# MODULATION OF ENZYME ACTIVITIES FOLLOWING THE CO-ADMINISTRATION OF POTASSIUM BROMATE AND CHLOROQUINE IN SELECTED TISSUES AND SERUM OF ALBINO RATS

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**ABSTRACT**

***The effect of administration of potassium bromate, chloroquine (membrane labilizers) and their co-administration on some cellular enzymes was investigated. The levels of activity of these enzymes were measured in the liver, kidney and serum 24 hours after 21 days of daily oral administration of potassium bromate, chloroquine and the co-administration of potassium bromate and chloroquine. The experimental animals were divided into four groups randomly. Group 1 rats were administered with distilled water and served as control. Group 2 were rats administered with 25 mg/kg body weight of potassium bromate. Group 3 were rats administered with 25 mg/kg body weight chloroquine, while rats in group 4 were co-administered with 25 mg/kg body weight of potassium bromate and chloroquine. This study investigated the effects of repeated administration of two potent membrane disruptors either alone or in combination on liver and kidney cellular enzymes and serum enzymes. Rats administered with potassium bromate exhibited some physical observations which include rapid breathing, diarrhoea and difficulty in movement, while rats administered with chloroquine exhibited hyperactivities. Results of enzyme activity determination showed significant decreases ($p<0.05$) in activity of kidney and liver dehydrogenases (lactate and glutamate) as well as in the transaminases (ALT and ASP) when potassium bromate and chloroquine were separately administered. Similarly, alkaline phosphatase activity was significantly reduced ($p<0.05$) in both tissues. Nonetheless, acid phosphatase (ACP) activity was not appreciably affected in both tissues. Corresponding significant increases ($p<0.05$) in activity of these enzymes in the serum was observed. However, unexpected high values of enzyme activities in both tissues when both potassium bromate and chloroquine were co-administered were observed. The elevated level of enzyme activities in serum confirmed further the properties of potassium bromate and chloroquine as membrane labilizers causing the cellular enzymes to leak into the blood. Furthermore the results obtained pointed to a probable synergy in the properties of the two compounds when they were administered concurrently, thereby creating a kind of modulatory effect on the enzymes, hence the observed increases in enzymes activity in the tissues studied. It could be inferred from the results therefore that the intrinsic properties of chemical substances could be modulated or modified intracellularly when in interaction with other compounds and even with the cell system.***

**Keywords:** Food additives, Chloroquine, Potassium bromate, Co-administration, Enzyme activity, Modulation

## INTRODUCTION

In the food industry, food (chemical) additives are added purposely to enhance quality (Abdulmumeen *et al*., 2012). While some are intentionally added, some others become part of food unintentionally occurring only in trace amount due to food packaging, storage and other handlings (Cavanaugh, 2002). Potassium bromate ($KBrO_3$), a white crystalline salt, soluble in water and only slightly soluble in alcohol but insoluble in ether (Kurokawa *et al.,* 1990) is a chemical food additive intentionally used in food industries, for baking and in confectionaries for improved product quality (Achukwu *et al*., 2009; Abdulmumeen *et al*., 2012). Desirable as it may, as a flour improver, there are many reported cases of toxicity involving potassium bromate. Being a strong oxidizing agent, potassium bromate is reported to cause disruption of the plasma membrane of cells (Akanji *et al*., 2008). Also Kazeem (2009) reported the toxicity of acute oral administration of potassium bromate thereby supporting the work of (Akanji *et al*., 2008) who reported the general organ toxicity of this compound. Mechanistic studies have also proposed that exposure to bromate causes renal toxicity in man and experimental animals (Uchida *et al*., 2006) through peroxidation of membrane lipids and DNA damage (Adekoya *et al*., 2011). Several other studies have established that potassium bromate is capable of causing damage to the plasma membrane of cells thereby causing such cells to release their internal contents to the extracellular environment (Akanji *et al*., 2008; Olajide *et al.,* 2015).

Due to its common use, relatively large numbers of people are exposed to the compound just as incident of occupational exposure to potassium bromate may occur during its production and its use as food additive (Dennis *et al*., 1994). From dietary exposure survey on potassium bromate in retail bread samples the presence of bromate was revealed in breads selected for such analysis (Denies *et al*., 1994; Achukwu *et al*., 2009).

