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

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ABSTRACT

Potassium bromate is a white crystalline salt of bromate ion and is soluble in water. Bromate is not an inherent component of water but traces of bromate can be found in drinking water. It is found in groundwater due to cross penetration of salt water when water source is close to sea or industrial effluent facilities. It is generated due to the conversion of bromide into bromate, containing raw waters that undergo ozonization and chlorination. The main aim of this study is to evaluate the toxic effects of Potassium bromate on the highly palatable freshwater fish *Labeo rohita.* LC50 for 96 hrs was determined by Probit analysis method. Sub-chronic doses of $KBrO_3$ such as 100mg/l, 130mg/l, 160mg/l, 190mg/l was taken for study. The experimental set up was maintained for 30 days. Enzymes such as Alkaline phosphatase, Lactate dehydrogenase, Serum Glutamate Oxaloacetate Transaminase and Serum Glutamate Pyruvate Transaminase were estimated and the histopathology of the liver was studied. It is evident from the present study that fishes exposed to potassium bromate are found to have damaged liver tissues and also increased serum enzyme levels.

KEYWORDS: Potassium bromate, Alkaline phosphotase, Lactate dehydrogenase, Serum Glutamate Oxaloacetate Transaminase, Serum Glutamate Pyruvate Transaminase, Probit analysis.

INTRODUCTION

Potassium bromate [KBrO3, CAS No: 7758-01-2, molecular weight: 167.01; density: 3.27] is a white crystalline salt of bromate ion and is soluble in water. It decomposes at about 370ºC and has a melting point of 350ºC. Bromate is not an inherent component of water but traces of bromate can be found in drinking water. It is found in groundwater due to cross penetration of salt water when water source is close to sea or industrial effluent facilities. It is generated due to the conversion of bromide into bromate, containing raw waters that undergo ozonization and chlorination and is frequently detected in tap water and bottled water. The rate of formation of bromate ion may increase with temperature (Siddiqui and Amy, 1993).

Bromate is currently regulated in treated drinking water at a maximum containment level of 10µg/l in the U.S.A. and Europe. Potassium salt of bromate is classified as a category I group 2B carcinogen (possibly carcinogenic to humans) by the International Agency for Research on Cancer. The Government of India on June 21st, 2016 banned the use of Potassium bromate as a food additive following a Centre for Science and Environment (CSE) study that found its presence in the bread caused cancer.

Laba, (2003) studied that Potassium bromate has harmful effects on the nutritional qualities of bread by lowering vitamins A1, B1, B2, E and niacin, the main vitamins in bread. Ueno *et al.,* (2000) and K.C., (2006) found bromate in some drinking water samples as by-product of ozone disinfection. Norris (1965), Paul (1966) and Stewat (1969) observed degeneration of kidney tubules and liver parenchymal cells and acute myocarditis due to acute human intoxication by accidental ingestion. Okolie and Ikewuchi (2004) indicated that Potassium bromate induced oxidative stress on some cataractogenic indices in lens, cornea and retina of white rabbits.

The subchronic effects of bromate were evaluated by Kurokawa *et al.,* (1990) who observed significant inhibition of body weight in males. Significant increase of serum parameters glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, alanine transaminase, lactate dehydrogenase, alkaline phosphatase, blood urea nitrogen, Na+ and cholinesterase was observed in both sexes. Khan *et al.,* (2003) studied that intake of Potassium bromate or exposure to it causes production of oxygen free species in living cells. Robert William (1996), corroborated that Potassium bromate is extremely irritating and injurious

to tissues especially those of the central nervous system and kidney.

Aquatic pollution, through discharge of agricultural pesticides, domestic wastes, trade effluents and oil spills has very adversely affected aquaculture. The environmental chemical toxins are many, and can exert their effects indiscriminately on both man and animals. There is an every chance of accumulation of these toxic substances in the cultured fishes which is going to affect the human health on consumption.

Thus the main aim of this study is to evaluate the toxic effects of Potassium bromate on the highly palatable freshwater fish *Labeo rohita*, on the enzymes and liver of the experimental animal. Extensive study has been made on mammals and limited literature is available on the impacts of bromate on the fresh water organisms.

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 10 to 12cm and weighed about 10-12g. They were acclimatized in cement tanks in the laboratory for 2 weeks. They were fed with the normal fish farm food consisting of oil cake and wheat flour. The tanks were maintained clean and neat to avoid contamination.

LC 50

The fishes were stocked in five plastic tubs, containing 14 liters of water. Each tub was provided with different concentrations of Potassium bromate viz., 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, 50% mortality was observed in 1000mg/l concentration of $KBrO_3$. The LC 50 value was found by Probit Analysis Method (Finney, 1971).

