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Research Article

Dose and Time-Related Toxic and Carcinogenic Effects of Potassium Bromate on Kidneys in Albino Rats

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ABSTRACT

Potassium bromate ($KBrO_3$) is one of the food additives which often used in bakeries as flour improver and dough conditioner. The salt of bromate ion, is a nephrotoxic in humans and carcinogenic in rats. Bromate is classified as a probable human carcinogen due to its kidney carcinogenicity in male and female rats following exposure in drinking water. The present study was conducted to investigate the toxic and carcinogenic effects of various levels of potassium bromate in male and female rats. The criteria for assessment measured through the biochemical, histopathological, and immunohistochemical alterations.

A total of 105 albino rats of both sexes, weighing 120 gm were used in this experiment, they were kept under standard conditions and housed in a metallic cages for two weeks as an adaptation period, and had free access to water and standard diet. The animals 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 and 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. Kidneys' functions markers showed significant variation of parameters than normal control cases. Histological examination of kidneys revealed congestion of its blood vessels, various degenerative and necrotic alterations. Carcinogenic and dysplastic alterations were recorded in some cases which depend mostly on the time and given doses, especially after 9 and 14 months of treatment. Immunohistochemical studies by using CD10 and Proliferating cell nuclear antigen (PCNA) gave positive results for the carcinogenic alterations that occurred in the renal tubules. Our study concluded that Potasium bromate has toxic and carcinogenic effects on kidneys and in turns in different tissues.

Key words: Toxic effect, Carcinogenic effect, Potasium Bromate, Pathology, Immunohistochemistry, Albino rats.

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). Studies have also shown that it possessed the potential of inducing cancer, kidney failure, deafness, redness and pains of the eye and skin (Mack, 1988; De Angelo *et al*., 1998). Potassium bromate, a salt of bromate ion, is nephro- and neurotoxic in human and carcinogenic in rodents when given orally (IARC, 1986; Kurokawa *et al*. 1990). 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. It also classified by the International Agency for Research on Cancer (IARC) as a category 2B carcinogen (possibly carcinogenic to humans) based on sufficient evidence of its kidney carcinogenicity in in male and female rats following exposure in drinking water (Kurokawa *et al*. 1983, 1986a, 1986b; DeAngelo *et al*. 1998; Wolf *et al*. 1998). Glaze, 1986 found that the incidence of neoplastic lesions in male and female rats fed on bread containing KBrO3 for 2 years was very rare, but in drinking water potassium bromate was found to be carcinogenic in the rats after two years of administration (Kurokawa *et al*., 1982 and Ohno 1982).

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Long term exposure to KBrO has been studied in rat (Kurokawa *et al.*, 1982, 1983, 1986, 1987 and 1990 and Ohno 1982). In rat kidneys a dose-response relationship and progressive severity of potasium bromate on renal dysplastic foci, preneoplastic lesions, renal adenomas, and finally renal carcinoma were also studied (Kurokawa *et al*. 1986a; DeAngelo *et 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 investigation was conducted to investigate the toxic and carcinogenic effects of various concentrations of dietary potassium bromate in renal tissues of male and female albino rats.

MATERIALS 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. 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 two weeks, 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 and was administered to male and female groups of rats daily till the end of the experiment. The rats were observed throughout the experimental periods to record the signs and deaths occurred. After 6, 9 and 14 months, sera have been collected from ten animals from each group (5 male and 5 female) and five from the control, then rats were sacrificed by cervical dislocation.

Biochemical study

Sera were analyzed for blood urea and creatinine which were colorimetrically measured by the method of Lyman. 1986.

Histopathological examinations

At the end of the experiment, kidneys were removed carefully, washed and fixed in neutral buffered formalin 10%, dehydrated in grades of alcohol, cleared and embedded in paraffin, sectioned at 5 µ thickness and stained by H & E and examined microscopically, some sections were stained by PAS technique *(Bancroft et al. 1996)*.

Immunohistochemical method

For immunohistochemical study CD10 and Proliferating cell nuclear antigen (PCNA) were used (Hall *et al.*,1990).

Statistical analysis

The significance of differences between means was compared at each time point using Duncan's multiple range test after ANOVA for one-way classified data (Snedecor and Cochran, 1989).