Chloroquine is an antimalarial agent widely accepted all over the world (Sharma and Mishra, 1999; Izunya *et al*., 2010; WHO, 2012; Swagata *et al*., 2014). It is also indicated in the treatment of rheumatoid arthritis and systemic lupus erythematosus (Dubois, 1978; Ducharme and Farinotti, 1996). Available data have shown that chloroquine is usually concentrated in some tissues such as the liver and kidney, following its oral administration (Adelusi and Salako, 1982; Ajani *et al*., 2009; Izunya *et al*., 2010). In toxic doses chloroquine had been reported to cause appreciable cellular damage to liver, kidney and heart muscle (Ngaha and Akanji, 1982; Izunya *et al*., 2010), thereby affecting the activities of the tissues cellular enzymes (Malomo *et al*., 1993).

Experimental reports have shown the toxic effect of chloroquine on kidney function when taken either during treatment or prophylaxis of malaria and even when administered acutely or chronically to rats. This is an action suggested to be probably due to its accumulation in kidney cells (Musabayane *et al*., 1993; 2000a; Cooper and Magwere, 2008) or due to its deposition in the adrenal glands which may indirectly affect the kidney functions through modulation of the secretion patterns of aldosterone causing a reduction in tubular $Na^+$ handling (Cooper and Magwere, 2008). The co-administration of chloroquine with other drugs or chemicals have been investigated and have been found to result in adverse effect to the kidney (Musabayane *et al*., 2000b). For example, concurrent administration of chloroquine and ethanol was discovered to induce extensive damage to the proximal tubule and collective duct cells of the kidney (Musabayane *et al*., 2000b; Cooper and Magwere, 2008)

The paucity of information on the effects of co-administration of bromate and chloroquine on liver and kidney enzymes aroused our interest in this study. Therefore, the study investigated some of the biochemical implications of the co-administration of bromate and chloroquine on rat tissues because the two compounds are often encountered in Africa sub regions in particular as component of processed food and as anti-malarial respectively.

This study therefore investigated the activities of some important enzymes (phosphatases, transaminases and

dehydrogenases) in diagnosis of organ functions following the co-administration of potassium bromate and chloroquine to rats. The liver tissues play vital role in drug metabolism because it houses most of the drug metabolizing enzymes while the kidney functions in maintenance of cellular homeostasis. The serum is the physiological fluid in which lost substances of tissue origin due to damage are deposited.

## MATERIALS AND METHODS

**Animals:** Twenty male white albino rats (*Rattus norvegicus*) of wistar strain with an average weight of 200 ± 5.0 grams were purchased from the Small Animal House of the University of Nigeria, Nsukka, Nigeria, and used for the study. The animals were kept in separate aluminium made metabolic cages in a well-ventilated room and were subjected to 12 hours light/12 hours darkness with relative humidity 45 – 60 % at temperature of 26 ± $3^{O}$ C. They were allowed free access to feed (Vital Feeds Nigerian Limited) and good drinking water on which they adapted to the environment for three weeks. The rats were handled and used in accordance with guidelines of the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (Council of Europe, 1986).

**Assay kits:** The assay kits for Lactate and glutamate dehydrogenases, alkaline and acid phosphatases, aspartate and alanine aminotransferases were obtained from Labkits - South Africa, Chemelex - Poland and Canovelles - Barcelona Spain. The potassium bromate used was a product of Labtech Chemicals Nigeria Limited, Lagos, CAS No. 7758-01-2, while the chloroquine used is a product of Evans Medical PLC, Agbara, Nigeria. All other reagents used were of analytical grade and were prepared in glass distilled water and stored in reagent bottles until required for use.

**Bioassay:** The rats used for this study were randomly divided into four groups, replicated thrice with five rats per replicate. Potassium bromate and chloroquine solutions were prepared in sterile distilled water to obtain the concentrations required and the solutions were given orally into rat groups as single daily dose as indicated below. Group 1 rats were administered with 1.0 ml water only and represent the control. Group 2 rats were administered with 1.0 ml solution 25 mg/kg body weight of potassium bromate. Group 3 rats were given 1.0 ml solution 25 mg/kg body weight chloroquine (CHQ) and group 4 rats were concurrently administered 1.0 ml each of 25 mg/kg body weight potassium bromate and 25 mg/kg body weight chloroquine. Administration of solutions of the compounds lasted for 21 days after which the rats from each groups were sacrificed 24 hours after the last dose.

**Preparation of Serum and Tissue Homogenates:** After 21 days post doses administration, rats were fasted overnight and when due for sacrifice they were anaesthetized in desiccator containing cotton wool soaked in ether. The rats were quickly brought out of the desiccator and dissected. 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 4000 rpm for 30 minutes (Yakubu *et al*., 2005; Akanji *et al*., 2008) using Heraus-Christ GMBH Osterode refrigerated centrifuge. Sera were collected by aspiration into clean, dry sample bottles using Pasteur pipette. This was stored frozen until required for use (within 12 hours of preparation) (Yakubu and Musa, 2012). Thereafter, the rats were dissected and the organs of interest (liver and kidney) were excised into beakers containing ice-cold 0.25M sucrose solution.

