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Original Article

Comparative study between the effect of Potassium Bromate and Calcium Carbonate on the tongue and parotid gland of adult male albino rats and the possible interaction between them (Histological and Immunohistochemical study)

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Abstract

Background: Potassium bromate (KBrO3) is widely used as a flour enhancer to improve dough quality and as an oxidizing agent in baked goods. An essential inorganic mineral in geological and biological processes is calcium carbonate (CaCO3) that is utilized in construction materials, cosmetics, and food.

The purpose of this study was to investigate the effects of calcium carbonate and potassium bromate on the parotid gland and tongue of adult male albino rats, as well as any potential interactions between the two.

Materials and methods: 40 male albino rats were used. The animals were equally divided into four groups. Group I "control" were given distilled water, group II were given KBrO3 at a dose of 100 mg/kg/day, group III were given CaCO3 at a dose of 50 mg/kg/ day and group IV rats were given KBrO3 then after one hour CaCO3, treatments extends for 14 days, then tongues and parotid were collected for histological study.

Results: KBrO3 caused distortion of tongue papillae, detached keratin layer, and distortion of the parotid architecture, atrophy and shrinkage of the acini, massive cytoplasmic expression of Bax in both organs. CaCO3 treatment caused no changes in both tongue and parotid with minimal expression of Bax in both. KBrO3+ CaCO3 treatment showed atrophied tongue papillae, detachment of the keratin covering, as regards the parotid there were little atrophy and shrinkage of acini with moderate Bax expression.

Conclusion: KBrO3 induced cellular damage in the rat tongue and parotid glands that can be decreased by concomitant administration of CaCO3.

Keywords: Potassium bromate, calcium carbonate, tongue, parotid glands

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Introduction:

A common food ingredient is potassium bromate (KBrO3), it considered the preferred ingredient in bread production worldwide due to its affordability, accessibility, and dependability in the creation of various baked goods and cheese. [1&2] It has a major detrimental impact on numerous organs, including the kidney, [3] liver, [4] thyroid gland, [5] and central nervous system. [6]

Since oxidative DNA damage was also identified as one of the major negative effects [7] it was deemed a possible human and animal carcinogen [8] and prohibited from being used in produced possessions in many nations. [9]

The most prevalent mineral in the body, calcium (Ca), can be obtained as a food supplement and in certain medications. To maintain vital metabolic processes like vascular contraction, vasodilatation, muscular contraction, nerve transmission, intracellular signalling, and hormone secretions, the body requires less than 1% of its total calcium content. [10]

The body can use bone tissue as a source and reservoir of calcium to maintain steady amounts in blood, muscles, and intracellular fluids. Serum calcium is also highly and tightly regulated and does not change in response to variations in dietary intakes. [10]

Although carbonate and citrate are the two primary forms of calcium used in supplements, calcium carbonate (E 170) is more widely accessible, less expensive, and more practical. While calcium citrate is easily absorbed with or without food, calcium carbonate is best absorbed when taken with food since it depends on stomach acid to be absorbed. [11]

The Food and Drug Administration (FDA) has certified CaCO3 for use in quantities that are consistent with acceptable manufacturing practices to colour medications generally, and it is listed among the food additives that are generally recognized as safe (GRAS) for use in dietary and nutritional supplements. [12]

There is no information on CaCO3's long-term toxicity or carcinogenicity. However, considering that both calcium and carbonate are naturally occurring components of the body, normal metabolites of people, animals, and plants, and have a long history of safe use as a source of calcium supplementation for humans, it is highly improbable that CaCO3 is accomplished of causing cancer. Additionally, there have been findings that link the use of calcium to health issues when other chemicals are present. [13]

This study aimed to compare the effect of potassium bromate and calcium carbonate on the tongue and parotid gland of adult male albino rats and the possible interaction between them.

Material and methods:

Ethical approval

The work was authorized by the research ethics and use of laboratory animals' registration number (Soh-5-12-4/2024-03) and adhered to the norms of Sohag University Animal Ethics.

Chemical and preparation:

Potassium Bromate (KBrO3) and calcium carbonate (CaCO3) were obtained from Sigma Chemicals Company and dissolved in distilled water before administration.

KBrO3 at a dose of 100 mg/kg/day was dissolved in 0.5 ml of distilled water and given by using a gastric tube for 4 weeks. The KBrO3 dose was calculated to reflect the amount normally utilized in the human diet. [14]

Calcium carbonate at a dose of 50 mg/kg/day, dissolved in 0.5 ml of distilled water and given using a gastric tube for 4 weeks . [15]

Animals and treatments: A total number of 40 adult male albino rats were used, the animals were brought from the animal house of Sohag faculty of medicine, and they were reared under the standard conditions of feeding, light-dark ratio and temperature. The animals were subdivided equally into four groups with 10 animals in each group:

Control group: The rats were given distilled water and the standard food.

Group II (KBrO3 group): The rats were given KBrO3 at a dose of 100 mg/kg/day, dissolved in 0.5 ml of distilled water using a gastric tube for 4 weeks

Group III (CaCO3 group): The rats were given calcium carbonate (CaCO3) at dose of 50

mg/kg/day, dissolved in 0.5 ml of distilled water using a gastric tube for 4 weeks

Group IV (KBrO3+ CaCO3) group: The rats were given KBrO3 then after one hour calcium carbonate (CaCO3) as the previously mentioned doses for 4 weeks.

Scarification and Tissue sampling

At the end of the experiment, all animals were anesthetized with co2; the tongue and parotid were obtained and processed for light microscopic (LM) examination.

