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Assessment of Potassium Bromate, Calcium Sulfate and Synergistic Toxic Effects in Male Mice

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Abstract :

Food additives are the basis of the modern food industry, and play an important role in improving the color, smell, and taste of food, altering its nutritional structure, perfecting its processing conditions, and extending its shelf life. Twenty four adult male Swiss albino mice were used in our experiment : Group (1) mice were Intraperitoneally injected of normal saline 0.5 ml (as control). Group (2) mice were intraperitoneally injected of well dispersed Potassium Bromate 320 mg/kg of body weight for two months (twice weekly). Group (3) mice were faded Calcium sulfate as chew of food 0.03% w/w for two months (daily intake). Group (4) mice were intraperitoneally injected of well dispersed Potassium Bromate 320 mg/kg of body weight for two months (twice weekly) and also given Calcium sulfate as chew of food 0.03% W/W for two months (daily intake). After the end of project protocol the mice had been sacrificed and blood collocated and this parameters was evaluated white blood cells (WBC) , red blood cells (RBC) ,platelet (PLT),hematocrit (HCT), and hemoglobin concentration (Hgb). The result showed there is highly significant decrease for WBC for the (group 2 and group 4) , Red blood cell count show that there is highly significant decrease of (group 2 and4), In contrast the result for Hemoglobin concentration show there were highly significant increase in group 2 and 4 by comparing with control group , also highly significant increase for Hct percentage for the (group 2 and 4) by comparing with control group and group 3. Finally platelets count result show that there is highly significant decrease in group 2 and group 4. From current study We can conclude that there are different harmfully effects of KBrO3 and the synergistic effect with CaSo4 on the animal model and according to that on human due to the daily uptake of this compounds in our life style ,according to the hematological indices (WBC),(RBC), (Hgb), (Hct), and finally (PLT). Thus the using of KBrO3 and CaSo4 should be limited and used specific documented concentration to reduce its unfavorable effects.

Keywords : Potassium Bromate, Calcium Sulfate, Male Mice, food industry

Introduction

Food additives are the basis of the modern food industry, and play an important role in improving the color, smell, and taste of food, altering its nutritional structure, perfecting its processing conditions, and extending its shelf life (1,2). Although the use of additives during food processing has become common practice on a global scale, consumers' concerns about their potential risks have not abated (3,4).

In recent years, with continuous improvement in consumers' quality of life, the demand for all-natural, no-additive food has kept increasing. As a result, a number of synthetic food additives have been included in the list of potential food-safety hazards, and many customers believe that the use of food additives is actually unnecessary or unwarranted (5,6).

In China, food-safety issues that are related to food additives, such as their misuse or overuse due to anthropogenic stressors, or the reckless use of nonedible chemical substances, have become increasingly prominent. Studies have shown that, between 2006 and 2015, a total of 253,617 food-safety incidents were reported in China, of which 75.5 % were caused by anthropogenic factors. Moreover, the highest number of food-safety incidents was caused by the illegal use of food additives which accounted for 34.36 % of the total (7). Indeed, the latest information that has been released by China's State Food and Drug Administration revealed that, out of 257,000 batches of food samples that were collected nationwide, there were 8224 batches of substandard food, 33.6 % of which was caused by the misuse or overuse of food additives by food production and processing organizations. What is worse, some illegal enterprises used nonedible chemical substances in order to pursue their economic interests, which have caused real or potential damage to consumers' health. (8)

Food additives are also used to increase nutrient value or to protect decaying nutrients during the preparation of factory-made foods. So, humans are unavoidably exposed to these complex mixtures in their foods. Some food additives have been prohibited from use due to their toxicity. For example, AF-2 (2-[2-furyl]-3- [5-nitro-2-furyl] acrylamide) is proved to induce DNA damage in bacteria and human cells and to cause mutations in bacteria, fungi, insects and mammalian cells *in vivo* and *in vitro*. It also causes chromosomal anomalies in mammalian cells, including human cells. AF-2 has been used in Japan since 1965 but it is banned presently (9). Another food additive, butter yellow (p-dimethylamino-azobenzene-an azo compound) was also banned first in the USA and following in Europe, because of its carcinogenicity in experimental animals (10).

Citric acid lead to necrotic changes such as vacuolated and glassy cytoplasm in hepatocytes, chromatin decrease and increase of collagen fibers amongst hepatocytes in mouse liver (11). Citric acid significantly increased micronucleus frequency in the erythrocytes of *Tinca tinca* at all doses used (12).