Known weight (1.0 g) of the liver and kidney were respectively chopped into small pieces and then homogenised in 0.25 M sucrose solution using Tissues Tearor homogenizer Model 985370-375. The homogenates were diluted with 0.25 M sucrose solution and stored frozen until required.

**Determination of Biochemical Parameters:** Enzymes that were assayed include lactate

dehydrogenase (Pesce, 1984), glutamate dehydrogenase (Delma, 1970), aspartate and alanine aminotransferases (Murray, 1984), alkaline phosphatase (Wenger *et al*., 1984) and acid phosphatase (Abbort *et al*., 1984).

**Statistical Analysis:** Data collected were analysed for their central tendencies and subjected to one way analysis of variance (ANOVA). The data were expressed as mean ± standard error of mean. Graph Pad Instat (Data set 1. SD) was used for all analysis. The Duncan Multiple Range Test (DMRT) was used to separate significant differences among treatment means at 95 % level of confidence. For all the tests, values with $p<0.05$ were considered to be of statistically significant (Mahajan, 1997; Yakubu and Musa, 2012).

## RESULTS

The data on the effects of oral administration of 25 mg/kg body weight of potassium bromate ($KBrO_3$), chloroquine (CHQ) and their combination on activities of some enzymes in the liver, kidney and serum of rats are presented in Tables 1, 2 and 3, respectively.

Following the administration of potassium bromate to rats, both liver and kidney recorded significant decreases ($p<0.05$) in lactate dehydrogenase (LDH) activities 60.19 % and 33.38 % respectively with corresponding significant increase ($p<0.05$) (49.37 %) in the serum compared with the control. Similarly the oral administration of chloroquine produced significant decreases ($p<0.05$) in activity of LDH in both tissues when compared with the control (Table 1).

Administration of chloroquine to rats also led to significant decrease in all the enzyme activities. However, when potassium bromate and chloroquine were co-administered the activity of LDH though lower than the control values but became insignificantly different in the liver when compared with the control but the activity was significantly reduced in the kidney (Table 1). The activities of glutamate dehydrogenase (GDH) provided a similar trend as LDH in both tissues and the serum (Tables 1, 2 and 3). However, the extent of loss recorded in GDH activity in the tissues was not as pronounced as for LDH. The GDH activity values were almost comparable to the control value in the kidney tissues (Table 2).

Both alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities recorded significant reduction ($p<0.05$) in both tissues (>50 %) in all cases, following the separate administration of potassium bromate and chloroquine. However with the co-administration of the two compounds increased, enzyme activity was elicited by the compounds when the values were compared results obtained following the administration of bromate and chloroquine alone. The activity values for both enzymes were higher than the control value in the liver but lower in the kidney (Table 1 and 2). The serum values of all enzyme activities were significantly elevated in all the groups ($p<0.05$).

Alkaline phosphatase recorded significant decreases ($p<0.05$) in activities in both the liver and the kidney. However, the activity of acid phosphatase was not appreciably affected in both tissues in all the groups following all categories of test administrations (Tables 1, 2 and 3).

## DISCUSSION

Upon acute or chronic administration of chemical substances such as drugs and other xenobiotics to experimental animals, both observable physical changes and non-observable biochemical changes manifest usually in the form of alterations in the normal cell functions of tissues, particularly those involved in active cellular metabolism. Such alterations in cell functions could eventually lead to altered structural integrity of the cells, thereby resulting to loss of the cell components to the extra cellular environment. Therefore enzyme activity changes in the liver, kidney and serum of albino rats was followed conventionally in this work to assess the effect of administration of bromate, chloroquine and their co-administration on the tissues. Enzyme assay is a rapid and revolutionary biochemical parameter for obtaining information about

