RENAL TOXICITY OF THE FOOD ADDITIVE POTASSIUM BROMATE ON THE FRESH WATER FISH LABEO ROHITA

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ABSTRACT
Potassium bromate (KBrO3), a white crystalline solid and a widely reactive food additive is often used in bakeries as flour improver, yielding higher bread volume and used as a dough conditioner for flour. The problem ofPotassium bromate started with ozonation of drinking water to form bromate as a major by product. When research was done to confirm the safety of ozonated water, it was found that Potassium bromate causes renal cancer in rats when they drank water with Potassium bromate. The aim of the present study is to estimate the renal toxicity of the food additive potassium bromate on the freshwater fish Labio rohita. The study includes determining the LC50 concentration of Potassium bromate in fish, the enzyme level, SGOT and SGPT which are functional markers of kidney, urea and creatinine level of the serum for ascertaining the function of the kidney and to study the histopathological changes in the kidney.

INTRODUCTION
Potassium bromate (KBrO3), a white crystalline solid and a widely reactive food additive (WHO,1996) is often used in bakeries as flour improver yielding higher bread volume (Kurokawa et al.,1990) and used as a dough conditioner for flour (Diachenko and Warner, 2002). Potassium bromate is slightly soluble in ethanol, and almost insoluble in acetone; it is very stable when dissolved in water at room temperature, and at drinking water pH, it should exist almost exclusively in the ionic form (USEPA, 1993A). The problem of Potassium bromate started with ozonation of drinking water to form bromate as a major by product (WHO, 1993). When research was done to confirm the safety of ozonated water, it was found that Potassium bromate causes renal cancer in rats when they drank water with Potassium bromate.

Potassium bromate is not allowed as an additive in packaged drinking water but its permissible limit as a contaminant has been fixed because its traces are found in ground water or when water undergoes treatment. It is also found in groundwater due to cross penetration of salt water when water source is close to sea or industrial effluent facilities. Toxicity of Potassium bromate has been reported in experimental animals. Mark (1988) reported that the lethal oral doses of KBrO3 in human is estimated to be 154-385 mg/kg body weight, while serious poisoning results at doses of 46-92 mg/kg bodyweight. In another study performed by Khan et al., (2003) in rats treated with 125 mg/kg bodyweight KBrO3 intra-peritoneally, the results showed marked increase in the level of blood urea nitrogen, serum creatinine, reduction of anti-oxidant enzymes, enhanced xanthine oxidase and lipid peroxidation. The carcinogenic and mutagenic effects of KBrO3 have also been reported in experimental animals (Ishidate et al., 1984 and Kurokawa et al., 1987).
Histopathological damage to the different parts of the kidney is mainly due to the presence of free radicals, generated because of the oxidative stress induced by Potassium bromate. The pars rectum of the proximal convoluted tubule is the segment most sensitive to oxidative stress and hence most affected. These degenerative changes in the proximal convoluted tubules reinforce the view of Koechel et al., (1984) and Damjanov (1996) who found that many chemicals have a direct nephrotoxic action and exerted their effects principally on the proximal convoluted tubule.

The aim of the present study is to estimate the renal toxicity of the food additive potassium bromate on the freshwater fish Labio rohita. To determine the LC50 concentration of Potassium bromate in fish, the enzyme level of Aspartate Transaminase (AST) and Alkaline Transaminase (ALT) which are functional markers of kidney, urea and creatinine level of the serum for ascertaining the function of the kidney and to study the histopathological changes in the kidney.

MATERIALS AND METHODS

The fish was collected from Aliyar dam, near Pollachi, Coimbatore District, Tamil Nadu. They were transported in polythene bags which were oxygenated. The fingerling ranged from 10cm to 12cm and weighed about 10-12g. They were acclimatized in cement tanks in the laboratory for 2 weeks. The fishes were stocked in five plastic tubs, containing 14 litres of water. Each tub was provided with different concentrations of Potassium bromate, 700mg/l, 900mg/l, 1000mg/l, 1300mg/l, and 1500mg/l for 96 hours. Ten fishes were stocked in each tub and mortality was recorded after 24 hours, 48 hours, 72 hours and 96 hours. The LC 50 value was determined by Probit Analysis Method (Finney 1971).

