Effect of Potassium Bromate on the Liver of Wistar rats


By

Alrasheid Abdalla Hamad Hassan
Omdurman Islamic University B.Sc.Biotechnology (2009)

Supervisor

Prof: Mohammed Elsheikh Barri

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قال تعالى:

{ والذي خلقني فهوى يهدٍن * والّذي هو يطعمني ويسقين *}

{ وآدآ مرضت فهوى يشفين }

صدق الله العظيم

سورة الشعراء الآيات: (78-80)
Dedication

To my loving parents

Those who lightened my way with their care

To my mother, and aunt Alwia,

To Those who supported me in this bright station in my life

To my brother and sisters

To Those who shared me the best moments of my life

To my large family members

Those who gave me the respect as a way to the people heart

To my dear mother basmat

Your words like a candle that lightened my way to success

I dedicate this work

Alrasheid
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Thanks at first to Allah, the greatest who gave me the ability and power to carry out this work

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Prof. Mohammed Elsheikh Barri

Who advised and helped me to construct this work.

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<td>Potassium bromated</td>
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Abstract

The present study was carried out to investigate the toxic effect of exposure to potassium bromate (KBro₃) on liver of albino rats. Thirty rats were divided randomly into 6 groups and treated with potassium bromate at doses of 0, 140, 170, 170, and 200mg/kg body weight respectively for 21 days. and rats in group C were treated for two more weeks. The results showed normal weight gain in group A control group, and weight reduction in third weeks in groups (B, C, D, and F), but in group E with vit E showed a significant gain weight, the mortality was 100% in rats treated with 170 mg/kg Bwt after 5th week, at the end of week two 50% from rats were dead which received (140, 170 and 200mg/kg Bwt). In the rats treated with 140, 170 and 200mg/kg Bwt showing a significant elevated of alanine transaminase (ALT) and aspartate transaminase (AST) and a significant decrease in total protein. In group treated with 200mg/kg Bwt of KBro₃ with vit E showed a significant decrease in ALT & AST and increase in total protein. Histopathological examination showed severe congestion of central veins and sinusoids, dilatation of liver sinusoids. Loss of hepatic architecture, vesicular nuclei, relative increase in Kupffer cells in liver of the groups treated with 140, 170, and 200 mg/kg Bwt, in the kidney, glomeruli were dilated with some glomerular tuft shrinking or segmented congestion of blood vessel with some areas of hemorrhage, Necrosis of lobular epithelioid, group 200 mg/kg Bwt with vitE showed less necrosis compared to other groups. These results suggested that the toxic effects of potassium bromate were more severe in Long-term exposure.
المستخلص

تعطي الدراسة الحالية لتقسي الأثر السمي لبرومات البوتاسيوم على كبد الفئران من نوع ألبينو عندما تعطي برمات البوتاسيوم بجرعات مختلفة.

طبقت هذه الدراسة على ثلاثين فئراً وزعت عشوائياً إلى 6 مجموعات، وأعطيت برمات البوتاسيوم عن طريق الفم بجرعات 0.140، 0.170، 0.200 ملجم/كجم من وزن الجسم على التوالي لمدة 21 يوماً، الفئران في المجموعة C عولجت لمدة أسبوعين زيادة. لوحظ زيادة معنوية في وزن الجسم في مجموعات F و G، ولكن مجموعة 200 ملجم/كجم فايتمين E لوحظ زيادة معنوية في وزن الجسم A و C.

حدثت الوفاة بنسبة 100% في الفئران التي تم تجربتها بـ 170 ملجم/كجم وزن الجسم لمدة 5 أسابيع. في نهاية الأسبوع الثاني حدثت الوفاة بنسبة 50% من الفئران التي أستقبلت (200، 170، 140، 200 ملجم/كجم وزن الجسم). هذا وقد لوحظ زيادة معنوية في الفئران التي جرعت بـ 140، 170، 200 ملجم/كجم وزن الجسم في نشاط اللينتين ترانز امينز W و الامينيزات ترانز امينز و نقصان معنوي في البروتين الكلي. المجموعة التي عولجت بـ 200 ملجم/كجم وزن الجسم مع فايتمين E لوحظ نقصان معنوي في نشاط اللينتين ترانز امينز W و الامينيزات ترانز امينز وزيادة معنوي في البروتين الكلي. في اختيارات الهيستوباثولوجي لوحظ أن الفئران التي تم تجربتها بـ 140، 170، 200 ملجم/كجم وزن الجسم حدث فيها احتقان حاد في الوريد المركزي وتجويف الكبد ونقصان في خلايا الكبد الدفاعية وفي الكلي، ووحظ توضع في مصفى الكليه وإنكماش في مجرى الدم ونزيف؛ وفي المجموعة التي جرعت بـ 200 ملجم/كجم وزن الجسم مع فايتمين E لوحظ أقل سمية مقارنة مع المجموعات الأخرى. من هذه النتائج يتضح أن الأثر السمي لبرومات البوتاسيوم كان أكثر حدة كلما زاد زمن التعرض لمادة البوتاسيوم بروميد.
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Food additives are substances added directly and intentionally to food, generally in small quantities, for improvement of specific purpose, however, a non-intentional additives may be an integral part of food. The practice of adding chemicals to food originated thousands of years ago and involved, for example, the use of flavors, spices, preservatives and repining agents (1). Our ancestors used to preserve meat 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.

