.50 ± 0.04^{b}	0.15 ± 0.02^{a}
$KBrO_3$	21.65 ± 0.12^{a}	34.20 ± 0.21^{c}	0.13 ± 0.06^{a}
ASA	19.40 ± 0.04^{a}	27.50 ± 0.20^{b}	0.15 ± 0.05^{a}
$KBrO_3$ + ASA	18.97 ± 0.08^{b}	25.35 ± 0.27^{a}	0.14 ± 0.01^{a}

Values are presented as mean ± S.D, n=5. Values with superscript a, b and c different from their controls are significantly different (p<0.05). ASA= acetylsalicylic acid, $KBrO_3$ = Potassium bromate.

Table 3: Effect of chronic administration of 20mg/kg body weight each of potassium bromate, acetylsalicylic acid and combination of potassium bromate and acetylsalicylic acid on lactate dehydrogenase (LDH) activities of liver, kidney and serum of rats.

Organs → / Groups↓	Liver LDH (U/l)	Kidney LDH (U/l)	Serum LDH (U/l)
Control	15.45 ± 0.09^{a}	120.30 ± 0.46^{a}	8.44 ± 0.07^{a}
$KBrO_3$	6.15 ± 0.04^{c}	80.24 ± 0.22^{c}	12.60 ± 0.14^{b}
ASA	14.90 ± 0.04^{a}	116.45 ± 0.33^{a}	8.52 ± 0.08^{a}
$KBrO_3$ + ASA	14.87 ± 0.07^{b}	114.10 ± 0.77^{b}	7.80 ± 0.14^{a}

Values are presented as mean ± S.D, n=5. Values with superscript a, b and c different from their controls are significantly different (p<0.05). ASA= acetylsalicylic acid, $KBrO_3$ = Potassium bromate.

Table 4: Effect of chronic administration of 20mg/kg body weight each of potassium bromate, acetylsalicylic acid and combination of potassium bromate and acetylsalicylic acid on glutamate dehydrogenase (GDH) activities of liver, kidney and serum of rats.

Organs → / Groups↓	Liver	Kidney	Serum
Control	39.20± 0.06[a]	32.25± 0.15[a]	0.23± 0.008[a]
$KBrO_3$	24.86± 0.11[b]	30.62 ± 0.03[b]	0.35± 0.016[b]
ASA	38.54± 0.05[a]	32.40± 0.11[a]	0.25± 0.013[a]
$KBrO_3$ + ASA	36.68 ± 0.12[a]	32.86± 0.11[a]	0.24± 0.013[c]

Values are presented as mean ± S.D, n=5. Values with superscript a, b and c different from their controls are significantly different ($p<0.05$). ASA= acetylsalicylic acid, $KBrO_3$ = Potassium bromate.

Administration of $KBrO_3$ alone caused significant reduction ($p<0.05$) in GDH activities in both tissues showed when compared with the control. The serum value of GDH activity showed a corresponding increase by (52%) of the control value. But administration of ASA singly or in combination with $KBrO_3$resulted in values comparable with the control in both tissues compared with when $KBrO_3$ alone was administered (Table 4).

Under the concurrent administration of $KBrO_3$ and ASA, the activity of GDH was down-regulated by 26% of the value obtained when $KBrO_3$ was administered, bringing its activity towards that of the control group (Table 4).

DISCUSSION

Enzyme activity measurement in tissues and body fluids is the most sensitive and earliest indicator of changes in the biochemical activities in tissues and organs. It is also useful in the detection and diagnosis of diseases (Price and Stevenson, 1982; Kim *et al.,* 2004). Similarly, the effects of ingestion or administration of xenobiotics on the normal physiology and metabolism in organs and tissues of animals can be detected and monitored by changes in enzyme level in the serum. Enzyme assay, one of the biochemical parameters of organs/tissues normo-function, indicates tissues/ cellular damage long before structural damage can be picked up by any conventional histology technique (Akanji *et al.,* 1993, 2008; Nnodim *et al.,* 2012).

