Biochemical Effect of Potassium Bromate on Wistar Albino Rats

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Dedication

To

My Family

To

My Friends
Acknowledgement

I would like to express my special praise and thanks to Allah, who gave me the necessary health, strength and patience to conduct this work.

I am greatly indebted to my supervisor Dr. Afaf Izzeldin for the valuable advice, guidance, encouragement and patience throughout the work.

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ملخص الأطروحة

لذا، وُضعت بعض الإجراءات مثل ترطيب التغذية وزيادة الحركة البدنية، والتي أظهرت أن هذه الإجراءات تزيد من نسبة الشفاء. كما أن المرضى الذين تلقوا العلاج في المراكز الصحية الأخرى تمكنوا من توحيد صحة اليوم إلى نصف اليوم، مما يبرز أهمية هذه الإجراءات في تحسين صحة المرضى. وفقاً للدراسات الحديثة، فإن هذه الإجراءات قد تساعد على تحسين صحة المرضى وزيادة نسبة الشفاء.
Abstract

The present study was carried out to investigate the toxic effect of potassium bromate on Wistar albino rats. Thirty rats were divided into 5 groups and treated with potassium bromate at doses of 0, 50, 100, 200 and 400 mg/kg body weight respectively for 21 days. The mortality was 100% in rats treated with 400 mg/kg Bwt. In rats treated with 100 and 200 mg/kg Bwt a significant increase was observed in the activity of alanine transaminase (ALT), urea, creatinine and potassium, and significant decrease in total protein, albumin and Na level. A significant decrease was also observed in Hb and PCV values in the group treated with 200 mg/kg Bwt, while no significant changes were observed in the group treated with 50 mg and 100 mg/kg Bwt. Histopathological examination showed haemorrhage and degenerative changes in kidney and liver of the group treated with 100, 200 and 400 mg/kg Bwt. These results suggested that the toxic effect of potassium bromate were more sever at higher doses.
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Introduction

Food additives are substances added directly and intentionally to food, generally in small quantities, for improvement of specific purpose; however, non-intentional additives may be an integral part of food. The practice of adding chemical to food originated thousands of years ago and involved, for example, the use of flavors, spices, preservatives and repining agents (Fennema, 1987). Our ancestors used to preserve meats and fish with salt, fruit with sugar and cucumber with vinegar solution. With the advent of processed food in the second half of the 20th century, many more additives have been introduced.

The additives play a vital role in today's bountiful and nutritive food supply and are carefully regulated by various international organization to ensure that additives introduced into food intended for human consumption are safe. However, toxic compound may be formed through metabolism of non-toxic additives either during food processing or after ingestion. Hence substances should be considered as additive if it has no ill effects and its margin of safety is adequate.

Potassium bromate has been used as food additive and was listed as flour treatment agent by FAO/WHO (1964). Later on
deleterious effects have been realized and it was claimed that potassium bromate is carcinogenic as well (Chipman et al., 1998 and Sai et al., 1992). Several safety evaluations have been done and they were controversial. The final decision that have been taken is that potassium bromate have to be banned and should not be listed as food additive.

In Sudan, however, food adulteration and indiscriminate addition of food additives, sometimes toxins, continue to be a problem. Despite the precaution undertaken potassium bromate is still in use although it was ruled unsafe.

The present study was aimed to evaluate the toxic effects of potassium bromate in wistar albino rats.
Chapter One

Literature Review

Food additives are substances other than a basic foodstuff which are present in food as a result of any aspects of producing, processing, storage or packaging (Food Protection Committee, 1961).

The use of food additives did not engender controversy until the early 1800s when intentional food adulteration became appallingly common. This problem continued until about 1920 when regulatory pressure and effective method of analysis reduces the frequency of food adulteration (Fennema, 1987).

The use of food additives have changed dramatically during the course of history (Fennema, 1987). In 1820 the addition of a chemical to food was done for respectable reason and since early 1800s intentional food adulteration increased greatly in frequency and seriousness. During this period the public develop increased concern about the quality of food (Accum, 1966). In 1920 regulatory pressure and effective method of analysis reduce the frequency and seriousness of food adulteration to acceptable level and the safety of the food supply has steadily improved since that time. In 1950 new problem began when food containing legal chemical additives were prevalent and the use of processed food increased and
contamination of some food with the byproduct of industrial activity became more common.

Now the uses of food additives are more strictly regulated and the regulation required evidence that each substance is safe at its intended level before usage; also continuing governmental surveillance of the food supply with regulatory judgments on the basis of emerging scientific information has resulted in the occasional banning of previously allowed food additives or the imposition of the new restriction on its use (Fennema, 1987).

Food additives are used for different purpose: as preservative used to retard or hinder undesirable flavor in food and may also serve as antioxidant that prevent rancidity of fat and oil (Frazier, 1955). To enhance or maintain nutritional values vitamins and minerals are added, to enhance color or favor, to improve cooking or backing quality and to bleach food like flour and certain cheese (Fraizer, 1955).