RESULTS

Biochemical Results (Urea and Creatinine mg/dl)

From table (1) it is clear that after 6, 9 and 14 months there's statistically significant increase in the mean value of urea and creatinine of various levels of potsium bromate (200, 400, and 600 ppm) as compared with the control group.

Clinical signs

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

Histopathological results

Hematoxylin and Eosin (H&E) stained sections of control kidney showed classical renal histological structure of the glomerulus, proximal and distal convoluted tubules, loop of henle and collecting tubules.

After six months of the beginning of the experiment, the kidneys of both male and female rats exposed to different doses of KBro3 showed congestion of the intertubular blood vessels and capillaries as well as capillary tufts of the glomeruli. Mild to moderate perivascular mononuclear inflammatory cells aggregation mainly in the form of lymphocytes, macrophages and plasma cells were observed. In some cases perivascular and peritubular area of edema and hemorrhage were also observed. Glomerular congestion in most cases with mild periglomerular mononuclear cells aggregation was also seen (Fig.1A).

Hypercellularity of the glomeruli represented by increase in the endothelial and inflammatory cells. Renal tubules showed disorganization of the epithelial lining with swelling and marked vacuolation of the proximal and distal renal tubular epithelium (Fig.1B) with dense peritubular mononuclear cells infiltration and increase in the number of degenerated renal tubular epithelium with pyknotic and lysed nuclei were seen, the lumina of some tubules contained hyaline cast. Fibroblastic cells proliferation in the interstitial tissue were noticed in some cases, in addition regenerative tubules were observed, characterized by cytoplasmic basophilia and vesicular nuclei in the cytoplasm of some renal tubules light brown fine pigments was observed (Fig.1C). The interstitial tissue and the peritubular areas of some cases were infiltrated with mononuclear inflammatory cells.

The renal capsule in some cases was covered by proteinaceous material and infiltrated by large number of mononuclear inflammatory cells. In some cases the renal tubules showed degenerative changes with individual necrosis of some cells, the lumen of some proximal and distal convoluted tubules contained flocculated and cellular casts, with severe necrosis and destruction of the epithelial lining of proximal convoluted tubules.

Table 1: The mean differences between urea and creatinine in the serum of control and treated groups of rats by KBrO3

Treatment / Variables	Control (1) Mean ±Std.	200ppm Mean ±Std.	400ppm Mean ±Std.	600ppm Mean ±Std.	F-test	p-value
1. UREA 6	25.09± 2.51	42.27± 2.92	45.28 ± 4.78	50.35± 8.30	22.561	0.001**
2. creatinine6	1.08± 0 .57	2.56 ± 0.60	2.40 ± 0.48	2.48 ± 0.86	5.828	0.007**
1. UREA 9	30.06± 4.15	37.79± 6.85	43.11 ± 3.72	53.49± 6.35	16.324	0.001**
2. creatinine9	1.18± 0.64	1.78 ± 0.64	2.94 ± 0.59	3.37 ± 0.51	14.148	0.001**
1. URE A 14	25.74± 1.84	38.61± 7.05	44.91± 3.66	53.49± 6.35	25.500	0.001**
2.creatinine4	1.36 ± 0.38	1.78 ± 0.64	3.16 ± 0.45	3.60 ± 0.15	29.207	0.001**

