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Pathological and Immunohistochemical study of potassium bromated on the liver of rat

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Abstract

Food additives are substances that become part of the food when added during the processing or production of that food. Potassium bromate ($KBrO_3$) is one of the food additives which often used in bakeries as flour improver and dough conditioner. It is a nephro and neurotoxic in humans and carcinogenic in rodents. In addition, experiments aimed at elucidating the mode of carcinogenic action have revealed that KBrO3 is a complete carcinogen.

The objective of this study was to investigate the toxic and carcinogenic effects of various levels of dietary potassium bromate in male and female rats. The criteria for assessment include its effects on growth, pathological, immunohistochemical, histochemical, and biochemical alterations.

In this experiment a total of 105 albino rats of both sexes, weighing 120 g were used, they were kept under standard conditions and housed in a metallic cages under standard conditions and had free access to water and standard diet. The animals were left for a week, as an adaptation period. The rats used were 45 male, 45 female and 15 rats of both sexes used as control. Both male and female rats were divided into three groups. KBrO3 dissolved in water at concentrations of 200, 400 and 600 ppm respectively was administered to male and female group rats daily till the end of the experiment. After 6, 9 and 14 months ten animals from each group (5 male and 5 female) and five from the control were sacrificed by cervical dislocation.

Histological examination of liver revealed congestion of its blood vessels, various degenerative and necrotic alterations with toxic hepatitis and bile duct hyperplasia. Carcinogenic and dysplastic alterations were recorded in some cases especially after 9 and 14 months of potassium bromate treatment which gave positive results by using Proliferating cell nuclear antigen (PCNA) as an immunohistochemical stain. Liver function markers showed significant increase in the level of Alanine Aminotransferase (ALT) and Aspartate aminotransferase (AST).

Key words: Rat , liver, KBrO3 ,Pathological, Immunohistochemical, Histochemical effect.
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Introduction

Potassium bromate ($KBrO_3$), a white crystalline solid and a widely reactive food additive **(WHO,1996)**, it is often used in bakeries as flour improver yielding higher bread volume **(Kurokawa *et al.*, 1990)** and used as a dough conditioner for flour **(DIACHENKO and WARNER 2002)**.

Potassium bromate is generated as a contaminant in drinking water due to conversion of bromide found naturally in water to bromate by ozone which is used as disinfectant **(Ueno *et al.*, 2000)**. Several researches on safety evaluation of potassium bromate were carried out and also It was found to be a genotoxic and carcinogenic. Studies have also shown that it possess the potential of inducing cancer, kidney failure, deafness, redness and pains of the eye and skin **(Mack, 1988; De Angelo *et al.*, 1998)**. The agent is carcinogenic in rats and nephrotoxic in both man and experimental animals when given orally. It has been demonstrated that KBrO3 induces renal cell tumors, mesotheliomas of the peritoneum, and follicular cell tumors of the thyroid. In addition, experiments aimed at elucidating the mode of carcinogenic action have

ISSN 1110-1423

revealed that KBrO3 is a complete carcinogen, possessing both initiating and promoting activities for rat renal tumorigenesis. It is classified as a category 2B carcinogen (possibly carcinogenic to humans) based on sufficient evidence of kidney carcinogenicity in rats by the International Agency for Research on Cancer (IARC).

The international Agency for Research on Cancer (IARC) recently evaluated all of the data on KBrO3 and concluded that there is sufficient evidence for the carcinogenicity of KBrO3 in experimental animals. Potassium bromate (KBrO3), a salt of the bromate ion, is nephro and neuro-toxic in human and carcinogenic in rodents **(IARC, 1986; Kurokawa et al. 1990)**. Bromate is classified as a probable human carcinogen due to its kidney carcinogenicity in male and female rats following exposure in drinking water **(Kurokawa et al. 1983, 1986a, 1986b; DeAngelo et al. 1998; Wolf et al. 1998)**. A dose-response relationship in rat kidneys was observed in progressive severity from renal dysplastic foci, preneoplastic lesions, through renal adenomas, and finally renal carcinoma **(Kurokawa et al. 1986; DeAngelo et al. 1998; Wolf et al. 1998)**. KBrO3 has been also classified as a compound belonging to the group 2B, a possible human carcinogen. Bromate is the BrO3- ion, a combination of bromine and oxygen. Bromate is listed as a B2 probable human carcinogen by the U.S. EPA **(Iris, 2004)**. In rat kidneys a dose-response relationship in progressive severity of renal dysplastic foci, preneoplastic lesions, renal adenomas, and finally renal carcinoma were studied **(Kurokawa et al. 1986a; DeAngelonet al. 1998; Wolf et al. 1998)**. In addition, studies by **Umemura et al. (2004, 2006)** demonstrated dose-dependent changes in cell proliferation of potassium bromate in male and female rat kidneys.