**Potassium Bromate**

**1.1 Physicochemical Properties**

Potassium bromate is found as white crystalline odourless powder, its molecular formula is KBrO3 with molecular weight of 167.01gm. Potassium bromate decomposes at 370°C with density of 3.27 g/cm
at 20°C and its solubility in water is 133 gm/L at 40°C and 498 gm/L at 100°C (Weast, 1986). Potassium bromate is a strong oxidizing agent. Biological bromate reduction has occurred with bromate utilized as terminal electron acceptor in the absence of oxygen and nitrate (Hijnen et al., 1995).

1.2 Metabolism of potassium bromate in animal and human

Following oral administration KBrO3 is rapidly absorbed from gastrointestinal tract. Fujie et al. (1984) administered rats with 100 mg/kg Bwt KBrO3 and it was detected in the plasma within 15 minutes. Bromate is very stable in the body and only small amount reduced to bromide by glutathione processes in the liver and kidney (Kutom et al., 1990). Potassium bromate is excreted in urine either as bromate or bromide (Fujie et al., 1984).

1.3 Uses and action

KBro3 is used widely as a maturating agent in flour and a dough conditioner in the bakery industrial (IARC, 1986 and Chipman et al., 1998). KBrO3 was evaluated for flour treatment to be at level of 62.5 mg/kg flour (FAO/WHO, 1964). This is because bromide arising from flour treated with KBrO3 at level less than 62.5 mg/kg did not present toxicological hazard; moreover, the data indicated that no detectable level of KBrO3 were found in bread
baked from treated flour. Potassium bromate is a strong oxidizing agent, its action in bread is the formation of disulphide bond between the wheat flour protein (gluten) and strip the hydrogen atom from the sulphydryl linkage so make more sulphur available for the gluten strengthened disulphide bond; hence strengthened the dough and more elastic gluten network was produced which is capable of expanding and increasing gas retaining capacity. This has economic advantage by shortening make up time or compensation for low protein level in flour (www.foodproductdesign.com 1999). The accepted equation for KBrO3 reaction as proposed by Tkachuk and Hlynka (1961) is:

$$\text{BrO}_3^- + 6\text{RSH} \rightarrow \text{Br}^- + 3\text{RSSR} + 3\text{H}_2\text{O}$$

(Bromate) (ProteinThiol) (Protein Disulfide)

Potassium bromate is also used for improvement of fish paste, as oxidizing agent in manufacture of certain cheese and in treating barely in beer making (IARC 1986 and Chipman et al., 1998).

Potassium bromate is a constituent of cold wave hair formulation. The formulation kits consist of ammonium thioglycolate solution that reduces the keratin disulfide bond to make the hair flexible and neutralizing solution which contain potassium bromate or iodate as an oxidizing agent that convert the sulphydryl group back to disulphide bond which stabilize the new form of the hair (DeVriese
Potassium bromate is generated as a contaminant in drinking water due to conversion of bromide found naturally in water to bromate by ozone when the ozone is used to disinfect water. It is frequently detected in tap and bottled water (Ueno et al., 2000). It is currently regulated in treated drinking water at a maximum contaminant level of 10µg/l in USA and Europe (U.S. EPA 2001).

1.4 Toxicity and Safety

Potassium bromate is a highly reactive substance which breaks down to the inactive bromide during dough fermentation and baking. It was considered that this break down was complete (www.archive.food.gov 1993). However, analytical techniques are now available to detect bromate up to a level of few part per billion (PPb) (IARC 1986). JECFA (1989) recommended that there should be no residue of KBrO₃ in food when it is used in food processing to avoid its toxicity.

Toxicity of potassium bromate have been reported in experimental animals. Mark (1988) reported that the lethal oral doses of KBrO₃ in human estimated to be 154-385 mg/kg body weight, while serious poisoning result at doses of 46-92 mg/kg Bwt. He also stated that
oral doses of 185-385mg/kg result in irreversible toxic effect mainly renal failure and deafness in human and lower doses associated with vomiting, diarrhea, nausea and abdominal pain. Watanabe et al. (2004) studied acute exposure of mice to KBrO3 administered orally at a rate of 1.2 mmol/kg body weight and sacrificed 3 hours after administration. He found significant elevation of serum uric acid, serum creatinine level, xanthine oxidase activity, relative kidney weight and renal oxidative stress which is an indicator of kidney damage.

In another study Okolie and Ikewuchi, (2004) indicated that KBrO3 induced oxidative stress on some cataractogenic indices in lens, cornea and retina of white rabbits receiving 60 mg/kg body weight orally for 28 days. They also stated a significant decrease in the activities of Na, K ATPase, catalase, superoxide dismutase and anti-oxidant vitamins A and C. Several reactive oxygen species (ROS) generated from KBrO3 (peroxy nitrite ONOO⁻, hydrogen peroxide H₂O₂, super oxide anion O₂⁻ and hydroxyl radical OH⁻) that might be a principle agent for KBrO3 provoked oxidative stress (Okalie and Ikewuchi, 2004). Potassium bromate brings about serious oxidative modification to protein, lipid and DNA (Chipman et al., 1998 and Watanabe et al., 2001).
In another study performed by Khan et al. (2003) in rats treated with 125 mg/kg Bwt KBrO3 intraperitoneally the results showed marked increase in the level of blood urea nitrogen (BUN), serum creatinine, reduction of anti-oxidant enzymes, enhanced xanthine oxidase and lipid peroxidation.

