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Teratogenic Effects of Potassium bromate On *Drosophila melanogaster*

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Abstract

In recent years, there has been growing concern about the toxic potential of substances consumed by humans. One such substance that has attracted attention is Potassium bromate ($KBrO_3$) used as a food additive, water disinfectant, and in pharmaceutical industries, has been extensively studied due to its detrimental impact on human health. It could potentially cause serious teratogenic effects on embryonic development. Certain pharmacological and physiological characteristics of fruit flies (*Drosophila melanogaster*) are comparable to those of humans. So, to study the teratogenic potentials, different concentrations of $KBrO_3$ (15mM to 30mM) were exposed to the egg to adult stages of fruit fly. This study assessed life-history parameters and revealed significant increases in larval and pupal mortality, extended developmental durations, smaller larval, pupal, and adult sizes, deformities in pupae and aberrant phenotype in adults, all in a concentration-dependent manner. The results showed that $KBrO_3$ treatment (15mM to 30mM) increased the developmental time of egg to pupae ($p < 0.0001$) and egg to adult ($p = 0.0017$). The viability rate of pupae ($p < 0.0001$); adults ($p < 0.0001$), the length ($p< 0.0001$) and width ($p < 0.0001$) of larvae and pupae decreased significantly as compared to the control group. These findings suggest that prenatal exposure to $KBrO_3$ has clear teratogenic effects on fruit flies, indicating potential risks to human health if $KBrO_3$ is used in any application.

Keywords: Teratogen, Potassium bromate, Developmental time (DT), Viability, Drosophila melanogaster and aberrant phenotype.

Introduction

Teratogenesis is the process that leads to developmental defects in a fetus. Chemical substances that can cause abnormalities in the offspring when ingested in prenatal stages are called teratogens. The impact of teratogens on fetal development causing various abnormalities depends on the amount of exposure, timing, and the stage of developing embryo or fetus during the exposure (Harden et al., 2009; Mayshar et al., 2011; Zomerdijk, et al., 2015). The abnormality caused by teratogens may manifest as anatomical, morphological, physiological, biochemical, or behavioral changes (Coyle et al., 1976). Teratogens are classified into four types: drugs and chemicals, metabolic conditions, physical agents, and infection (Lim et al., 2011).

In recent years, there has been greater attention paid to the possible toxic effects of substances consumed by humans (Allam et al., 2019). Food additives are chemicals used to enhance the quality, quantity, flavor, color, and food production. Numerous studies have shown that harmful food additives and preservatives are linked to various health problems, including heart disorders, neurological issues, cancer, and obesity (Fewtrell, 2004; Simmons et al., 2014; Shahidi and Ambigaipalan, 2015; Carocho et al., 2017; Sambu et al., 2022). Potassium bromate ($KBrO_3$) is a food additive commonly used in the production of cheese, beer, and fish paste products. It is also found in the pharmaceutical industry and cosmetics, such as cold-wave hair treatments (IARC, 1986). Bromate is a notable byproduct formed when surface water is treated with ozone, and it can be present in drinking water (Jahan et al., 2020). $KBrO_3$ is primarily used in bread making to strengthen and soften the dough by enhancing the flour and improving the action of gluten (Kurokawa et al., 1983; Chuhan and Jain, 2016). Many countries, including the European Union, Brazil, the UK, and Canada, banned the use of Potassium bromate in food production starting in the 1990s. Sri Lanka and China followed ban in 2001 and 2005, respectively (Chauhan and Jain, 2016). In 2016, India also prohibited Potassium bromate as a food additive (FSSAI, 2016), yet it continues to be illegally used in various food industries (Grace, 2016; Chavan et al., 2019; Bello and Sani, 2023). A study by the Centre for Science and Environment (CSE) revealed that around 84% of 38 popular bread and bun brands tested positive for Potassium bromate.
Traditional mammalian teratology tests are challenging due to their complex mating procedures, treatment schedules, need for trained personnel, and high costs. However, *Drosophila* has the potential to serve as an alternative screening method (Schuler et al., 1982). According to Wilson (1978), *Drosophila* is notable for its ability to absorb, circulate, metabolize, and excrete chemicals, while also efficiently producing a large number of offspring in a short amount of time. Toxicity studies have shown many deleterious effects following consumption of $KBrO_3$ either in adult rodents or even in humans (Ahmad et al., 2014). To assess the potential impact of $KBrO_3$ on embryonic development, the fruit fly model was used, focusing on the various stages of development from egg to adult. Previous studies on developmental toxicity have indicated that fruit flies are sensitive to mammalian teratogens, making them a valuable model for examining teratogenic effects (Sharma and Kumar, 1999).

