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Protective Effect of Pomegranate Juice on Potassium Bromate Induced Lifespan Reduction in *Drosophila melanogaster*

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Abstract: Lifespan is influenced by a complex interplay of genetic, environmental, and lifestyle factors, including susceptibility to diseases and survival mechanisms. Early nutritional interventions, such as, the use of food additives, herbal remedies, and mineral supplements, play a pivotal role in shaping growth, survival, and aging outcomes. This study investigated the ameliorative effect of Pomegranate Juice (PJ) a nutrient-rich "Pathya" fruit symbolizing life, health, and longevity against Potassium Bromate ($KBrO_3$) induced toxicity on lifespan using *Drosophila melanogaster* as a model organism. $KBrO_3$, a widely used as food additive and a major water disinfection by-product, was examined for its impact on lifespan. The experiment consisted of four groups: Group I (Control), Group II (20 mM $KBrO_3$), Group III (Pomegranate Juice), and Group IV (combination of PJ + 20 mM $KBrO_3$), with each group comprising 3 replicates of twenty flies. Results revealed a significant difference in lifespan in $KBrO_3$ treated flies, with reduction of 73% in males and 68% in females as compared to the control. Remarkably, administration of PJ improved longevity by 55% in males and 51% in females exposed to $KBrO_3$, highlighting its protective effect. These findings underscore the potential of PJ in promoting longevity, and improving the quality of life. The observed toxicity of $KBrO_3$ on lifespan suggests its avoidance as a food additive and raises concerns about potential risks to human health if $KBrO_3$ is used in any application.

Keywords: Nutritional interventions, Potassium Bromate, Pomegranate juice, *Drosophila melanogaster* and Longevity

Introduction

In recent decades, research has provided critical insights into the biological and molecular mechanisms of ageing, a universal process marked by the progressive, time-dependent decline in physiological functions that ultimately leads to death (Kaeberlein, 2013). Lifespan is influenced by a complex interplay of genetic,

environmental, and lifestyle factors, including susceptibility to diseases and survival rates (Hjelmborg et al., 2006; Chmielewski et al., 2016). Notably, early-life conditions, such as maternal and juvenile nutrition, have profound effects on ageing patterns and longevity, as they shape developmental and metabolic pathways critical for long-term health (Metcalfe and Monaghan, 2003; Gungormuş et al., 2010; Barnes and Ozanne, 2011). Diet has emerged as a particularly influential factor, with evidence linking its molecular effects to lifespan modulation (McKay and Mathers, 2011). Studies on rodents demonstrated, how early nutritional interventions, including food additives, herbal remedies, and mineral supplements, can influence growth, survival, and ageing outcomes (Sayer and Cooper, 2002). These findings highlight the pivotal role of diet in lifespan modulation, underscoring its potential as a tool for enhancing health and promoting longevity.

Building on the understanding of diet impact on health and lifespan, the use of food additives has become a subject of growing concern. Food additives, synthetic substances introduced to enhance food appearance, taste, and preservation, are widely used to attract consumers (Gungormuş and Kılıç, 2012). However, over the past two decades, increasing evidence has linked certain additives to adverse health effects, including endocrine disruption (Trasande et al., 2018). One notable example is Potassium Bromate ($KBrO_3$), a compound commonly employed in the production of bread, cheese, beer, and fish paste products. Beyond food, Potassium Bromate is also used in the pharmaceutical and cosmetics industries, including cold-wave hair treatments (Kurokawa et al., 1983; IARC, 1986; Chuhan and Jain, 2016). Despite its utility, $KBrO_3$ has been identified as a chemical of concern due to its potential health risks, including its byproduct formation in ozone-treated water (Jahan et al., 2020). This has led to its prohibition as a food additive in several countries, including India in 2016 (Chauhan and Jain, 2016; FSSAI, 2018). Nevertheless, its illicit use persists in some food industries, posing ongoing public health risks (Grace, 2016; Chavan et al., 2019; Bello and Sani, 2023).