El-sokarry (2000) reported the protective effect of melatonin for the kidney and liver of rats treated with 100 mg/kg Bwt KBrO3 for 24 hours, significant increased in malondialdehyde (MDA) was observed as an indicator of lipid peroxidation. Histopathological examination revealed a typical tubules, a typical hyperplasia, hyaline droplets degeneration and necrotic changes.

Farombi et al. (2002) examined the effect of kolaviron in oxidative stress in kidney and liver of rats treated with KBrO3 intragastrically as a single dose of 300 mg/kg B wt. The result showed significant increase in relative kidney weight while the body weight and relative liver weight did not affected, decreased in superoxide dismutase, glutathione peroxidase and catalase in kidney were also reported.

Kurokawa et al. (1990) reported that when rats received KBrO3 for 13 weeks at rates of 150, 300, 600, 1250, 2500 and 5000 mg/l of drinking water, all animals given doses greater than 1250 mg/l died within 7 weeks. However, significant increase in alanine
transaminase (ALT) aspartate transaminase (AST), blood urea nitrogen (BUN) were reported in rats dosed with 600 mg/kg Bwt.

The carcinogenic and mutagenic effects of KBrO₃ have been also reported in experimental animals (Ishidate et al., 1984 and Kurokawa et al., 1987). It was classified as group 2B possible human carcinogen by IARC (1986). DeAngelo et al. (1998) studied the carcinogenicity of KBrO₃ in mice and rats. Mice were treated with 0.08, 0.4 and 0.8 g/l in the drinking water for 100 weeks and rats were treated with 0.02, 0.1, 0.2 and 0.4 g/l for 100 weeks. The results showed that KBrO₃ is carcinogenic to the rats kidney, thyroid and mesothelium and is renal carcinogen to mice.

Another study by Umemura et al. (1993) about carcinogenicity in rats exposed to KBrO₃ in drinking water revealed an increased cell proliferation in the proximal tubules. The genotoxic potential of KBrO₃ was also tested in Chinese hamster cell (Speit et al., 1999). Detection of oxidative DNA damage were observed. Molecular analysis of deletion mutation indicated a high portion of G to T transversion which arises after replication of 8-oxodeoxyguanosine generated from deoxyguanosine upon oxidation by KBrO₃.

Chipman et al. (1998) have examined the DNA damage with KBrO₃ following incubation of calf thymus DNA with KBrO₃, the
result showed significant increase in concentration of 8-oxodeoxyguanosine relative to deoxyguanosine. The role of oxidative stress in carcinogenesis were also shown in study conducted by Umemura et al. (1995). 8-oxodeoxyguanosine level were measured in the kidney and liver of rats received doses of 100, 200 and 400 mg/kg Bwt in the drinking water for 30 weeks. 8-oxodeoxyguanoine levels in kidney were significantly elevated with doses of 200 and 400 mg/kg Bwt. No significant changes were found in liver. Number of a typical tubules, a typical hyperplasia and renal cell tumor were significantly higher.

1.5 Detection of potassium bromate

Himata et al. (2000) showed that KBrO$_3$ in bread and baked good as low as 5 ppb (ng/g) can be detected using liquid chromatography. In another study Dennis et al. (1994) detected KBrO$_3$ to a limit of 12µg/kg bread using gas chromatography.
Chapter two

Materials and Methods

2.1 Materials and Experimental Design

2.1.1 Potassium Bromate (KBrO3)

Potassium Bromate in a powder form was supplied by Sudanese Consumer Protection Association at Khartoum.

2.1.2 Animals

Thirty healthy wistar albino rats of both sex weighing from (60-70gm) were supplied by Medicinal and Aromatic Plant Research Institute (MAPRI), National Research Center. They were housed under standard conditions within the premises of (MAPRI) and have free access to water and standard diet. They were left for a week as an adaptation period.

2.1.3 Experimental Design

At the end of the adaptation period the rats were divided randomly into five groups (6 in each). Group I was left as control, while groups II, III, IV and V were orally administered with KBrO3 using nasogastric tube (HFR [8] K AMOTO CORPORATION OSAKA JAPAN) daily at concentration rate of 50, 100, 200 and 400 mg/kg body weight respectively for 21 days.

2.1.4 Parameters

Clinical signs and mortality were recorded. Body weights were recorded
regularly every week. Blood samples were obtained for haematological investigation which included red blood cells (RBCs) count, white blood cells (WBCs) count, packed cell volume (PCV), haemoglobin concentration (Hb), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV).

Serum investigation included, total serum protein concentration, albumin concentration, globulin concentration, alanine transferase (ALT) activity, urea, creatinine, sodium and potassium concentrations. Organ weights, namely, liver, kidney, heart, lung and spleen were taken after death or slaughter. Slices from liver, kidney, stomach, heart, lung, spleen and brain were collected after death or slaughter and fixed in 10% neutral buffered formalin for histopathological investigation.