Potassium bromate (KBrO3) is a food additive which exists as a white crystal, granules or powder, which is colourless, odourless and tasteless. It has no medicinal value but is added to flour as a maturing agent, to dough, to fish paste as a conditioner, and also to cheese (2).

Food additives play a vital role in today’s bountiful and nutritive food supply and are carefully regulated by various international organizations to ensure that additives introduced into food intended for human consumption are safe (3). Potassium bromate has been used as food additive and was listed as flour treatment agent by FAO/WHO (1964) (4). Later on deleterious effects have been realized and it was claimed that potassium bromate is carcinogenic as well (5). 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, some times toxins, continue to be a problem. Despite the precaution undertaken potassium bromate is still in use although it was ruled unsafe.

Liver lobule is composed of hepatocytes or parenchymal cells that occupy almost 80% of the total liver volume and non parenchymal liver cells that contribute
to only 6.5% of the liver volume and to 40% of the total number of liver cells (6). Non parenchymal liver cells, including Kupffer cells (KC), pits cells and hepatic stellate cells (HSCs) are localized in the walls of the hepatic sinusoids. HSCs, also known as perisinusoidal cells or I to cells and were earlier known as lipocytes or fat-storing cells (7). Quantitative estimates indicated that numbers of HSCs in rat liver were about 10–12% of hepatocytes. Further, many researchers have detected much more stellate cells around the portal areas than around the central areas (8, 9). However, Baratta (10) stated that stellate cells were distributed heterogeneously all over the liver.

Limited information exists concerning the cellular alterations associated with exposure to KBrO3 in liver tissues. Impairment of liver function is a direct consequence of changes in the histological structures of the organ, which depends on the degree of exposure to toxic substances (11). These facts necessitate the need for more studies to demonstrate the hepatotoxic effects of KBrO3.
1.2. Rationale of the study:

Potassium bromate is a toxic substance to human body if used in small amounts. However, some people reuse it to improve bread size.
1.3. Objectives

1.3.1. General Objective:
To study the effect of potassium bromate on the liver and biochemical changes in the blood of wistar rats.

1.3.2. Specific Objectives:
To assess the blood levels of:

- Alanine transaminase (ALT)
- Aspartate transaminase (AST)
- Serum total proteins
- The hepatotoxicity of potassium bromate.
Chapter Two

2. Literature review

Food additives are substances other than a basic food stuff which are present in food as a result of any aspect of production, processing, storage or packaging \(^\text{12}\). 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 methods of analysis reduces the frequency of food adulteration \(^\text{1}\) .

Potassium bromate is a white crystal, granule or powder, which is colourless, odourless and tasteless. It has no medicinal value but is added to flour as a maturing agent, to dough, to fish paste as a conditioner, and also to cheese \(^\text{2}\) . Potassium bromate (KBrO\(_3\)) is an oxidizing agent. Studies have shown that potassium bromate has harmful effects on the nutritional qualities of bread by lowering vitamins A, B1, B2, E and niacin, the main vitamins in bread \(^\text{13}\) . Studies have also shown that it possess the potential of inducing cancer, kidney failure, deafness, redness and pains of the eye and skin \(^\text{14}\) . Strong oxidizer, Contact with other material may cause fire. harmful if swallowed, may cause kidney damage, and cause cancer based on animal studies, it may cause nervous system effects. explosive when mixed with combustible material. causes eye, skin, and respiratory tract irritation. It is harmful to Kidneys, central nervous system and liver. It is harmful to eye where it causes moderate eye irritation, and may cause transient corneal injury. It causes skin irritation. In the presence of moisture, this material may be absorbed through the skin.

Potassium bromate is harmful if swallowed, it causes gastrointestinal irritation with nausea, vomiting and diarrhea, may cause liver and kidney damage, and may cause central nervous system depression. Hearing loss and deafness have been reported. May form methemoglobin which in sufficient concentration causes cyanosis (bluish discoloration of skin due to deficient oxygenation of the blood).
It causes respiratory tract irritation, chronic, liver and kidney damage, and causes cancer according to animal studies.

Vitamin E (α-tocopherols) are multifaceted antioxidants, that scavenge oxygen free radicals, lipid peroxides and singlet oxygen (15). They act as membrane stabilizers by their positive influence on membrane lipid organization (16). The oral lethal dose (LD50) of potassium bromate has been established in wistar rat as 160-190 mg/kg Bwt (17). Bromate is reduced to bromide in body tissues (18).