The fishes were randomly divided into 5 groups, 12 fishes in each group. Potassium bromate of various concentrations, 100mg/l, 130mg/l, 160mg/l, 190mg/l were taken in four tubs and one tub was kept as control.

CALCULATION

Concentration of pyruvate in test (µg per litre) = \( \frac{\text{O.D Test}}{\text{O.D Standard}} \times \frac{\text{Concentration of Standard in µg}}{\text{Volume of Sample in ml}} \times 1000 \)

UREA (Monica, 1992)

The prepared sera and reagent were placed at room temperature. The colour intensity was measured using spectrophotometer at wave length 600 nm and urea concentration was calculated as follows:

\[
\text{Urea (mg/dl)} = \frac{\text{Tested sample}}{\text{Standard sample}} \times 50
\]

Where 50 is the standard concentration

The fishes were fed twice a day with the common fish farm food. The water was changed daily in order to prevent contamination and occurrence of pathogens. They were maintained for 30 days.

SGOT (Reitman and Francle, 1957)

For the estimation of SGOT, two test tubes were taken and marked as Control (C) and Test (T). To the ‘Control’ and ‘Test’ tubes 0.125ml of sample was added, mixed well and incubated at 37°C for 5 minutes. To both the test tube 0.025ml of Reagent -2 was added, mixed well and allowed to stand at room temperature for 20 minutes. Finally 1.25ml of solution -1 was added to both the test tubes, mixed well and allowed to stand at room temperature. The values of ‘Control’ and ‘Test’ were measured against distilled water on Spectronic-20 D + at 505nm.

CALCULATION

Concentration of oxaloacetate in test (µg) per litre

\[
= \frac{\text{O.D Test}}{\text{O.D Standard}} \times \frac{\text{Concentration of Standard in µg}}{\text{Volume of Sample in ml}} \times 1000
\]

SGPT (Reitman and Francle, 1957)

For the estimation of SGPT, two test tubes were taken and marked as Control (C) and Test (T). To the ‘Control’ and ‘Test’ tubes 0.125ml of Reagent-1 was added and incubated at 37°C for 5 minutes. Then 0.025ml of sample was added, mixed well and incubated at 37°C for 60 minutes. To both the test tubes 0.025 ml Reagent-2 was added, mixed well and allowed to stand at room temperature for 20 minutes. Then 1.25ml of solution 1 was added to both the test tubes, mixed well and allowed to stand at room temperature for 10 minutes. The OD values of ‘control’ and ‘test’ were measured against distilled water on spectronic-20 D+ at 505nm.

CREATININE (Monica, 1992)

The prepared sera and reagent were mixed and placed at 37°C. The absorbance \(A\) of the sample and standard were read at 510 nm after 30 seconds \(A_1\), and after 90 second later \(A_2\), and creatinine concentration was calculated as follow:

\[
\text{Creatinine (mg/dl)} = \frac{A \text{ sample}}{A \text{ standard}} \times 2
\]

Where 2 is the standard concentration.

\[
A = A_2 - A_1
\]
HISTOPATHOLOGY
After the experimental period, fishes were killed, the liver was excised, made into pieces and fixed in a fixative (10% buffered formalin) transformed into specimen bottles. After proper dehydration by graded alcohol, they were embedded in paraffin wax and thin sections of 4-5µm thick were made with the help of Rotator Microtome. It was then stained by Hematoxylin and Eosin and examined microscopically (Bancroft et al., 1996).

STATISTICAL ANALYSIS
Mean, Standard deviation, one way ANOVA and DMRT was done for enzyme studies.

RESULTS AND DISCUSSION
LC50
Potassium bromate poses toxic effect on *labeo rohita* which is evident by the findings of the present investigation and the calculated LC50 value (Figure1) (Table 1). Fish mortality may have resulted by the absorption of Potassium bromate and greater activity of chemicals in the body of fishes. The exact cause of death due to Potassium bromate poisoning are multiple and depend mainly on time and concentration combination. Mortality is tabulated in Table 2.