2.2 Methods

2.2.1 Haematological Methods

Blood samples were collected by puncturing retro-orbital plexus, with heparinized capillary tube (SUPE-PIRO-GERMAN code N-4361), into dry clean tube containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. The haematological procedure was done according to (Schalm, 1965).

2.2.1.1 Red Blood Cells (RBCs) Count

RBCs were counted using improved Neubauer haemocytometer (Hawksly and son Ltd, England). Hayem's Solution was used as a
diluent (Sodium chloride 1.0gm, sodium sulphate 0.5gm, mercuric chloride 0.5gm and made up to 200ml with distilled water). RBCs were expressed in millions/mm³ blood.

2.2.1.2 White Blood Cells (WBCs) Count

WBCs were Counted using improved Neubaur hemocytometer (Hawksly and son Ltd, England). Turk's Solution was used as diluent. WBCs were expressed in thousand/mm³ blood.

2.2.1.3 Packed Cell Volume (PCV)

PCV was measured using hematocrit method. Bloods sample were placed in the capillary haematocrit tube and centrifuged using hematocrit centrifuge (Hawksly and son Ltd, England). The PCV percent was read on the scaling instrument provided with microhaematocrit.

1.1.2.4 Haemoglobin (Hb) concentration

The determination of Hb concentration was based on the conversion of Hb to cyanomethaemoglobin by mean's of Drabkin's solution (Potassium cyanide 0.2gm, potassium ferricyanide 0.2gm and sodium bicarbonate 1.0gm in a liter of distilled water). The coloured solution was read with spectrophotometer (JENWAY 6305 UV/Vis) at wave length 540 nm and compared against standard Hb(cromatest). Hemoglobin concentration was expressed as follow:
Hb (g/dl) = \frac{\text{tested sample} \times 15}{\text{Standard sample}}

Where 15 was the standard concentration.

### 2.2.1.5 Mean Corpuscular Hemoglobin Concentration (MCHC)

MCHC percentage was calculated from Hb and PCV values as follows:

\[
\text{MCHC} \, (\%) = \frac{\text{Hb} \, (g/dl) \times 100}{\text{PCV}\%}
\]

### 2.2.1.6 Mean Corpuscular Volume (MCV)

MCV in cubic microns was calculated from RBC and PCV values as follows:

\[
\text{MCV} \, (\text{cubic microns}) = \frac{\text{PCV} \, \text{percent} \times 10}{\text{RBCs count} \, (\text{million}/\text{cmm})}
\]

### 2.2.2 Chemical Methods

Blood samples were taken in a similar procedure as haematology into dry clean tubes and allowed to clot at room temperature for 30 minutes then centrifuged (Hittich EBA35) at 3000 r.p.m. Sera were separated and stored at – 20°C until analyzed for biochemical investigation.

#### 2.2.2.1. Total protein determination

The determination of total protein concentration was done according to Biuret method described by Reinhold (1953).

Principle of the method:-
The principle of this method is based on the reaction of protein peptide bond with cupric ion in alkaline media found in Biuret solution (sodium potassium tartarate 9.0gm Cuppric sulphate 3.0 gm, potassium iodide 5.0 gm, sodium hydroxide 2N and made up to one liter distilled water) and resulting in formation of coloured complex.

Protein + cupric ion → protein cupric ion complex

Procedure:

The prepared sera and reagent were placed in water path at 37°C. Changes in scaling reading of spectrophotometer (JENWAY 6305 UV/VIS) at wave length 540 nm were recorded. The concentration of total protein was calculated as follow:

\[
\text{Total protein (g/dl)} = \frac{\text{tested sample} \times 6}{\text{Standard sample}}
\]

Where 6 was the concentration of the standard.

2.2.2.2. Albumin determination

Albumin concentration was measured using Bromo Cresole Green (BCG) method described by Spencer and Prince (1977).

Principle of the method:

The principle of this method is based on quantitative binding of serum albumin to BCG.

Procedure:

The prepared sera and reagent were mixed and incubated at 37°C
and were read at wave length of 670 nm using spectrophotometer.

Albumin concentration was calculated as follow:

\[
\text{Albumin (g/dl)} = \frac{\text{tested sample} \times 5}{\text{Standard sample}}
\]

Where 5 was the concentration of the standard.

### 2.2.2.3 Globulin determination

This parameter is obtained by subtracting Albumin concentration from total protein concentration.

### 2.2.2.4 Alanine Transaminase (ALT)

This enzyme is formerly known as Glutamate Pyruvate Transaminase (GPT). The determination of serum ALT activity was done according to the method of Reitman and Franklin (1957). ALT catalyzes the transfer of the amino group of glutamate to pyruvate in a reversible reaction:

\[
\text{Oxaloacetate} + \text{alanine} \rightarrow \text{glutamate} + \text{pyruvate}
\]

ALT is measured by monitoring the concentration of pyruvate hydrozone formed by reaction with 2-4-dinitrophenyl hydrazine (DNPH) in alkaline solution. ALT substrate was incubated for 5 minutes at 37°C then the sera were added, mixed and left at 37°C for 30 minutes, then a colour reagent were added and mixed at room temperature for 20 minutes. The optical density was measured at 505nm with spectrophotometer.
ALT values was expressed in U/L

2.2.2.5. Urea determination

Urea concentration in sera was measured using enzymatic colorimetric method described by Monica (1992).