Materials and Methods

Maintenance of stock culture

The wild-type strain of *Drosophila melanogaster* (Oregon K) was used for the experiment, which was collected from the *Drosophila* Stock Centre, Department of Zoology, University of Mysore. Flies were reared on a standard wheat cream agar medium, following the protocol described by Shivanna et al., (1996) at a constant temperature of 22 ± 1°C.

Preparation of different concentrations of $KBrO_3$

$KBrO_3$ (Purchased from HiMedia, Catalog no- GRM1092 , MW- 167) stock solution of 100mM was prepared, and subsequent dilutions were made to make various concentrations ranging from 5mM to 100mM, each containing 3% yeast.

Synchronization of age

To ensure consistency in age, egg collection followed a modified method outlined by Nusslein-Volhard, (1977). Approximately 200-250 well-fed 7-day-old adults were transferred to a bottle and were starved for 4 hours. At the end of the starvation period, the flies were transferred to fresh media bottles supplemented with yeast and allowed to lay the egg for 4 hours. The first batch of eggs was discarded, and the second batch of eggs was used for the experiments. 100 eggs were introduced in each of the fresh culture bottles (4 replicates each with 100 eggs) and left in the bottles to continue to hatch. After a 24-hour incubation period, newly hatched larvae were treated using varying concentrations of $KBrO_3$ with 3% yeast.

Productivity and Developmental Time (DT)

There were no observed effects at 5mM and 10mM. However, complete mortality was recorded at and above 40mM. Hence, the four concentrations (15, 20, 25, and 30mM) were finalized. For each concentration (15mM to 30mM), four replicates were maintained consisting of 100 eggs on culture bottles. A negative control was maintained in the same manner as treatments, with the addition of only 3% yeast. Daily records were maintained throughout the incubation period for each treatment group, documenting the numbers of formed pupae and hatched adults until the completion of development in all the flies. The developmental time

(DT) (Egg to pupa and Egg to adult) was calculated according to the following formula (Jovanovic et al., 2018).

$$DT = \sum_{d=1}^{X} \mathrm{nd} * \mathrm{d/nt}$$

Where 'nd' is the number of pupae /emerging flies in'd' days after the eggs were laid, and 'nt' is the total number of individuals (pupae/emerged flies) at the end of experimental time.

Morphometric assay

Morphometry of 12 randomly picked larvae which were treated with different concentrations of $KBrO_3$ along with Control for 48hrs, 72hrs, 96hrs, and pupae was done. The length and width of the larval body have been measured according to the procedure described by Roberts, (1998). Morphology of treated flies was also observed and recorded.

Statistical Analysis

The collected data was analyzed by using Graph Pad Prism software. A one-way analysis of variance (ANOVA) was conducted, and in cases where a significant difference was identified in the ANOVA, Tukey's multiple comparison tests were executed.

Results

Developmental time (DT)

The results in Table 1 revealed the time necessary for pupariation and emergence at different concentrations (15mM, 20mM, 25mM, and 30mM) compared to the control group. There was a significant difference in developmental time (DT) from egg to pupae ($F = 41.32$, $df = 4$, $p < 0.0001$) and from egg to adults ($F = 8.52$, $df = 4$, $p = 0.001$) among the different concentrations of $KBrO_3$. The mortality was slower at 20mM therefore the mean DT (egg to adult) for 20mM was longer than that of the control and other concentrations (Fig. 1).

