Protective activity of acetylsalicylic acid on biochemical markers of hepatic and renal function in rats administered with potassium bromate

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ABSTRACT

The effect of administration of acetylsalicylic acid, a membrane stabilizer, on markers of hepatic and renal function parameters in potassium bromate-induced damage in rats tissue cellular system was investigated. The levels of these markers were measured in the serum 24hours after 15 days of daily oral administration of potassium bromate and concurrent administration of potassium bromate and acetylsalicylic acid. The experimental animals were randomly divided into four groups as those administered distilled water (control), those administered 20mg/kg body weight potassium bromate (Group 2), those administered 20mg/kg body weight acetylsalicylic acid (Group 3) and those administered 20mg/kg body weight potassium bromate and acetylsalicylic acid concurrently (Group 4). Physical observations of rats administered with potassium bromate include rapid breathing, diarrhoea and impaired locomotion. The serum level of both the hepatic and renal function markers were significantly elevated, (p<0.05) compared with group administered with potassium bromate only. Serum electrolytes level were also significantly increased (p<0.05) compared with the control. This indicates the extent of damage to the organs by potassium bromate. However, these trends were reversed with the concurrent administration of potassium bromate and acetylsalicylic acids. These observations reveals the ability of acetylsalicylic acid to protect the organs whose malfunction result from the damage inflicted by potassium bromate. The photomicrograph of the organs revealed observable alterations to the normal structural architecture of the organs when potassium bromate was administered. The trend was however reversed when potassium bromate and acetylsalicylic acid were co-administered. Therefore, it is conclusive to say that the dose of acetylsalicylic acid that was employed in this work could prevent liver and kidney damage through protection of the membrane of the tissues cellular system. A role probably attributable to the ability of acetylsalicylic acid as a membrane stabilizer and which could be as a result of its anti-inflammatory property.

Keywords: Food additive, Potassium bromate, Acetylsalicylic acid, Function Indices
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INTRODUCTION

Food additives are substances added to food to preserve flavour or enhance its taste and appearance (Abdulmumeen et al., 2012).

Many research studies have revealed the potential toxicity of some commonly used food additives and these have been reported in various research publications (Sai et al., 1992; Akanji et al., 2008; Olajide et al., 2009). The mode of toxicity of some of the additives have also been postulated from several studies (Akanji et al., 2008) to be by the interaction of the compounds with the membrane of cells of tissues. Potassium bromate, KBrO3, has been in this regard confirmed by (Akanji et al., 2008) to labialize cell membranes of the liver and kidney of rats. Potassium bromate is an odourless, white crystalline salt, soluble in water, but slightly soluble in alcohol and insoluble in ether (Kurokawa et al., 1990). It is widely used as an
additive in food industries, baking and confectionaries for improved product quality (Achukwu et al., 2009; Abdulmumeen et al., 2012).

Despite the widespread use and because of its affirmed toxicity, the use of the compound has been prohibited in countries like the EU, Canada, Brazil, Sri Lanka in 2001 and China in 2005. In Nigeria, the National Agency for Food and Drug Administration and Control (NAFDAC) banned the use of potassium bromate in 2003 (Achukwu et al., 2009).

Exposure to additives may be through direct or indirect (intentional and unintentional) applications respectively, to maximize profit, improve quality and to increase aesthetic value (Magnus, 1982; Abdulmumeen et al., 2012) as well as to preserve excess (Akanji, 2002). Therefore, relatively large number of people are exposed to potassium bromate (National Institute for Occupational Safety and Health, 1998). The incident of occupational exposure to potassium bromate may occur during its production and during its use in any form as food additive (Dennis et al., 1994).

For example, dietary exposure survey on potassium bromate in retail bread samples in United Kingdom revealed presence of bromate in all six unwrapped breads selected for analysis (Dennis, et al., 1994). Consequently, whatsoever is the aim of the technology of food additives, it is important to consider even while achieving specific technical result, whether such compounds affect the quality of the commodity and the health of the consumer (Magnus, 1982, Achucikuwu et al., 2009).

The metabolism of a chemical substance causes it to elicit its biochemical nature at a target cell in the body, the metabolic product of which is either detoxified or eliminated or accumulates in cells and cause cellular damage.

Toxicity of acute oral administration of potassium bromate at doses ranging from 85-602 mg/kg body weight to both male and female rats has been reported (Kawanah et al., 1991, Kazeem, 2009) with general organ toxicity by (Kurokawa et al., 1990; Sai et al., 1992, Akanji et al., 2008). Results of mechanistic studies proposed that exposure to bromate causes renal toxicity in man and experimental animals (Uchida et al., 2006) through lipid peroxidation and DNA damage (Kasai et al., 1987; Adekoya et al., 2011).