Principle of the method:

The principle of this method is that urea is hydrolyzed by urease to ammonia and carbon dioxide. The intensity of the color formed when ammonia reacts with alkaline hypochlorite and sodium salicylate is proportional to the concentration of urea in sera samples.

\[
\text{Urea} + \text{H}_2\text{O} \rightarrow 2\text{NH}_3 + \text{CO}_2
\]

Procedure:

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

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

Where 50 was the standard concentration.

2.2.2.6. Creatinine determination

Concentration of serum creatinine was measured by an enzymatic colorimetric method (Monica, 1992). This procedure is based on picrate reaction method.

Principle of the method:
The principle is that creatinine in alkaline solution react with picrate ion forming a redish coloured complex. The amount of the colour formed is directly proportional to the creatinine concentration in sera samples.

Creatinine + picric acid → red colour complex

Procedure:

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 (A₁), and after 90 second later (A₂), and creatinine concentration was calculated as follow:

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

Where 2 was the standard concentration.

\( A = A₂ - A₁ \)

2.2.2.7 Sodium determination

Serum sodium concentration was determined using flame Photometer (Varley, 1967 and 1980).

Principle of the method:

The principle of this method is that; the flame emission photometer is used to measure the light emitted by exited atoms (alkali metals Na, K and Li are easy to excite). The solution of sodium is aspirated into the flame. Firstly the flame breaks the molecular bonds and converts it to the atomic state. As the temperature rises the atoms
absorb energy and enter the exited electronic state. The exited atom return to the ground state by emitting light of characteristic wave length. The emission flame photometer (corning flame photometer-400) was used with wave length 590 nm.

Procedure:

0.1ml of sera was diluted with 9.9 ml distilled water, the diluted sample was read against the low standard of sodium. Sodium concentration was calculated as follow:

\[
\text{Sodium (mEqu/L)} = \frac{\text{reading of test} \times 140}{\text{reading of standard}}
\]

Where 140 was the standard concentration

2.2.2.8. Potassium determination

Serum potassium concentration was measured using flame photometer according to (Varley, 1967 and 1980).
**Principle of the method:**

The principle is similar to that of sodium except using solution of potassium and wave length of 770 nm.

**Procedure:**

0.1ml of serum was diluted with 9.9 ml distilled water and the diluted sample was read against the low standard of potassium. Potassium concentration was calculated as follow:

\[
\text{Potassium (mEqu/L)} = \frac{\text{reading of test} \times 5}{\text{reading of standard}}
\]

Where 5 was the standard concentration

---

**2.2.3. Histopathological Methods**

Slices from organs liver, kidney, heart, lung, spleen, stomach, intestine and brain were collected from dead or slaughtered rats and fixed in 10% neutral buffered formalin (sodium hydrogen 6.5 gm/L and sodium dihydrogen 4.0gm/L) then embedded in paraffin wax and sectioned at 5 μm and stained by Hemotoxyline and Eosin (H&E) using Drury and Wallington (1980) method.

**2.2.4 Statistical Analysis**

Data were analyzed statistically by student t-test (Mendehall, 1971).
Chapter three

Results

3.1 Clinical Signs and Mortality

Rats received 50 mg/kg B wt showed no clinical signs, whereas depression and difficulty in breathing were observed in rats which received 100 and 200 mg/kg Bwt. The mortality was 100% in rats which received 400 mg/kg Bwt after the third day of dosing.

3.2 Body Weight and Relative Organs Weights

Table (1) shows the effect of various doses of potassium bromate on rats body weights. No significant differences were observed between control rats and treated ones throughout experimental period in the body weight. There were no significant differences in the relative weights between control rats and those treated with 50 mg/ kg Bwt, while significant (P<0.05) increases were observed in the relative weight of liver and kidney in those received 100 and 200 mg/kg Bwt (Table 2).

3.3 Haematological Findings

The haematological findings in rats treated with potassium bromate are summarized in table (3). There were no significant differences in
Table [1]: The mean body weights (gm) of rats orally treated with various levels of potassium bromate

<table>
<thead>
<tr>
<th>Doses mg/kg Bwt</th>
<th>Week1</th>
<th>Week2</th>
<th>Week3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>69.5±7.2</td>
<td>79.0±8.3</td>
<td>86.5±9.4</td>
</tr>
<tr>
<td>50</td>
<td>67.0±3.1</td>
<td>76.0±6.1</td>
<td>83.0±6.1</td>
</tr>
<tr>
<td>100</td>
<td>68.8±3.1</td>
<td>77.3±4.6</td>
<td>79.1±5.6</td>
</tr>
<tr>
<td>200</td>
<td>66.6±5.0</td>
<td>71.7±3.7</td>
<td>74.0±4.2</td>
</tr>
</tbody>
</table>