The present study was conducted to investigate the toxic and carcinogenic effects of various levels of dietary potassium bromate in male and female rats. The criteria for assessment includes its effects on growth, pathological, immunohistochemical, histochemical, biochemical and hematological alterations.

Material and Methods

Chemicals:

Potassium bromate (KBrO3) in the form of powder with a purity of greater than 99.5%. It was supplied by a private chemical company at Cairo Egypt.

Animals:

A total of 105 albino rats of both sexes, weighing 120 g were supplied by faculty of Veterinary medicine, Cairo University.

A total of 105 albino rats of both sexes, weighing 120 g were supplied by faculty of Veterinary medicine, Cairo University. They were kept under standard conditions and were housed in metabolic cages under standard conditions and had free access to water and standard diet. The animals were left for a week, as an adaptation period.

Experimental Design:

In this experiment; the rats used were 45 male, 45 female and 15 rats of both sexes used as control. Both male and female rats were divided into three groups. KBrO3 dissolved in water at concentrations of 200, 400 and 600 ppm respectively was administered to male and female group rats daily till the end of the experiment. The rats were observed throughout the experimental periods to record the signs and deaths occurred till the end of the experiment. After 6, 9 and 14 months ten animals from each group (5 male and 5 female) and five from the control were sacrificed by cervical dislocation.

Histopathological examination:

Tissue samples of the liver were removed carefully, and fixed in neutral buffered formalin 10%, dehydrated in ascending grade of alcohol, cleared and embedded in paraffin, sectioned at 5 µm thickness and stained by H & E and examined microscopically **(Bancroft et al. (1996)**.

Immunohistochemical method :

For immunohistochemical study Proliferating cell nuclear antigen (PCNA) was used was applied according to **(Hall et al., 1990)**.

Biochemical method:

Serum samples were collected and analyzed to determine the level of Aspartate aminotransferase (AST)), and Alanine Aminotransferase (ALT) using the method of **Reitman and Frankel (1957).**

Results

Clinical signs of some rats due to the effects of pot. bromate included dullness, ataxia and loose their appetite, sometimes circling with paddling movements and hyper-excitability were also recorded in some animals.

Biochemical results: From table 1and Figs A and B it is clear that:

1- ALT 6:

There's statistically significant difference between treatment and control, as (**F**) value equal (16.032) with P-value level less than (0.01) for (600ppm), (200ppm), (400ppm), (Control), which the **Mean** equal (59.99), (55.69), (55.08), (42.89), response, also (**F**) value equal (6.950) with P-value level less than (0.01) which the **Mean** equal (66.43), (64.76), (56.58), (43.45), response.

2- ALT 9:

There's statistically significant difference between treatment and Control as (**F**) value equal (6.294) with P-value level less than (0.01) for (200ppm), (600ppm), (400ppm), (Control), which the **Mean** equal (53.04), (50.07), (46.70), (37.77), response, meanwhile there's no statistically significant difference between treatments and Control, as (**F**) value equal (1.860) with P-value more than (0.05).

3- ALT 14:

There's statistically significant difference between treatment and Control, as (**F**) value equal (3.789) with P-value level less than (0.05) for (200ppm), (600ppm), (400ppm), (Control), which the **Mean** equal (50.59), (49.35), (47.64), (43.05), response, while There's no statistically significant difference between treatment and Control, as (**F**) value equal (1.328) with P-value more than (0.05).

Table -1: Showing the differences between more than two groups of variable ALT and AST mg/dl by using One way ANOVA (F test).