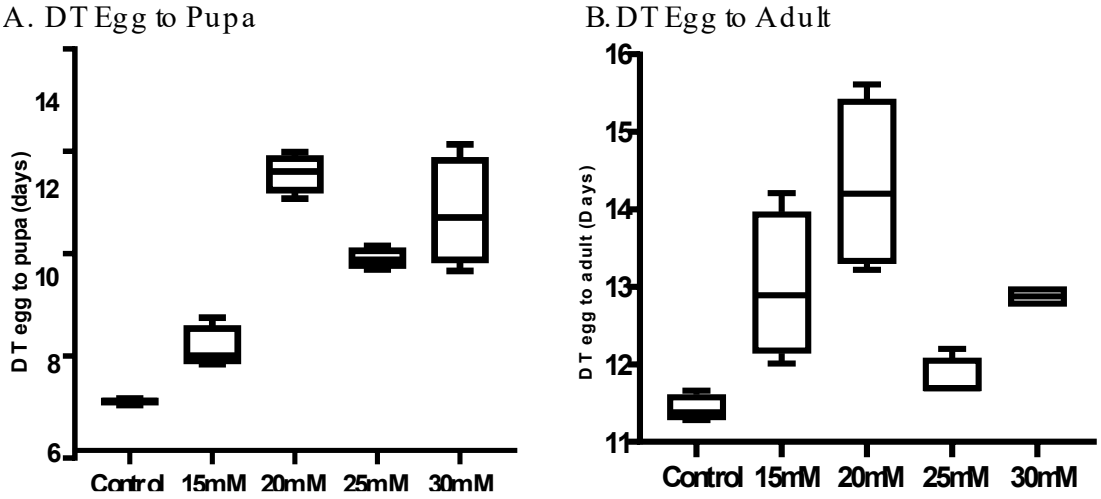

Fig. 1. The developmental time (DT) of *Drosophila melanogaster* exposed to varying concentrations of $KBrO_3$ shows a concentration-dependent delay.

Table 1. Developmental Time of *D. melanogaster* in days (Mean± SE) with respect to different concentrations of $KBrO_3$. (*) Indicates mortality in larvae and adults.

S.No	Concentrations	Developmental time of egg to pupae	Developmental time of egg to adult
1	Control	7.12±0.03	11.4±0.08
2	15mM	8.33±0.24	13.00±0.47
3	20mM	12.33±0.21*	15.25±0.55*
4	25mM	10.59±0.11***	13.82±0.05***
5	30mM	10.91±0.37***	13.81±0.56***

Table 2. Showing viability and productivity of *D. melanogaster* treated with different concentrations of $KBrO_3$.

Sl. No	Concentrations	No. of Eggs	No. of pupae	Total no. of adults	Viability of egg to pupae (%)	Viability of Pupae to adult (%)
1	Control	400	365	345	91.25	94.52
2	15mM	400	323	246	80.75	76.16
3	20mM	400	207	132	51.75	63.76
4	25mM	400	104	58	26	55.76
5	30mM	400	86	36	21.5	41.86

Productivity Assay

The DT and productivity assay data in Table 1 and 2 respectively indicate that mean productivity (egg to pupae and egg to adult) and viability were significantly affected by treatments ranging from 15mM to 30mM compared to the control group (Fig. 2). Significant differences were observed among groups for pupal ($F = 91.39$, $df = 4$, $p < 0.0001$) and adult productivity ($F = 199.1$, $df = 4$, $p < 0.0001$). A multiple comparison test showed no significant difference in pupal productivity between 15mM and control groups ($p = 0.183$), but adult productivity differed significantly ($p < 0.0001$). Productivity of both pupae and adults was significantly different for 20mM and 15mM ($p < 0.0001$). The 15mM group exhibited the highest pupal viability (80%), while the 30mM group showed the lowest viability (21%). The 20mM group demonstrated about 51% viability. Mortality was high before pupation, particularly at the second and third instar stages, and continued during the pupal stage, with difficulties primarily at tail eclosion, resulting in many pupae failed to emerge as an adults (Fig. 5 a-e).