Table [2]: The mean relative organ weight (%) of rats orally treated with various levels of potassium bromate

<table>
<thead>
<tr>
<th>Doses mg/kg Bwt</th>
<th>Relative organ weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kidney</td>
</tr>
<tr>
<td>0</td>
<td>0.72±0.08</td>
</tr>
<tr>
<td>50</td>
<td>0.82±0.07</td>
</tr>
<tr>
<td>100</td>
<td>0.99±0.02*</td>
</tr>
<tr>
<td>200</td>
<td>1.20±0.09*</td>
</tr>
</tbody>
</table>

* P<0.05

RBCs and WBCs, count Hb concentration and PCV value between control rats and those dosed with 50 and 100 mg/kg B wt. However,
there was a significant decrease in Hb concentration and PCV values in group treated with 200 mg/kg B wt KBrO3 after the second week (P<0.05). Also there were no significant differences in MCHC concentration and MCV in groups treated with 50 and 100 mg/kg Bwt potassium bromate, but in the group treated with 200 mg/kg Bwt a significant decrease was observed in MCHC concentration after the second week (P<0.05).

3.4 Changes in Serum Constituents

The effects of various doses of potassium bromate on concentrations of total protein, albumin, globulin, albumin:globulin ratio and activity of alanine transaminase are given in table (4).

There were no significant differences between control rats and the group treated with 50 mg/kg Bwt in total protein and albumin concentrations. However, in the group treated with 100 mg/kg Bwt significant decrease was observed after the second and third weeks (P<0.05), in the group treated with 200 mg/kg Bwt a significant decreases were observed in total protein concentration at the second week and in albumin concentration throughout experimental period (P<0.05).

Table [3]: The mean haematological changes of rats orally treated with various levels of potassium bromate

<table>
<thead>
<tr>
<th>Duration</th>
<th>Doses</th>
<th>RBCs</th>
<th>WBCs</th>
<th>Hb</th>
<th>PCV</th>
<th>MCHC</th>
<th>MCV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(week)</td>
<td>mg/kg B wt</td>
<td>×10^6/mm³</td>
<td>×10^5/mm³</td>
<td>g/dl</td>
<td>%</td>
<td>%</td>
<td>fl</td>
</tr>
<tr>
<td>-------</td>
<td>------------</td>
<td>------------</td>
<td>------------</td>
<td>------</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>4.9±0.25</td>
<td>6.3±0.85</td>
<td>13.0±0.72</td>
<td>33.7±0.96</td>
<td>38.6±0.80</td>
<td>69.0±0.3</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>4.9±0.69</td>
<td>6.9±1.10</td>
<td>12.9±0.33</td>
<td>33.8±1.60</td>
<td>37.8±0.25</td>
<td>68.0±0.6</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>4.8±0.60</td>
<td>6.6±1.00</td>
<td>11.9±0.97</td>
<td>31.5±2.70</td>
<td>37.7±0.56</td>
<td>66.0±1.3</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>5.3±0.58</td>
<td>7.2±1.40</td>
<td>11.5±1.00</td>
<td>34.0±1.80</td>
<td>33.8±0.19</td>
<td>64.0±1.8</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>5.1±0.28</td>
<td>6.2±0.28</td>
<td>13.4±0.70</td>
<td>35.8±0.96</td>
<td>37.4±0.65</td>
<td>70.0±1.8</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>4.9±0.40</td>
<td>5.6±0.44</td>
<td>13.2±0.66</td>
<td>36.2±1.20</td>
<td>36.4±0.30</td>
<td>74.0±1.2</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>4.8±0.48</td>
<td>5.9±0.09</td>
<td>13.8±0.24</td>
<td>35.6±1.80</td>
<td>38.7±1.10</td>
<td>74.0±0.5</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>4.3±0.36</td>
<td>5.6±0.51</td>
<td>10.4±0.50*</td>
<td>29.3±0.2*</td>
<td>35.4±2.50*</td>
<td>68.0±1.1</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>5.3±0.82</td>
<td>6.7±0.36</td>
<td>13.3±0.53</td>
<td>34.3±1.50</td>
<td>38.7±0.40</td>
<td>65.0±0.8</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>5.2±0.66</td>
<td>6.2±0.47</td>
<td>12.7±0.85</td>
<td>32.8±1.50</td>
<td>39.6±0.50</td>
<td>63.0±1.2</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>4.3±0.48</td>
<td>6.2±0.54</td>
<td>11.3±1.40</td>
<td>30.3±2.50</td>
<td>37.2±1.50</td>
<td>70.0±3.1</td>
</tr>
</tbody>
</table>

* P<0.05

There was no significant differences in globulin concentration between control and treated groups. While significant decreases were observed in albumin:globulin ratio in those treated with 100
mg/kg B wt at the third week and the group treated with 200 mg/kg B wt throughout experimental period (P<0.05). There was no significant changes in ALT activity in the group treated with 50 mg/kg B wt, but significant increases were observed in the groups treated with 100 and 200 mg/kg B wt throughout the experimental period (P<0.05).