Preparation for light microscopic:

For general histological analysis, specimens intended for light microscopy inspection were prepared into 5µm paraffin slices, fixed in 10% buffered formalin, and stained with haematoxylin and eosin (HX&E) .[16]

Immunohistochemical examination :

The avidin-biotin complex (ABC) method was used for immunohistochemical staining. 5µm paraffin slices were placed on positively charged slides, deparaffinized, rehydrated, and then treated for 10 minutes in a 3% hydrogen peroxide solution in methanol to block endogenous peroxidase activity. Sections were then washed in PBS and exposed to 10% goat serum for 10 minutes at room temperature to avoid nonspecific binding. To recover the antigen, the slides were boiled for 10 minutes in 10 mmol/L citrate buffer at pH 6 (cat. no. AP 9003) and then allowed to cool for 20 minutes. Sections were then incubated with the primary antibodies; Rabbit polyclonal anti-BAX protein marker 1:1000, (to detect the presence and location of the BAX protein within tissue which plays a role in apoptosis)

Finally, the immunohistochemical reaction was detected using 3,3′-diaminobenzidine (DAB) hydrogen peroxide chromogen followed by counterstaining with Mayer's haematoxylin .[17]

Morphometric study: The following measurements were taken

1. Mean length and width of the fungiform and filiform papillae (H&E X200).
2. Number (H&E X200) and diameters of acini ((H&E X400).
3. Mean area percentage of immunohistochemical reaction of BAX (X200).

Five non-overlapping fields from each slide were selected for each measured parameter. From each animal, five slides were chosen. After calculating the mean for each animal, the mean for each group was estimated.

Statistical analysis: The data statistics analysed using SPSS software version 16 (SPSS Inc., Chicago, IL, USA). The results were expressed as mean ± Standard deviation (SD). One-way ANOVA test, the post-hoc test (LSD) was used to compare values among various groups. The differences were considered statistically significant if value P value < 0.05. [18]

RESULTS

1.H& E results

1.1 tongue:

Normal tongue papillae architecture was found when the dorsal surface of the sham group's tongue was examined. The filiform papillae are long, finger-like projections with wholly formed epithelial coat that are made up of a lamina propria core covered by a keratinized stratified squamous epithelium. Taste buds were located on the superior surfaces of the fungiform papillae, which had a distinctive mushroom-shaped appearance. **(Figure1a).** KBrO3 group showed distorted filiform and fungiform papillae, some papillae were markedly atrophied with detached taste buds, marked widening and disturbance of the lamina propria and muscle layer **(Figure1b).** CaCO3 group revealed normal tongue tissue layers similar picture like control group with normal filiform and fungiform papillae, normal epithelial lining, intact lamina propria and muscle layer **(Figure1c).** As regards the KBrO3+ CaCO3 group the tongue showed atrophied tongue papillae with detached keratin layer, lamina propria and muscle layer appeared normal **(Figure1d).**

1.2 Parotid:

Histological analysis of the control group's parotid gland showed that it was made up of collecting ducts and secretory acini. Each of the spherically organized acini was made up of pyramid-shaped serous cells with rounded nuclei at the base. The cuboidal cells that lined the intercalated ducts had compressed, narrow lumens **(Figure2 a & b).**

The KBrO3 group showed disturbed glandular architecture, the acini showed numerous vacuolations in the cytoplasm, most nuclei appeared atrophied and shrunken. Some blood vessels were congested with blood and haemorrhage between the acini was also noticed. **(Figure3 a& b)**. The parotid glands of the CaCO3 group showed nearly normal structure like control with normal arrangement of acini and intralobular ducts. **(Figure4 a& b)**. Parotid gland of the KBrO3+ CaCO3 group showed some improvement in architecture, still vacuolations in the acini and congested vessels with haemorrhage were also seen **(Fig5 a& b).**

2.Immunohistochemical results:

2.1. tongue

Examination of the control group revealed minimal BAX expression limited to keratin layer, the fibroblastic cells and blood vessels in the lamina propria **(Figure6a).** KBrO3 group showed massive cytoplasmic expression of BAX in all layers of tongue tissue in keratinocytes, blood vessels and fibroblastic cells of the lamina propria **(Figure6b).** CaCO3 group revealed minimal expression of BAX limited to keratin layer and the fibroblastic cells and blood vessels in the lamina propria **(Figure6c).** KBrO3+ CaCO3 group showed moderate expression of BAX in all layers; keratinocytes, fibroblastic cells and blood vessels in the lamina propria **(Figure 6d).**

2.2. Parotid gland

Immunohistochemical examination of the parotid glands of the control group showed mild cytoplasmic immunoreaction for BAX in acinar cells **(Figure7a).** The Parotid gland of KBrO3 group showed intense cytoplasmic expression of BAX in acinar and ductal cells. **(Figure7b)**. Parotid gland of CaCO3 group showed minimal expression for BAX in acinar cells **(Figure7c).** KBrO3+ CaCO3 group IV slides revealed moderate expression of BAX in the acinar and ductal cells of the parotid gland **(Figure7d)**.