Akanji et al., (2008) revealed the effect of chronic administration of potassium bromate on some 'marker' enzymes of rat cellular system. The result showed that bromate was capable of disrupting the plasma membrane of cells. The presence of high oxygen content of potassium bromate was speculated to be partly responsible. In this study we have tried to exploit the property of acetylsalicylic acid (ASA) as a membrane stabilizer (Ngaha and Akanji, 1982) to protect the membrane of cells in order to prevent bromate induced cell damage.

As a member of the salicylates, acetylsalicylic acid is a non-steroidal anti-inflammatory agent (Vane and Bonting, 2003, Holmes et al., 2010) with the potentiality to stabilize membrane of cells (Hudson et al., 2008). This is a role that has been reported by many researchers (Ngaha and Akanji, 1982; Olajide et al., 2005, 2009). Therefore the ability in acetylsalicylic acid to prevent damage to the liver and kidney tissues in the presence of potassium bromate was investigated in this study. It is imperative because potassium bromate is a strong oxidizer and is implicated in excess oxygen poisoning and having ability to produce reactive oxygen species (ROS) when metabolised, and so may be a probable potential membrane disruptor.

MATERIALS AND METHODS

Animals
A total of twenty male albino rats (Rattus norvegicus) of wistar strain with an average weight of 180±4.5g were procured from the Small Animal Holding Unit of the Department of Biochemistry, Kogi State University, Anyigba, Kogi State, Nigeria.

The animals which were kept in aluminium floor cages placed in a well-ventilated room (Temp.
28±3°C, 12 hours natural light and 12 hours darkness, humidity 45-50%) were allowed free access to food (Vital Feeds Nig. Ltd) and clean drinking water on which they were stabilized for three (3) weeks.

The rats were handled and used in accordance with the guidelines of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes – ETS-123 (2005).

**Assay kits**
The assay kits for albumin, bilirubin (total and direct), Uric acid, Urea and Creatinine were obtained from Randox Laboratories Ltd, UK. Potassium bromate was supplied by Labtech Chemicals Nig. Ltd, Lagos, Nigeria while acetylsalicylic acid is a product of Tega Laboratories, Chelsea, London.

All other reagents used were of analytical grade and were prepared in glass distilled water and stored in reagent bottles until required for use.

**Animal grouping and administration of compounds**

**Bioassay**
The rats were randomly assigned to four groups of 5 rats each. The compounds (potassium bromate and acetylsalicylic acid) solutions were prepared in sterile distilled water to obtain the specified concentration. The solutions were administered orally as a single dose daily into rat groups as indicated below.

- **Group A** constitutes rats administered with distilled water only and represents the control group.
- **Group B** were rats administered with 20mg/kg body weight of potassium bromate.
- **Group C** were rats administered with 20mg/kg body weight acetylsalicylic acid while
- **Group D** rats were concurrently administered 20mg/kg body weight of potassium bromate and 20mg/kg body weight acetylsalicylic acid.

The administration lasted for 21 consecutive days after which the rats from each group were sacrificed 24 hours after the last dose.

**Preparation of serum**
At the end of the treatment period, all rats were fasted overnight and anaesthetized by keeping in a desiccator containing cotton wool soaked in ether. Blood was then withdrawn into clean and dried sample bottles by cardiac punctures. The blood was allowed to clot for 10 minutes at room temperature and thereafter centrifuged at 4000rpm for 30 minutes (Yakubu et al., 2005; Akanji et al., 2008) Heraus-Christ GMBH Osterode refrigerated centrifuge. Sera was collected by aspiration into clean, dry sample bottles using pasteur pipette and kept frozen until required per use. The sera was used for liver and kidney function tests within 12 hours of preparation (Yakubu and Musa, 2012).

**Determination of biochemical parameters**
The parameters evaluated include albumin (Doumas et al., 1971), bilirubin (total and direct) (Kaplan et al., 1984) for liver function tests, and Urea (Kaplan et al., 1984), creatinine and uric acid (Schultz et al., 1984) and electrolytes, (sodium, potassium, carbonate and chloride ions (Krieg et al., 1986) for kidney function test.

**Statistical analysis**
Results are expressed as mean ± SDM. n values were the same (5) for both control and test groups. The data were analysed using Graph pad instat (Data set 1. SD) soft-ware and Duncan Multiple Range Test (DMRT) was conducted for the pair-wise comparisons to determine the significant difference at 95% level of confidence. For all the tests, values of results with p<0.05 were considered to be of statistical significance (Mahajan, 1997; Yakubu and Musa, 2012).