Sub-Chronic Toxicity Test

Sub-chronic doses of $KBrO_3$ such as 100mg/l, 130mg/l, 160mg/l, 190mg/l was taken in four tubs. Each tub was stocked with 12 fishes and one tub served as control. 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. The experimental set up was maintained for 30 days.

Enzyme Study

After 30 days of treatment, blood was drawn using a syringe by direct heart puncture for enzyme studies. The blood was collected in vials containing anti coagulant.

Alkaline phosphatase

The procedure described by Bassey *et al.*, (1946), modified by Wright *et al.*, (1972), using Randox kits was used for the assay. In a cuvette, 10 µl of sample was mixed with 500 µl of the reagent. The initial absorbance was read at 405 nm and subsequently over 3 minutes. The mean absorbance per minute was used in the calculation:

ALP activity (IU/l) = 2742 × ΔA 405 nm/min.

Where: 2742 = Extinction coefficient;
ΔA 405 nm/min = change in absorbance per minute for the homogenate sample.

Lactate dehydrogenase

The quantitative analysis of lactate dehydrogenase was assayed according to the method described by King (1965b). One ml of buffered substrate and 0.1 ml of enzyme extract was added and the tubes were incubated at 37ºC for 5 minutes. After the incubation period, 0.2 ml of NAD+ solution was added to the test and 0.2 ml of distilled water to the control and the incubation was continued for another 15 minutes. The reaction was then arrested by the addition of 1 ml of DNPH reagent and the tubes were incubated for a further period of 15 minutes at 37ºC. After the last incubation period, 7 ml of 0.4 N NaOH solution was added and the colour development was measured at 420 nm in a UV spectrophotometer.

SGOT (AST)

SGOT activity was estimated by 2,4-DNPH method (Reitman and Francle,1957) using diagnostic Reagent kit. 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 37C for 5 minutes. Then 0.025ml of sample was added, mixed well and incubated at 37C for 60 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 tube , mixed well and allowed values of 'Control' and 'Test' were measured against distilled water on Spectronic-20 D + at 505nm.

Calculation

$$\text{Concentration of oxaloacetate in test (µg) per litre} = \frac{\text{O.D T}\times\text{st}}{\text{O.D of Standard}} \times \frac{\text{concentration of standard in µg}}{\text{Volume of sample in ml}} \times 1000$$

SGPT (ALT)

SGPT activity was estimated by 2,4-DNPH method (Reitman and Francle,1957) using diagnostic Reagent Kit. For the estimation of SGPT, 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 37ºc for 5 minutes. Then 0.025ml of sample was added, mixed well and incubated at 37ºc 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.

Calculation:

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

Histopathology

After the experiment, fishes were killed; the liver was excised, cut into pieces and fixed in 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 taken 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 pose toxic effect on *Labeo rohita* which is evident by the findings of the present investigation and the calculated LC50 value observed for 96 hours was found to be 1000mg/l (Table 1). Fish mortality may have resulted by the absorption of Potassium bromate and greater activity of chemicals in the body of fishes. Mortality is tabulated in Table 1. The LC50 graph with regression equation is depicted in Table 2 and Figure 1.

Enzyme study

Serum Glutamic Oxaloacetic Transaminase

The mean of Serum Glutamic Oxaloacetic Transaminase (SGOT) has increased in all the treatments, 100mg/l, 130mg/l, 160mg/l and 190mg/l $KBrO_3$ treated fish (411.68 ± 0.3083, 314.62 ± 0.0723, 474.80 ± 0.0559, 318.41 ± 0.0576 P<0.01) (Table 3 and Figure 2) in comparison with the control (250.60 ± 0.3873 P<0.01). One way ANOVA (Table 4) is significant at 1% level. DMRT is significant at 5% level (Table 5).

Serum Glutamate Pyruvate Transaminase

The biomarker enzyme Serum Glutamate Pyruvate Transaminase (SGPT) showed a very significant increase in all the treatments, 100mg/l, 130mg/l, 160mg/l and 190mg/l $KBrO_3$ treated fish (72.45 ± 0.356, 60.55 ± 0.268, 99.12 ± 0.286, 50.69 ± 0.276 P<0.01) when compared to control (0.1 ± 0.002) (Table 3 and Figure 3). One way ANOVA is significant at 1% level (Table 4) and DMRT is significant at 5% level (Table 5).