Treatment Variables	Control (1)	200ppm	400ppm	600ppm	F-test	p-value
	Mean ±Std.	.Mean ±Std	.Mean ±Std	.Mean ±Std		
1. ALT 6	42.89± 1.72	55.69 ± 6.06	55.08± 4.95	59.99 ±1.75	16.032	0.001**
2. AST 6	43.45 ± 6.09	56.58 ±16.12	66.43 ±2.15	64.76± 3.89	6.950	0.003**
1. ALT 9	37.77± 6.35	53.01 ± 5.80	46.70 ± 5.25	50.07 ± 6.07	6.294	0.005**
2. AST 9	42.88± 4.50	52.77± 8.44	53.43± 9.69	54.28± 9.33	1.860	0.17
1. ALT 14	43.05 ± 0.68	50.59± 3.29	47.64± 4.69	49.35± 4.91	3.789	0.03*
2. AST 14	44.57 ± 2.87	52.58± 5.01	54.03 ± 10.00	52.20±11.81	1.328	0.30

**Significant at the (.01) level
*Significant at the (.05) level

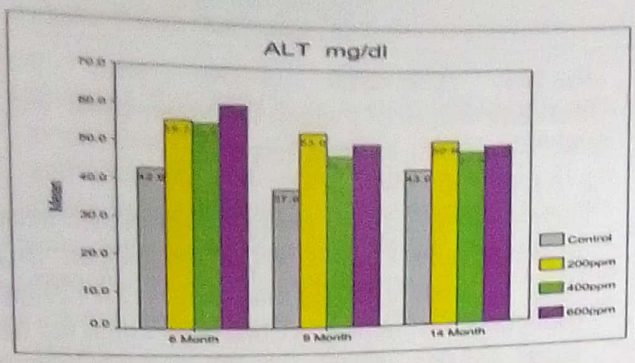

Fig. (A): The differences between the level of Alanine Aminotransferase (ALT) treatment.

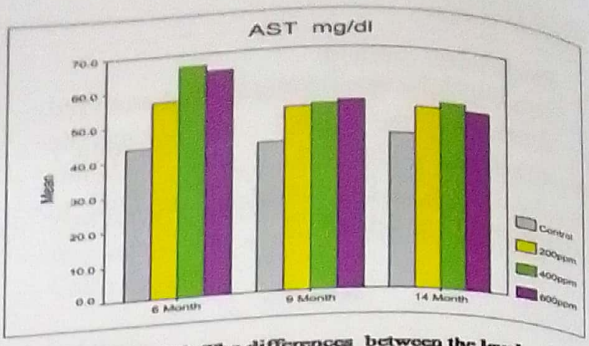

Figure (B): The differences between the level of Alanine Aminotransferase (AST) treatment.

Pathologic Changes:

Six months after potassium bromate treatment, liver sections showed enlargement and darkening of nuclei with clumping of chromatin. Evidence of dilatation and proliferation of bile ducts as well as dilatation of hepatic sinusoids with kupffer cell hyperplasia were noticed. Disruption of hepatic architecture to the degree of complete disorganization with increase in inflammatory cells were noticed here and there. Changes in the hepatic parenchyma varied from mild to moderate vacuolar degeneration of hepatocytes. Some hepatic cells were either atrophied with deeply stained eosinophilic cytoplasm or necrotic with pyknotic nuclei and others were apoptotic. Generalized necrosis of the hepatic cells was seen especially in the periportal areas (Fig. 1A). In addition; the hepatic cells of some cases lose its normal shape and became irregular or elongated with slightly vacuolated cytoplasm and their nuclei haphazardly located. Hypertrophic and cytomegalic hepatocytes were present especially in the centrilobular and midlobular areas in some cases. Increase in the number of binucleated hepatocytes with clear variations of nuclear size was observed (Fig. 1B). Kupffer cells were activated, manifested by increasing in their number and swelling of their nuclei.

Some cases showed different forms of coagulative necrosis, the most common form was multifocal minute necrotic foci replaced by mononuclear cells scattered throughout the hepatic parenchyma, confluent necrosis was also observed which involves masses of hepatic cells distributed in a certain region of the hepatic lobules together with individual hepatic cells necrosis were seen. The remaining hepatic cells were suffered from pressure atrophy and severe degenerative changes. Variable extent of micro and macro-vesicular steatosis were also observed.

Portal areas revealed congested blood vessels with slight perivascular and periportal mononuclear cells infiltration mostly lymphocytes and macrophages and increase in fibrous tissue proliferation with hyperplasia of the lining epithelium of some bile ducts together with oval cells proliferation. These cells were restricted to periportal areas and few cells extended and observed in between the hepatic parenchyma (Fig.1C). In few cases the hyperplastic bile ducts and the newly formed bile ducts arranged in an adenoid pattern (Fig. 1D).

After 9 and 14 months post treatment the lesions were more severe and the severity of the lesions was mostly dose related and the more the given pot. bromate concentration the more the severity of the lesions.