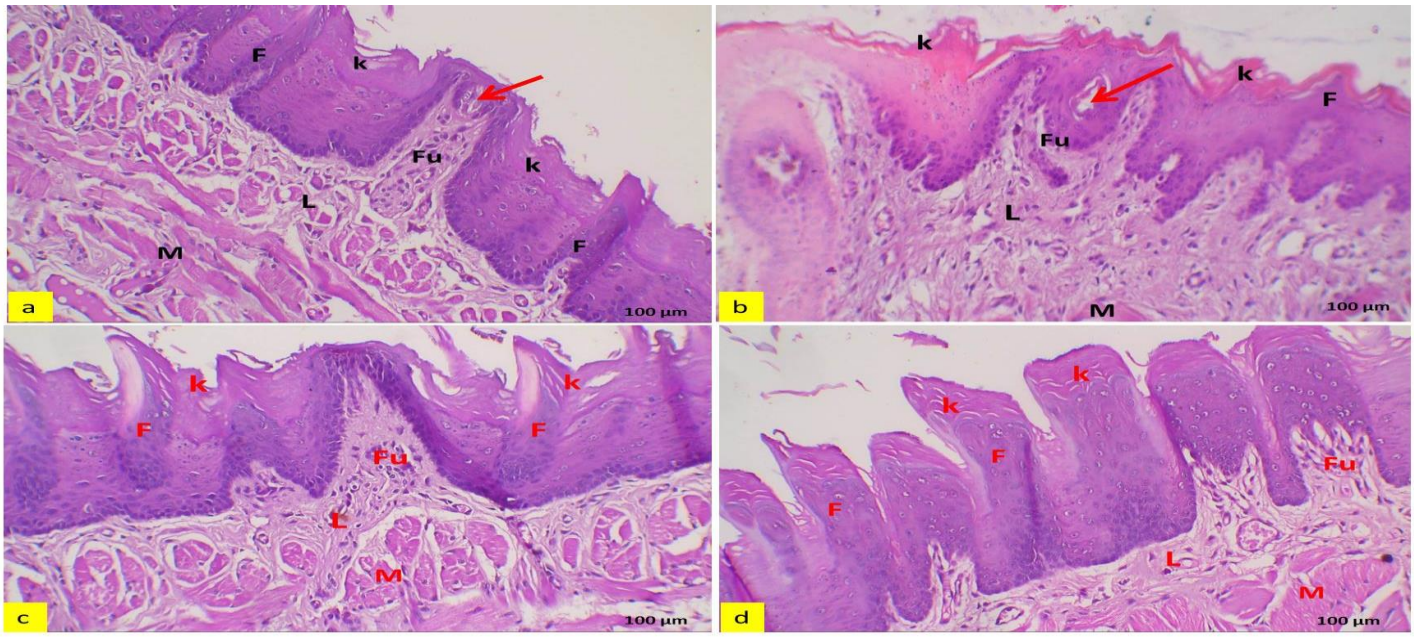


Figure1 : Histological photomicrographs showing rat tongue dorsal mucosa **(a)** control group revealing stratified squamous epithelium which is lightly keratinized (K) with conical filiform papillae(F), mushroom shaped fungiform papilla(Fu) with intact taste buds(arrow), intact lamina propria (L) and muscle layer (M). **(b)** KBrO3group showing detachment of the keratin layer (K), distorted filiform(F) and fungiform papillae (Fu with detached taste buds (arrow), wide lamina propria (L) with abnormal picture and disturbed muscle layer (M). **(c)** CaCO3 group showing normal stratified squamous epithelium which is lightly keratinized (K) with normal filiform papillae(F) and fungiform papilla (Fu), intact lamina propria (L) and muscle layer (M). **(d)** KBrO3+ CaCO3 group revealing atrophied filiform papillae(F) and fungiform papilla (Fu) with detached keratin layer (K), normal lamina propria (L) and muscle layer (M)**.** (H&E X 200)

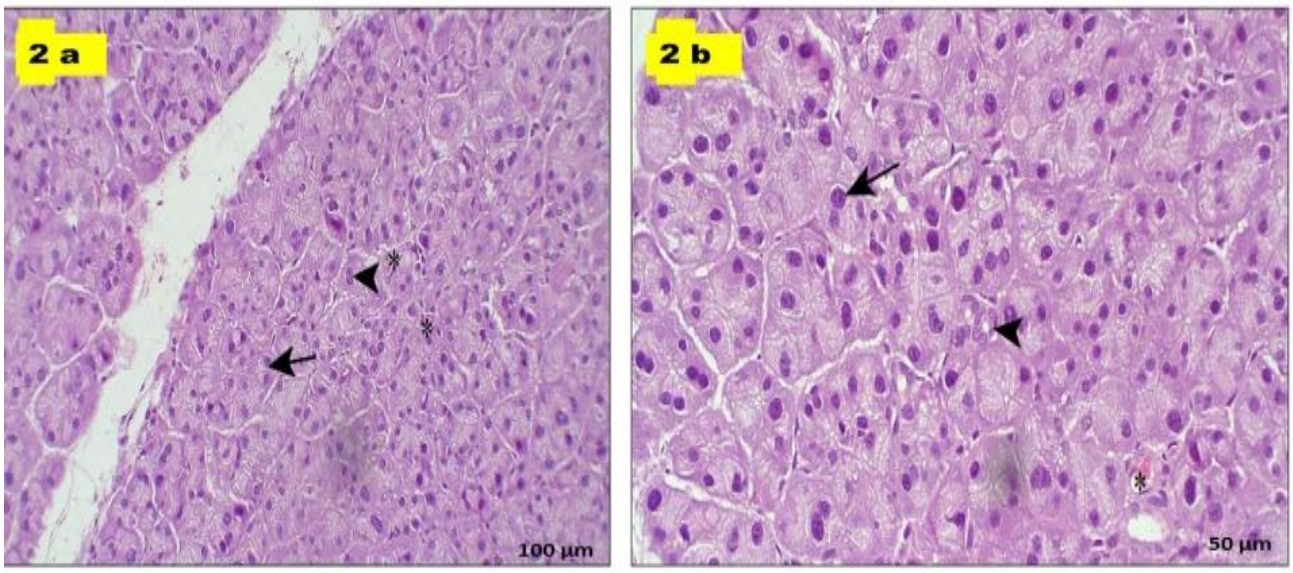


Figure.2 (a&b): A photomicrograph of parotid gland of control group showing normal appearance of serous acini (arrows), rounded vesicular nuclei (arrow head) and normal blood vessels (star) (H&E X 200 &400).