Lactate Dehydrogenase

Lactate dehydrogenase in the control is 2957.20 ± 3.834. A significant increase has been observed in 100mg/l treated fish (3140.6 ± 3.209, P < 0.01). A significant decrease has been observed in 130mg/l, 160mg/l and 190mg/l $KBrO_3$ treated fish (1440.90 ± 2.702, 2216.50 ± 2.500, 1414.20 ± 3.094 P<0.01) (Table 3 and Figure 4). One way ANOVA in all the treated samples is significant at 1% level (Table 4). DMRT is significant at 5% level (Table 5).

Alkaline Phosphatase

The marker enzyme alkaline phosphatase in the control is 0.09± 0.003. In the various concentration of 100mg/l, 130mg/l, 160mg/l and 190mg/l $KBrO_3$ treated fishes, a significant increase was recorded as 0.88 ± 0.062, 0.82 ± 0.031, 0.79 ± 0.030 and 2.32 ± 0.058, P<0.01 respectively (Table 3 and Figure 5). The highest value was found in fishes treated with 190mg/l of $KBrO_3$. One way ANOVA showed significant results at 1% level in all treatments (Table 4). The DMRT result is significant at 5% level. DMRT for 130mg/l and 160mg/l treated fishes are not significant (Table 5).

Histopathology

The histopathological examination of liver 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 liver. The control liver shows normal liver architecture with normal portal triad, dilated bile ducts and sinusoids (Figure 6 and 7).

Treatment with 100mg/l $KBrO_3$ fish tissue showed congestion, central vein dilatation, congestion of sinusoid and diffuse lymphocytic infiltrate in the parenchyma with bile stasis (Figure 8 and 9).

130mg/l of Potassium bromate treated fishes show liver tissue with congestion, bile stasis, loss of hepatocytes and architecture, central vein dilatation and ballooning degeneration (Figure 10 and 11).

Treatment with 160mg/l of $KBrO_3$ show focal dilatation of bile ducts and widening of sinusoids (Figure 12 and 13), while in 190mg/l $KBrO_3$ treated fish in additional to the above observations bile duct proliferation and diffuse mononuclear cell infiltrate in the entire parenchyma is seen (Figure 14 and 15).

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

S.NO	NO. OF FISH	TOXICANT CONCENTRATION IN mg/l	MORTALITY IN TEST ANIMALS	
			96 HOURS	%
1	10	700	0	0
2	10	900	3	30
3	10	1000	5	50
4	10	1300	8	80
5	10	1500	10	100

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

LC50 (Log Concentration)	95% confidence		Probit equation	Chi-square
	Lower limit	Upper limit		
3.007083	2.935025	3.07914	Y= -24.5005 + 9.810188	8.623268

Table 3: Enzyme level of the blood of freshwater fish *Labeo rohita* treated with various concentration of $KBrO_3$

SAMPLE	SGOT (U/L)	SGPT (U/L)	LDH (U/L)	ALP (U/L)
CONTROL	250.60±0.3873**	0.1.±0.002**	2957.20±3.834**	0.09±0.003**
A (100mg/l)	411.68±0.3083**	72.45±0.356**	3140.60±3.209**	0.88±0.062**
B (130mg/l)	314.62±0.0723**	60.55±0.268**	1440.90±2.702**	0.82±0.031**
C (160mg/l)	474.30±0.0559**	99.12±0.286**	2216.50±2.500**	0.79±0.030**
D (190mg/l)	318.41±0.0576**	50.69±0.276**	1415.20±3.094**	2.32±0.058**

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 on the blood of freshwater fish *Labeo rohita* treated with various concentration of $KBrO_3$

SAMPLE	df	SS	MS	F	P	CV%
SGOT	4	156542.286776	39135.571694	1472701.5791	0.000**	0.05
SGPT	4	26511.807093	6627.951773	314456.3980	0.000**	0.26
LDH	4	13223445.340000	3305861.335000	7387399.6350	0.000**	0.03
ALP	4	13.204158	3.301040	3778.1651	0.000**	3.02

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 freshwater fish *Labeo rohita* treated with various concentrations of $KBrO_3$

TEST	CONTROL	A(100mg/l)	B(130mg/l)	C(160mg/l)	D(190mg/l)	MEAN
SGOT	250.600^{e}	411.678^{b}	314.616^{d}	474.302^{a}	318.412^{c}	353.922
SGPT	0.0966^{e}	72.4520^{b}	60.5520^{c}	99.1200^{a}	50.6940^{d}	56.5829
LDH	2957.20^{b}	3140.60^{a}	1440.90^{d}	2216.50^{c}	1415.20^{e}	2234.08
ALP	0.0942^{d}	0.8780^{b}	0.8190^{c}	0.7930^{c}	2.3160^{a}	0.9800

Means followed by a common letter are not significantly different at the 5% level by DMRT.