In some cases of both male and female rats there were areas with lightly stained eosinophilic cells type, composed of cells slightly larger than the normal hepatocytes, with light eosinophilic cytoplasm and prominent nuclei. The cells were also arranged haphazardly, and had lost the normal arrangement of hepatic cords. The cytoplasm of some cells, were coarsely granular giving a ground glass appearance of the cells. Vacuolated hepatocytes (clear cells) were more

pronounced revealed honey comb appearance of the cytoplasm due to presence of very small and fine vacuoles and appear in trabecular arrangement of small eosinophilic cells and large vacuolated cells with hyperchromatic nuclei (fig. 2A). In few cases large dysplastic foci of basophilic cells surrounded by eosinophilic cells were observed. The basophilic foci composed of varying sized hyperplastic hepatic cells with darkly stained nuclei and basophilic cytoplasm. The cells of these foci did not retain the normal arrangement of hepatic cells (Fig.2B). Mixed types of vacuolated, eosinophilic and basophilic in addition to cytomegalic and karyomegalic cells were observed in some animals. The above mentioned foci were seen in some cases after 9 and 14 months of the treatment in both male and female but appeared to be more frequent in the male rats which were much more progressed by time (especially in the group treated by 600 ppm) after 14 months. The cells of eosinophilic foci showed marked degree of atypia represented by cellular and nuclear pleomorphism, hyperchromacia, cytomegally and karyomgally and presence of prominent one or more nucleoli.

In two male animals after 9 months of group 3 (treated by 600 ppm pot. Bromate) the cells were arranged in the form of trabeculae of variable thickness (2-4 cell rows) and the cells were small with eosinophilic cytoplasm and deeply stained nuclei (Fig.2C). In other cases dysplastic appearance of the hepatocytes with hyperchromatic nuclei and clear mitotic cells and giant cells formation were seen (Fig.2D), variations of nuclear size, binucleation and hyperchromatic nuclei with clear large nucleoli were also observed (Fig.2E).

Immunohistochemical results:

PCNA immuno-reactivity was localized in the nucleus and the nucleolus together with the nuclear membrane of the dysplastic cells of the hepatocytes. Its expression was estimated as the percentage (number) of positively stained cells by the antibody. In control liver sections there was a very few number of PCNA positive cells as shown in figure (1). The number of PCNA positive cells was increased in the nuclei of dysplastic cells of treated groups. After 6 months of treatment the number of positive cells was few (Fig.2&3). After 9 and 14 months it reached to high positive cells number with strong immune-staining (Fig.4-6).

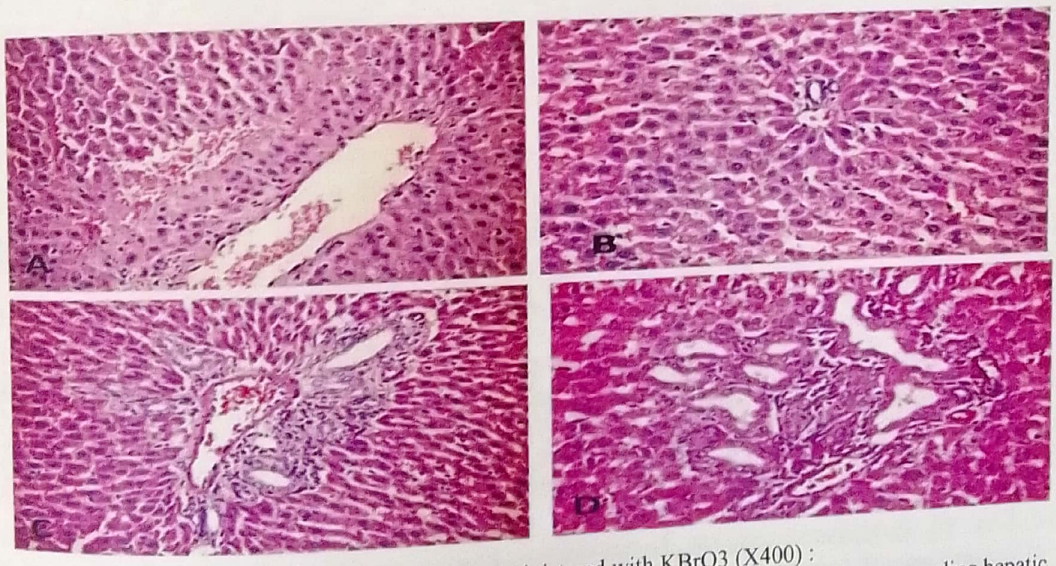

Fig. (1): H &E, Stained sections of male and female liver administered with KBrO3 (X400) :

A) Mal rat liver 6 months, showing congested and dilated blood vessel and necrosis of its surrounding hepatic cells with areas of hemorrhages.