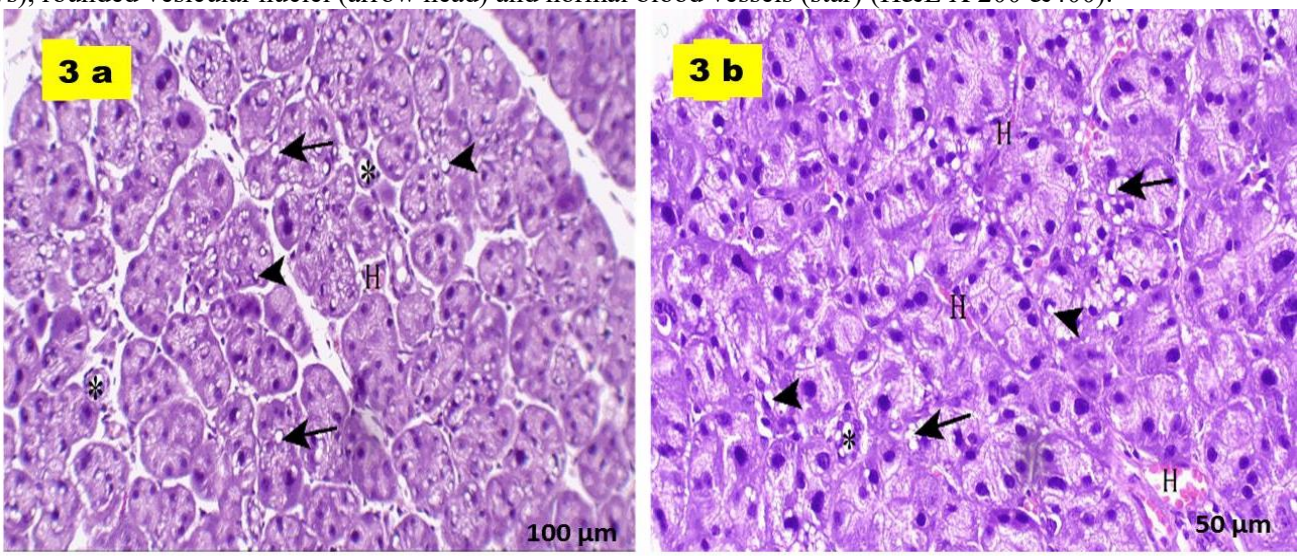


Figure.3 (a&b): A photomicrograph of parotid gland of KBrO3 group showing disturbed serous acini, multiple vacuolation within acinar cytoplasm (arrow), shrunken atrophied nuclei (arrow head), small atrophied intralobular ducts (star) and dilated congested blood vessel with haemorrhage (H). (HX&E 200 &400).

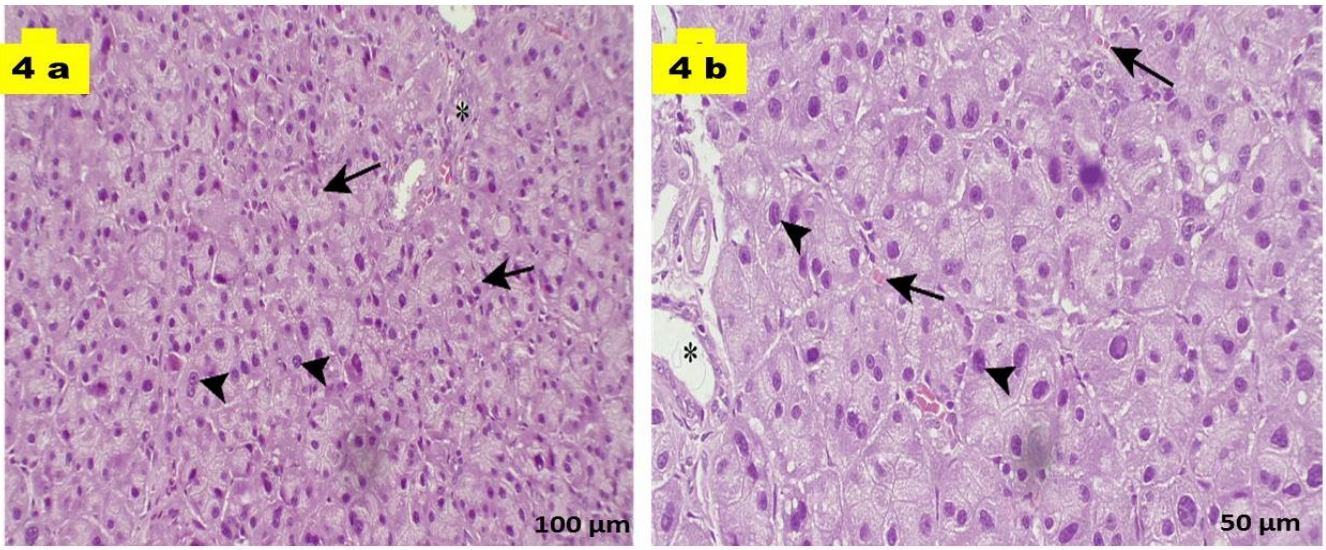


Figure4 (a&b): A photomicrograph of parotid gland of CaCO3 group showing a near normal structure of parotid gland in the form of normal serous acini (arrow), rounded vesicular nuclei (arrow head) and normal appearance of intralobular ducts (star) (HX&E 200 &400).