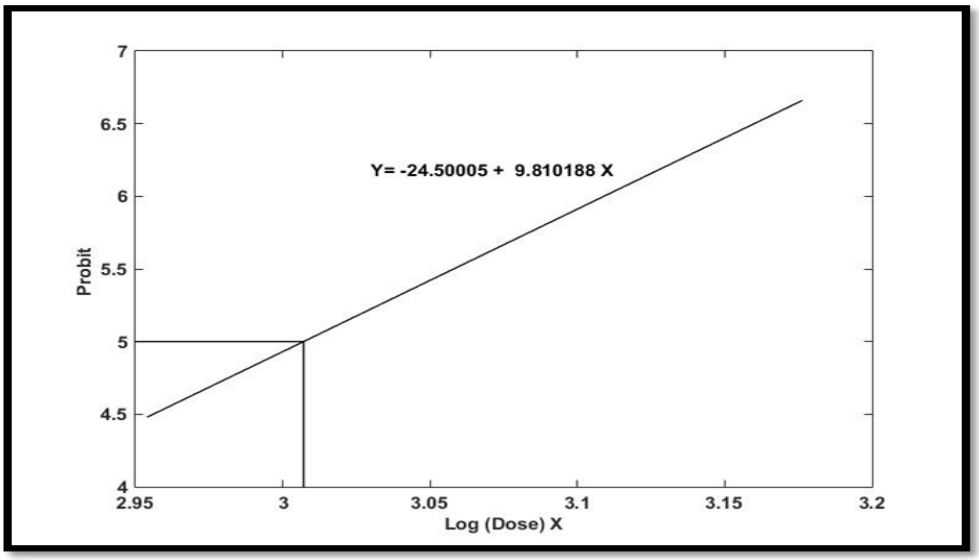


Figure 1: Regression graph showing LC50 for fishes treated with different concentrations of Potassium bromate.