B). Mal rat liver 6, showing increase number of binucleated hepatocytes with variation of nuclear size.

C).Female rat liver for 6 months, showing dilated and hyalinized blood vessel wall with hyperplasia of the bile ducts and formation of newly formed bile ducts and ductules in the portal tract that extended between the hepatic cells which appeared atrophied and deeply stained. Note oval cell proliferation.

D) Male rat liver administered for 9 months, showing hyperplasia of the bile ducts with the formation of newly formed bile ducts which arranged in an adenoid pattern .

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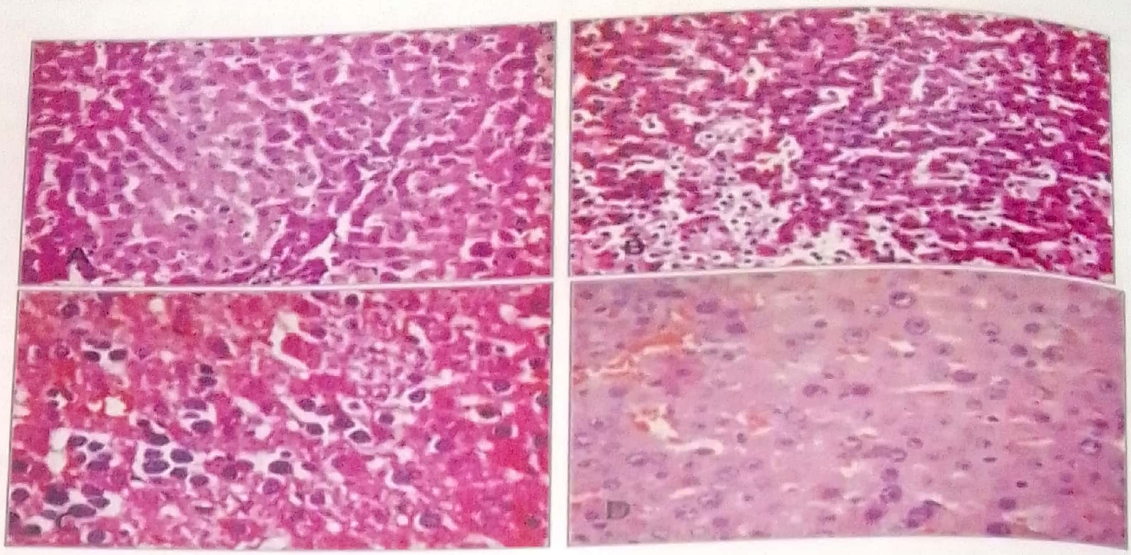

Fig. (2): Photomicrograph of stained sections of female and male rat liver administered with KBrO3:
A) Female rat liver administered for 9 months G2, showing large dysplastic foci of basophilic hepatic cells surrounded by eosinophilic cells.
B) Female rat liver administered for 12 months G3, showing trabecular arrangement of small eosinophilic cells and vacuolated cells with hyperchromatic nuclei.
C) Female rat liver administered for 12 months G3, showing dysploastic appearance of the hepatocytes with hyperchromatic nuclei and clear mitotic cells (arrow) and giant cells (Arrow head).
D) Photomicrograph of male rat liver administered for 12 months G3, showing dysplastic changes manifested by variations of nuclear size, binucleation and hyperchromatic nuclei with clear large nucleoli. Stain H&E, X400.