Potassium Bromate

Potassium bromate (KBrO3) is a well-known flour improver that acts as a maturing agent (13). It has been in use as a food additive for the past 90 years (14) . It acts principally in the late dough stage giving strength and elasticity to the dough during the baking process while also promoting the rise of bread. KBrO3 is also used in beer making, cheese production and is commonly added to fish paste products (15). Additionally, it is used in pharmaceutical and cosmetic industries and is a constituent of cold wave hair solutions (14). Moreover, KBrO3 can appear as a byproduct in an ozonization of water containing the nutritional quality of bread as the main vitamins available in bread are degraded (16). It is known that KBrO3 induces oxidative stress in tissues (17, 18, 19) that could be the basis of bromate-induced carcinogenesis (20).

Also it is an oxidizing agent that has been used in analytical chemistry , is not a naturally occurring compound and is synthesized by passing elemental bromine through a solution of potassium hydroxide (21,22). In an industrial setting, KBrO, is considered a respiratory irritant, with a time-weighted average workplace exposure of 5 mg/m3 for a standard work week (21).

Most serious human exposures to KBrO, solutions have been through the accidental or purposeful ingestion of permanent hair-wave solutions and human patients who ingest KBrO, solutions develop renal disease and neuropathy (23, 24).

KBrO3 in various consumer items poses mild to severe toxicity to critical organs, like the kidney, liver, and brain in the living systems . It has been categorized as a potential

class II B carcinogen for humans , while it is a confirmed carcinogen in the experimental animals attributed to its extensive oxidizing property and mutagenicity (25-27).
For these harmful effects, its usage in food products is banned in many countries of the European Union, Canada, and many south American, African, and Asian countries, including India, China, and Sri Lanka, yet it is used in countries like the USA and Japan with certain limitations. Also, it is restrictively or illegally used in many other countries.

Calcium Sulfate :

Calcium sulfate, has a long history of use in medicine and dentistry (28–30). It exists in three forms, calcium sulfate dihydrate, calcium sulfate hemihydrate or anhydrous calcium sulfate, which differ greatly in physical properties (31- 32) .
The most commonly used form is calcium sulfate dihydrate (CSD). The earliest start application was in the 1890s and then it was widely used in bone regeneration and tissue regeneration.(33-34). Calcium sulfate can be used as a delivery vehicle for growth factors and antibiotics, although this application has not been thoroughly exploited in the clinical setting.
Calcium sulfate (E 516) is authorized food additives in the EU, in accordance with Annex II and Annex III to Regulation (EC) No 1333/2008 and specific purity criteria have been defined in the Commission Regulation (EU) No 231/2012, They were previously evaluated by JECFA several times, the latest in 2010 (35) and the Scientific Committee on Food (SCF) in 1991 (36). Both committees established a group acceptable daily intake (ADI) .
Calcium sulphate was evaluated by EFSA in 2003, 2004 and 2008 for use in foods for particular nutritional uses (37), as a mineral substance in foods intended for the general population (38) and for use as a source of calcium in food supplements (39). The overall conclusion in the evaluations was that calcium sulphate as a source of calcium or as a mineral substance in foods is of no safety concern (37-39).
Functional uses of calcium sulphate also include, Yeast food, dough conditioner, firming agent, sequestrant, anhydrous Chemical formula CaSO4 dihydrate: CaSO4·2H2O calcium sulphate is permitted as a source of calcium in food supplements, in foods in general and in foods for special nutritional purposes , the majority of sulphate intake from the diet comes from protein-derived methionine and cysteine (40). WHO reported that however, in areas with high levels of naturally occurring sulphate in the drinking water supply, drinking water may constitute the principal dietary source (41).
In the processing of soy protein, calcium sulfate, dihydrate is added as a coagulator in soy protein, and it is reacted with soy protein. From the reaction, soy protein is curdled and there is no residue of calcium sulfate, dihydrate in food.(42)
Ferguson used biodegradable calcium sulphate antibiotic carrier containing tobramycin in the surgical management of patients with chronic osteomyelitis and the infection was resolved in 97.9% cases.(43) and LD50 was 3000 mg/kg (Rat) ,in Canada This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations (CPR) (44).