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

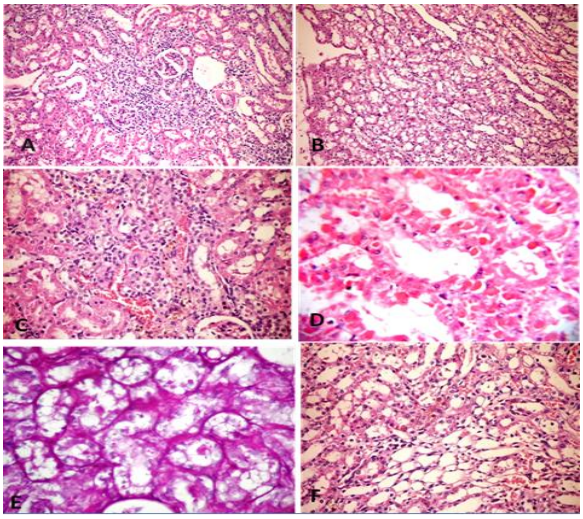

Fig. 1: Sections of rats' kidneys administered with KBrO3 showing; **A-** Aggregation of mononuclear cells between the renal tubules and glomeruli, (Female from G2 for 6 months), (X200). **B-** Marked vacuolation of the renal tubular cells, (G1 for 6 months), (X 200). **C-** Congested intertubular blood vessels, regenerated renal tubules and presence of light brown fine pigments in the cytoplasm of some renal tubules, (Male from G2 for 6 months). (X400). D- Eosinophilic hyaline bodies in the proximal convoluted tubules, (Male from G1 for 9 months), (X400). E- Eosinophilic hyaline bodies in the proximal convoluted tubules, (Male from G1 for 9 months), (PAS X400). F- Congestion of the peritubular blood vessels and capillaries, Note the presence of fine light brown pigment granules in some renal tubules, (Female from G1 for 6 months), (X 200).

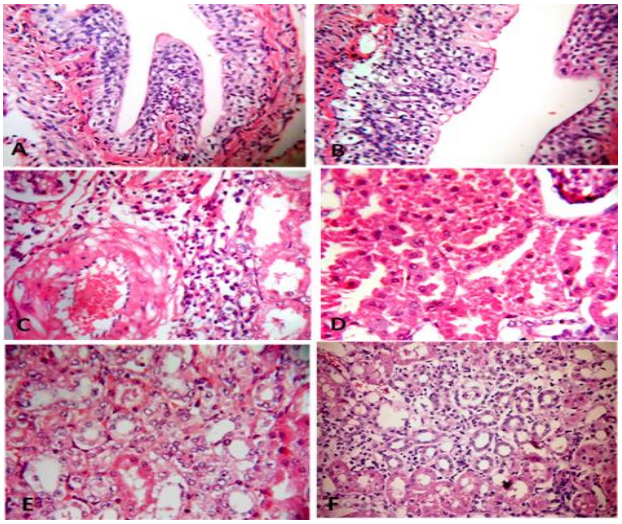

Fig. 2: H&E stained sections of rats' kidneys administered with KBrO3 showing; A. Hyperplasia and papillary projections of the transitional cells lining the renal pelvis, (Male from G2 for 14 months), (X400). B. Marked vacuolation of the hyperplastic transitional cells lining the renal pelvis, (Male from G2 for 14 months), (X400). C. Congestion, with marked perivascular mononuclear cells infiltration and severe vacuolation of the vessel wall, (Female from G3 for 9 months), (X400). D. Increase granularity of the cytoplasm of renal tubules with irregularity of the cells lining and appearance of apoptotic cells, (Male from G3 for 12 months), (X400). E. Newly formed renal tubular cells with basophilic cytoplasm and deep basophilic pleomorphic nuclei which arranged in clumps or acini, (Male from G2 for 12 months), (X400). F. Dysplastic changes of the lining epithelium of the proximal convoluted tubules, (Male from G2 for 6 months), (X 200).

In some cases intracytoplasmic homogenous eosinophilic granules of variable size and shape (hyaline droplet degeneration) were observed in the cytoplasm of renal tubular cells (Fig.1D) which stained positive by PAS reaction (Fig.1E). Brown pigments in the tubular epithelium of both cortex and medulla were also observed, and the pigment granules were commonly seen within the cortical tubular cells than the other tubular cells (Fig.1F). They were more extensive in both degree and distribution especially in males treated rats after 9 and 14 months. Regarding microscopic examinations of the renal pelvis, there were hyperplasia of its transitional cells lining (urothelial hyperplasia**)** which had a dose-dependent**.** Urothelial hyperplasia was characterized by marked increase in the number of layers of the cells lining the renal pelvis, especially in treated males after 9 and 14 months (Fig.2A). Destruction and sloughing together with marked vacuolation of the hyperplastic transitional cells lining the renal pelvis was also observed (Fig.2B). All the above mentioned lesions were related to the dose and time related manner in rats exposed to Pot. Bromate and the lesions appeared to be in a focal, multifocal, and diffuse manner. After 9 and 14 months, the renal blood vessels and peritubular blood capillaries were congested with marked dilatation of the renal tubules which lined by low cuboidal and flattened cells in the corticomedullary area. Severe destruction and necrosis of the renal tubules with marked mononuclear cells infiltration in the interstitial tissue and perivascular area with vacuolation of the blood vessel wall were also recorded (Fig.2C). Some renal tubular cells showed marked granularity of its cytoplasm and the granularity appeared to be more pronounced in the proximal convoluted tubules with irregularity of the cells lining and appearance of apoptotic cells (Fig.2D). The proximal convoluted tubules and the renal tubules in the cortico-medullary area were vacuolated, with marked cystic dilatation and elongation of some and appearance of eosinophilic hyaline cast in some tubules. Some tubules showed marked cystic dilatation with papillary projections of its lining wall.