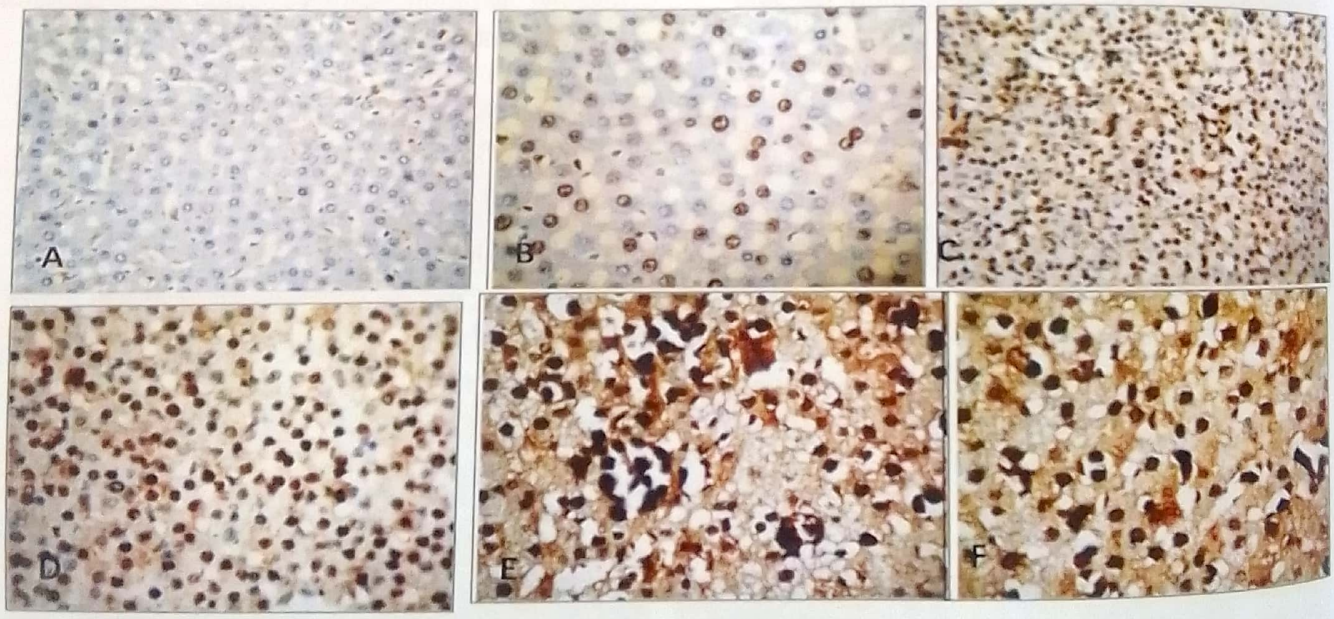

Fig. (3): Paraffin stained section of rat liver receiving KBrO3:
A): Control rat liver after 9 months showing negative (PCNA) immunostaining. PCNA, X400.
B): Rat liver after 6 months showing moderate intensity of immunostaining of the nuclei and nucleoli of dysplastic cells. PCNA, X 400.
C): Rat liver after 6 months of showing strong intensity of immunostaining (PCNA) positive nuclei and nucleoli. PCNA, X200.
D): Rat liver after 9 months showing strong intensity of immunostaining (PCNA). Note the deeply positive nuclei and light cytoplasmic staining. PCNA, X 400.
E): Rat liver after 9 months showing strong intensity of immunostaining (PCNA) positive nuclei. Note the light cytoplasmic staining. PCNA, X 450.

F): Rat liver after 9 months showing strong intensity of immunostaining (PCNA) positive nuclei. Note the light cytoplasmic staining. PCNA, X450.

Discussion

The present study revealed that long-term oral administration of KBrO3 in drinking water at doses of 200, 400, 600 ppm for 6, 9, 14 months leading to degenerative and destructive effects in liver tissue, carcinogenicity was also established which was dose and time related, evidence of sinusoidoidal dilatation with kupffer cells hyperplasia were noticed. Disruption of hepatic architecture to the degree of complete disorganization with increase in inflammatory cells was noticed here are there. Changes in the hepatic parenchyma varied from mild to moderate vacuolar degeneration of hepatocytes. Generalized necrosis of the hepatic cells were seen especially in the periportal areas and there was multifocal minute necrotic foci replaced by mononuclear cells scattered throughout the hepatic parenchyma, confluent necrosis was also observed which involves masses of hepatic cells distributed in a certain region of the hepatic lobules together with individual hepatic cells necrosis were also seen. Portal areas showed slight perivascular and periportal mononuclear cells infiltration mostly lymphocytes and macrophages, with hyperplasia of the bile ducts and formation of newly formed bile ducts and ductules. So the intense damage to the hepatic tissue and its distortion in its architecture, with congestion of the hepatic blood vessels and sinusoids as well as cell necrosis which were recorded in most animals of all administered groups agreed with that recorded by **Abuelgasim, Omer and Elmahdi (2008)** and **Oyewo et al,. (2013)**. The present research is also supported by **Akanji, et al., (2008)** where Potassium bromate caused infiltration of the interstitial tissue of the liver and portal tracts by mononuclear cells. In contrast to our findings, **Umemura *et al.* (1995)** reported no pathological change in the liver which was mostly due to the low dose given and the short time of administration. **Khan *et al.* (2003)** reported during their study a reduction of antioxidant enzymes and enhancement of xanthine oxidase and lipid peroxidation when rats were treated with potassium bromate administered intraperitoneally. Meanwhile **El-Sokkary (2006)** observed significant increase in malondialdehyde as an indicator of lipid perioxidation. These mentioned findings support the histological changes occurred in the liver during the present study as lipid peroxidation play a role in liver injury.