In this study administration of $KBrO_3$resulted in significant reduction ($P<0.05$) in the ALP, LDH and GDH activities in both tissues studied. However, ACP activity were significantly elevated ($P<0.05$) in both tissues with no significant difference in the serum ($P>0.05$). The observation could be explained by a possible complete metabolism and eventual elimination of $KBrO_3$ before getting to the lysosomes where the enzyme is localized, or that the enzyme is being protected from the compound by the lysosomal. The increased activity of the enzyme in the tissues may be due to the de-novo synthesis of the enzyme molecule *in situ* (Yakubu *et al.,* 2001). The finding of this study was in support of results of earlier workers who reported toxicity of $KBrO_3$ in tissues and organs of rat (Sai *et al.,* 1992); Uchida *et al.,* 2006; Akanji *et al.,* 2008).

Results of mechanistic studies have shown that exposure of rats to bromate causes renal toxicity by the formation of reactive intermediates which in-turn cause lipid peroxidation in their kidneys (Kasai *et al.,* 1987; Adekoya *et al.,* 2011). The increased levels of activities of enzymes (ALP, LDH and GDH) in the serum when $KBrO_3$was administered confirmed the likelihood of damage being done to the membrane of cells of organs where they are located, which could cause leakage of these enzymes into the extracellular fluid. The loss of LDH activity is expected by virtue of its localization in the cytosol, giving it a close proximity to the plasma membrane of the cell (Philip, 1995; Akanji and Yakubu, 2000).

It is expected that damage to the plasma membrane of cell will certainly result in release of these enzymes to the extracellular fluid such as it was observed by the significant increase in activities of these enzymes in the serum. The observed significant reduction in GDH activities following the administration of $KBrO_3$ is an indication of the toxicity potential of the compound to the mitochondria where

the enzyme is located. This will impart a negative effect on the energy management by the cell because the mitochondrion (where GDH resides) is the power house where energy is generated in the cell (Akanji *et al.,* 2008).

Conversely, when acetylsalicylic (ASA) was administered to rats and enzymes activities of the organs were assayed, it was observed that the activities of the enzymes were not affected appreciably in the two tissues (Table 1, 3 and 4). The activity values of these enzymes were relatively close to their control values. This showed that ASA is capable of stabilizing the membrane of the tissues/organs thereby preserving the structural integrity of the tissues. The concurrent administration of $KBrO_3$ and ASA to the rats, culminated in a distinct set of result from what was observed when $KBrO_3$ alone was administered (Table 1, 3 and 4). The conspicuously low values of activities of ALP and LDH (Figure 1 and 3) when $KBrO_3$ was administered was eliminated and enzyme activity values were brought close to the control values with the introduction of ASA. The serum enzymes activity values obtained when both $KBrO_3$ and ASA were administered presupposes a likely repair to the damage done to cell membrane following administration of $KBrO_3$.

Several workers (Miller and Smith, 1966; Ngaha and Akanji, 1982; Olajide *et al.,* 2009) have reported on the membrane stability property of acetylsalicylic acid and so it is expected to prevent any form of damage either to the plasma membrane in the form of membrane leakage or membrane structural alteration/disruption when it is administered with a membrane disruptor such as potassium bromate. The results in Table 1-4 confirmed the ability of ASA as a membrane stabilizer and its ability to prevent the loss of activities of ALP, LDH and GDH in the tissues studied.

This study confirmed the stabilizing effect of ASA by preventing membrane disruption induced by $KBrO_3$. This role could be associated with the property of ASA, as a membrane stabilizer which lock itself (its molecule) or probable its metabolic intermediate into the cell's permeability thereby blocking the spaces created between molecules in the ordered lipid bilayer of the membrane, and therefore restoring the integrity of the cell membrane structure.

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