Materials and methods

Experimental Animals

Twenty four adult male Swiss albino mice were used *Mus musculus*, weight 20–30 g, and 8 to 12 weeks of age, were used in the study. The animals were placed in the animal house of Faculty of Science, University of Kufa, with the use of a standard pellet diet and water ad libitum and standard environment situations temperature (25 ±2C°) and 12

hour light-dark cycle. The procedure for using live animals in research was first reviewed, approved and accepted according to the Central Committee for Bioethics of University of Kufa (Institutional Animal Care And Use Committee (IACUC)).

Study Protocol

A total number of 24 Swiss albino mice used, Animals have been divided into four groups, (n= 6) mice as the following :

Group (1) mice were Intraperitoneally injected of normal saline 0.5 ml (as control) for two months (twice weekly) . Group (2) mice were intraperitoneally injected of well dispersed Potassium Bromate 320 mg/kg of body weight for two months (twice weekly). Group (3) mice were faded Calcium sulfate as chew of food 0.03% w/w for two months (daily intake). Group (4) mice were intraperitoneally injected of well dispersed Potassium Bromate 320 mg/kg of body weight for two months (twice weekly) and also given Calcium sulfate as chew of food 0.03% W/W for two months (daily intake).

Animals Sacrificing

The mice were sacrificed at the end of two months , and used anesthetic drugs to anesthetized mice, ketamine (80mg/kg) and xylazine (10 mg/kg) (45). Following a heart puncture , and blood collection, it is collected and placed in a test tube including 1% EDTA at room temperature and then used for getting Hematological parameters complete blood count

Blood Test

Blood was collected by heart puncture using 1% EDTA tubes. The blood levels of white blood cells (WBC) ,and red blood cells (RBC) ,platelet (PLT),hematocrit (HCT), and hemoglobin concentration (Hgb) were evaluated using a Beckman Coulter hematology analyzer (German).

Statistical Analyses

The data was analyzed using the Graphpad prism v7 (GraphPad Software Inc.). Statistical analysis of variance was used to compare the treated groups and control group, and one way Anova used to determine statistical significance (P-value < 0.05) (46).

Result:

The result of the table 1 and the figures (1-3) show that there is highly significant decrease for WBC for the (group 2 and group 4) by comparing with control group and other groups respectively (2.783 ±0.437) and (2.415 ±0.156).

Red blood cell count show that there is highly significant decrease of (group 2 and4) by comparing with control group (3.320 ±0.4342) and (2.415±0.1567) respectively, while there are no significant difference between group 3 with control group.

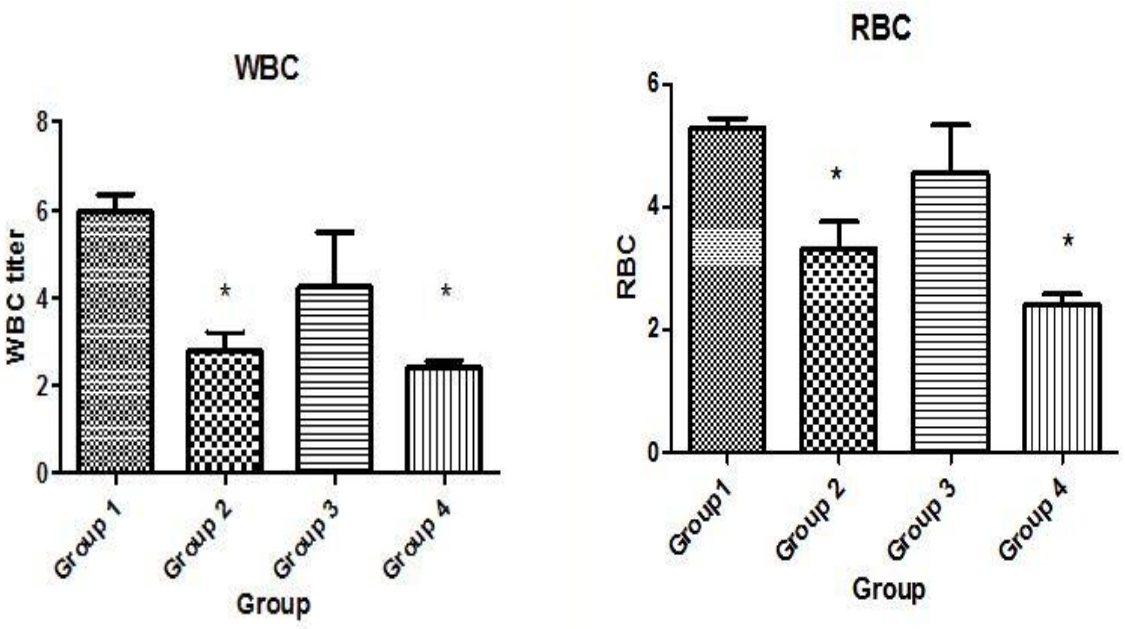
In contrast the result for Hemoglobin concentration show there were highly significant increase in group 2 and 4 by comparing with control group (12.42 ±0.2884) and (11.33 ±0.1314) respectively while there is slightly significant increase between group 3 with control group.