The increase in ALT parameter suggests high permeability of hepatocytes due to liver damage which was supported in the histopathological results observed during the present study. ALT is consequent with hepatic cell damage and injured cell membrane permeability. This finding is parallel with **Kurokawa *et al.* (1990) and Omer et al,. (2008)** who reported an increase in ALT in rats received 600 mg kg^{-1} potassium bromate in drinking water. Some degeneration of endothelial cells observed in rats administered with potassium bromate, may be an indication of the destruction of the capillary endothelium of the liver by the chemical substance. This may result in reduction in total protein and albumin synthesis and increase in alanine transaminase (ALT), which are consequent with hepatic cell damage and injured cell membrane permeability **(Omer et al., 2008)**. The portal tracts of some cases infiltrated with chronic inflammatory cells and increase in fibrous tissue proliferation with hyperplasia of the lining epithelium of some bile ducts agreed with **Akanji, et al., (2008)**.

The present results showed that the lesions were mostly dose and time related and the severity of the lesions were more seen in groups of rats given high doses of KBrO3 in drinking water. After 9 and 14 months post treatment we found that in some cases of both sexes an areas with dysplastic foci, some with lightly stained eosinophilic cells type, which arranged haphazardly, with coarsely granular cytoplasm. The cells of eosinophilic foci showed marked degree of atypia represented by cellular and nuclear pleomorphism, hyperchromacia, and presence of prominent one or more nucleoli.The vacuolated type (clear cells) were more pronounced and appear in trabecular arrangement of small eosinophilic cells and large vacuolated cells with hyperchromatic nuclei. In few cases

ISSN 1110-1423

large dysplastic foci of basophilic cell foci, composed of varying sized hyperplastic hepatic cells with darkly stained nuclei and basophilic cytoplasm. The cells of these foci did not retain the normal arrangement of hepatic cells.

The above mentioned foci were seen in few cases after 9 and 14 months of treatment in both male and female but appeared to be more frequent in the male rats after 14 months. The dysplastic hepatocytes showed hyperchromatic nuclei, binucleation and variations of nuclear size with clear large nucleoli were also observed. The lesions described were much more progressed by time and in the animals received the doses (especially in the group treated by 600 ppm) after 14 months.

In two male animals after 9 months of group 3 (treated by 600 ppm pot. Bromate) the cells were arranged in the form of trabeculae of variable thickness (2-4 cell rows) and the cells were small with eosinophilic cytoplasm and deeply stained nuclei.

The present study showed a distinct nuclear staining for PCNA in liver cells of some sacrificed male and femal rats treated with KBrO3 after 6, 9, 14 months. The number of positive cells and nuclei of dysplastic cells were increased significantly with the increased treated dose which were more at the high doses (600 ppm) as compared to control group.

The dysplastic foci of hepatocytes that observed by using the high dose administration of KbrO3 during the present work is an indication of cell proliferations and the carcinogenic effects of the liver cells, which agreed with **Umemura et al. (2003)** who reported that high dose treatment will induced cell proliferation associated with the DNA damage, mutations and induction of HCC. Our results was confirmed by using PCNA-immunostain which gave strong positive results especially the dysplastic cells. **Bravo (1986) and Celis (1987),** has been identified that PCNA is an auxilliary protein of DNA polymerase delta and is synthesized in correlation with the proliferative state of the cell. Our results were coincided with that of **Pizem et al. (2001)** who stated that PCNA was useful for proliferative activity assessment of hepatocytes and their expressions was higher in liver with carcinogenic characters. **Goodman, (2007)** distinguished a dysplastic nodule from a hepatocellular carcinoma by histological examination and they are further classified dysplastic cells as low-grade or high grade, based on morphologic features. They added that low-grade dysplastic nodules composed of liver cells that are minimally abnormal, showing mild increase in cell density, and they have no cytologic atypia, though they may have large cell change, formerly referred to as large cell dysplasia and or atipia **(Anthony, 1973)**. The high-grade dysplastic nodules are defined as having architectural and/or cytologic atipia, but the atypia is insufficient for a diagnosis of hepatocellular carcinoma. These lesions most often show increased cell density, often with an irregular trabecular pattern **(ICGHN, 2009)**. Small cell dysplasia or atipia was the most frequently seen form of cytologic atypia in high-grade dysplastic nodules **(Watanabe, 1983)**. He added that in case of Small cell atipia the clusters of cells showed features that suggest increased cellular proliferation, also plates more than two cells thick, pseudo-gland formation, cytoplasmic basophilia, higher nuclear/ cytoplasmic ratio, nuclear hyperchromasia are the most suggestive diagnostic elements **(Goodman, 2007).**