**Table 1: Effects of chronic administration of 25 mg/kg body weight potassium bromate, chloroquine and their combination on some selected liver enzymes in albino rats**

| *Activities in IU/L* / *Groups* | LDH | GDH | AST | ALT | ALP | ACP |
|---|---|---|---|---|---|---|
| **Control** | 15.45±0.09$^{a}$ | 39.20±0.06$^{a}$ | 4.18±0.17$^{a}$ | 6.01±0.35$^{a}$ | 3.80±0.04$^{a}$ | 19.50±0.04$^{a}$ |
| **$KBrO_3$** | 6.15±0.04$^{d}$ | 24.86±0.11$^{d}$ | 2.36±0.16$^{c}$ | 1.67±0.18$^{b}$ | 1.25±0.12$^{b}$ | 19.15±0.12$^{a}$ |
| **CHQ** | 8.15±0.03$^{c}$ | 28.22±0.02$^{c}$ | 3.60±0.33$^{c}$ | 2.40±0.12$^{b}$ | 1.65±0.10$^{b}$ | 10.80±0.06$^{b}$ |
| **$KBrO_3$ + CHQ** | 13.85±0.05$^{a}$ | 35.24±0.23$^{b}$ | 5.53±0.14$^{b}$ | 6.95±0.23$^{d}$ | 3.57±0.03$^{a}$ | 19.30±0.01$^{a}$ |

*Test values with different superscripts across the column are significantly different ($p<0.05$). $KBrO_3$ = Potassium bromate; CHQ = Chloroquine*

**Table 2: Effects of chronic administration of 25 mg/kg body weight potassium bromate, chloroquine and their combination on some selected kidney enzymes in albino rats**

| *Activities in IU/L* / *Groups* | LDH | GDH | AST | ALT | ALP | ACP |
|---|---|---|---|---|---|---|
| **Control** | 120.30±0.46$^{a}$ | 32.25±0.15$^{a}$ | 2.74±0.25$^{a}$ | 5.60±0.42$^{a}$ | 21.35±0.03$^{a}$ | 28.50±0.04$^{b}$ |
| **$KBrO_3$** | 80.24±0.22$^{c}$ | 33.60±0.11$^{a}$ | 1.05±0.10$^{b}$ | 2.60±0.28$^{c}$ | 18.30±0.06$^{b}$ | 34.20±0.21$^{a}$ |
| **CHQ** | 87.25±0.25$^{b}$ | 30.85±0.03$^{b}$ | 0.50±0.06$^{c}$ | 2.15±0.13$^{c}$ | 10.96±0.10$^{c}$ | 22.46±0.62$^{c}$ |
| **$KBrO_3$ + CHQ** | 42.60±0.11$^{d}$ | 32.05±0.09$^{a}$ | 1.20±0.13$^{b}$ | 4.50±0.36$^{b}$ | 8.71±0.44$^{d}$ | 26.73±0.22c |

*Test values with different superscripts across the column are significantly different ($p<0.05$). $KBrO_3$ = Potassium bromate; CHQ = Chloroquine*

**Table 3: Effects of chronic administration of 25 mg/kg body weight potassium bromate, chloroquine and their combination on some selected serum enzymes in albino rats**

| *Activities in IU/L* / *Groups* | LDH | GDH | AST | ALT | ALP | ACP |
|---|---|---|---|---|---|---|
| **Control** | 8.44±0.07$^{c}$ | 0.23±0.008$^{b}$ | 5.96±0.25$^{b}$ | 6.37±1.04$^{b}$ | 0.17±0.02$^{b}$ | 0.15±0.02$^{a}$ |
| **$KBrO_3$** | 12.60±0.14$^{a}$ | 0.30±0.016$^{a}$ | 14.76±0.80$^{a}$ | 15.08±0.50$^{a}$ | 0.34±0.03$^{a}$ | 0.15±0.06$^{a}$ |
| **CHQ** | 10.24±0.27$^{b}$ | 0.32±0.013$^{a}$ | 13.74±0.99$^{a}$ | 7.30±2.05$^{b}$ | 0.36±0.04$^{a}$ | 0.17±0.04$^{a}$ |
| **$KBrO_3$ + CHQ** | 10.36±0.93$^{b}$ | 0.34±0.013$^{a}$ | 14.46±0.61$^{a}$ | 5.89±1.59$^{c}$ | 0.36±0.08$^{a}$ | 0.16±0.07$^{a}$ |

*Test values with different superscripts across the column are significantly different ($p<0.05$). $KBrO_3$ = Potassium bromate; CHQ = Chloroquine*

tissues cellular integrity and it also plays significant role in disease investigation and diagnosis (Malomo, 2000; Yakubu *et al*., 2003; Nnodin, 2012).

In relatively high doses both potassium bromate and chloroquine employed in this work have been reported to cause appreciable cellular damage to body tissues (Kukoyi *et al.,* 2000; Ajani *et al*., 2009; Izunya *et al.,* 2010; Olajide *et al.,* 2014).