While there is highly significant increase (31.02 ±1.723), (31.15±0.3708) for Hct percentage for the (group 2 and 4) respectively by comparing with control group and group 3.

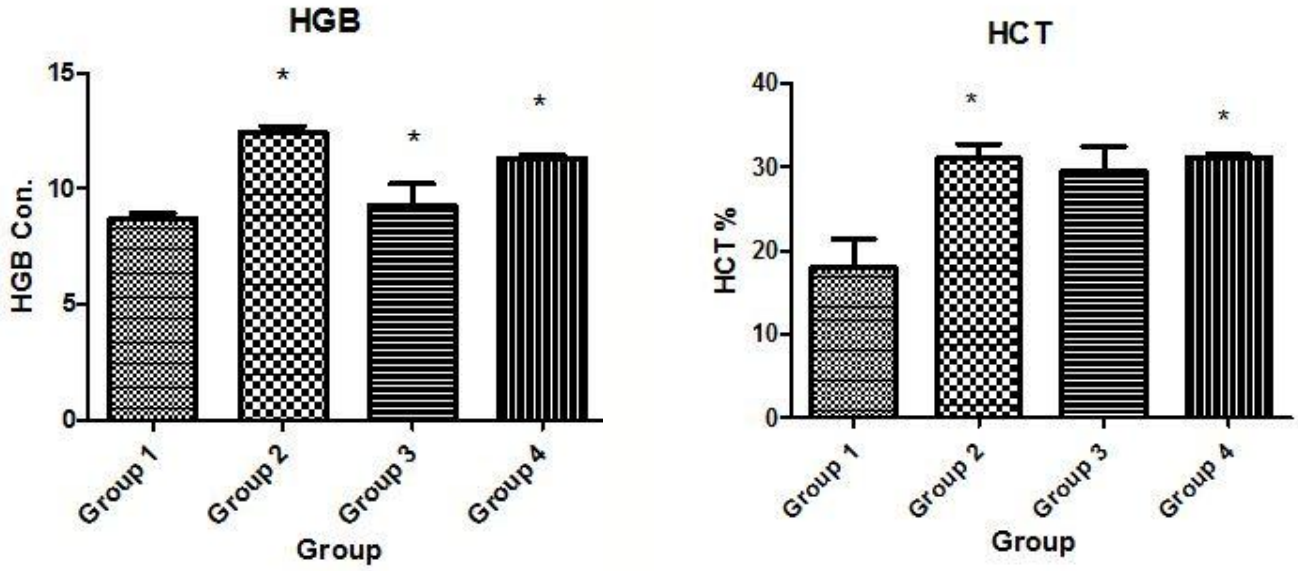
Finally platelets count result show that there is highly significant decrease in group 2 and group 4 (378.6 ±100.1), (325.4 ±140.7) respectively by comparing with control group.

TABLE 1: Effects of Potassium Bromate (group 2), Calcium Sulfate (group 3) and Synergistic effect (group 3) in blood compounds. Showed the complete blood count (CBC) include: White blood Cell (WBC), Red Blood Corpuscles (RBC), Hemoglobin (Hgb), Hematocrit (Hct), and platelets count (PLT) Mean ± Standard error (S.E.) , significant differences (P< 0.05) between means .