In contrast, traditional health systems like Ayurveda promote the natural approaches to enhancing health and longevity. Through practices such as Rasayana, which emphasize herbal supplements, balanced diets, and rejuvenative regimens, these systems aim to delay ageing and promote holistic well-being (Udapa, 2004). This highlights the importance of adopting safer dietary practices that address modern health concerns while drawing on traditional wisdom to promote longevity. Traditional health systems, such as Ayurveda, emphasize natural approaches to promoting longevity and well-being through dietary and herbal interventions. Among the many Rasayanas identified in Ayurveda, Pomegranate (*Punicagranatum*) holds a prominent place (Sharma, 2004; Udapa, 2004; Balasubramani et al., 2011). Ayurvedic texts describe Pomegranate as a "wholesome" (Pathya) fruit, valued for its ability to support tissue regeneration, promote strength, and enhance overall vitality (Chunekar, 2004). Similarly, other traditional systems like Unani and Traditional

Chinese Medicine regard Pomegranate as a powerful symbol of life, health, longevity, fertility, intellect, and spirituality (Mahdihassan, 1984). This shared recognition across diverse medical traditions highlights potentials of Pomegranate as it naturally fostering health and extending lifespan.

Consideration on the exploration of diet, natural remedies, and their roles in promoting health and longevity, modern research has increasingly turned to model organisms to study the molecular mechanisms of ageing. Ageing pathways are remarkably conserved across species, including *Saccharomyces cerevisiae*, *Caenorhabditiselegans*, *Drosophila melanogaster*, and rodents (Iliadi et al., 2012; Johnson et al., 2013; Lee et al., 2016). Among these, *D. melanogaster* has gained prominence as a key model organism due to its genetic similarities with humans, sharing approximately 60% genome homology (Adams et al., 2000). This makes *Drosophila* an invaluable tool for investigating the biological impacts of dietary factors, toxicants, and genetic modifications on ageing and health. Using *Drosophila*, researchers can assess critical biomarkers such as stage-specific lethality and lifespan changes, enabling the evaluation of interventions ranging from food additives to herbal supplements (Carey et al., 2006; Olcott et al., 2010; Landis et al., 2020). By integrating toxicological insights with an understanding of dietary and metabolic influences, studies on *Drosophila* contribute significantly to uncovering the factors that shape ageing and longevity.

Exploring the effects of harmful additives alongside holistic interventions, such as Rasayana, provides valuable perceptions into strategies for promoting health and longevity. While Potassium Bromate ($KBrO_3$) is well-documented for its carcinogenic effects in rodents, research on its toxic impact on *Drosophila melanogaster* and the potential protective benefits of Pomegranate juice (PJ) remains limited. This study aims to address this gap by investigating the effect of $KBrO_3$ on *D. melanogaster* and evaluating whether PJ can mitigate its toxicity and enhance longevity. The findings provide a comprehensive perspective on the interplay between harmful exposures and natural remedies in shaping life-history traits.

Materials and Methods

Maintenance of stock culture

Drosophila melanogaster (Oregon K) were sourced from the *Drosophila* Stock Centre at the Department of Zoology, University of Mysore. These stocks were cultured using Wheat cream agar medium, following the method outlined by Shivanna et al., (1996). The cultures were then raised and kept at a consistent temperature of 22±1°C.

Standardization of $KBrO_3$ concentration

Data of previous studies were used to quantify the egg to pupae lethality due to exposure of different concentrations of $KBrO_3$ (Aiwale and Shivanna, 2024). LC_{50} can be calculated for the egg-to-pupae and pupae-to-adult fly transition (Nanda and Firdaus, 2022; Kumari, P. et al., 2023). The LC_{50} for the Egg to Pupae transition was calculated using the Probit assay, while the EC_{50} for developmental time from Egg to Pupae was determined using the AAT Bioquest online platform. To study the ameliorative effect of Pomegranate juice on longevity, a 20 mM concentration of $KBrO_3$ was used, based on the egg-to-pupae transition results.

Pomegranate Juice (PJ) preparation

The Pomegranate pulp (Aril) was manually crushed and squeezed using a muslin cloth, ensuring that only the pulp was utilized and seeds were excluded. The freshly extracted juice was subsequently filtered using Whatman filter paper No. 1 to remove any remaining particulates. The filtered juice was then used for the experiment.