Potassium bromate (KBrO3) is an oxidizing agent is generated as a contaminant in drinking water due to conversion of bromide found naturally in water to bromate by ozone which is used as a disinfectant (19). Studies have also shown that it possess the potential of inducing cancer, kidney failure, deafness, redness and pains of the eye and skin (20). Its toxicity has led to its ban in most countries. Potassium bromate (KBrO3) is an oxidizing agent that has been used as a food additive mainly in the bread-making process (17) and primarily as a dough conditioner for flour (21). The maximum concentration of KBrO3 allowed in bread is (0.02ug/g) according to USFDA (22). The Potassium bromate is rapidly absorbed from gastrointestinal tract and can be detected in plasma. It is very stable in body and small amount was reduced to bromide by a process involving glutathione in liver and kidney (23).

2.1 Physiochemical Properties of potassium bromate:

Potassium bromate is found as a white crystalline odourless powder, its molecular formula is KBrO3 with molecular weight of 167.01gm. Potassium bromate decomposes at 37°C with a 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 (24).

Potassium bromate is a strong oxidizing agent. Biological bromate reduction has occurred with bromate utilized as terminal electron accepter in the absence of oxygen and nitrate (25).
2.2 Metabolism of potassium bromate in animals and humans:
Potassium Bromate is rapidly absorbed in gastrointestinal tract and small amount is reduced to bromide by glutathione process in the liver and kidney (\textsuperscript{23}). The concentration of Potassium Bromate starts to be reduced in plasma at 15 min after administration. This concentration was quickly reduced to approximately one third within another 15 min and was not detectable in plasma at 2 h (\textsuperscript{17}). Concentration of bromate in urine peaked at approximately 1 h after administration. The very little amount of potassium bromate is not detected in urine until the dose reached 5 mg/kg of body weight. About 3–6\% of the higher doses were recovered in the urine. These observations are generally consistent with the observed elimination of bromate in human urine following poisoning (\textsuperscript{28}).

2.3 Uses and action:
Potassium bromate (KBrO\textsubscript{3}) is used widely in food and cosmetic industries to induce oxidation. It is used in bakeries as a food additive and flavor improver giving strength and sponge like characters to the dough. In the process of bread-baking, potassium bromate is reduced to potassium bromide (KBr) which is a more stable compound. However, this reaction may be incomplete, and some KBrO\textsubscript{3} remains, and leads to potential oxidative damage. So, the usage of KBrO\textsubscript{3} in bread production is prohibited in some countries. However, flour producers still use potassium bromate (\textsuperscript{27}). It is commonly added to some Japanese pastes of fish. Also, the usage of ozone as disinfectant in drinking water converts the bromide, which is present naturally in water into bromate causing water contamination by potassium bromate (\textsuperscript{28}). It has also been used as a constituent in cold wave hair solution (\textsuperscript{28}). Several researches were carried out on safety evaluation of potassium bromate. It was found to be a nephrotoxic, neurotoxic, genotoxic, and a carcinogenic agent as it causes lipid peroxidation (LPO) and oxidative DNA damage (\textsuperscript{29,30}).
Potassium bromate is generated as a contaminant in drinking water due to conversion to bromide which is 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. It is currently regulated in treated drinking water at a maximum contaminant level of 10μg/l in USA and Europe.

2.4 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. However, analytical techniques are now available to detect bromate up to a level of few part per billion (PPb). IARC and JECFA 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 reported that the lethal oral doses of KBrO3 in human was estimated to be 154-385 mg/kg body weight, while serious poisoning results 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 studied acute exposure of mice to KBrO3 administered orally at a dose 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 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 decreases 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 O−2 and hydroxyl radical OH−) that might be a principle agent for KBrO3 provoked
oxidative stress (37). Potassium bromate brings about serious oxidative modification to protein, lipid and DNA (5,38). In another study performed by Khan (39) in rats treated with 125 mg/kg Bwt KBrO3 intrapretoneally 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. Histopathological examination revealed a typical tubules, a typical hyperplasia, hyaline droplets degeneration and necrotic changes. Farombi et al (40) 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 was not affected, a decrease in superoxide dismutase, glutathione peroxidase and catalase in kidney were also reported. Kurokawa et al (17) reported that when rats received KBrO3 for 13 weeks at dose 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 (41). It was classified as group 2B possible human carcinogen by IARC (33,14) who 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 (42) 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 (43). Detection of oxidative DNA damage was observed. Molecular analysis of deletion mutation indicated a high portion transversion which arises after replication of 8-oxodeoxyguanosine generated from deoxyguanosine upon oxidation by KBrO3.
Chipman et al (44) 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 was also shown in a study conducted by (45). 8-oxodeoxyguanosine level was measured in the kidney and liver of rats received KBrO3 at a dose of 100, 200 and 400 mg/kg B wt in the drinking water for 30 weeks. 8-oxodeoxyguanosine levels in kidney were significantly elevated with the doses of 200 and 400 mg/kg Bwt. No significant changes were found in the liver. Number of atypical tubules, atypical hyperplasia and renal cell tumor were significantly higher. Several cases of acute bromate intoxication have been reported in humans following accidental or suicidal ingestion of permanent hair wave neutralizing solution. These products usually contain either 2% potassium bromate or 10% sodium bromate. The most common acute signs are severe gastrointestinal irritation (vomiting, pain, and diarrhea) and CNS depression (lethargy, hypotension, hypotonicity, and loss of reflexes). Anemia from intravascular hemolysis may also occur.