Fig 2: Effect of Potassium bromate on SGOT of the freshwater fish *Labeo rohita*

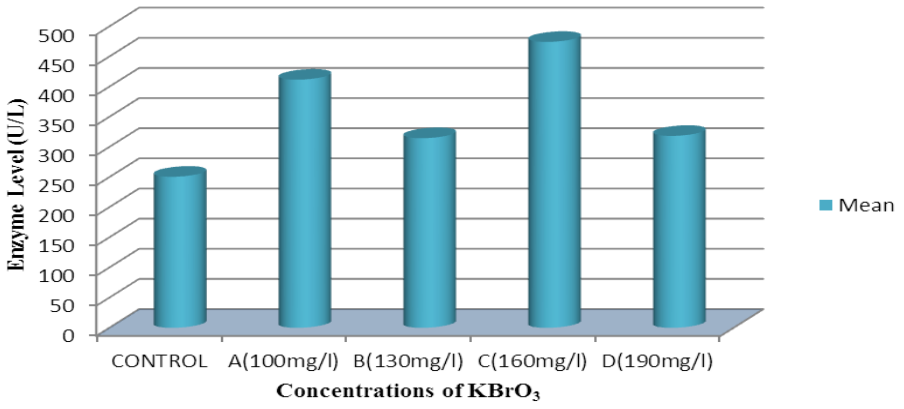


Fig 3: Effect of Potassium bromate on SGPT of the freshwater fish *Labeo rohita*

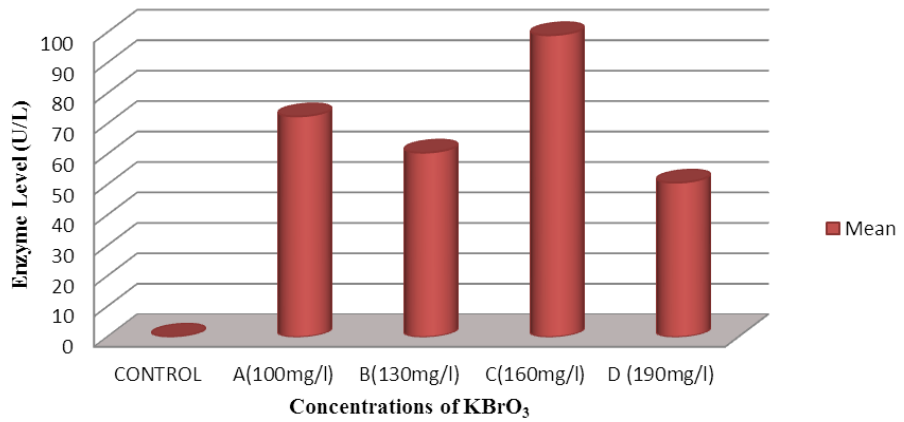
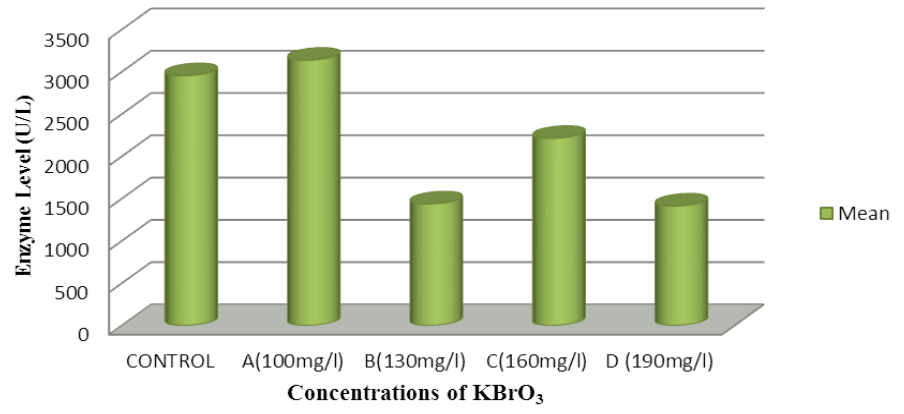
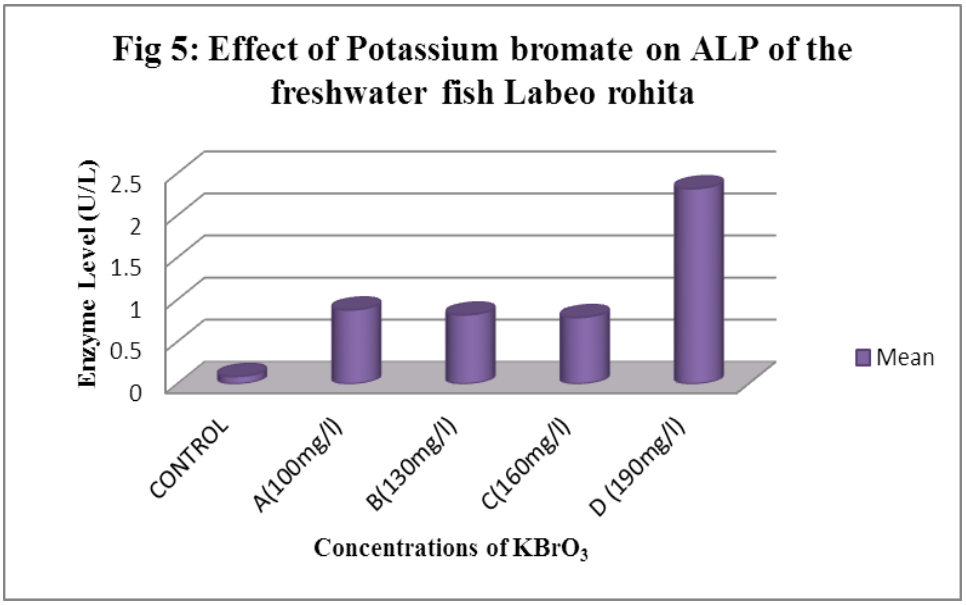


Fig 4: Effect of Potassium bromate on LDH of the freshwater fish Labeo rohita





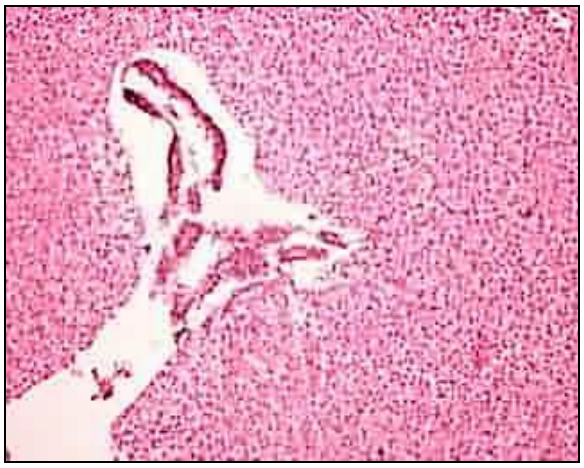


Fig 6: Liver of control fish (HE × 100).