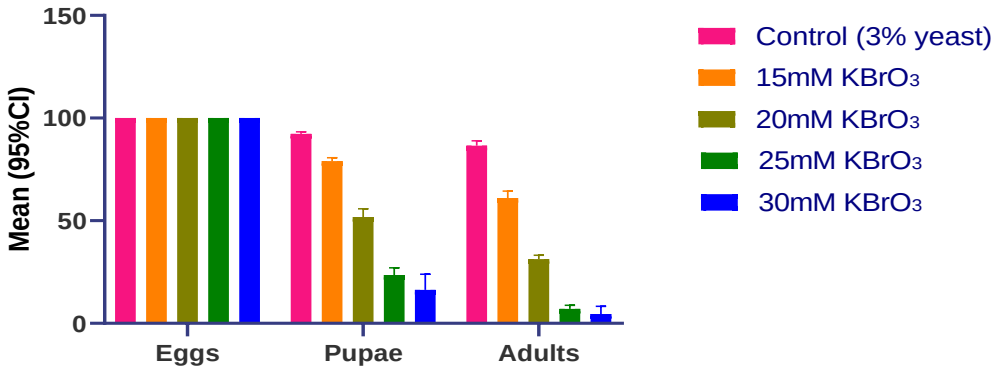

Fig.2. The productivity of pupae and adults in *Drosophila melanogaster* treated with various concentrations of $KBrO_3$ (Mean ± SE) showed a statistically significant difference ($F = 253.1$, $df = 4$, $p < 0.0001$).

Morphometric Assay

Larval length and width were measured at 48, 72, and 96 hours, and at the pupal stage, size was determined by multiplying these measurements (Table 3). Growth rates differed significantly among larvae exposed to varying concentrations (15mM to 30mM) of $KBrO_3$. There were significant differences in length ($F = 359.6$, $df = 4$, $p < 0.0001$) and width ($F = 124.3$, $df = 4$, $p < 0.0001$) between treated and control groups (Fig. 3). Treated larvae developed more slowly, were smaller than untreated larvae, indicating a negative correlation between developmental time and body size ($r = -0.80$, $p = 0.048$). This effect was continued in adult's size especially at concentrations from 20mM to 30mM (Fig. 4).

Table 3. Larval and pupal morphometry in terms of Size (Length× Width) in mm for each 24 hours of time intervals after treatment with different concentrations of $KBrO_3$ in *D. melanogaster* (Mean±SE).

Hours	Control	15mM	20mM	25mM	30mM
48 hours	0.59±0.01	0.52±0.03	0.35±0.01	0.23±0.01	0.16±0.01
72 hours	1.38±0.05	0.75±0.04	0.46±0.01	0.31±0.02	0.22±0.03
98 hours	2.33±0.08	1.59±0.05	0.76±0.03	0.52±0.02	0.28±0.02
Pupae	2.50±0.06	1.87±0.05	1.77±0.05	1.67±0.05	1.11±0.06

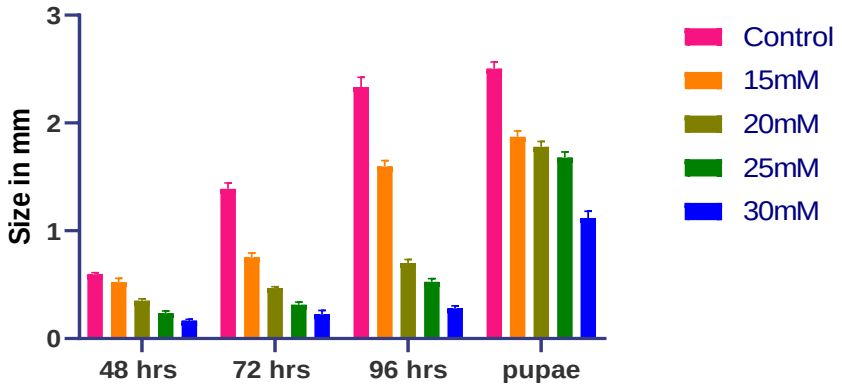

Fig.3. A bar graph illustrating morphometric changes in size at different concentrations of $KBrO_3$ showed a significant difference ($F = 502.5$, $df = 4$, $p < 0.0001$).