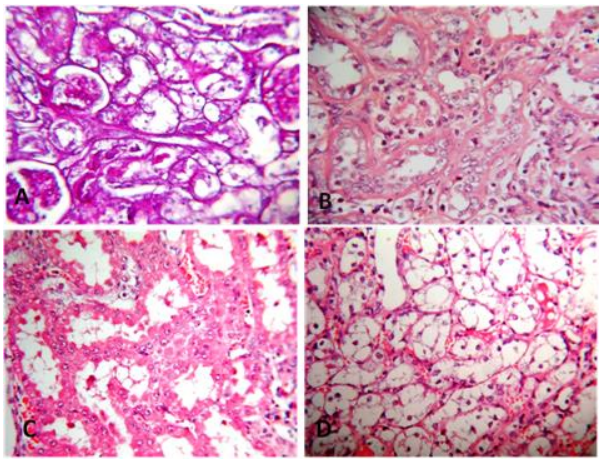

Fig. 3: PAS and H&E stained sections of rats' kidneys administered with KBrO3 showing; A. Thickening of the tubular basement membrane, with peritubular and periglomerular fibrosis, (Male ,from G1 for 12 months), (PAS X400). B. Disorganized growth pattern of the dysplastic tubules, Note the variation of nuclear size with general nuclear atypia, (Male from G3 for 12 months), (X400). C. Hyperplastic and dysplastic changes with eosinophilic granular cytoplasm (granular cell) and corrugation of its lining, (Male from G3 for 9 months), (X400). D. Hyperplastic and dysplastic changes with vacuolated cells type of dysplastic tubules, (Male from G3 for 9 months), (X400).

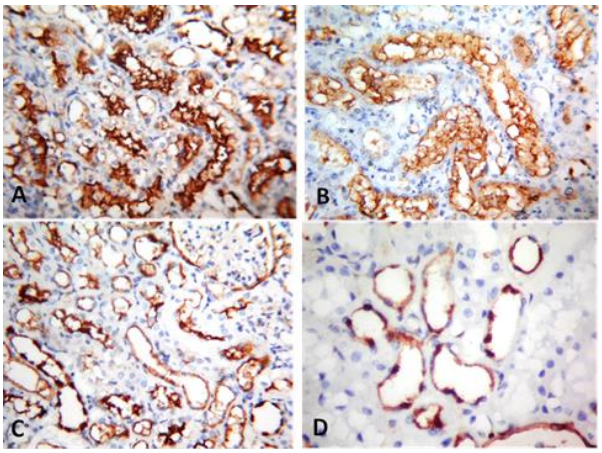

Fig. 4: CD10 stained Paraffin sections of rats' kidneys receiving KBrO3 showing; A. Strong intensity of immunostaining of renal tubules with marked cytoplasmic stain, (after 14 months), (X 400). B. Moderate to strong (light brown) intensity of immunostaining of renal tubules with clear cytoplasmic stain, (X 400). C. Strong intensity of immunostaining of the nuclei in dysplastic tubular cells with slight cytoplasmic stain, (X 400). D. Strong intensity of immunostaining of the nuclei in dysplastic tubular cells with slight cytoplasmic stain, (X 400).

The epithelial lining of some renal tubules contained cells with deeply eosinophilic cytoplasm and hyperchromatic nuclei together with the presence of binucleated cells some renal tubules.

Newly formed renal tubular cells with basophilic cytoplasm and deep basophilic pleomorphic nuclei were observed in some animals after 6 months especially in group 3 and in most animals after 9 and 12 months. Some nuclei were large vesicular with prominent nucleoli, others were deep basophilic with granular or compact chromatin. These newly formed cells appeared either in clumps or trying to form acini (Fig.2E).