Experimental groups

A developmental exposure method, as described by Kumari, P. et al., (2023), was employed to assess adult lifespan. This approach involved treating larvae and adults with specific experimental regimes and conditions to measure their longevity. The experiment was divided into four groups to evaluate the ameliorative effect of Pomegranate juice (PJ) on $KBrO_3$ induced toxicity affecting longevity. Group I: 1st instar larvae were treated with 3% yeast (prepared in distilled water) considered as control. Group II: 1st instar larvae treated with 20 mM $KBrO_3$ (Purchased from HiMedia, Catalog No. - GRM1092, MW- 167) + 3% yeast prepared in distilled water. Group III: Newly emerged flies of Group II (1 day old) were treated with Pomegranate Juice (PJ) + 3% yeast (prepared in PJ). Group IV: 1st instar larvae were treated with the mixture of PJ + 20 mM $KBrO_3$ + 3% yeast (prepared in PJ). The same respective diets were maintained for all newly emerged adults across all experimental groups until the death of all flies.

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 was discarded, and the second batch of eggs was used for the experiments. The eggs were introduced in fresh food bottles

and left in the bottles to continue to hatch. After a 24-hours incubation period, newly hatched larvae were treated using above said regimes.

Longevity assay

Newly eclosed male and female flies were separated into groups (3 replicates of 20 flies each per sex) and transferred to fresh vials containing the same diet used during their developmental stages (except for Group III). Flies were moved to a fresh medium every third day and any occurrences of mortality were recorded. This transfer process persisted until the entire fly population had perished, as indicated by Phom et al., (2014).

Statistical Analysis

To evaluate the lifespan of *Drosophila melanogaster* exposed to $KBrO_3$, survival curves were generated and statistical analysis was conducted using the Log-rank (Mantel-Cox) test in GraphPad Prism. LC_{50} and EC_{50} values were determined using the Probit assay and the AAT Bioquest online platform, respectively, for mortality and developmental time during the transition from egg to pupae.

Results

Ameliorative effect of Pomegranate Juice against $KBrO_3$ Toxicity

Using a developmental exposure method, stage-wise lethality and developmental time, specifically from Egg to Pupae, were used to determine the LC_{50} (Nanda and Firdaus, 2022; Kumari et al., 2023) and EC_{50}. The LC_{50} and EC_{50} for $KBrO_3$ was determined to be 20.22 mM based on larval lethality and 21.06 mM based on developmental time data respectively (Aiwale and Shivanna, 2024). Consequently, nearest value of LC_{50} (20 mM) was employed in subsequent experiments (Niveditha et al., 2017; Mishra et al., 2018; Krüger et al., 2021; Kumari, P. et al., 2023). To evaluate the protective effect of Pomegranate juice (PJ) against the detrimental impact of $KBrO_3$ on longevity, adult flies were reared and treated according to predefined groups and regimes throughout their lifespan.

Table 1. Protective effects of Pomegranate juice on lifespan modulation in *Drosophila melanogaster* exposed to Potassium Bromate.

Groups	No. of flies	Median lifespan (Day)		Maximum lifespan (Day)	
		Female	Male	Female	Male
Group I	60	47.5	43.5	75±2.4	62.1±3.1
Group II	60	15	11.5	21.3±1.5	16.8±1.3
Group III	60	31	26	38.4±0.9	36.3±0.7
Group IV	60	18.5	13	28.8±0.6	19.2±2.5

The present study showed significant difference between male (χ2- 161.3, df-3, *P*<0.0001) and female (χ2- 121.1, df-3, *P*<0.0001) lifespan (Fig. 1). In Group II, treated with $KBrO_3$ alone, the median lifespan decreased by 73% in males and 68% in females compared to the control. However, in the PJ-treated group (Group III), longevity increased by 55% in males and 51% in females compared to Group II (Table. 1). In Group IV, where flies were exposed to a combination of $KBrO_3$ and PJ, a partial amelioration effect of PJ was observed. While the survival pattern remained similar to Group II.