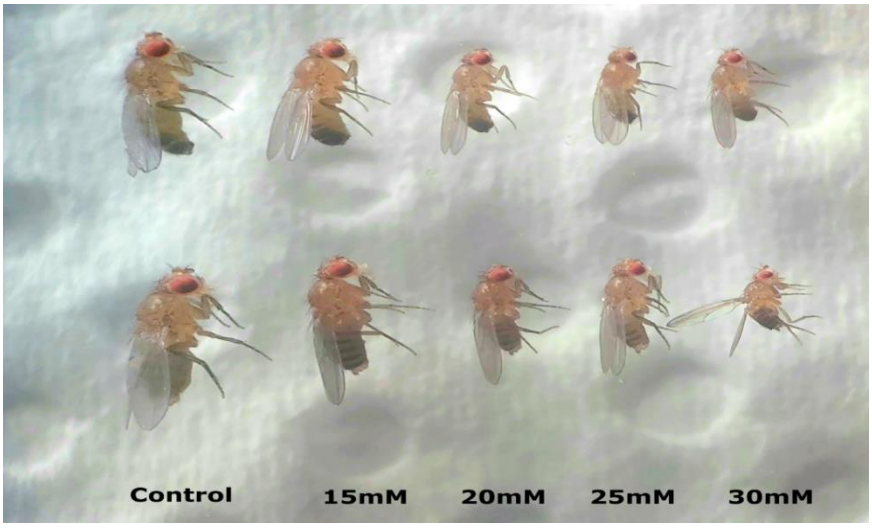

Fig.4. Concentration-dependent size variation in *Drosophila melanogaster* exposed to $KBrO_3$.

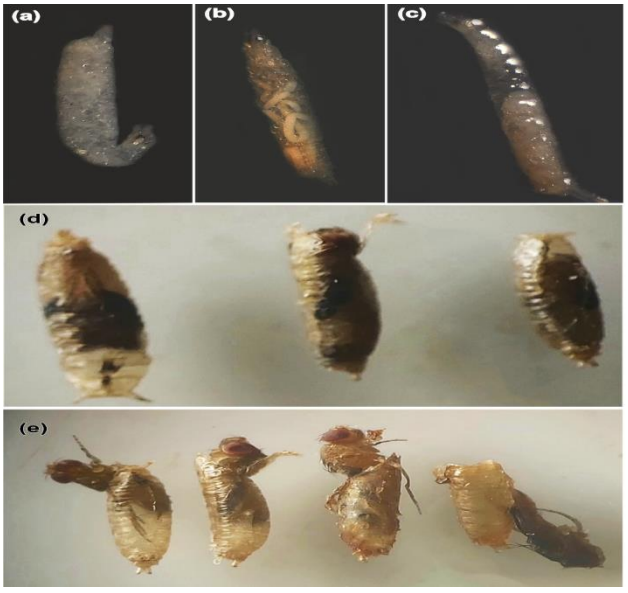

Fig.5. Effects of $KBrO_3$ on larvae and pupae of *Drosophila melanogaster*, (a-c) show deterioration of internal organs and fat bodies in larvae and pupae, (d) depicts abnormal pupal morphology with failure to emerge, while (e) shows pupae struggling during eclosion, resulting in mortality.

Aberrant phenotype

During the screening of flies that emerged from various concentrations of $KBrO_3$, several aberrant phenotypes were found exclusively in the treated groups, particularly in the 20mM population (Fig. 6). Some flies displayed abdominal deformities (Fig. 6, a), forming a "zebra body" (Fig. 6, b-c) and defective tarsals (Fig. 6, l). Most abnormalities occurred in the wings (Fig. 6, d-i) and eyes (Fig. 6, j) including uneven wings (Fig. 6, e), bubbled wings (Fig. 6, f), loss of wings (Fig. 6, g), unexpanded wings (Fig. 6, h), crumbled wings (Fig. 6, i), and sepia eye pigmentation (Fig. 6, j). Occasionally, mass development was observed on the ventral side of both male and female flies (Fig. 6, k-l).

Fig. 6. Aberrant phenotypes in *Drosophila melanogaster*, (a) show abdominal segmentation deformities, (b, c) Zebra body, (d-i) depict wing abnormalities, (j) shows a sepia eye, and (k, l) display mass development on the ventral part of the abdomen and defective tarsals.

