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Cytogenetic Effects of Potassium Bromate $KBrO_3$ Associated with Iraqi Baking Industry

KEYWORDS: Potassium bromated, DHFR, Bakery workers, MI, BI, CA

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ABSTRACT *This study was aimed to determin the potassium bromate content of most consumed loaves within Baghdad city, and evaluating the cytogenetic effects of potassium bromated at bakery workers by measuring mitotic index (MI) blastogenic index (BI) and chromosomal aberrations (CA) beside measuring of dihydrofolate reductase (DHFR) activity in both healthy and exposed groups. The findings referred to includes two type of Iraqi loaves KBrO3, electrical samun 10 ± 2.34 and loaf 0.3±0.12. The exposed workers have high chromosomal aberration represented by CB,RC and MN. While DHFR activity suffered graduated reduction depending on age group and years of services.*

Introduction:
Potassium bromate (KBrO3) is a nephrotoxic and carcinogenic substance used in food and cosmetics industry, and also found in drinking water as a by-product of disinfection by ozonaization [1]. Despite the ban placed on the use of potassium bromate as bread-enhancer in one type of bread and in bakery products, in Iraq it is commonly used by bakers to increase bread volume and texture. The maximum concentration of potassium bromate allowed in bread by the US Food and Drug Agency (FDA) is 0.02µg/g [2].

Pure potassium bromate is white crystals or granules, melting-point: About 350°C; decomposes at about 370°C with evolution of oxygen, density 3.27 g/cm3, Soluble in water, slightly soluble in acetone, dimethyl sulfoxide, ethanol, methanol and toluene [3]. Potassium bromate is highly toxic. It produces lipid peroxidation and oxidative DNA damage in rat kidney. There is also evidence that it increases the amount of α2u-globulin in male rat kidney. The available data, including evidence of genetic toxicity, indicate, however, that potassium bromate causes renal tumours through a mechanism involving oxidative damage [4]. No data were available on the absorption, distribution, metabolism or excretion of potassium bromate in humans. The present study aims to measured KBrO3 concentration for main consumed bread within Baghdad city and investigate some cytotoxic effects of this material in exposed people.

Materials and methods:
Bread sample collection:
Three of most consumed bread brands available in Baghdad markets were collected, the concentration of potassium bromate was determined to fifty random bread samples; spectrophotometric assay was used as described by [5]. 20 gm of each sample were dried at 80°C for one hour. The dried crust was pulverized and 1g of each powdered sample was weighed into 100 ml Pyrex beaker and 20 ml of distilled water was added. Well shaken mixture was filtered by Whatman 1 filter paper. 5 ml of the filtrate solution was mixed with 1ml of 0.01M promethazine. Few drops of 12 M HCl was added, all contents well shaken for 1minute and absorbance of the colored solution measured at 520nm. The concentration was calculated from the linear regression curve obtained from the standard solutions of potassium bromate.

Blood sample collection:
Thirty blood samples of bakery workers aged from 25-44 years old who used potassium bromate in preparation of dough were collected, compared with ten blood samples from healthy person as a control. cytotoxic effects were investigated by measuring MI, BI and CA according to [6] as well as Dihydropholate reductase (DHFR) activity was determined in both groups of blood cultures [7].

Results and discussions:
Potassium bromate determination:
Quantitative determinations of potassium bromate of three types of Iraqi bakery are shown in table 1. The type (A) shows negative results of KBrO3 assay, the highest concentration of potassium bromate was recorded in type B (electrical samun) 10 µg/g, while the type C (loaf) gave 0.3 µg/g. These levels are higher than 0.02µg/g, permitted by the US Food and Drug Agency (FDA) but lower than the levels permitted by China (50µg/g) and Japan (10µg/g). Unfortunately there are no local studies dealt with the impact of KBrO3 on the immunological and genetic aspects for comparison. In fact the first and third type of bakeries characterized by daily consumption without the need for storage periods for that we don't need to add a large quantity of KBrO3 to the dough, but the second type of products (electrical samun) needs a storage time reach to one week in show shops and super markets to ensure the maintain of the required appearance and softness for sale, need KBrO3 added. The real danger behind the use of KBrO3 in the preparation of loaves and pastries came from the daily consumption of this food in large quantities without determine the extent toxicity of this material on human health.