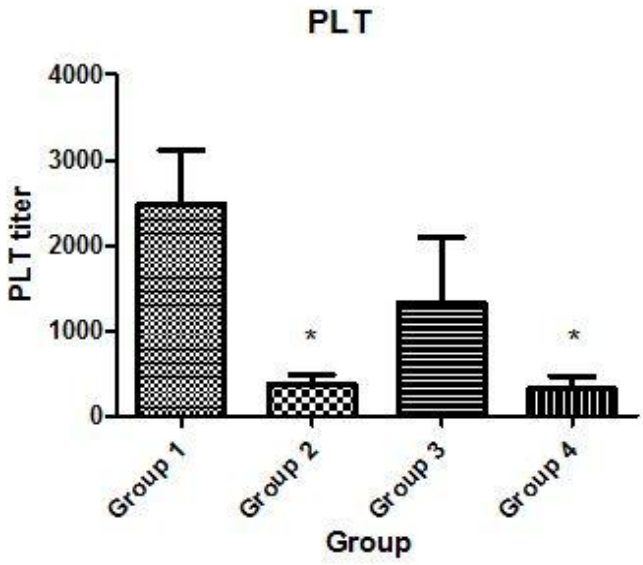
CBC indices Group	WBC $10^3/mm^3$ (Mean ± S.E.)	RBC $10^6/mm^3$ (Mean ± S.E.)	Hgb g/dl (Mean ± S.E.)	Hct % (Mean ± S.E.)	PLT $10^3/mm^3$ (Mean ± S.E.)
Control group (1) n = 6	5.978 ±0.387	5.278 ±0.1628	8.708 ±0.2328	17.96 ±3.343	2489 ±621.2
group (2) n = 6	2.783 ±0.437	3.320 ±0.4342	12.42 ±0.2884	31.02 ±1.723	378.6 ±100.1
group (3) n = 6	4.248 ±1.238	4.545 ±0.7863	9.260 ±0.9480	29.48 ±3.041	1323 ±772.6
group (4) n = 6	2.415 ±0.156	2.415±0.1567	11.33 ±0.1314	31.15±0.370	325.4 ±140.7



Figure(1): show WBC $10^3/mm^3$ count and RBC $10^6/mm^3$ titer in mice groups; control, Potassium Bromate , Calcium Sulfate , and both of them group. n = 6 for each group .



Figure(2): Show HGB g/dl **count and HCT** % **in mice groups; control, Potassium Bromate , Calcium Sulfate , and both of them group. n = 6 for each group .**



Figure(3): Show PLT $10^3/mm^3$ **count in mice groups; control, Potassium Bromate , Calcium Sulfate , and both of them group. n = 6 for each group .**

4 - Discussion :

The result show there is significant decrease in WBC count in group 2 that is due to KBrO3 induces oxidative stress in tissues (17, 47) and this lead to increase WBC count for acute period but for long term uses this lead to decrease them and this agree

with article reported by chipman (48). While in CaSo4 show there is no significant effect, for the group 4 there is highly significant effect due to the double effect of KBrO3 and CaSo4 that may occur due to ROS production and formation of a reactive calcium phosphate layer in biological system and this agree with by (49).

Red blood corpuscles and platelets result show that there is significant decrease in there count in group 2 due to $KBrO_3$ is strong oxidizing agent that generates free radicals (47,50) and also due to DNA strand breakage in these cells induced by the oxidative stress associated with KBrO3 this will lead to decrees in there count Furthermore, there could have been bone marrow suppression with selective megakaryocytic depression (51). So, the reductions in the RBCs, WBCs and platelets could imply selective systemic toxicity effect by KBrO3. In another study KBrO3 induced chromosomal aberrations and decreased both the cell proliferation index and the mitotic index of human peripheral lymphocytes in vitro (52).

While there is no significant effect of $CaSo_4$ on both Red blood corpuscles and platelets and this agree with (53), i*n vivo* mammalian erythrocyte micronucleus assay using male mice tested at the different dose levels gave negative results, while in group 4 the result show highly significant effect may be due to the accumulation of CaSo4 in tissues of body organs and this act Synergistically with KBrO3 to make this effect due to ROS effect or genetic damage as mention above.

Related to the Hemoglobin results that show there is significant increase in the Hgb concentration in all group by comparing with control thus due to increase the destruction of RBC and this correlation can be seen significantly reported by *Zhang et al*., (54).

Conclusion

From current study conclude that We can conclude that there are different harmfully effects of KBrO3 and the synergistic effect with CaSo4 on the animal model and according to that on human due to the daily uptake of this compounds in our life style ,according to the hematological indices (WBC),(RBC), (Hgb), (Hct), and finally (PLT). Thus the using of KBrO3 and CaSo4 should be limited and used specific documented concentration to reduce its unfavorable effects.

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