ENZYME STUDY
SGOT (Serum Glutamate Oxaloacetate Transaminase)
The value of SGOT in the control is 250.60 ± 0.8878. A notable increase has been found in 160 mg/l Potassium bromate treated fish (474.30 ± 0.0559, P<0.01). Decrease in SGOT has been observed in 100 mg/l, 130mg/l, and 190mg/l Potassium bromate treated fishes (411.68 ± 0.3083, 314.62 ± 0.0723, 318.41 ± 0.0576) (Table 3) (Figure 2). The one way ANOVA is significant at 1% level (Table 4). DMRT for control and experimental shows significance at 5% level (250.600, 314.62 ± 0.0723, 318.41 ± 0.0576) (Table 3). STABILIZATION and Eosin and examined microscopically (Ban

Wolf et al., (1972) stated that damage to liver cell will result in elevation of SGOT in the serum. Williamson et al., (1996) observed that serum lipid profile was elevated along with increase in marker enzyme concentration viz SGOT. Necrosis or membrane damage release the enzyme SGOT into circulation. Estimation of these enzymes reveal the extent and type of cellular damage.

SGPT (Serum Glutamate Pyruvate Transaminase)
The control value for SGPT is 0.1 ± 0.002. A notable increase has been found in 160 mg/l treated fish (99.12 ± 0.286, P<0.01), decrease in SGPT has been observed in 100 mg/l, 130mg/l and 190 mg/l Potassium bromate treated fishes (72.4 ± 0.356, 60.55 ± 0.268, and 50.69 ± 0.276) (Table 3) (Figure 3). The one way ANOVA is significant at 1% level (Table 4). DMRT shows significant results at 5% level (0.0966, 72.4520, 60.5520, 99.1200, and 50.6940).

Kurokawa et al., (1990) and Omer et al., (2008) reported an increase in SGPT in rat. Degeneration of endothelial cells observed in rats administered with Potassium bromate may be an indication of the destruction of the capillary endothelium of the liver by the chemical substance. This results in reduction in total protein and albumin synthesis and increase in SGPT which are consequent with hepatic cell damage and injured cell membrane permeability.

Urea
The value for urea in the control is 4.47 ± 0.040. A significant increase has been observed in 160mg/l Potassium bromate treated fishes (5.68 ± 0.030, P<0.01). Significant decrease has been observed in 100mg/l, 130mg/l and 190mg/l treated fishes (3.24 ± 0.072, 2.74 ± 0.045 and 1.02 ± 0.038) (Table 3) (Figure 4). The one way ANOVA is significant at 1% level (Table 4). DMRT is significant at 5% level (42.4652, 3.2430, 2.7350, 5.6772 and 1.0244) (Table 5).

According to Khan et al., (2003) the elevation in urea indicates its adverse effect on kidney functions. Hanley et al., (1986) found that increase of serum enzyme leads to tissue damage. The increase in serum level of urea is the indication of renal toxicity. De Angelo et al., (1998), Akanji et al., (2003) revealed that in general, increase in urea level is associated with nephritis, renal ischemia, urinary tract obstruction and certain extra renal diseases.

The observed nephrotoxity in the present study is similar to the above observations. According to Giri et al., (1999) Potassium bromate induces renal proliferative response and damage by elaborating oxidative stress.

Creatinine
The creatinine of control is 0.16 ± 0.022. Decrease in creatinine is observed in 100mg/l, 130mg/l, 160mg/l and 190mg/l Potassium bromate treated fishes (0.08 ± 0.009, 0.06 ± 0.008, 0.03 ± 0.006 and 0.08 ± 0.007) (Table 3) (Figure 5) respectively. At 1% level the one way ANOVA for creatinine between control and treatment is significant (Table 4). The DMRT result is significant between the control and 130mg/l and 160mg/l treated fishes at 5% level (0.1558, 0.0604 and 0.0314). 100 mg/l and 190mg/l treated fishes are not significant (0.0816 and 0.0814) (Table 5).

Copeland (2015) revealed that low muscle mass and advanced liver diseases is a leading cause of a low creatinine level, severe malnutrition that leads to muscle loss also cause low creatinine level. The liver is a primary site for protein manufacture and breakdown in the body. If the liver is not functioning well, proteins are not made or broken effectively, potentially causing low creatinine level.