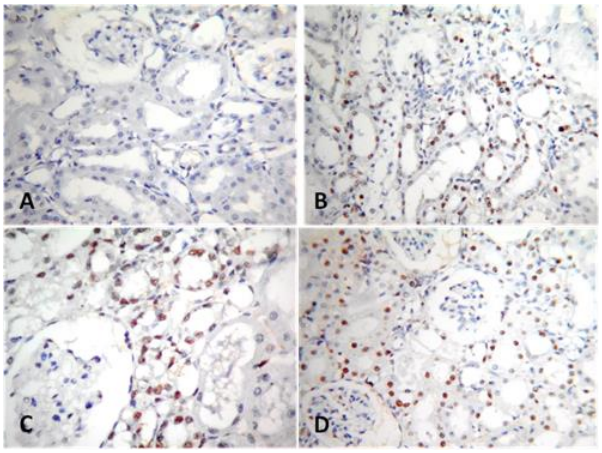

Fig. 5: PCNA stained Paraffin sections of rats' kidneys receiving KBrO3 showing; A. Negative immunostaining of renal tubules, (X 400). B. Increase intensity and number of immunostaining of the proximal convoluted tubules (After 9 months), (X400). C. Increase intensity and number of immunostaining of the dysplastic proximal convoluted tubules. (After 9 months), (X400). D. Strong intensity of immunostaining of the dysplastic tubules. (After 14 months), (X400).

In addition to the previously mentioned degenerative changes which were observed in all treated groups karyomegally and cytomegally of some renal tubular epithelium especially at the corticomedullary junction were seen.

Dysplastic renal tubules (Atypical Hyperplasia) were observed in male and female rats given potassium bromate at 400 ppm and at 600 ppm especially after 9 and 14 months. The dysplastic renal tubules were found in the periglomerular location especially in the proximal convoluted tubules of the outer cortex (Fig.2F). Dysplastic tubules were usually present in both kidneys. They consist of a single layer of basophilic dysplastic tubular cells. The cells possessed a basophilic cytoplasm and round or oval nuclei with diffusely distributed chromatin and prominent nucleoli of some cells, the tubular basement membrane often appeared thickened which give positive reaction by PAS stain (Fig. 3A). A brown pigment was sometimes present in some cells of the distal renal tubules. In some areas the lesions appeared to be distributed in a disorganized growth patterns with variation of nuclear size, nuclear hyperchromacia and general nuclear atypia (Fig.3B). Although the morphology of some of these cells was similar the normal cells, sometimes showed loss of polarity. Cell vacuolization was occasionally seen. These lesions were rarely seen in untreated controls. The cell size was increased with decrease in Nuclear/Cytoplasmic ratio. Cell borders were very distinct with the appearance of "windows" or separation of cells (Fig.3B). These changes differed from the normal proximal convoluted tubular cells, which have a less dense eosinophilic cytoplasm, indistinct cell membrane, and a preserved apical villous border. The nuclear size and chromatin staining was increased, vesicular and pleomorphic and the nucleoli appeared more prominent in the dysplastic tubules. Some dysplastic tubules showed multilayer of its epithelial cells, some others presented a cystic structure with papillary projections protruding into the lumen. In some cases the dysplastic cells appeared to have clear

cytoplasm (clear cell), eosinophilic granular cytoplasm (granular cell) with corrugation of its lining (Fig.3C), and basophilic cytoplasm (dark cell). There was slight nuclear pleomorphism without mitotic figures. with papillary growth pattern were also seen. The dysplastic tubular cells were composed of medium sized to large cells arranged in nests, trabeculae and solid sheets.

Some cases after 9 and 14 months showed clear vacuolation of the epithelial lining of the renal tubules in both cortex (Proximal convoluted tubules) and medulla (Vacuolated cells type of dysplastic tubules) (Fig.3D).

Immunohistochemical results

CD10 was expressed in most cases showed vacuolation of the lining epithelium of tubules (clear cell), the stain was highly positive in some cases in which the cytoplasm of the lining epithelial cells stained brown and the nucleus deep brown (Fig. 4A). Other cases appeared moderately positive (Fig. 4B) and some other slightly positive (Fig. 4C&D).

The dysplastic cases expression was also observed in the proximal convoluted tubules especially in the apical section of few cases after 6 months and some cases after 9 & 14 months. The cytoplasm of the dysplastic cells appeared as brown rim while the nuclei stained deep brown. There was marked staining by CD10 in proximal tubular cells. The control cases showed negative staining for CD10. The collecting tubular cells and urothelial epithelium negative stained by CD10.