The carcinogenicity of KBrO3 was clearly established in F344 rats after long-term oral administration in the drinking water at doses of 500 and 250 ppm **(Kurokawa et al.1983)**. It is highly probable that active oxygen radicals are involved in the demonstrated carcinogenic and toxic effects **(Kurokawa et al.1990)**. **Kurokawa et al. (1990)** added that the oxidizing properties of KBrO3 are the reasons for its use as a food additive and industrial chemical, but recently, the carcinogenic and promoting potentials of several oxidizing chemicals have been revealed by various in vivo and in vitro studies.

In conclusion, the present study indicated that a long-term exposure to s KBrO3 caused alterations in the histology of the liver of rats. Potasium bromate cause several degenerative,

destructive, necrotic and proliferative changes. In the present study carcinogenesis was recorded as basophilic, eosinophilic and vacuolated foci of hepatocytes, together with dysplastic changes of hepatocytes. The toxic and carcinogenic effects of $KBrO_3$ was dose and time-dependent.

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الملخص العربي

مضيفات الطعام هي المواد التي تصبح جزءا من الغذاء عند إضافتها أثناء معالجة أو إنتاج المواد الغذائية. برومات البوتاسيوم (KBrO3) هي احد هذه مضيفات الطعام التي غالبا ما تستخدم في المخابز كمحسن للدقيق ومكيف للعجين. ولقد ثبت أن هذه الماده لها تأثير سام على الكلية والأعصاب في البشر ومسرطنة في القوارض. بالإضافة إلى ذلك، فقد كشفت التجارب طريقة إحداث الأثر المسرطن لبرمات البوتاسيوم وهى تعتبر أنها مادة مسرطنة كاملة.

كان الهدف من هذه الدراسة التعرف على الآثار السامة والمسببة للسرطان من تناول مستويات مختلفة من برومات البوتاسيوم مع الغذاء في الفئران الذكور والإناث .واشتملت معايير التقييم على أثر هذه المادة على النمو، التغيرات المرضية، والكيمياء النسيجية المناعية والتعديلات البيوكيميائية.

في هذه التجربة تم استخدام ما مجموعه 105 جرذان من كلا الجنسين، ويزن كل منها 120 غرام. وضعت الفئران تحت الظروف القياسية في أقفاص معدنية مع حرية الوصول إلى المياه ونظاما غذائيا عاديا .تركت الحيوانات لمدة أسبوع كفترة تكيف .وكانت الفئران المستخدمة 45 من الذكور و 45 من الإناث و 15 فأراً من كلا الجنسين ككنترول . تم تقسيم الفئران الذكور والإناث إلى ثلاث مجموعات. أذيبت برومات البوتاسيوم في الماء في تركيزات 200 و 400 و 600 جزء في المليون على التوالي حيث كانت تدار بين الفئران مجموعة من الذكور والإناث يوميا حتى نهاية التجربة. بعد 6 و 9 و 14 أشهر من التجربه تم التضحية بعشرة حيوانات من كل مجموعة (5 ذكور و 5 إناث) مع خمسة من الكنترول.

كشف الفحص المجهرى لنسيج الكبد عن احتقان الأوعية الدموية به وظهورمختلف التغيرات التنكسية والميتة مع التهاب الكبد السام وتضخم القناة الصفراوية. سجلت تغييرات مسرطنة وخلل نسجى في بعض الحالات خصوصا بعد 9 و 14 شهرا من العلاج البوتاسيوم برومات التي أعطت نتائج إيجابية باستخدام الخلايا المتكاثرة مستضد النووي (PCNA) باعتباره صبغة هستومناعية .أظهرت دلالات وظائف الكبد زيادة كبيرة في مستوى الألانين أمينوترانسفيريز (ALT)واسبارتات الأمينو ترانسفيريز(AST) .