HISTOPATHOLOGY
The histopathological examination of kidney at the end of 30 days of treatment with 100mg/l, 130mg/l, 160mg/l and 190mg/l of Potassium bromate is compared with that
of the control kidney. In the kidney of control fish, the section shows kidney tissues with normal glomeruli (Fig 6, 7). Treatment with 100mg/l Potassium bromate shows kidney with normal glomeruli, congested tubules, interstitial inflammation composed of lymphocytes and few thick walled blood vessels (Fig 8, 9).

130mg/l treated fish shows shrunken glomeruli, congested tubules, interstitial inflammation and dilated blood vessels (Fig 10, 11). Treatment with 160 mg/l of Potassium bromate shows kidney with few normal congested glomeruli, congested tubules, interstitial inflammation and dilated blood vessels (Fig 12, 13). In the 190mg/l KBrO₃ treated fish, in addition to the above changes, the kidney show normal glomeruli, congested tubules, increased interstitial inflammation and dilated blood vessel (Fig 14, 15).

Kurokawa et al., (1990), Kitto and Dumars (1949) reported that the renal damage include direct tubular toxicity due to induction of active oxygen radicals. Niwa et al., (1974), Kuwahara et al., (1984) and Hamada et al., (1990) found that in the chronic phase, the changes are specific with either unchanged or sclerotic glomeruli, marked interstitial fibrosis and tubular atrophy.

Joseph et al., 1990, Kitto and Dumars (1949) also found that the proximal convoluted tubules are the most affected.

Koechel et al., (1984) and Damjanov (1996) found that the proximal convoluted tubules reinforce that many chemicals have a direct nephrotoxic action and exert their effects principally on the proximal convoluted tubules.

Table 1: Percentage (%) Mortality in Labeo rohita treated with different concentrations of Potassium bromate.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>NO. OF FISH</th>
<th>TOXICANT CONCENTRATION IN mg/l</th>
<th>MORTALITY IN TEST ANIMALS 96 HOURS %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>700</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>900</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>1000</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>1300</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>1500</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 2: LC 50 value of Potassium bromate and the 95% confidence limit in Labeo Rohita

<table>
<thead>
<tr>
<th>LC50 (Log Concentration)</th>
<th>95% confidence Lower limit</th>
<th>Upper limit</th>
<th>Probit equation</th>
<th>Chi-square</th>
</tr>
</thead>
</table>

Table 3: Enzyme level in the blood of freshwater fish Labeo rohita treated with various concentration of Potassium bromate.

<table>
<thead>
<tr>
<th>TEST</th>
<th>SGOT</th>
<th>SGPT</th>
<th>UREA</th>
<th>CREATININE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>250.60±0.3873**</td>
<td>0.1±0.002**</td>
<td>4.47±0.040**</td>
<td>0.16±0.022**</td>
</tr>
<tr>
<td>A (100mg/l)</td>
<td>411.68±0.3083**</td>
<td>72.45±0.356**</td>
<td>3.24±0.072**</td>
<td>0.08±0.009**</td>
</tr>
<tr>
<td>B (130mg/l)</td>
<td>314.62±0.0723**</td>
<td>60.55±0.268**</td>
<td>2.74±0.045**</td>
<td>0.06±0.008**</td>
</tr>
<tr>
<td>C (160mg/l)</td>
<td>474.30±0.0559**</td>
<td>99.12±0.286**</td>
<td>5.68±0.030**</td>
<td>0.03±0.006**</td>
</tr>
<tr>
<td>D (190mg/l)</td>
<td>318.41±0.0576**</td>
<td>50.69±0.276**</td>
<td>1.02±0.038**</td>
<td>0.08±0.007**</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation of the samples in each group; **-significant at p < 0.01.

Table 4: One way ANOVA for the enzyme analysis of the serum of freshwater fish Labeo rohita treated with various concentration of Potassium bromate.