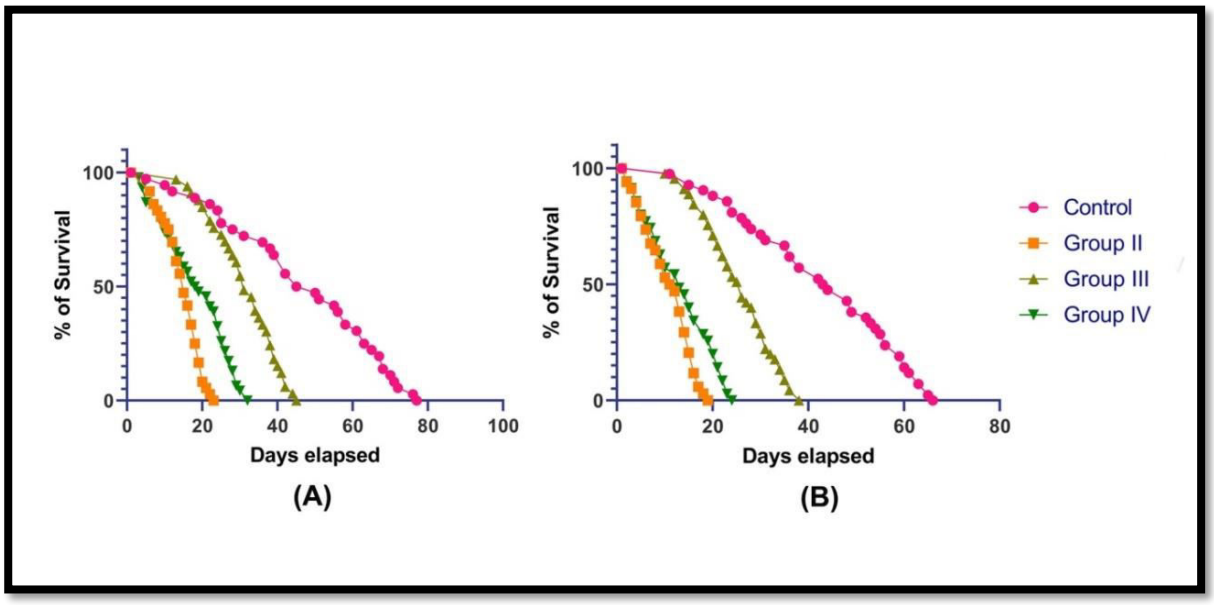

Figure. 1. Lifespan extension by Pomegranate juice (PJ) in *Drosophila melanogaster* (A) Female and (B) Male flies. PJ significantly (*P*< 0.0001) mitigated the toxic effect of $KBrO_3$ and extended the lifespan of treated flies.

Discussion

Several internal and external factors can influence the lifespan of *Drosophila melanogaster*, and in this study, we minimized the impact of these factors by maintaining constant environmental conditions. Photoperiod, known to affect egg laying, pupation, metabolic rate, and longevity (Lanciani et al., 1991; Qiu and Hardin, 1996; Sheeba et al., 2000), was controlled with a 12-hours light-dark cycle. Additionally, we used wheat cream agar as the standard medium to avoid variations in food that could impact longevity (David et al., 1975). To eliminate the influence of maternal age on offspring longevity (Sorensen and Loeschcke, 2002), males and females of the same age were used. The renewal of the medium every third day addressed the negative effect of metabolic waste on longevity (Bradley and Simmons, 1997). With these factors, the only variable in this experiment was the food regimens.

Our findings indicate that $KBrO_3$ significantly reduces the median lifespan of *D. melanogaster*, decreasing it by 65 to 75% compared to the untreated control group

(Group I). Pomegranate juice (PJ) was found to mitigate the toxic effects of $KBrO_3$(Group III) showing a threefold increase in longevity compared to the $KBrO_3$ only group (Group II). However, in Group IV, where flies were treated with both PJ and $KBrO_3$, PJ did not fully counteract the toxic effects on longevity, resulting in a lifespan reduction similar to that observed in Group II. These results suggest that while PJ can significantly improve longevity when administered alone, its protective effect was limited when combined with $KBrO_3$, highlighting the complexity of interactions between different treatments and the need for further investigation into the mechanisms involved.

It has been demonstrated across various species, ranging from yeast to mice and humans, that a key factor in aging is the accumulation of damage to genetic material (Moskalev et al., 2013; Pitt and Kaeberlein, 2015). The carcinogenic effects of $KBrO_3$, a substance used as a food additive, water disinfectant, as a neutralizer in permanent waving, and in pharmaceuticals, have been well documented from 1980 to 2023. However, research specifically examining the impact of $KBrO_3$ on lifespan in animals remains limited. $KBrO_3$ has been found to induce genetic damage, delay thedevelopmental time, cause oxidative stress, and inhibit the activity of antioxidant enzymes. These factors may play a key role in the reduction of lifespan observed with $KBrO_3$ exposure (Ahmad and Mahmood, 2012; 2016; Temel, 2023; Aiwale and Shivanna, 2024).