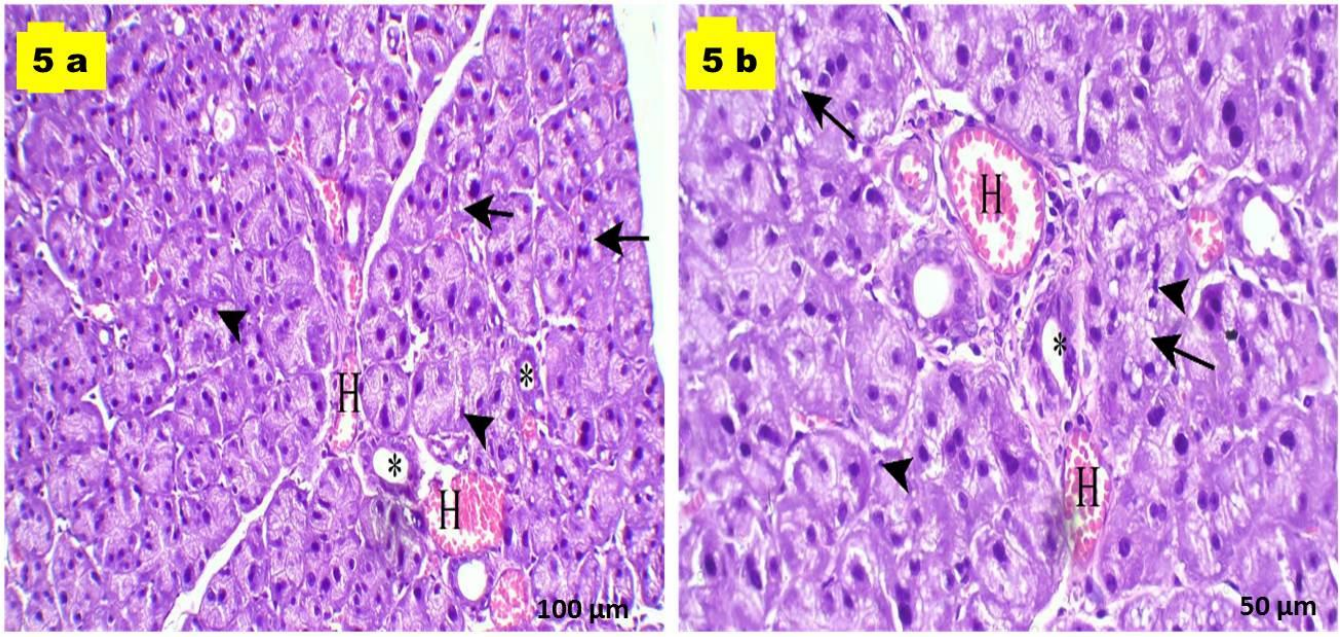


Figure5 (a&b): A photomicrograph of parotid gland of KBrO3+ CaCO3 group showing (a) improved arrangement of serous acini, (b) some vacuolation within acinar cytoplasm (arrow), (c) little atrophied nuclei (arrow head), (d) small atrophied intralobular ducts (star), (e) dilated congested blood vessel with haemorrhage(H). (HX&E 200 &400).

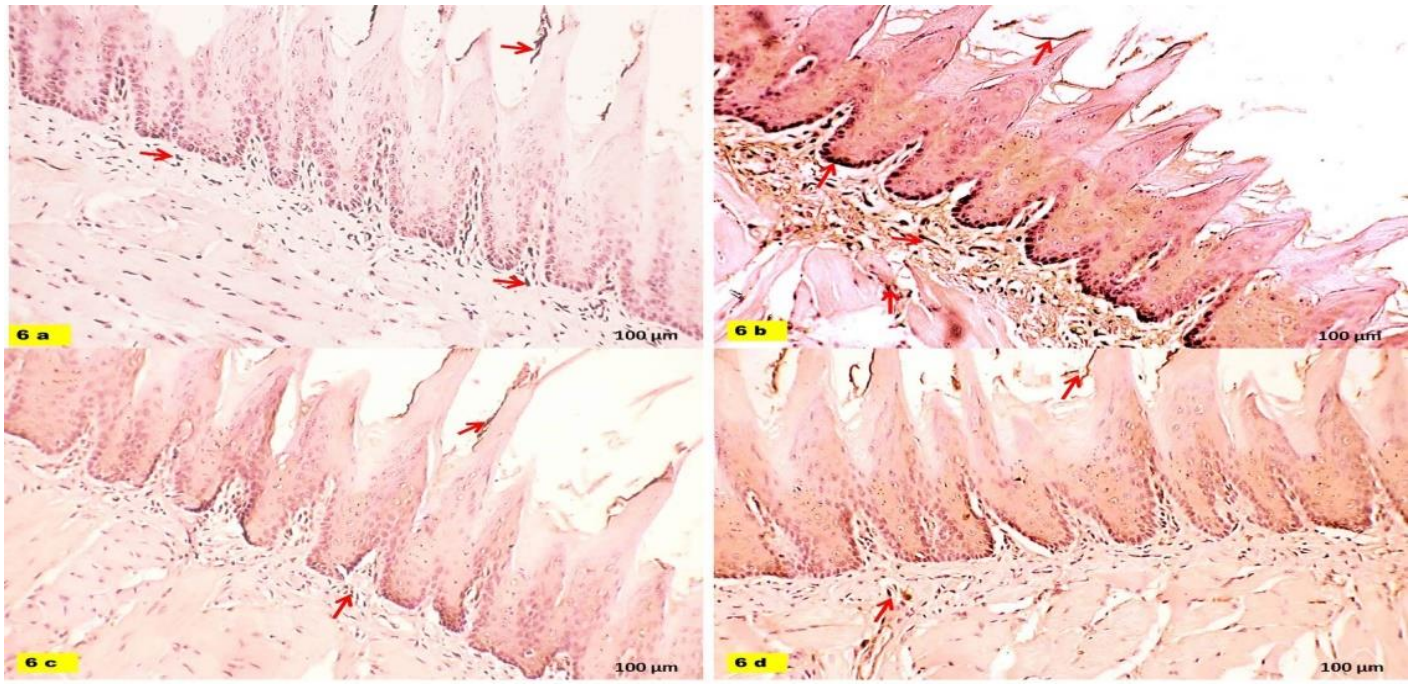


Figure6: A micrograph of the dorsum of the tongue of **(a)** control group revealing minimal expression of BAX limited to keratinized and the fibroblastic cells and blood vessels in the lamina propria**. (b)** KBrO3group showing massive cytoplasmic expression of BAX in all layers of tongue tissue in keratinocytes, blood vessels and fibroblastic cells of the lamina propria**. (c)** CaCO3 group showing light expression of BAX limited to keratinized and the fibroblastic cells and blood vessels in the lamina propria. **(d)** KBrO3+ CaCO3 group revealing moderate expression of BAX in all layers; keratinocytes, fibroblastic cells and blood vessels in the lamina propria. (BAX X 200).