The toxic effects of KBrO3 are attributed to its ability to induce oxidative stress (OS) leading to enhanced production of reactive oxygen species (ROS) which are important mediators of tissue injury. The ROS are widely thought to be generated in the cell due to reduction of KBrO3 to bromide by intracellular reductants. KBrO3 has been shown to induce oxidative modification of lipids and proteins in several animal tissues. Increased levels of intracellular ROS due to exposure to KBrO3 also result in DNA damage causing mutations, rearrangements, and transcriptional errors that impair important cellular functions, in many cases leading to cell death (46). Supporting the involvement of ROS in bromate action, several antioxidants (AO) have been shown to ameliorate the bromate-induced toxicity (47, 48, 49, 50).

Exposure to KBrO3 results in nephrotoxicity since kidney is the primary target organ of this compound. Chronic administration of KBrO3 causes renal cell
carcinomas in rats, hamsters and mice and thyroid and mesothelioma tumors in rats \(^3\) .

2.5 Detection of potassium bromate:

Himata \textit{et al.} \(^5\) showed that KBrO3 in bread and baked good as low as 5 ppb (ng/g) can be detected using liquid chromatography. In another study Dennis \textit{et, al} . \(^5\) detected KBrO3 to a limit of 12μg/kg bread using gas chromatography.

2.6 The liver:

the liver is highly specialized tissue consisting of mostly hepatocytes regulates a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions. The liver is responsible for the mainstay of protein metabolism, synthesis as well as degradation. Albumin is the major protein product of the liver and is used as an indicator of hepatic synthetic functions.
Chapter Three

3. Materials and Methods

3.1. Materials and Experimental Design

3.1.2 Potassium Bromate (KBrO3):

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

3.1.3 Experimental design:

The rats were divided randomly into six groups A, B, C, D, E and F each containing five rats. Group A was left as the control group, which were orally administered with 1ml of distilled water daily for 21 days. The other groups B, C, D, E, and F served as the experimental groups and were orally administered with potassium bromate (manufactured by Windia Speciality Chemicals LTD, Tamil Wadu, India) for 21 days respectively. The concentration of KBrO3 used was 25 g per liter of distilled water; the average bromate water consumption was 0.6 ml/day (equivalent to 15 mg KBrO3/day) and the average body weight was 0.2 kg. The corresponding daily dosages of KBrO3 were then calculated and measured as 140, 170, and 200 mg/kg b.w./day and administered to groups B, C, D, E and F respectively Kurokawa et al. The present experiment was designed to be dose-dependent with the different doses of KBrO3 administered daily to the experimental groups for a period of 3 weeks using nasogastric tube (HFR [8] K AMOTO CORPORATION OSAKA JAPAN) daily at a concentration rate of 140, 170, 170, 200 with vit E and 200 mg/kg body weight, respectively for 21 days and group D for 5 weeks.

Capsules of vitamin E (Eviol di-alpha-tocopheryl acetate, G.A. Pharmaceutical S.A Greece) were cut open and emptied into a clean container. Vegetable oil was added to prepare a suspension solution containing 34 mg of vitamin E in 0.1 mL
Vegetable oil. Each rats in group E was treated with 20mg/kg /day through oral gavage route for 3 weeks.
The suspension solution was kept from sunlight to avoid degradation, by stocking in a dark air-tight jar.

3.2 Experimental Animals:

Thirty (30) adult rats from the Faculty of Veterinary Medicine University of Khartoum Laboratory were used in this experiment. They were housed in the Laboratory at 27 ± 2 °C, relative humidity 50 ± 15% and normal photo period (12h dark/12h light).

3.3 Feeds:

The rats were fed with pelletized growers mash and water. The Pelletized growers mash is composed of the following, maize, soya, groundnut cake, corn brown palm kernel cake (PKC), fish meal, bone meal, oyster shell, vitamins and mineral, methionine, lysine, salt and blood meal. The rats were fed twice daily. The body weight of the rats were taken weekly and documented.