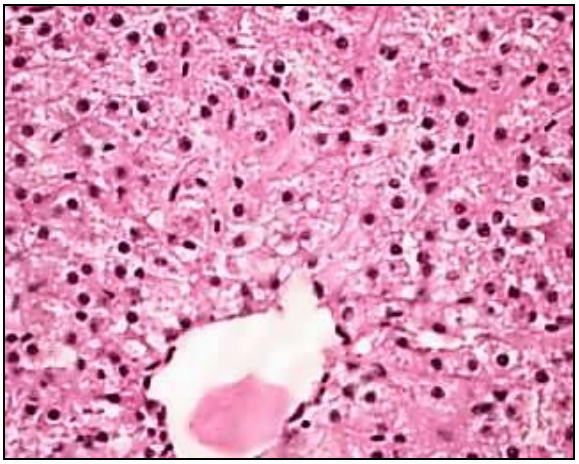


Fig 7: Liver of control fish (HE × 400)

Figure 6 and 7: Section shows normal liver architecture with normal portal triad, dilated bile ducts and sinusoids.

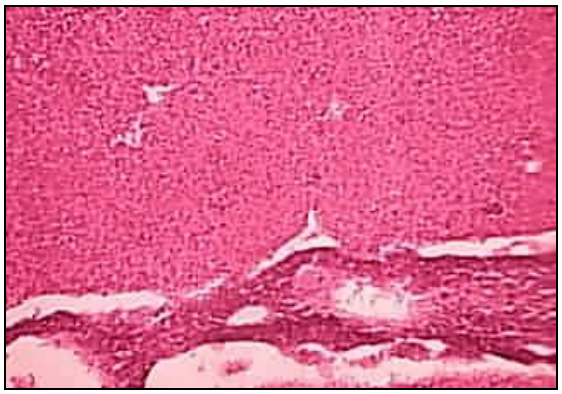


Fig 8: Liver of the fish treated with 100mg/l of $KBrO_3$ (HE x 100).

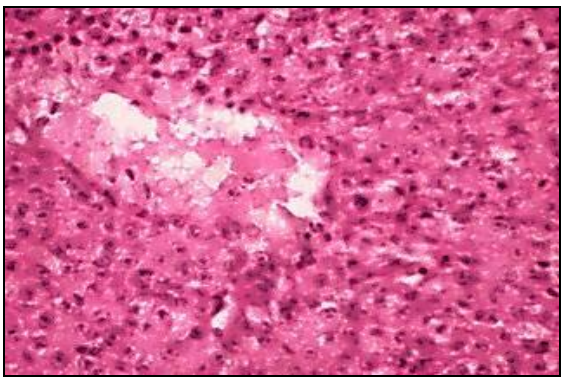


Fig 9: Liver of the fish treated with 100mg/l of $KBrO_3$ (HE x 400).

Figure 8 and 9: Section shows liver tissue with congestion, central vein dilatation, congestion of sinusoid and diffuse lymphocytic infiltrate in the parenchyma with bile stasis.

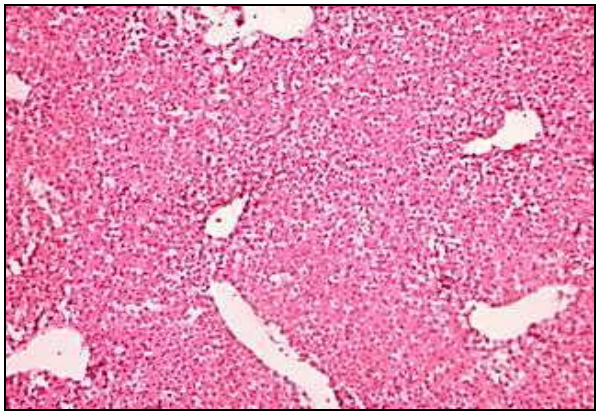


Fig 10: Liver of the fish treated with 130mg/l of $KBrO_3$ (HE x 100).

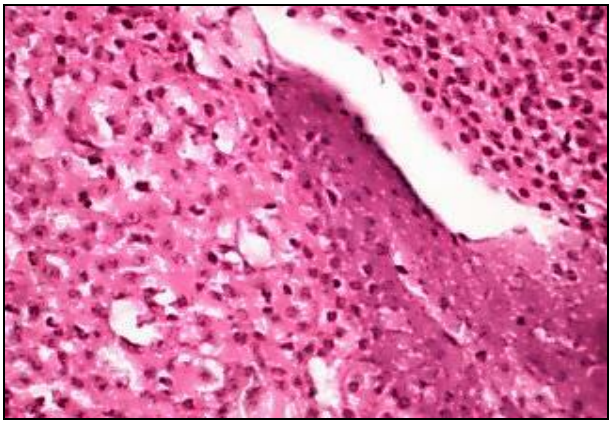


Fig 11: Liver of the fish treated with 130mg/l of $KBrO_3$ (HE x 400).

Figure 10 and 11: Section shows liver tissue with congestion, bile stasis, loss of hepatocytes and architecture, central vein dilatation and ballooning degeneration.