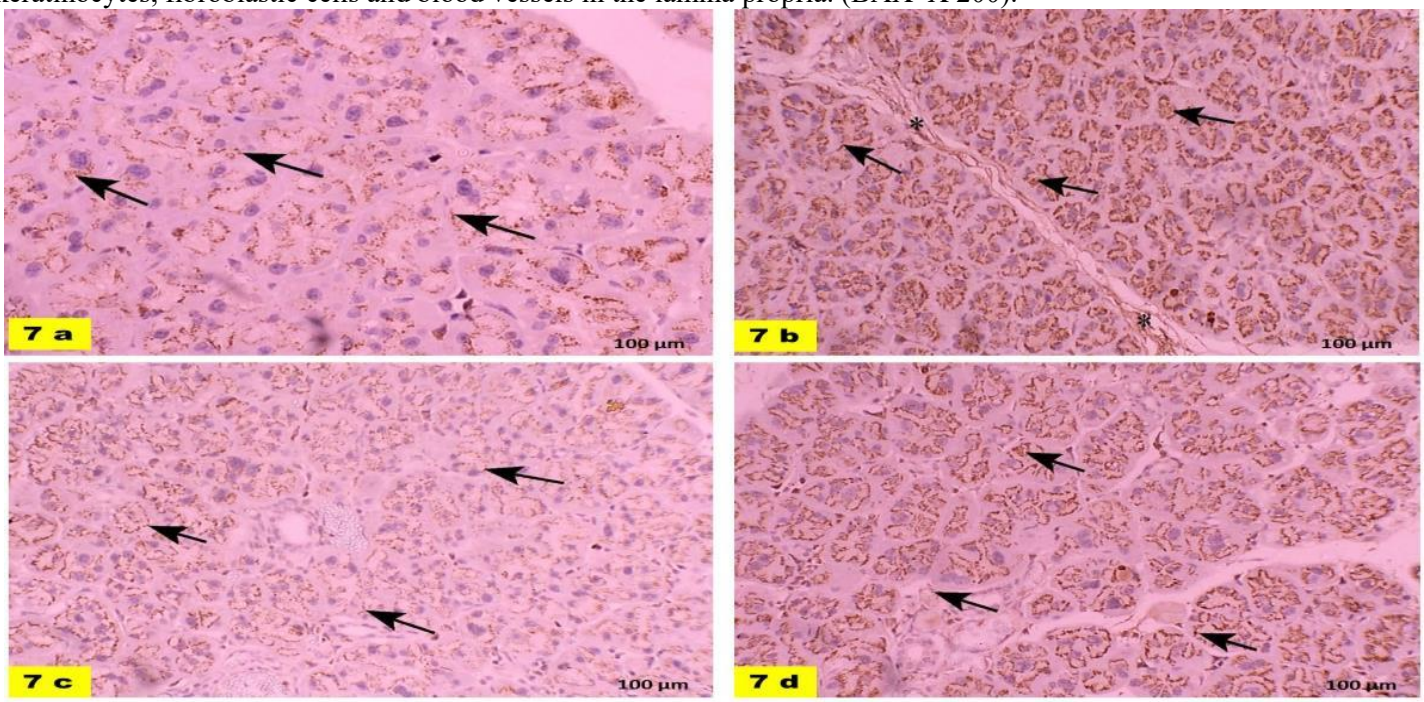


Figure7: A photomicrograph of parotid gland **(a)** control group showing little immune expression of BAX in the cytoplasm of serous acinar cells (arrow). **(b)** KBrO3group showing intense immune expression of BAX in the cytoplasm of serous acinar and ductal cells (arrow) and within the lobules (star). **(c)** CaCO3 group showing little immune expression of BAX in the cytoplasm of serous acinar cells same like control (arrow). **(d)** KBrO3+ CaCO3 group showing intense immune expression of BAX in the cytoplasm of serous acinar and ductal cells(arrow) (BAX X200).

Morphometric analysis results

Investigations of length and width of filiform and fungiform papillae revealed significant decrease in KBrO3 group in comparison with the control group, with non-statistically significant difference between CaCO3 and KBrO3+ CaCO3 groups in comparison to control group. **(Histogram 1&2)**

Investigations of number and diameters of acini revealed significant decrease in KBrO3 and KBrO3+ CaCO3 groups in comparison with the control group, with non-statistically significant difference between CaCO3 group in comparison to control group. **(Histogram 3)**

Investigations of the mean area of BAX reaction in tongue revealed highly significant increase in KBrO3 and KBrO3+ CaCO3 groups in comparison with control group, with non-statistically significant difference between CaCO3 group in comparison to control group**. (Histogram 4)**

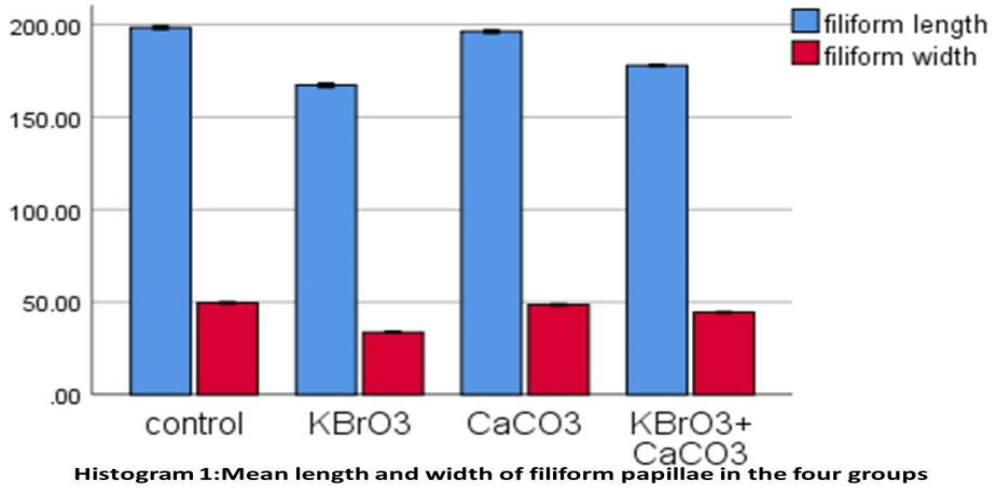
Investigations of mean area of BAX reaction in parotid revealed significant increase in KBrO3 group in comparison with control group, with non-statistically significant difference between CaCO3 and KBrO3+ CaCO3 groups in comparison to control group. **(Histogram 4)**