3.4 The Experimental groups:

**Group A** control: This group contained 5 rats which received distilled water and fed for 3 weeks.

**Group B**: This group contained 5 rats which received 140 mg/kg body weight of KBrO3 by nasogastric tube for 3 weeks.

**Group C**: This group contained 5 rats which received 170 mg/kg body weight of KBrO3 by nasogastric tube for 3 weeks.

**Group D**: This group contained 5 rats which received 170 mg/kg body weight of KBrO3 by nasogastric tube for 5 weeks.
Group E: This group contained 5 rats which received KBrO3 at a dose level of 200 mg/L in drinking water with co. administration of 20mg/kg B wt of vitamin E through oral gavage route for 3 weeks.

Group F: This group contained 5 rats which received 200 mg/kg body weight of KBrO3 by nasogastric tube for 3 weeks.

3.4 Biochemical methods:

3.4.1 Blood Sampling:

Blood samples were collected at the last day of the treatment period, all rats were fasted and blood was then withdrawn into clean and dried sample bottles by cardiac punctures, This procedure was performed after anesthetized the rats through inhalation of chloroform that soaked in a piece of cotton bedded in tidily closed desiccator. The blood was allowed to clot for 10 minutes at room temperature and there after centerifuged at 3000 rpm for 5 minutes and stored at 2-8°C until analyzed (53).

3.4.2 Biochemical Measurements:

Serum samples were analyzed for total serum protein concentration, alanine transferase (ALT) activity, Aspartate transaminase (AST) activity by spectrophotometer, using commercial kits (biosystems chemical, barceloa, Spain).

3.4.3 Total protein determination:

The determination of total protein concentration was done according to Biuret method as described by Reinhold et al (54).

3.4.3.1 Principle of the method:

The principle of this method is based on the reaction of protein peptide bond with cupric ion in alkaline medium found in Biuret solution, the intensity of color formed is due to total protein concentration in the sample.
3.4.3.2 Procedure:

Three tubes were labelled as (blank, standard and sample). In the corresponding tube, add 25µl for standard, 25µl for sample and 25µl for blank and add 1ml reagent in all tubes. All tubes were mixed and incubated at 37°C for 5 min. Read the absorption of the sample and standard against the blank. The coloured solution was read with a spectrophotometer (JENWAY 6305 UV/Vis) at a wavelength of 540 nm.

The concentration of total protein was calculated as follows:

Total protein (g/dl) = \frac{\text{Ab of sample} - \text{Ab of blank}}{\text{Ab of standard} - \text{Ab of blank}} \times \text{concentration of standard}

3.5 Estimation of alanine transaminase (ALT/GPT):

3.5.1 Principle of the method:

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 et al. The enzyme catalyzed the reversible transfer of an amino group from alanine to \( \alpha \)-ketoglutarate forming glutamate and pyruvate.

ALT is measured by monitoring the concentration of pyruvate hydrozone formed by the reaction with 2-4-dinitrophenyl hydrazine (DNPH) in alkaline solution, and mean absorbance per/min at 340 nm/min

3.5.2 Procedure:

Assaying conditions (wavelength 340nm, temperature at 37°C) are pipetted into a cuvetted 1.0 ml of working reagent then was mixed with 100 µl of sample after incubation for 30 seconds, this initial absorbance was measured then repeated three times at 1 minute interval, calculate the difference between absorbance and the average absorbance differences per minute (ΔA/min)
3.6 Estimation of aspartate aminotransferase (AST/GOT):

3.6.1 Principle of the method:

Aspartate aminotransferase (AST or GOT) catalyzes the transfer of the amino group from aspartate to 2 oxoglutarate forming oxalacetate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH; measure at 340nm by means of the malate dehydrogenase (MDH) coupled reaction.

\[
\text{Aspartate} + 2\text{-oxoglutarate} \rightarrow \text{oxalacetate} + \text{glutamate}
\]

\[
\text{Oxalacetate} + \text{NADH} + \text{H}^+ \rightarrow \text{Malate} + \text{NAD}^+
\]

4.6.2 Procedure:

Assaying conditions (wavelength 340nm, temperature at 37°C) is pipetted into a cuvette 1.0 ml of working reagent then was mixed with 100 µl of sample after incubation for 30 seconds, this initial absorbance was measured then at 1 minute interval for 3 minutes, calculate the difference between absorbance and the average absorbance differences per minute (ΔA/min).

3.6 Histopathological Methods:

Slices from the liver and kidney, were collected from dead or sacrificed 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 et al (56) method.

3.7 Statistical Analysis:

Data was expressed as mean ± standard deviation. Comparative analysis was done using analysis of variance (ANOVA). Comparative analysis was performed using comparison test. Statistical significance was set at P<0.05. All statistics were done using SPSS for windows (version 21.0).
4. Results

4.1 Observation:

There were no abnormal activities observed in the control group throughout the period of study. In the treated groups there was reduction in physical activities when compared with the control group, although for group three the physical activity was restored to minimal.