**Histological examination**
Tissues histological examination was as described by Krause (2001). The photomicrographs were observed using the Leitz, DIALUX research microscope at X100.
RESULTS

The effects of oral administration of 20mg/kg body weight of potassium bromate (KBrO₃), acetyl salicylic acid (ASA) and their combination on liver and kidney function parameters and the electrolytes composition are as presented in Table 1-3 respectively.

When potassium bromate was administered to rats, there was a significant increase (p<0.05) in albumin concentration compared with the control value. There was 33% increase in albumin concentration above the control value for the period the experiment lasted.

Conversely, administration of potassium bromate and acetylsalicylic acid in concurrence did not result in a significant difference (p>0.05) in serum albumin concentration when compared with the control (Table I).

Similarly both direct and total bilirubin concentration witnessed significant increases (P<0.005) following administration of potassium bromate to rats, but these values were brought toward the control when ASA was administered solely and with the combined administration respectively. The observed (25.5% and 15.0%) elevation in concentration of direct and total bilirubin respectively following administration of KBrO₃ alone were significantly reduced (P<0.05) and the values bear no further significant difference from the control (Table I).

Urea, uric acid and creatinine were measured for the kidney function tests. The values of these parameters in the serum following administration of potassium bromate to rats were significantly higher (P<0.05) than the control. The values increased by 13.46%, 13.52% and 31.20% respectively of the control value (Table 2).

TABLE 1: Effects of chronic administration of 20 mg/kg body weight each of potassium bromate, acetylsalicylic acid and combination of potassium bromate and acetylsalicylic acid on some liver function parameters in serum of albino rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver function parameters in serum of albino rats</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Albumin g/dl</td>
</tr>
<tr>
<td>Control</td>
<td>2.82 ±041ᵃ</td>
</tr>
<tr>
<td>KBrO₃</td>
<td>3.75±0.17ᶜ</td>
</tr>
<tr>
<td>ASA</td>
<td>3.06±0.11ᵇ</td>
</tr>
<tr>
<td>KBrO₃ + ASA</td>
<td>2.90±0.32ᵃ</td>
</tr>
</tbody>
</table>

Values are mean of 5 replicates ± SD. Test values carrying superscripts different from their controls across the column are significantly different (p<0.05). KBrO₃=potassium bromate; ASA=acetylsalicylic acid

TABLE 2: Effects of chronic administration of 20 mg/kg body weight each of potassium bromate, acetylsalicylic acid and combination of potassium bromate and acetylsalicylic acid on some kidney function parameters in serum of albino rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Kidney function parameters in serum of albino rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urea g/dl</td>
</tr>
<tr>
<td>Control</td>
<td>19.38±1.85ᵃ</td>
</tr>
<tr>
<td>KBrO₃</td>
<td>21.99±2.87ᶜ</td>
</tr>
<tr>
<td>ASA</td>
<td>19.35±1.15ᵃ</td>
</tr>
<tr>
<td>KBrO₃ + ASA</td>
<td>19.22±0.53ᵇ</td>
</tr>
</tbody>
</table>

Values are mean of 5 replicates ± S.D. Test values carrying superscripts different from their controls across the column are significantly different (p<0.05). KBrO₃=potassium bromate; ASA=acetylsalicylic acid
However the trend changed when acetylsalicylic acid was administered concurrently with potassium bromate. The observed increase in the serum concentration were no longer noticed rather the values showed no significant difference (p>0.05) when they were compared with the control (Table 2).

Following administration of potassium bromate to rats, the values obtained for electrolyte concentrations (Na$^+$, K$^+$ and HCO$_3^-$) showed significant increases when they were compared with the control. However, Cl$^-$ concentration was significantly reduced (p<0.05).

There was recovery from the observed trend when acetylsalicylic acid was co-administered with potassium bromate to rats. The values were close to the control value with those of K$^+$ and Cl$^-$ slightly higher than the control.

Administeration of potassium bromate to rats caused severe congestion of the central vein. There was severe vacuolar degeneration with hepatocytes surrounding portal vein showing clear spaces in the cytoplasm (plate 1). However, when acetylsalicylic acid alone and its combination with bromate were administered to rats the histology of the liver was normal (plate 2 and plate 4) as compared with the control (plate 3).

**DISCUSSION**

Measurement of biochemical parameters of organ function (hepatic, renal) provides a rapid means of information on the functional state of the organ.

It also enhances accurate diagnosis and risk assessment that can lead to adoption of therapy that can improve clinical outcome (Shivaray et al., 2010).

Both liver and kidney are two major organs involved in active metabolism and their malfunction could result in deleterious effects on the whole organism. The liver, aside of its synthetic roles, houses the chief metabolizing enzymes while the kidney play a major role in synthetic and regulatory mechanism (Shivaray et al., 2010).