PCNA immunoreactivity was localized in the nucleus and nucleolus together with the nuclear membrane of the dysplastic cells of proximal convoluted tubules. Its expression was estimated as the percentage of positively stained cells by the antibody. In control kidney sections there was a very few number of PCNA positive renal tubular cells as shown in (Fig. 5A). 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 were few. After 9 and 14 months reached to high positive cells number with strong immunostaining (Fig. 5 B,C&D).

DISCUSSION

The result of the present study reveals that the administration of potassium bromate caused some characteristic physical and clinical changes in rats as evident in the depression, difficulty in breathing, with hyper-excitability and paddling movements in some animals.

The long-term oral administration of KBrO3 in drinking water during the present study at doses of 200, 400, 600 ppm for 6, 9, 14 months leading to different Biochemical findings, degenerative and destructive effects together with generalized congestion and haemorrhages in the examined organs.

The degenerative, necrotic and regenerative changes observed during the present study come parallel with that recorded by Kurokawa *et al*. (1982a,b) and Kurokawa *et al.* (1990). The present findings agreed also with Abuelgasim *et al.* (2008), where they reported generalized congestion, haemorrhage and degenerative changes in the kidney and liver.

In some cases, homogenous eosinophilic granules of variable size and shape (hyaline droplet degeneration) were observed in the cytoplasm of renal tubular cells especially the proximal convoluted tubules, this finding agreed with that observed several authors {Onodera *et al.*, 1986, Nakano *et al.* (1989), Kurokawa *et al*., (1990) and Kurata *et al.* (1992)}. The droplets were stained positive by PAS reaction.

Dysplastic renal tubules (Atypical Hyperplasia) were observed in the proximal convoluted tubules of the outer cortex of male and female given K. bromate at 400 ppm and at 600 ppm especially after 9 and 14 months. Dysplastic tubules were usually present in both kidneys, It consists of a single layer of basophilic dysplastic tubular cells. The cells possessed a basophilic cytoplasm and round, oval or ovoid nuclei with diffusely distributed chromatin and prominent nucleoli of some cells, the tubular basement membrane often appeared thickened which gave positive reaction by using PAS stain.

It is well known that KBrO3 is a complete carcinogen, having both initiating and promoting activities for the development of renal cell tumors. It is highly probable that active oxygen radicals are involved in the demonstrated carcinogenic and toxic effects (Kurokawa *et al*, 1985 and 1990). The results showed also that the mean numbers of kidney dysplastic foci were significantly increased in a dose-related manner and Dees *et al*. (1980) and Kurokawa *et al* (1985) added that the incidences of dysplastic foci, considered to be preneoplastic lesions of renal cell tumors, were significantly higher in rats treated with >30 ppm. The present results are completely in accordance with that mentioned by (Hard, 1990 and 1992) which showed that there were different phenotypes of preneoplastic renal lesions or tumors which include basophilic, acidophilic, clear cells. Tumor cells also grow in papillary, papillary cystic, tubular, and solid patterns.

Our results were confirmed by using CD10 immunostain which was thought to be a tumor-specific antigen (Chu and Arber, 2000) including renal proximal tubular epithelial cells of the kidney (Avery *et al*., 2000 and Kim and Kim, 2002). The present results showed differences in the distribution of CD10 staining. The high dose groups of KBrO3 showed prominent and deep staining, whereas the dysplastic changes of the proximal convoluted tubules showed intense and intermediate staining of the high dose groups. Lee and Droller (2000) and Simon *et al.,* (2004) stated that the differences in CD10 expression correlate with the extent and distribution of genetic damage.

Our results were coincided with that of Pizem *et al.* (2001) who stated that PCNA was useful for proliferative activity assessment mainly of proximal convoluted tubular cells and hepatocytes and their expressions was higher in kidney and liver with carcinogenic characters.

As we mentioned that the carcinogenicity of KBrO3 was clearly established in 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). They 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.

Conclusions

Potassium bromate (KBrO3) has many dangerous and toxic effects on kidney tissues. Further studies should be encouraged in this regard. The physical properties of KBrO3 make it easy to be taken or administered as a poison to human, thus its use and handling should be highly regulated by the relevant authorities.

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