Discussion

$KBrO_3$ has been utilized as a food additive in the manufacturing processes of beer, cheese, bread, and fish paste products. Additionally, it is incorporated into pharmaceuticals and generated as an oxyhalide by-product during the process of water ozonation (IARC, 1986; Kurokawa et al., 1983; Chuhan and Jain, 2016; Jahan et al., 2020). Umemaru et al., (1995) reported that $KBrO_3$ causes oxidative stress in cells, which may be the basis for energy partitioning and alteration in gene expression. It has been shown that $KBrO_3$ is responsible for reactive oxygen species and causes carcinogenicity (Ishidate et al., 1981; Kurokawa et al., 1987). Genotoxicity is one of the possible causes of aberrant phenotype. There have been many reports suggested that $KBrO_3$ exposure increases the 8-OHdG (8-hydroxydeoxy guanosine) *in vivo* and *in vitro* studies which causes mutations (Ballmaier and Epe, 1995; Umemura et al., 1995).

The genetic connection between body size and developmental time in an organism is relevant. The larval period is considered a potentially crucial component of fitness (Robertson, 1960). In *D. melanogaster*, as in all animals, the phenotype is structured by the growth period (Mirth and Shingleton, 2012). Previous studies reported that Methylparaben (MP), Propylparaben (PP), Ethylparaben (EP), and Bisphenol A(BPA) act as Endocrine disrupting chemicals (EDCs) which are known estrogen agonists that can activate gene expression of estrogen receptors and affect the growth and development of *D. melanogaster* through distraction of signaling pathways (Liu et al., 2014; Weiner et al., 2014). Exposure to different types of nanoparticles was evident to variations observed in the abdominal segments, wings, thorax, eyes, and pigmentation in *D. melanogaster*. Among these, some aberrant phenotypes cause mutation and are passed down to the next generation whereas some of them cause somatic mutations and cause developmental abnormalities (Demir et al., 2011; Vecchio et al., 2012; Vales et al., 2013; Jovanovic et al., 2018; Pappus and Mishra, 2018).

A study conducted on teratogenicity of $KBrO_3$ using *D. melanogaster* revealed that life-history parameters (larval, pupal and adult mortality) were significantly affected along with delayed developmental duration, reduced size of pupae and adult along with phenotypic deformities in flies on exposure to $KBrO_3$ in concentration dependent manner may be the result of oxidative stress. The results of this study are consistent with numerous other research findings involving the treatment of *Drosophila melanogaster* with various nanoparticles, endocrine-disrupting chemicals (EDCs), pollutants, and heavy metals (Demir et al., 2011; Vecchio et al., 2012; Vales et al., 2013; Jovanovic et al., 2018; Pappus and Mishra, 2018). In *Drosophila*, the key developmental phases involve molting during the larval stage and the transformation from larva to pupa to adult, primarily driven by pulses of the 20-hydroxyecdysone (20E) hormone (Jindra et al., 2013; Mitchell et al., 2013; Niwa and Niwa, 2014; Yun mu et al., 2021). The juvenile hormone (JH) signal suppressed 20E production in the Prothoracic gland (PG) via Kr-h1, while the 20E signal inhibited JH synthesis in the Corpora allata (CA) through EcR/USP (Jia et al., 2017; Liu et al., 2018; Li et al., 2019). During the larval stages, elevated levels of JH triggered the activation of the 20E pulse signal, which initiated the molting process in larvae. However, in third instar larval stage, reduced JH levels triggered the 20E pulse, leading to begin the metamorphosis process in the flies (Di Cara and King-Jones, 2013). The previous study indicated that $KBrO_3$ induces oxidative stress, potentially contributing to the development of neoplasia in endocrine glands (Stasiak et al., 2009). Based on this information, it can be hypothesized that $KBrO_3$ cause mutations and may inhibit the production of both 20E and JH in larvae. This inhibition could delay the activation of the 20E pulse signal, thereby postponing the timing of pupation and eclosion.
The present study demonstrated that the prenatal treatment of $KBrO_3$ displayed clear teratogenic potentiality with various deformities in the fruit fly indicating potential risks to human health if $KBrO_3$ is used in any application.

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Conflict of interest

The authors declared no conflict of interest.

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