In this study, the observed significant increases (P<0.05) in the serum concentration of the parameters investigated following administration of potassium bromate (albumin, total and direct bilirubin for liver function, urea, uric acid and creatinine for kidney function) could imply damage to these organs; thereby adversely affecting both their synthetic, secretory and excretory functions.

Alterations in secretory, synthetic and excretory functions of the liver have been reported as a useful index of impaired organ function or dysfunction (Yuegang et al., 2008; Yakubu et al., 2012). Therefore the observed high serum
levels of these indices above the control when potassium bromate was added could be responsible for the alterations. This condition was however, changed when acetylsalicylic acid was administered concurrently with potassium bromate.

A functional role of albumin is as a binder and transporter of metal ions, drugs, bilirubin etc. Its level may therefore be used as a measure to assess the synthetic function of the liver (Mayne, 1994; Ekanem and Yusuf, 2007). Therefore the significant increase (P<0.05) in the level of albumin and bilirubin in the serum indicates damage to the liver, which is the site of synthesis of albumin. Moreso since bilirubin is transported to the liver bound to albumin, high plasma conjugated bilirubin concentration indicates impaired hepatic excretory function (Thapa and Walia, 2007; Mayne, 1994; Ekanem and Yusuf, 2007).
Though over production and, or over excretion of bilirubin are known causes of high bilirubinemia (Kaplan 1987, Yakubu et al, 2012), it is more probable and arguable to link the observed increase in the parameters investigated in the present study to hepatocellular damage caused by administration of potassium bromate.

This study shows that there is significant (P<0.05) changes in urea, uric acid and creatinine levels in the serum of potassium bromate treated rats and these changes were reversed following the concurrent administration of acetylsalicylic acid. Histological changes in the glomerulus such as irregular dilation of the tubules, and necrosis resulting in the distortion of the glomerula basement and severe infiltration of the membrane interstitial cells were observed in potassium bromate treated rats.

Such changes may result in physiological effects such as alterations in renal heamodynamics (Montenegro et al., 1996; Sarika et al., 2009), decrease in renal blood flow and glomerular filtration rate (GFR) and hence reduced
creatinine and uric acid clearance (Sarika et al., 2009). Hence the observed significant increase (P<0.05) in the levels of renal function parameters studied in this work.

Complementing to the observed changes above is the elevated levels of the serum electrolytes particularly Na⁺, K⁺ and Cl⁻. Na⁺ and K⁺ are major components of extracellular and intracellular fluids respectively whose levels are regulated by the kidney. Therefore high serum level of the electrolyte could indicate renal dysfunction particularly at tubular and glomerular levels. Renal regulation of electroneutrality usually results in an inverse relationship between Cl⁻ and HCO₃⁻ (Ibrahim 2001). Therefore, this may account for the observed levels of Cl⁻ and HCO₃⁻ when potassium bromate was administered. These observation however, respond to treatment with acetylsalicylic acid.

We have presented the histological examinations of the tissues to serve to complement the evidences from the organ function tests by revealing the damage, if any, to the normal architecture of the structure of these tissues when potassium bromate alone was administered to rats and when it was administered concurrently with acetylsalicylic acid. The various photomicrographs showed that the observed structural distortions when bromate was administered were no longer seen when acetylsalicylic acid was administered, confirming the capability of acetylsalicylic acid to protect the organs from damage that could arise following the administration of potassium bromate.

Acetylsalicylic acid was found to prevent liver damage (Imaeda et al., 2009) in acetaminophen induced hepatocytes damage. Hepatotoxicity of acetaminophen triggered an increase in the inflammatory cascade involving cytokine Pro-IL-κB which was prevented by acetylsalicylic acid through the “down” regulation of the pro-inflammatory cytokine (Holmes, 2010).

The salicylates are known to be able to accumulate at the mildly acidic environment such as occurring at sites of inflammation and at low pH salicylates are uncharged and can enter the cell membrane with subsequent deprotonation in the cell (Friedrich, 2001).

We conclude therefore, that acetylsalicylic acid was able to reverse the effect of potassium bromate on the functional indices of liver and kidney in rats. This role may probably be due to its attribute as a membrane stabilizer arising probably from its anti-inflammatory property. Both Ignarro, (1971); Satyalatha (2014); and Krasopoullos et al.,(2008) had earlier reported the potency of certain anti-inflammatory drugs as membrane stabilizers. Acetylsalicylic acid irreversibly blocks the action of the cyclooxygenase enzyme system in the synthesis of prostaglandins, in a process that is responsible for the phenomenon of inflammation.

REFERENCES


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