Previous research has highlighted the complexity of the relationship between developmental time and lifespan, with findings varying across species. While some studies reported a positive correlation, others found a negative correlation, and some observed no significant relationship at all (Mayer and Baker, 1984; Lee, W et al., 2013; Lee, Y et al., 2016). This suggests that the interaction between developmental duration and lifespan is influenced by species-specific factors. In line with this, a recent study by Aiwale and Shivanna, (2024) demonstrated that Potassium Bromate ($KBrO_3$) delayed developmental duration in a dose-dependent manner. Present study supporting the idea that lifespan and developmental time may negatively correlated, particularly under the influence of $KBrO_3$ in *D. melanogaster*.

The mitochondrial free radical theory of aging suggests that mitochondrial DNA is highly vulnerable to oxidative damage from reactive oxygen species (ROS), which accelerates aging (Moskalev et al., 2013). However, the relationship between ROS and lifespan is complex, as ROS can have both harmful and beneficial effects depending on the species and conditions (Shields et al., 2021). Studies have shown that a single oral dose of $KBrO_3$ induces oxidative stress in rats by increasing levels of methemoglobin, nitric oxide, and hydrogen peroxide, while disrupting antioxidant enzymes and reducing blood antioxidant capacity (Ahmad and Mahmood, 2012; 2016).

Activating the pentose phosphate pathway has shown neuroprotective benefits, supporting brain health and longevity by reducing oxidative damage (Tang, 2019). However, recent studies indicated that $KBrO_3$ inhibits this pathway and intracellular

antioxidant enzymes (Temel, 2023), potentially explaining the reduced lifespan observed in *Drosophila melanogaster* in the present study. Research suggests that $KBrO_3$ induced oxidative stress and cellular damage may indirectly reduce both lifespan and healthspan (Ahmad and Mahmood, 2012; 2016).

Pomegranate consists of approximately 80% juice and 20% seeds (Newman et al., 2007), with the juice containing high levels of polyphenols, including anthocyanins (387 mg/L), punicalagins (1561 mg/L), ellagic acids (121 mg/L), and hydrolyzable tannins (417 mg/L) (Seeram et al., 2006). These polyphenols, particularly anthocyanins, are known for their antioxidant properties, which helps to protect against oxidative stress, reduce the risk of chronic diseases, prevent their progression (Calín-Sánchez et al., 2013; Cano-Lamadrid et al., 2017) and increases the longevity in mice (Aires et al., 2012) and, *C. elegans* (Wilson et al., 2006). Pomegranate juice has been shown to reduce oxidative stress, inflammation, and infection rates in hemodialysis patients (Shema-Didi et al., 2012). Our findings align with previous studies demonstrating that Pomegranate juice supplementation extends longevity in *Drosophila melanogaster* (Balasubramani et al., 2014), *C. elegans* (Wilson et al., 2006; Zheng et al., 2019) and mice (Aires et al., 2012; Spindler et al., 2013). The antioxidant potential of Pomegranate juice likely plays a critical role in mitigating the free radical damage possibly induced by $KBrO_3$, a key factor in aging, thereby increasing lifespan.

Conclusion

Longevity and lifespan are fundamental aspects of biological research, encompassing both the duration and quality of life in all living organisms. While lifespan refers to the total duration of organism lives, from birth to death, longevity involves factors that contribute to the extension and enhancement of life, often focusing on resilience and health during that time. This study explored the effects of Potassium Bromate ($KBrO_3$), an oxidizing agent used in the food and cosmetic industries, and Pomegranate juice (PJ), known for its health benefits, on the lifespan of *Drosophila melanogaster*. The results demonstrated that $KBrO_3$ significantly reduced the lifespan of *D. melanogaster*, while PJ administration notably improved longevity, mitigated the harmful effect of $KBrO_3$, and extended the healthy lifespan of the treated flies. These findings emphasize the role of PJ in enhancing longevity by promoting resilience thereby improving both the quality and duration of life. Conversely, observed toxicity of $KBrO_3$ on lifespan suggests its avoidance as a food additive and raises concerns about 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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