Table 1: Concentration of potassium bromate (µg/g) in bread samples

Bread samples	NO. of positive samples	Potassium bromated concentration (µg/g)
A (Samun)	0/15	0.0
B (electrical samun)	20/20	10 ± 2.34
C (loaf)	3/15	0.3 ± 0.12

***Mean ± SD**

Cytogenetic assessment:
The second aim of this study was investigation the effects of KBrO3 on PBL from bread industry workers. From table 2 we can observe significant reduction in both MI and BI with the increase in age and length of service in years accompanied with elevated in CA, represented by chromatid breaks CB, micronuclei MN and ring chromosome RC. The highest proportion of TCA has been observed in the last age group (< 40) reached to (0.149±0.03), while the reduction percent in MI from control to the (< 40) group was 56%, as well as reduction percent in BI was 30.15%. Many chemical, physical and biological effects play an important role in the stability of genetic material and DNA causes inhibition in both MI and

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BI [8, 9, and 10]. From the studies that KBrO3 has potential effects on tumor incidence [11,12] associated with Significant decreases in serum chemistry, including glutamate pyruvate transaminase, albumin-to-globulin ratio, potassium, and cholinesterase were observed in female rats in the high-dose group. From [13] dose-dependent increased tumor incidence was observed in the kidney (adenomas and carcinomas combined and carcinomas alone), the thyroid (adenomas and carcinomas combined and carcinomas alone), and tunica vaginalistestis (mesotheliomas). Furthermore, potassium bromate clearly induced gene mutations at the HPRT locus. Molecular analysis of potassium bromate-induced mutations indicated a high proportion of deletion mutations. Three out of four point mutations were G-to-T transversions, which typically arise after replication of 8-oxoguanine [14] in the number of aberrant metaphase cells were observed following single oral doses of potassium bromate to Long-Evans rats, as well as KBrO3 caused significant increases in the number of micronuclei following either IP injection [15].

Table2: Cytotoxic effects of potassium bromated on peripheral blood lymphocytes from bakery workers

Age groups	No. of samples	BI	MI	CB	MN	RC	TCA
Control	10	51.23±4.2 a	2.5±0.34 a	0.0 a	0.01±0.001 a	0.0 a	0.01±0.001 a
25-30	11	44.56±3.5 b	1.6±0.11 b	0.02±0.002 b	0.02±0.01 b	0.01±0.001 b	0.05±0.021 b
30-35	7	41.34±0.54 c	1.25±0.12 c	0.03±0.001 c	0.031±0.02 c	0.031±0.02 c	0.092±0.03 c
35-40	5	36.12±2.21 d	1.02±0.12 d	0.03±0.01 d	0.05±0.001 d	0.34±0.01 d	0.114±0.01 d
< 40	7	35.78±3.66 e	1.1±0.23 e	0.042±0.01 e	0.065±0.001 e	0.042±0.02 e	0.149±0.03 e

CB: chromatid break MN: micronuclei RC: ring chromosome TCA: total chromosomal aberration

Each number represent M±SD for 100 lymphocytes, different letters within the same column refer to significant differences at p≤0.05.

Specific activity determination of DHFR:
The specific activity of DHFR was measured in PBL cultures of both exposed and healthy peoples. From table 3 the enzyme activity suffered from gradual decline depending on age group and years of services as compared as with control group. DHFR play important role in nitrogenic base builds that are necessary in DNA formation and cell division [16].

In exposed peoples the DHFR activity was reduced, these explain the reduction in both MI and BI in PBL cultures for them, so KBrO3 like in effects MTX drug which is depressing the DHFR activity in PBL for chronic myelogenous leukemia CML, and causes inhibition in each MI,BI,RI and CCP [16]. In other word KBrO3 have inhibition effects on DNA and RNA replication enzymes and causes obstruction in cell cycle progression.

Table 3: Specific activity of DHFR enzyme in the PBL of exposed workers to KBrO3

Age groups	No. of samples	Specific activity U/ml (Max)	Specific activity U/ml (Min)	SD
Control	10	12.5	9.16	3.13
25-30	11	11.23*	8.6	4.11
30-35	7	9.54*	7.43	2.45
35-40	5	7.89*	5.45	1.37
< 40	7	6.56*	4.54	1.34

*** Significant differences at p≤0.05, SD (standard deviation)**

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