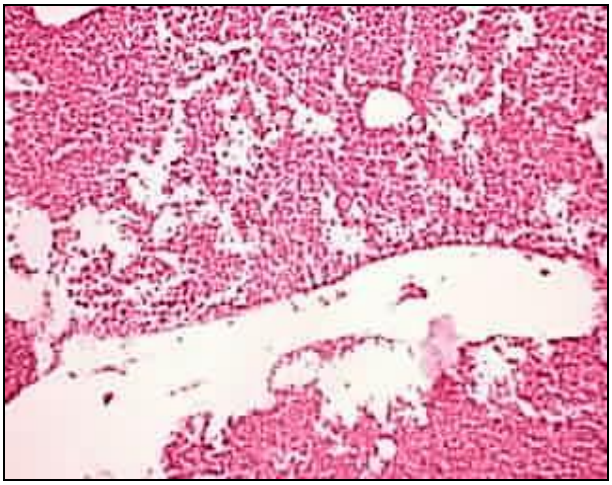


Fig 12: Liver of the fish treated with 160mg/l of $KBrO_3$ (HE x 100).

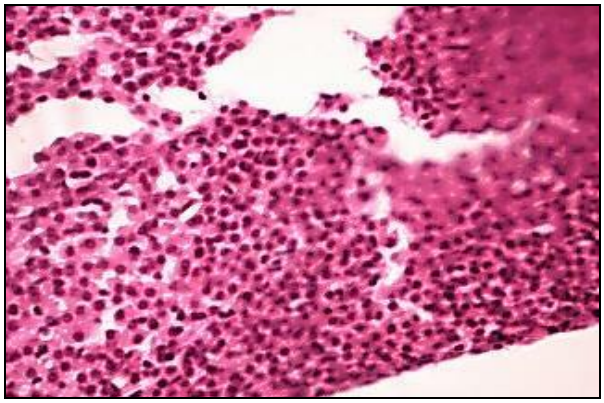


Fig 13: Liver of the fish treated with 160mg/l of $KBrO_3$ (HE x 400).

Figure 12 and 13: Section shows liver tissue with congestion, bile stasis, focal dilatation of bile ducts, widening of sinusoids and ballooning degeneration.

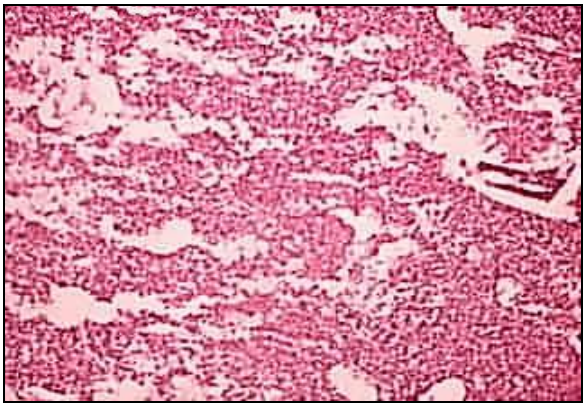


Fig 14: Liver of the fish treated with 190mg/l of $KBrO_3$ (HE x 100).

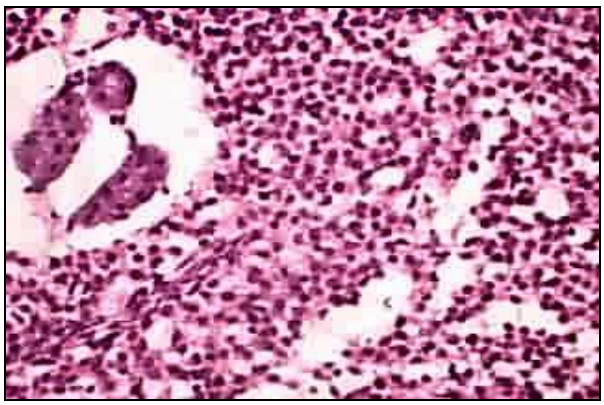


Fig 15: Liver of the fish treated with 190mg/l of $KBrO_3$ (HE x 400).

Figure 14 and 15: Section shows liver with loss of architecture, bile duct proliferation and diffuse mononuclear cell infiltrate in the entire parenchyma.

Oloyede *et al.,* (2009), Papiya Bigonia *et al.,* (2013) observed a significant increase in the SGOT level in serum of the rat. Rahman *et al.,* (2001) quoted SGOT to be a well-known biomarker enzyme to predict possible toxicity. Wolf *et al.,* (1972), Singh *et al.,* (2001) explained that the elevation of transaminase enzyme is

the prediction of damage and diseased condition of liver by the toxicants.