4.1.1 Clinical Signs and Mortality:

Rats received 200 mg/kg B wt with vitamin E showed no clinical signs, whereas depression and difficulty in breathing were observed in rats which received 140 and 200 mg/kg Bwt. The mortality was 100% in rats which received 170 mg/kg Bwt after 5 weeks, at the end of 2nd week 50% of rats were dead which received 140 and 170 and 200mg/kg Bwt, on the last day of the 3rd week the rats in group F has no appetite for eating, as compared to the corresponding control group, at the end of the 3rd week blood sample was taken in plane vacutainer to measure the total protein and ALT and AST activity, and all rats had been dissected and a sample from the liver and kidney had been taken, except group D that received 170mg/kg Bwt.

4.1.2 Body Weight

Table (1) & fig (1) clearly showed the effect of various doses of potassium bromate on rats body weights. Analysis of variance (ANOVA) showed significant difference (P<0.05) on body weight between the groups for the three weeks of administration of potassium bromate, The control group showed normal weight gain as well as weight reduction in third week in groups (B ,C ,D and F). In G E (200mg/kg Bwt) supplemented with vit E a significant gain in weight in the third week as compared to the corresponding control group was seen. The results showed that potassium bromate had duration – dependent effect on weight.
Table [1]: The mean body weights (mg) of rats orally treated with various levels of potassium bromate

<table>
<thead>
<tr>
<th>Doses mg/kg</th>
<th>Week0</th>
<th>Week1</th>
<th>Week2</th>
<th>Week3</th>
<th>Week4</th>
<th>Week5</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 0</td>
<td>92.20±13.44</td>
<td>98.20±20.90</td>
<td>99.20±22.25</td>
<td>116.00±22.25</td>
<td>00.00</td>
<td>00.00</td>
</tr>
<tr>
<td>B 140</td>
<td>117.00±16.78</td>
<td>117.00±13.20</td>
<td>86.60±49.19</td>
<td>70.60±65.30</td>
<td>00.00</td>
<td>00.00</td>
</tr>
<tr>
<td>C 170</td>
<td>110.40±9.09</td>
<td>116.20±10.16</td>
<td>106.60±17.10</td>
<td>63.60±58.78</td>
<td>00.00</td>
<td>00.00</td>
</tr>
<tr>
<td>D 170</td>
<td>132.60±1.95</td>
<td>120.80±26.57</td>
<td>99.80±60.40</td>
<td>78.60±72.59</td>
<td>28.00±62.00</td>
<td>00.00</td>
</tr>
<tr>
<td>E 200+E</td>
<td>90.20±11.23</td>
<td>96.00±17.97</td>
<td>98.20±16.30</td>
<td>105.40±12.58</td>
<td>00.00</td>
<td>00.00</td>
</tr>
<tr>
<td>F 200</td>
<td>92.80±19.99</td>
<td>80.20±11.32</td>
<td>63.00±37.67</td>
<td>51.40±29.56</td>
<td>00.00</td>
<td>00.00</td>
</tr>
</tbody>
</table>

Data are expressed as mean± standard deviation, P value

Fig 1. mean body weights

4.2 Biochemical Results:

The effects of various doses of potassium bromate on activity of alanine transaminase (ALT) and aspartate transaminase (AST) and the concentration of total protein are given in table (2) & fig (2). The results showed significant elevation in serum enzymes and differences between control group and the other groups (B(P< 0.003) , C (P<0.001) , and F(P<0.000)) in group E treated with
200mg/kg Bwt with vit E there was a significant decrease in (ALT & AST), and the total protein levels showed a significant decrease in the treated groups (B(P<0.005),C(P<0.001),F) compared to the control, but in group E treated with 200mg/kg Bwt with vit E the total protein level was same as control group.

**TABLE 2. Serum levels of liver enzymes activity and total protein level in rats treated with various doses of potassium bromate**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>140mg/kg Bwt</th>
<th>170mg/kg Bwt</th>
<th>200+E</th>
<th>200mg/kg Bwt</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT level (IUL)</td>
<td>26.19±13.80</td>
<td>40.72±23.34</td>
<td>29.34±8.06</td>
<td>15.90±4.40</td>
<td>96.03±36.52</td>
</tr>
<tr>
<td>AST level (IUL)</td>
<td>45.09±10.36</td>
<td>100.80±12.26</td>
<td>110.49±24.68</td>
<td>71.58±29.20</td>
<td>128.55±39.45</td>
</tr>
<tr>
<td>Protein</td>
<td>9.33±1.04</td>
<td>6.39±0.36</td>
<td>5.51±1.14</td>
<td>9.39±1.60</td>
<td>6.06±2.38</td>
</tr>
</tbody>
</table>