The effects of various doses of potassium bromate on concentrations of urea, creatinine, sodium and potassium are given in table (5).

There were no significant differences in the concentration of urea, creatinine, Na, and K levels between control and rats received 50 mg/kg B wt. However, significant increases were observed in urea and creatinine levels in the groups treated with 100 and 200 mg/kg B wt throughout the experimental period (P < 0.05). Meanwhile significant decreases in sodium levels were observed in the group treated with 100 mg/kg B wt after the second and third week and the group treated with 200 mg/kg B wt throughout the experimental period (P < 0.05). Significant increases were observed in potassium levels after the second and third week in the group treated with 100 mg/kg Bwt but throughout experimental period in the group treated with 200 mg/kg B wt.

3.4 Histopathological Finding
In the group treated with 400 mg/kg B wt the kidney showed haemorrhage, proteinaceous material in the lumen of the tubules beside tubular degeneration fig (1). The liver was congested and haemorrhagic fig(2). There were haemorrhage and degeneration in the stomach fig(3), haemorrhage in the heart and depletion of lymphocyte in the spleen.

In the group treated with 200 mg/kg Bwt the kidney is haemorrhagic and congested. Congestion of the liver, Haemorrhage and area of degeneration in the heart, fig (4). The intestine showed an increase of goblets cell fig (5). Sloughing and necrosis of the epithelium were evident in the stomach. The spleen showed depletion of lymphocyte (fig 6). Congestion and vaculation were seen in the brain fig (7).

In the group treated with 100 mg/kg B wt the kidney was haemorrhagic and congested. The liver showed congestion and vaculation. The lung was haemorrhagic, congested and pneumonic fig (8), also sloughing of epithelium of stomach was evident fig (9).

There were no pathological changes seen in the group treated with 50 mg/kg Bwt.

Table [4]: The mean changes in serum constituents of rats orally treated with various levels of potassium bromate
<table>
<thead>
<tr>
<th>Duration (week)</th>
<th>Doses mg/kg B wt</th>
<th>Total protein g/dl</th>
<th>Albumin g/dl</th>
<th>Globulin g/dl</th>
<th>Alb/Glb ratio</th>
<th>ALT U/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>6.6±0.53</td>
<td>3.8±0.67</td>
<td>2.9±1.10</td>
<td>1.3±0.90</td>
<td>23.7±5.30</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>6.8±0.46</td>
<td>3.6±0.46</td>
<td>3.3±0.79</td>
<td>1.09±0.63</td>
<td>26.6±0.50</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>6.2±0.40</td>
<td>3.1±0.48</td>
<td>3.0±0.86</td>
<td>1.03±0.70</td>
<td>32.2±3.70*</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>6.0±0.43</td>
<td>2.9±0.22*</td>
<td>3.0±0.16</td>
<td>0.97±0.20*</td>
<td>42.3±0.43*</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>7.8±0.68</td>
<td>3.7±0.55</td>
<td>4.2±1.20</td>
<td>0.88±0.90</td>
<td>28.5±4.7</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>7.0±0.69</td>
<td>3.1±0.65</td>
<td>3.9±0.50</td>
<td>0.79±0.60</td>
<td>30.2±4.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>6.0±0.49*</td>
<td>2.7±0.37*</td>
<td>3.5±0.53</td>
<td>0.77±0.45</td>
<td>44.5±2.20*</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>5.3±0.53*</td>
<td>2.3±0.15*</td>
<td>3.1±0.37</td>
<td>0.74±0.30*</td>
<td>56.3±3.2</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>7.3±0.96</td>
<td>3.7±0.50</td>
<td>3.6±1.10</td>
<td>1.03±0.80</td>
<td>31.0±3.9</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>6.3±0.45</td>
<td>3.0±0.14</td>
<td>3.3±0.46</td>
<td>0.91±0.30</td>
<td>37.4±4.6</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>5.8±0.54*</td>
<td>2.5±0.42*</td>
<td>3.0±0.61</td>
<td>0.83±0.52*</td>
<td>57.0±6.5</td>
</tr>
</tbody>
</table>

* P<0.05

Table [5]: The mean changes in serum constituents of rats orally treated with various levels of potassium bromate
<table>
<thead>
<tr>
<th>Duration (weeks)</th>
<th>Doses mg/kg B wt</th>
<th>Urea mg/dl</th>
<th>Creatinine mg/dl</th>
<th>Sodium mEq/L</th>
<th>Potassium mEq/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>26.7±5.4</td>
<td>1.0±0.40</td>
<td>137.5±3.8</td>
<td>3.5±0.40</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>28.6±4.2</td>
<td>1.2±0.50</td>
<td>134.8±2.9</td>
<td>3.6±0.12</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>33.4±2.8†</td>
<td>2.4±0.48†</td>
<td>130.0±4.6</td>
<td>3.9±0.50</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>41.7±5.0†</td>
<td>3.0±0.82†</td>
<td>123.0±3.5†</td>
<td>5.2±0.40†</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>35.5±9.0</td>
<td>1.2±0.3</td>
<td>136.0±3.3</td>
<td>3.4±0.45</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>34.0±4.7</td>
<td>1.4±0.25</td>
<td>136.0±7.5</td>
<td>3.9±0.50</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>49.0±1.0†</td>
<td>2.8±0.96†</td>
<td>127.2±5.2†</td>
<td>4.4±0.30†</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>57.0±2.9†</td>
<td>4.8±0.40†</td>
<td>117.3±4.3†</td>
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</tr>
<tr>
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<td>0</td>
<td>31.5±6.5</td>
<td>1.6±0.4</td>
<td>134.0±5.9</td>
<td>3.3±0.38</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>39.3±4.5</td>
<td>1.8±0.27</td>
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<td>3.8±0.50</td>
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<tr>
<td></td>
<td>100</td>
<td>50.0±5.0†</td>
<td>3.9±0.63†</td>
<td>123.8±3.3†</td>
<td>5.7±0.50†</td>
</tr>
</tbody>
</table>