Studies revealed damage to the hepatocytes and liver necrosis due to reduction in transaminase activity (Abdel-Tawwab *et al.*, 2001 and Mousa and Khattab, 2003). Observation of Karmen *et al.*, (1995) found that changes in protein metabolism and enzyme inhibition action in the cells led to the reduction of SGOT activities in the liver and also leakage of enzyme into the serum which showed to be elevated in the serum. Willianson *et al.*, (1996) showed the loss of functional integrity of liver such as that of viral hepatitis, as well as cardiac infraction and muscle injury which indicates the high level of SGOT.

Willianson *et al.*, (1996), Abdel-Tawwab *et al.*, 2001 and Mousa and Khattab, 2003), Singh *et al.*, (2001) studied marker enzyme SGPT concentration to be elevated, the release of these enzymes occur along with necrosis or damage of the cell membrane. The conversion of alanine to pyruvate and glutamate is catalysed by SGPT which is released in the serum.

The change in protein metabolism and inhibition of enzyme may be reasons for the reduction of enzyme activity in the liver (Karmen *et al.*, 1995). Oloyede *et al.*, (2009) also observed a significant increase in the serum SGPT level and decreased activity in the liver tissue when compared to control. The actual elevated level of SGPT is due to the leakage of enzyme from the damaged tissues.

Akanji *et al.*, (2008) observed a significant increase in LDH in the kidney and small intestine in a single dose. The recovery period revealed reduction in LDH activities of all the tissue. They observed a concomitant increase in serum enzyme in the initial dose. Philip (1995) showed a significant loss of LDH due to its close proximity to the plasma membrane. Slight damage to the plasma membrane will lead to leakage of LDH from the cell interior to the extracellular environment (Akanji *et al.*, 1993).

The increase in the serum LDH activity at 100mg/l of Potassium bromate indicates the leakage of cytolic enzyme from the tissue into the serum due to a labialized plasma membrane (Akanji *et al.*, 2008).

Hanley *et al.*, (1986), Akanji *et al.*, (2008) and Papiya Bigoniya *et al.*, (2013) found elevated levels of Alkaline Phosphatase enzymes in the study. Oloyede *et al.*, (2009) observed a significant reduction in the activity of ALP in the liver tissues when compared to control liver. This reduction was due to leakage of enzymes into the serum. The observation was supported by Fleisher and Schwartz (1917) who found leakage of ALP due to cell membrane damage. Oloyede *et al.*, (2009) also reported that marker enzyme will not be usually found in the serum without any damage in the tissues or organ which secretes the enzyme.

Wright Plummer (1974) and Shahjahan *et al.*, (2004) reported Alkaline Phosphatase as a marker enzyme of the plasma membrane and endoplasmic reticulum. The loss of membrane components into the serum was observed by Malbia and Hart (1971) and insitu inactivation of the enzyme molecules was studied by Umezawa and Hooper (1982).

According to Akanji *et al.*, (1993), the detectable quantity of ALP in the serum may be due to the disruption of ordered lipid-bilayer caused by oxygen containing chemical compound which oxidize the lipid bilayer and disrupt that layer.

Dimkpa *et al.*, (2013) observed perisinusoidal fibrosis and centrilobular sinusoidal dilatation in the liver of rats, treated with $KBrO_3$. Omer *et al.*, (2008) and Khan *et al.*, (2003) studied liver cells with vaculation, sinusoidal dilation which is associated with enhancement of Xanthine oxidase and Lipid peroxidise and reduced antioxidant enzymes by $KBrO_3$. They also reported the destruction of the capillary endothelium of liver, degenerative changes and fenestration of endothelial cells. This results in the reduction of total protein, albumin synthesis and increase in alanine transaminase which is closely related with hepatic cell damage and injured cell membrane permeability.

Elmahdy *et al.*, (2015) examined the liver of albino rats and found clumped chromatin in a darkened and enlarged nuclei. The dilatation and proliferation of bile ducts, dilatation of hepatic sinusoids with kuffer cell hyperplasia were also noticed along with disrupted hepatic architecture, loss of shape, slightly vacuolated cytoplasm and haphazardly located nuclei. The presence of hypertropic and cytomegatic hepatocytes in centri lobular areas were also observed with oval cell proliferation in the lining epithelium of bile ducts and an infiltration of mononuclear cells in the portal revealed congested blood vessels.

CONCLUSION

The benefits of drinking water disinfection are well recognized, however an undesirable side effect is in the production of “disinfection by-products”. Potassium bromate is one such toxicant that is formed in water. On the other hand, fish is a vital source of food for people, providing approximately 16% of the animal protein consumed by the world’s population. It is clear from the present study that fishes exposed to potassium bromate are found to have damaged liver tissues and also increased serum enzyme levels.

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