The significant level of decreases ($p<0.05$) observed in the activity of alkaline phosphatase, ALP; (a membrane-localised enzyme) lactate dehydrogenase, LDH; aspartate and alanine amino-transferases (cytosol enzymes) in the organs following administration of potassium bromate pre-supposes damage to the organised membrane structure of cells of the tissues under study. This is in support of the work of (Akanji *et al.,* 2008). The observation established possible damage to the cells structures and hence may be responsible for the loss by leakage of the enzymes to the extra cellular environment or its inhibition. However, the corresponding significant increase in activity of these enzymes in the serum supports loss of enzyme molecules from these tissues rather than inhibition of enzymes activities.

We observed similarly that when chloroquine was administered alone to the rats, the activities of the enzymes when compared with the control were significantly reduced in

the tissues, but with the level of reduction being more pronounced with administration of potassium bromate alone. Earlier loss in some of tissue enzymes was reported when chloroquine was co-administered with insulin to rats (Ajani *et al.,* 2009).

In most cases as reflected in this work, we observed that the liver enzymes were relatively more affected than the kidney following the administration of these compounds either alone or when in co-administration. However, acid phosphatase was by the least affected among the enzyme when potassium bromate and chloroqunine phosphate were administered separately. The serum value of acid phosphotase (ACP) showed no significant difference ($p>0.05$) compared with the control. This observation may be explained by the possession of individual organelle membrane by the lysosomes (Wright *et al.,* 1979; Akanji *et al.,* 2008), the organelle which houses the enzyme, or acid phosphatase due to the lysosomotropic status of chloroquine resulting in increase in size and number of liver lysosomes (Alfonso *et al.,* 1980; Greenspan and Dong, 1989; Zahid and Abidi, 2003; Andrey *et al*., 2014).

The toxicity of potassium bromate and chloroquine and their abilities to cause damage to both cell membranes and tissues have been severally reported (Savarino *et al.,* 2006; Akanji *et al.,* 2008; Ajani *et al.,* 2009; Olajide *et al.,* 2014). The magnitude of loss of activity of glutamate dehydrogenase (GDH), a mitochondrial indicator of structural or membrane integrity (Yakubu *et al.,* 2003) vis-à-vis the loss of alanine and aspartate aminotransferases (cytosolic enzymes) may imply a reduction in the amount of energy made available to the cells (Akanji *et al.,* 2008) as well as impaired amino acid/protein metabolism. Chloroquine has been reported to cause decrease in activities of some cellular enzymes such as cytochrome $aa_3$ and b, by acting as an uncoupler of oxidative phosphorylation. This role may adversely affect that of the mitochondria in energy transduction (Cooper and Magwere, 2008).

In the present study, we expected based on the individual intrinsic role of the two compounds as membrane labilizers, an attendant significant decreases in enzyme activities in the tissues following the co-administration of the two compounds to rats.

However, contrary to our expectation when the two compounds were co-administered, it was of striking interest, our observation of relatively significant increase ($p<0.05$) in values of enzyme activities in the tissues. These values were even higher than when each of potassium bromate and chloroquine was administered alone and this observation was more conspicuous in the liver than the kidney. This observation nullified the expected negative synergy effect of the co-administration of the two compounds. Furthermore, we observed that despite the high values of enzyme activities in the tissues, the activity of the enzymes are still relatively high in the serum. From the results of this study, since the serum enzyme activities were convincingly raised to the level that may suggest the leakage of enzymes from the tissues, due to damage, the following may be the most probable mechanisms to explain the observations in the tissues when both compounds were administered concurrently.

i. The two compounds may probably through coupled action caused the activation of these enzymes in the tissues resulting in the observed high values when compared with the control.

ii. Chloroquine while acting in its capacity may have enhanced the increased secretion of these enzymes. Chloroquine is known to impair receptor recycling and impairments of receptor recycling favours secretion (Alfonso *et al.,* 1980).

iii. A probable modulatory role exerted on the enzymes as a result of synergy effect of the co-administration through the generation of metabolite that may act as effector molecules or by providing a medium in which the binding of one molecule facilitates the binding of another substrate molecule leading to high activity of the enzymes in the tissues.

iv. Possible production of intermediate by the interaction of the two drugs that may favour the induction or denovo synthesis of the enzymes.

In our opinion and from the trend of the results obtained we conclude by considering a probable synergistic action of the two compounds (when co-administered). Such action possibly could have created a form of positive homotropic response (Conn and Stumpf, 1989; Richard and Dennis, 2011; Weil, 2013) causing the binding of one substrate molecule to probably influence or facilitate the binding of the next molecule by increasing the affinities of the vacant binding sites or by the direct effects of the chemical substances combined.

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