<table>
<thead>
<tr>
<th>ENZYME</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>PROB</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGPT</td>
<td>4</td>
<td>26511.807093</td>
<td>6627.951773</td>
<td>3.14456.3980</td>
<td>0.000**</td>
<td>0.26</td>
</tr>
<tr>
<td>SGOT</td>
<td>4</td>
<td>156544.287677</td>
<td>39135.571694</td>
<td>147270.51791</td>
<td>0.000**</td>
<td>0.05</td>
</tr>
<tr>
<td>UREA</td>
<td>4</td>
<td>612.132234</td>
<td>15.533059</td>
<td>43250.7060</td>
<td>0.00**</td>
<td>0.55</td>
</tr>
<tr>
<td>CREATININE</td>
<td>4</td>
<td>0.042369</td>
<td>0.010592</td>
<td>228.1094</td>
<td>0.000**</td>
<td>8.30</td>
</tr>
</tbody>
</table>

df - Degrees of freedom; SS - Sum of Squares; MS - Mean Square; F - F-test; P-Probability; CV – Coefficient of Variation; ** - Significant at P < 0.01 level.

Table 5: DMRT for the enzyme analysis of the blood of fresh water fish Labeo rohita treated with various concentration of Potassium bromate.

<table>
<thead>
<tr>
<th>TEST</th>
<th>CONTROL</th>
<th>A (100mg/l)</th>
<th>B (130mg/l)</th>
<th>C (160mg/l)</th>
<th>D (190mg/l)</th>
<th>MEAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGOT</td>
<td>250.60°</td>
<td>411.67°</td>
<td>314.61°</td>
<td>474.30°</td>
<td>318.41°</td>
<td>353.922</td>
</tr>
<tr>
<td>SGPT</td>
<td>0.0966°</td>
<td>72.45°</td>
<td>60.55°</td>
<td>99.12°</td>
<td>50.69°</td>
<td>56.5829</td>
</tr>
<tr>
<td>UREA</td>
<td>4.4652°</td>
<td>3.2430°</td>
<td>2.735°</td>
<td>5.6772°</td>
<td>1.0244°</td>
<td>3.4290</td>
</tr>
<tr>
<td>CREATININE</td>
<td>0.1558°</td>
<td>0.0816°</td>
<td>0.0604°</td>
<td>0.0314°</td>
<td>0.0814°</td>
<td>0.0821</td>
</tr>
</tbody>
</table>
Means followed by a common letter are not significantly different at the 5% level by DMRT.

Figure 1: Regression graph showing LC50 for fishes treated with different concentrations of Potassium bromate.

Figure 2: Effect of Potassium bromate on SGOT level of the freshwater fish, *Labeo Rohita*.

Figure 3: Effect of Potassium bromate on SGPT level of the freshwater fish, *Labeo rohita*.
Figure 4: Effect of Potassium bromate on the Urea level of the freshwater fish, *Labeo rohita*.

Figure 5: Effect of Potassium bromate on the Creatinine level of the freshwater fish, *Labeo rohita*.

Figure 6: Kidney of Control Fish (HE x100) HH(HE×100) (HE×100) (HE×100)
Section shows kidney tissue with normal glomeruli.

Figure 7: Kidney of Control Fish (HE×400)

Fig 8: Kidney of fish treated with 100 mg/l of KBrO₃ (HE×100)

Fig 9: Kidney of fish treated with 100 mg/l of KBrO₃ (HE×400)

Section shows kidney with normal glomeruli, congested tubules, interstitial inflammation composed of lymphocytes and few thick walled blood vessels.

Fig 10: Kidney of fish treated with 130 mg/l of KBrO₃ (HE×100)

Fig 11: Kidney of fish treated with 130 mg/l of KBrO₃ (HE×400)

Section shows kidney with shrunken glomeruli, congested tubules, interstitial inflammation and dilated blood vessels.
Section shows kidney with few normal congested glomeruli, congested tubules, interstitial inflammation and dilated blood vessels.

Section shows kidney with few normal glomeruli, congested tubules, increased interstitial inflammation and dilated blood vessels.

CONCLUSION
The fact that the use of Potassium bromate as a food additive in the manufacturing of bread is proven to be hazardous for human health. It has to be avoided from consumption as some of the dangers it poses are disruption of thyroid function. It slows neural and cognitive development, causes skin disorders, DNA damage, proves to be toxic to kidney and is potentially carcinogenic. Strict regulation on the incessant and illegal use of this lethal chemical agent is mandatory by the support of Health and Food Safety Committees.

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REFERENCES