Table 1 showed the morphometric changes among the four studied groups

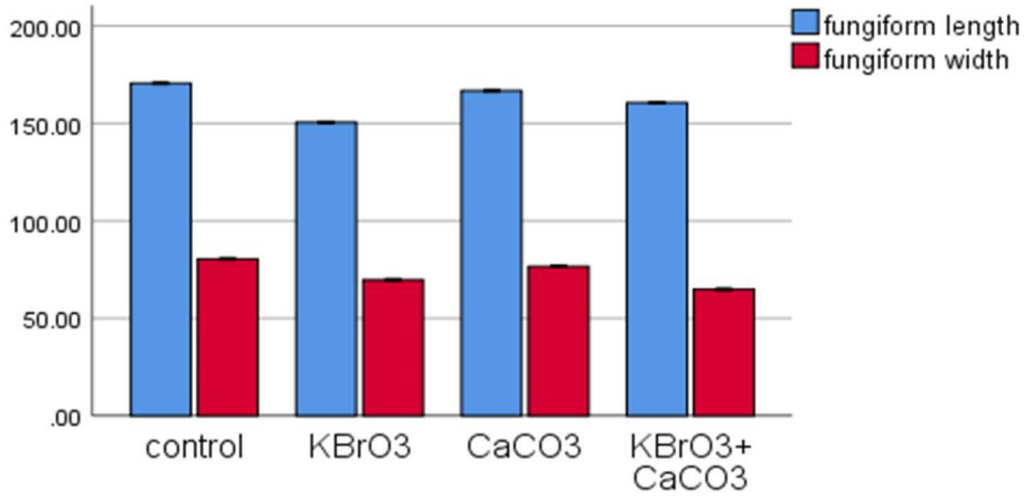
	Mean length of filiform papillae	Mean width of filiform papillae	Mean length of the fungiform papillae	Mean width of the fungiform papillae	Diameter of acini (AD)	Number of acini (AN)	area % of BAX reaction in tongue	area % of BAX reaction in parotid
Control group	198.3±1.1	49.5±.4	170.5±.4	80.4±.2	30.54 ±.2	42.5±1	3.08±.09	6.8±.10
KBrO3 group	167.19±1.5[@]	33.6±.37[@]	150.5±.4[@]	69.7±.6[@]	27.5±.3[@]	33.9±.87[@]	22.6±.38[@]	30.2±.2[@]
CaCO3 group	196.26±1	48.5±.38	166.7±.6	76.6±.4	29.4±.2	40±.81	3.13±.25	6±.3
KBrO3+ CaCO3	177.9±.56	44.4±.31	160.5±.4	64.8±.5	28.5±.3[@]	37±.8[@]	20.17±.11[@]	6.25±.5
P Value (Between groups)	<0. 05*	<0. 05*	<0. 05*	<0. 05*	<0. 05*	<0. 05*	<0. 05*	<0. 05*

Table 1: Morphometric changes in the four experimental groups (mean ± standard deviation) [@] Statistically significant when compared with control group *(P*<0.05).

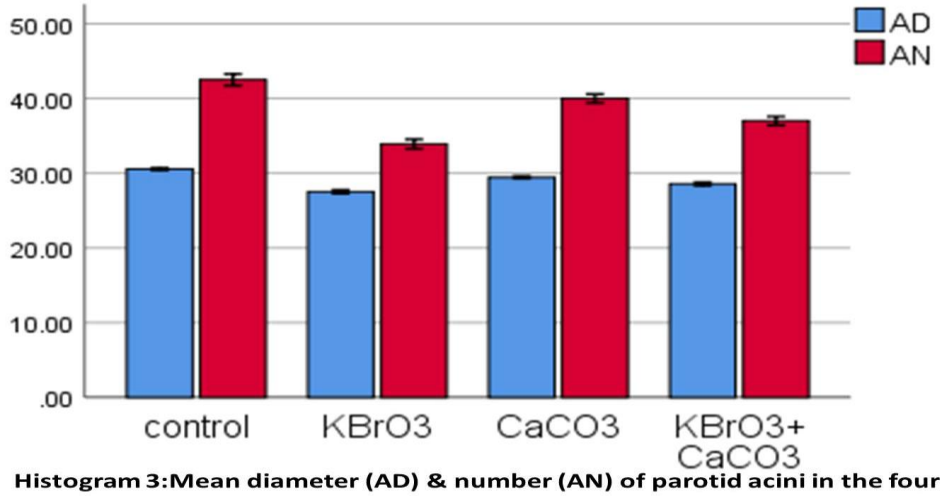
* Statistically significant between groups



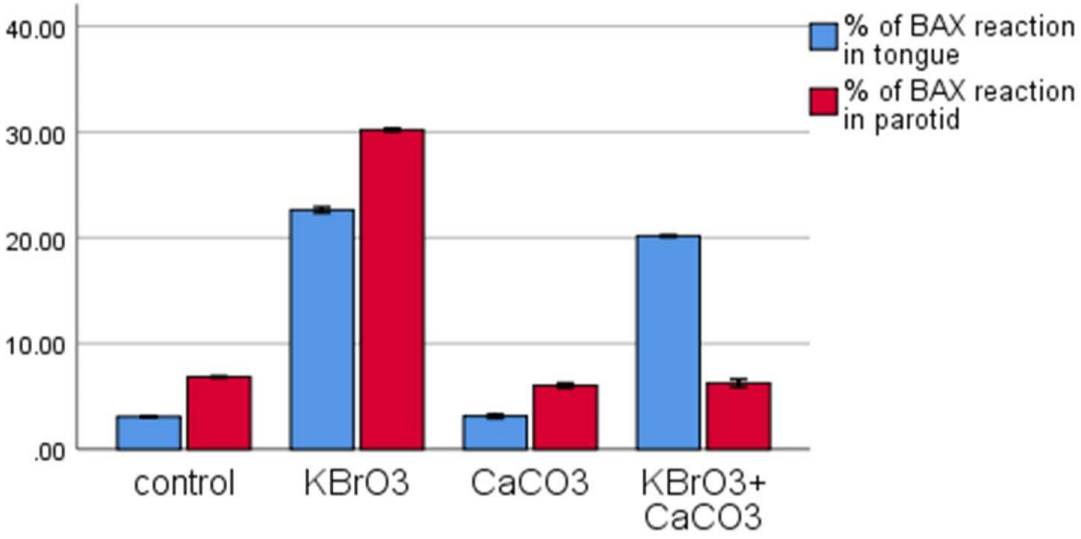
Histogram 1:Mean length and width of filiform papillae in the four groups



Histogram 2:Mean length and width of fungiform papillae in the four groups



Histogram 3:Mean diameter (AD) & number (AN) of parotid acini in the four groups



Histogram 4:Mean area % of BAX reaction in the four groups

Discussion

Saliva plays a crucial role in both oral mucosal protection and digestion. Unsuitable salivary flow can have detrimental implications concerned with the health of the mouth and throat. [19]

Numerous studies have been conducted to evaluate the harmful effects of KBrO3 on different body organs, but not many have examined the tongue and salivary glands, particularly the parotid gland. For this reason, we are interested in examining the harmful effects of KBrO3 at residual dosages observed in bread and cakes. In agreement with Turati et al. [20] found that the tongue is considered one of the most anatomical sites for the progression of oral cancer.