* P<0.05
Fig (1) Kidney from rats received 400 mg/kg B wt of KBrO3. Note, tubular degeneration and congestion. H&E x10.

Fig (2) Liver from rats received 400 mg/kg B wt of KBrO3. Note, congestion, haemorrhage and fatty changes. H&E x20.
Fig (3) Stomach from rats received 400 mg/kg Bwt of KBrO3. Note, degeneration of the mucosa. H&E x20.

Fig (4) Heart from rats received 200 mg/kg Bwt of KBrO3. Note, haemorrhage and degeneration of cardiac muscles. H&E x20.
Fig (5) Intestine from rats received 200 mg/kg B wt of KBrO₃. Notice increased goblet cells. H&E x20.

Fig (6) Spleen from rats received 200 mg/kg B wt of KBrO₃. Notice lymphocyte depletion. H&E x20.
Fig (7) Brain from rats received 200 mg/kg B wt of KBrO3. Note, congestion and vaculation. H&E x10.

Fig (8) Lung from rats received 100 mg/kgB wt of KBrO3. Note, congestion and atelactasis. H&E x20.
Fig (9) Stomach from rats received 100 mg/kg B wt of KBrO3. Note, sloughing of the epithelial mucosa. H&E x20.
Chapter Four

Discussion

The present study has shown that more rapid death occurred when potassium bromate has been given at the rate of 400 mg/kg B wt. This pointed out the correlation of potassium bromate effects with the dose. This finding is similar to that reported by Kurokawa et al. (1990) who found the lethal dose of potassium bromate in rats range between 280-495 mg/kg B wt.

When potassium bromate given to rats at a dose of 50 mg/kg B wt there were no signs of toxicity as indicated by the non significant alteration in haematology, serum constituents or histopathology.

However, doses of 100, 200 mg/kg Bwt potassium bromate exhibited signs of poisoning which was illustrated by depression and difficulty in breathing. The generalized congestion and pneumonia correlate the appearance of clinical signs.

There were no significant effects on the body weights in rats dosed with potassium bromate. This indicates that potassium bromate has no effect on the growth rate. This is correlated to Farombi et al. (2000) and Watanabe et al. (2004) who reported that no
effect on the body weight, but in contrast to Okolie and Ikewuchi (2004) who reported a significant decrease in the body weight.

The relative kidney and liver weights were increased in rats received 100 and 200 mg/kg potassium bromate. This finding is in line with the results obtained by Farombi et al. (2000) and Watanabe et al. (2004).

The transient decrease in Hb and PCV values in rats received 200 mg/kg B wt potassium bromate at the second week is an indicator of anemia. Similar results were reported by Chipman et al. (1998) and Watanabe et al. (2001) who claimed that potassium bromate was known to induce methaemoglobinaemia and cyanosis that resulted from oxidation of ferrous ion to ferric by reactive oxygen species (ROS) generated from potassium bromate.

The increase in serum creatinine and urea levels are indication of renal toxicity. This is illustrated by the pathological changes seen in the kidney. This is in harmony with Khan et al. (2003) and Watanabe et al. (2004). The decrease of sodium beside the increase of potassium levels may be reflected by the toxic effects of potassium bromate on the kidney as sodium and potassium levels are regulated by the kidney. The effects are either on the permeability of the tubular cell membrane or on the tubular epithelium where minerals are reabsorbed. Sodium and potassium
are also regulated by Na-K ATPase, the activity of this enzyme is reported to decrease by the oxidative effect generated by (ROS) (Okolie and Ikewuchi, 2004).

The reduction of total protein and albumin may be due to liver damage which resulted in reduction of protein synthesis. The increase of ALT may be attributed to the high permeability of the hepatocyte membranes or its destruction as confirmed by the pathological changes seen in the liver. This finding is in agreement with Kurokawa et al. (1990).
Conclusions

From the result obtained, we can conclude that;

1- The toxicity of potassium bromate is dose dependent. In Sudan the problem to ban KBrO3 is difficult as the technical equipments for measuring potassium bromate in small amount is not available in a wide range.

2- More studies is needed for exposure of KBrO3 for a longer time to Confirm its carcinogenicity.
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