Data are expressed as mean±standard deviation, P value probability using one-way ANOVA

**Fig 2.** Serum levels of liver enzymes activity and protein level treated with different doses of potassium bromate
4.3 Histopathological Findings:

The Histopathological findings in rats treated with potassium bromate were shown in the coming figures. In group B treated with 140mg/kg Bwt, the Liver showed severe congestion & dilation of central veins and sinusoids, Loss of hepatic architecture, vesicular nuclei with necrosis in liver cells (Fig 3&4), figure (5&6) the Kidney showed congestion, hemorrhages, dilation of glomerular capsules and segmentation of glomerular tuft. Necrosis of renal tubules with eosinophilies in the cytoplasm in the capsular areas in medulla. In group C treated with 170mg/kg Bwt fig (7&8 and 9) the liver showed Loss of normal hepatic architecture, pyknotic nuclei of hepatocytes dilatation of central veins and hepatic sinusoids, severe congestion of central veins and sinusoidal necrosis of hepatocytes with complete loss of hepatic cells, some vasodilation in some hepatic areas, relative increase in Kupffer cells and infiltration of inflammatory cells especially in portal areas.

Fig (11&12) of the kidney showed dilated glomeruli, with some segmented glomerular tuft, severe necrosis of tubules of the kidneys, some tubules showed eosinophils in the cytoplasm, with infiltration of inflammatory cells in the medullary areas with some pyknotic nuclei. In group F treated with 200mg/kg Bwt fig (13&14) the liver showed Loss of normal hepatic architecture and the hepatocytes with pyknotic nuclei, some of hepatic cells were replaced by debris and remained as necrotic cells, relative increase in Kupffer cells congestion and dilatation of central veins and sinusoids. In group F treated with 200mg/kg Bwt fig (15) the liver showed necrotic areas appearance as (empty space). Fig (16&17) illustrated the kidney showing dilated glomeruli, with some glomerular tuft, Shrinking or segmented congestion of blood vessel with some areas of hemorrhage, necrosis of lobular epithelial cells which appeared more eosinophilic. In group E treated with 200mg/kg Bwt with vit E fig (18) the kidney showed less necrosis.
compared with the group treated with 200mg/kg Bwt only, showed hypertrophy of cells with vesicular nuclei and no segmentation of glomerular tuft.

**Fig3**: Photomicrograph of a Liver section in group one treated with 140mg/L KBrO₃ showing severe congestion in central veins with dilated sinuosids and haemorrhage (H&E x10).

**Fig4**: Photomicrograph of a Liver section in the group treated with 140mg/L KBrO₃ showing severe congestion of central vein & sinuosids, pyknotic hepatic cells and dilated sinusoid (H&E X40).
**Fig 5:** Photomicrograph of a kidney section in the group treated with 140mg/L KBrO₃ showing Congestion, haemorrhage, dilated glomeruli & segmented glomerular tuft (H&E x10).

**Fig 6:** Photomicrograph of a kidney section in the group treated with 140mg/L KBrO₃ showing dilated glomeruli, segmented glomerular tuft, congestion, haemorrhage, necrosis & inflammatory cells infiltration (H&E x40).
Fig 7: Photomicrograph of a Liver section in the group treated with 170mg/L KBrO$_3$ showing loss of architecture of normal liver lobes. Congestion & haemorrhage, vacuoles in hepatocytes, dilation of sinusoids, prominent kupffer cells (H&E x 10).

Fig 8: Photomicrograph of a Liver section in the group treated with 170mg/L KBrO$_3$ showing congestion, vesicular nuclei, vacuoles in hepatocytes, dilation of sinusoids (H&E x40).
Fig 9: Photomicrograph of a Liver section in the group treated with 170mg/L KBrO₃ showing congestion, necrotic areas (empty spaces) (H&E x10).

Fig 10: Photomicrograph of a Liver section in the group treated with 170mg/L KBrO₃ showing congestion and necrotic areas (H&E x40).
Fig 11: Photomicrograph of a kidney section in the group treated with 170mg/L KBrO$_3$ showing Segmentation of glomerular tuft and congestion (H&Ex10).

Fig 12: Photomicrograph of a kidney section in the group treated with 170mg/L KBrO$_3$ showing necrosis of renal tubules, congestion and infiltration of inflammatory cells (H&Ex40).
Fig 13: Photomicrograph of a Liver section in the group treated with 200mg/L KBrO₃ showing loss of normal hepatic architecture with dilatation of central vein & sinusoids(H&Ex10).

Fig 14: Photomicrograph of a Liver section in the group treated with 200mg/L KBrO₃ showing pyknotic nuclei of hepatic cells, increase number of kupffer cells & dilatation of blood vessels(H&Ex40).
**Fig 15:** Photomicrograph of a Liver section in the group treated with 200mg/L KBrO₃ showing necrotic areas appearance as (empty space) (H&Ex10).