In our study the histological examination of haematoxylin and eosin-stained sections of KBrO3 group II rats showed distorted filiform and fungiform papillae, some papillae were markedly atrophied with detached taste buds, vacuolisations in all layers of the tongue, The lamina propria had marked cellular infiltrates.

In agreement with Moubarak et al. [21] who proved that the histological studies of rat tongue treated with KBrO3, revealed many histological changes that worsen thru increasing the time and dose of the KBrO3. In suggestion with previous studies. [22&23] which stated that the effects of KBrO3 on the rat tongue papillae in form of atrophy or distortion was due to its lowering effect on iron, folic acid and important vitamins especially vitamin B complex, filiform papillae were higher atrophied than the fungiform papillae due to its higher metabolic rate so it more affected with any nutritional deficit. The role of KBrO3 in apoptosis was via synthesis of 8-hydroxydeoxyguanosine, a marker of oxidative damaging to the DNA by formation of bromine free radicals that induce the cellular oxidation and damaging. [24] In accordance with this study Boonyarom and Inui [25] stated that the muscle atrophy are due to amplified degree of oxidative damage and resultant protein deprivation, so the oxidative stresses can cause muscle spacing and atrophy in the tongue of rats.

The present study revealed that administration of KBrO3 had hazardous effect on parotid gland as revealed by Histological examination which demonstrated disturbed serous acini with vacuolated cytoplasm and wide spaces between acinai. Most nuclei were shrinked and atrophied. The intralobular ducts appeared atrophied and congested blood vessels with apparent haemorrhage. These results were in agreement with Moubarak, et al., 2020. [21] who observed extensive loss of normal parotid architecture in response to KBrO3 treatment in the form of acinar irregularity, nuclear hyperchromatism, pleomorphism, wide inter-acinar spaces with congested blood vessels.

Disturbed cellular architecture could be attributed to oxidative stress induced by KBrO3, as in the previous study. [26] confirmed that oxidative stress could induce radical-mediated damage to cellular bio-membranes that resulted in lipid peroxidation. Extensive lipid peroxidation always correlated with the disintegration of membrane integrity. [27]

wide spaces between acinai in response to KBrO3 treatment could be attributed to accumulation of interstitial oedema fluids as suggested by Krishnan et al.[28] Increased connective tissue spacing and cellular infiltrates proved by previous research. [29] which denoted that cellular toxicity resulted from tissue hypoxia and oedema formation which leads to increasing dissemination of protective plasma proteins and exodus of cellular infiltrates into the affected tissue.

Our result is also supported by studies. [30 & 31] revealed hepatocyte vacuolation in the liver of rats treated with KBrO3, also Elsheikh et al., 2016 [32] reported vacuolation of testicular cells of male rats that received 400 mg/L KBrO3 in drinking water for 8 weeks.

Histological examination of CaCO3 group revealed that both tongue and parotid gland showed near normal picture by H&E examination this is supported by karnad et al. [33] who supposed significant anti-inflammatory activity of CaCO3 in acute as well as in sub-acute models of inflammation in rat organs.

On the other hand this study revealed that tongue and parotid gland of KBrO3+ CaCO3 group showed minimal changes in structure of both organs in comparison to control, still some vacuolations in the cytoplasm and congested blood vessels with apparent haemorrhage were seen, this in line with Mohamed [34] who revealed that administration of

calcium carbonate for 30 or 60 days during fluoride treatment minimized hazardous effect on the rat organs.

KBrO3-induced apoptosis was reported by Ali et al.[35] which were seen in this study in KBrO3 and KBrO3+ CaCO3 groups in the tongue and KBrO3 group in the parotid gland. Reactive oxygen species (ROS) are thought to be a key mediator of the apoptosis caused by KBrO3 and are frequently in charge of the mitochondrial apoptosis signalling pathway. Cytochrome c is released from mitochondria in response to apoptotic stimuli, which sets off a chain of events that activates caspase. [36]

As regards CaCO3-treated rats there were non-significant changes in comparison to control. By promoting macrophage activity and the release of inflammatory mediators such as TNF-α and IL-8, calcium carbonate (CaCO3) usually causes pro-inflammatory reactions. Nevertheless, it has been demonstrated that tannylated CaCO3 materials—which contain tannic acid—have anti-inflammatory and antioxidant properties, and that altered CaCO3 particle forms and surface characteristics, like phosphate coating, can inhibit pro-inflammatory reactions. [37]

Morphometric results demonstrated decrease in the width and length of both filiform and fungiform papillae in the tongue, decrease in the diameter and number of acini in the parotid gland as regards the KBrO3 group. There were some decreases in these measurements in the KBrO3 +CaCO3, with no morphometric changes as regards CaCO3 group.

The imbalance between antioxidants and excessive free radical production may be the primary cause of KBrO3's detrimental effects. Tissue damage results from the free radicals' reaction with lipids, proteins, and nucleic acids. [38,39] Induction of the immune complex is another potential mechanism for KBrO3's harmful effects. [40]

Conclusion: KBrO3 induced histological alterations in tongue and parotid glands of adult male albino rat, CaCO3 induced no changes as regards the tongue and parotid glands, the KBrO3 +CaCO3combination minimally decrease the harmful effects of KBrO3 treatments.

Recommendations: Re-evaluation of calcium carbonate (E 170) as a food additive for medical use as a supplement or antacid, recommendations depend on individual needs.

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