**Fig 16:** Photomicrograph of a kidney section in the group treated with 200mg/L KBrO₃ showing dilated glomeruli with segmental tuft, infiltration of inflammatory cells and congestion (H&Ex40).
**Fig 17:** Photomicrograph of a kidney section in the group treated with 200mg/L KBrO₃ showing necrosis of renal tubules, dilated glomeruli (H&Ex40).

**Fig 18:** Photomicrograph of a kidney section in the group treated with 200mg/L KBrO₃ with vitamin E showing less necrosis compared with the group treated with 200mg/l, hypertrophy of cells with vesicular nuclei. No segmentation of glomerular tuft (H&Ex40).
5.1 Discussion

The present study was conducted to study the effect of potassium bromate on albino rats when administered at different concentrations.

Potassium bromate is a crystalline white powder it has no medicinal value and it has been used as food additive and was considered as flour treatment agent by FAO/WHO (1964). The use of potassium bromate as a food additive created many health problems and it was claimed that potassium bromide is carcinogenic and several safety evaluation have been done but unfortunately the results are controversial. However, doses of 140, 170 and 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.

In the present study, there were significant reductions in body weights of rats administered with KBrO3 compared with the controls. This was more evident in the 3\textsuperscript{rd} weeks of study where rats administered with 140, 170 and 200 mg/kg b.w./day KBrO3 had significantly lower body weights compared with the controls. This agrees with a previous study of (\cite{57}), which reported a significant reduction in bodyweight of rats administered with potassium bromate. In contrast, other studies of \cite{58, 59} have reported absence of KBrO3 effect on body weights of rats.

The present study showed death that occurred when potassium bromate has been given at the rate of 140 & 170 & 200 mg/kg B wt, in the end of week two 50\% from rats were dead, and 100\% of all rats in group 170mg/kg Bwt were dead in five weeks, in the last day of the 3\textsuperscript{rd} week, the rats in group F 200mg/kg Bwt had no appetite for eating.

The reduction of total protein observed in this study was due to liver damage which resulted in reduction of protein synthesis due to toxicity of potassium bromate. This finding is in agreement with \cite{21}.
The increase of ALT & AST might be attributed to the high permeability of the hepatocyte membranes or its destruction as confirmed by the pathological changes seen in the liver and kidney. This finding is comparable with the result of (17). That the increased activities of serum AST and ALT are often used as a marker of hepatic injury as they indicate cellular leakage of intracellular enzymes and loss of liver cell membrane stabilization (60). Destruction of the hepatocytes induced by KBrO3 caused significant (P<0.05) rise in AST and ALT in (Group B&C&F) which was supported by the histological alterations that appeared. This agreed with the earlier studies which reported an increase in ALT and AST in rats administered with 20, 50, 100, and 200 mg/kg Bwt of KBrO3 (48, 61).

Administration of KBrO3 with vitamin E markedly reduced hepatic histoarchitecture and biochemical abnormalities. The significant reduction in enzyme activities of rats treated with vitamin E suggests its capability to improve the deleterious effects of KBrO3. Decreased levels of transaminases indicate restoration of the integrity of plasma membrane and protection of hepatocytes against damage, that caused by hepatotoxin. This in concurrence with the frequently recognized viewpoint that serum levels of transaminases come back to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes. This agreed with (62).

KBrO3 administration in this work caused distortion and damage to the liver tissue architecture. Congestion of the central vein and sinusoidal dilatation, as well as the significant degrees of cell necrosis and degeneration with inflammatory cellular infiltration, were recorded in all treatment. This study is similar to the work carried out by (3)
5.2 Conclusions:

From the results obtained, we can conclude that:

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

(2) Further study is needed to confirm the carcinogenicity of KBrO₃ when added to bread for a longer time.

(3) Potassium bromate is toxic causing damage to the liver and kidney with significant increase in liver enzymes and significant decrease in protein and reduction of body weight.

(4) Vitamin E neutralizes the toxicity of potassium bromate in rats.

(5) The present study indicated that a short-term exposure to small and high doses of KBrO₃ cause alterations in the histology of the liver and kidney of Wistar rats.
5.3 Recommendations

1- Prohibiting the use of KBrO$_3$, as an improving substance in bread kneading, as it is harmful and toxic, and it damages the liver and kidney, as showed and proved by the findings of this study.

2- Conducting further and comprehensive studies in this field, is required as it is important for human health.

3- The concerned officials, in the field of specifications and standards Lab. analysis and investigation should look into consideration in improving and updating, developing methods related to the food stuff analysis, namely the bread.
5.4 References


44. Chipman, J.K., Davies, J.K., Parson, J.L., Nair, J., O'Neill, G. and Fawell, J.K. (1998). DNA oxidation by potassium bromate; a direct mechanism or linked to lipid peroxidation. Toxicol. 126, 93-102


