

# Chromium picolinate enhances reproductive success and development in *Drosophila melanogaster* by reducing oxidative stress

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**ABSTRACT:** Chromium picolinate (Cr(Pico)<sub>3</sub>) is widely recognized for its ability to enhance insulin sensitivity. However, its broader effects, particularly on reproductive and developmental parameters, have not been extensively explored. The present study evaluated the impact of Cr(Pico)<sub>3</sub> supplementation on reproductive physiology and developmental outcomes in *Drosophila melanogaster*, with an emphasis on oxidative stress modulation. The study involved treating *Drosophila melanogaster* with four doses of Cr(Pico)<sub>3</sub> (5, 10, 15, and 20 µg/ml of diet) and monitoring key reproductive and developmental parameters, such as larval and pupal periods, pupation rates, adult fly emergence, and egg hatching. The results demonstrated that supplementation with Cr(Pico)<sub>3</sub> at 15 µg/ml significantly shortened larval and pupal periods by 8.0% and 6.4%, respectively ( $p < 0.001$ ), and improved the percentage of pupae and adult fly emergence by 7.8% and 7.4%, respectively. Additionally, Cr(Pico)<sub>3</sub> enhanced fecundity by 13.9% and fertility by 13.6% at 15 µg/ml, compared to the control group. Antioxidant enzyme activities, including catalase (CAT), superoxide dismutase (SOD), and glutathione-S-transferase (GST), were also significantly elevated in Cr(Pico)<sub>3</sub>-treated flies ( $p < 0.05$ ), suggesting a strong defence against oxidative stress. These results indicate that Cr(Pico)<sub>3</sub> can enhance reproductive success and developmental efficiency by promoting antioxidant defence mechanisms. Further studies are warranted to explore the potential of Cr(Pico)<sub>3</sub> in other model organisms, with broader implications for its use in addressing human oxidative stress-related reproductive dysfunction.

**KEYWORDS:** Chromium picolinate; Dietary supplement; oxidative stress; fertility; fecundity; antioxidant enzymes

## 1. INTRODUCTION

Chromium, an essential trace element, has long been recognized for its role in regulating metabolic processes, particularly those involving glucose, lipid, and protein metabolism. The biologically active form, trivalent chromium (Cr(III)), is a crucial cofactor in insulin signalling pathways and is widely used as a dietary supplement to improve glucose tolerance and overall metabolic health, especially in patients with type 2 diabetes and metabolic syndrome [1,2]. Among the various forms of Cr(III), chromium picolinate (Cr(Pico)<sub>3</sub>) has gathered significant attention due to its superior bioavailability and its capacity to enhance insulin sensitivity by facilitating more efficient uptake of chromium by tissues [3]. While the effects of Cr(Pico)<sub>3</sub> on metabolic health have been well-documented, its role in other physiological processes, such as reproduction and development, has received comparatively less attention.

Oxidative stress plays a significant role in disrupting reproductive and developmental processes [4]. In reproductive tissues, oxidative stress can impair oocyte maturation, sperm viability, and fertilization, ultimately leading to decreased fertility and reproductive success [5]. Reactive oxygen species (ROS) can damage cellular structures, proteins, lipids, and DNA, impairing cell function and viability [6]. Several studies have shown that antioxidants can help mitigate this damage, improving reproductive outcomes by restoring the balance between ROS and antioxidants [6]. Chromium picolinate, known for its antioxidant properties, has the potential to modulate oxidative stress in reproductive systems, thereby improving reproductive efficiency and developmental success.

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*Drosophila melanogaster* serves as an excellent model organism for studying oxidative stress and reproductive health, as it shares conserved metabolic and genetic pathways with higher organisms, including humans. Its short life cycle and large reproductive output make it ideal for high-throughput experiments that assess the effects of nutritional supplements on reproduction and development [7]. Previous studies in *Drosophila melanogaster* have demonstrated that nutritional and environmental factors can significantly influence reproductive success, making it a suitable model for investigating the role of Cr(Pico)<sub>3</sub> in these processes [8]. Furthermore, *Drosophila melanogaster* has been extensively used in oxidative stress studies due to its well-characterized antioxidant defence system, including key enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione-S-transferase (GST) [9].

Given the growing body of evidence linking oxidative stress to reproductive dysfunction, this study aims to evaluate the effects of Cr(Pico)<sub>3</sub> on reproduction and development in *Drosophila melanogaster*. By exploring Cr(Pico)<sub>3</sub>'s ability to modulate oxidative stress and improve reproductive health, this research could offer valuable insights into the broader applications of chromium supplements in mitigating oxidative stress-related reproductive issues in higher organisms, including humans.

## 2. RESULTS

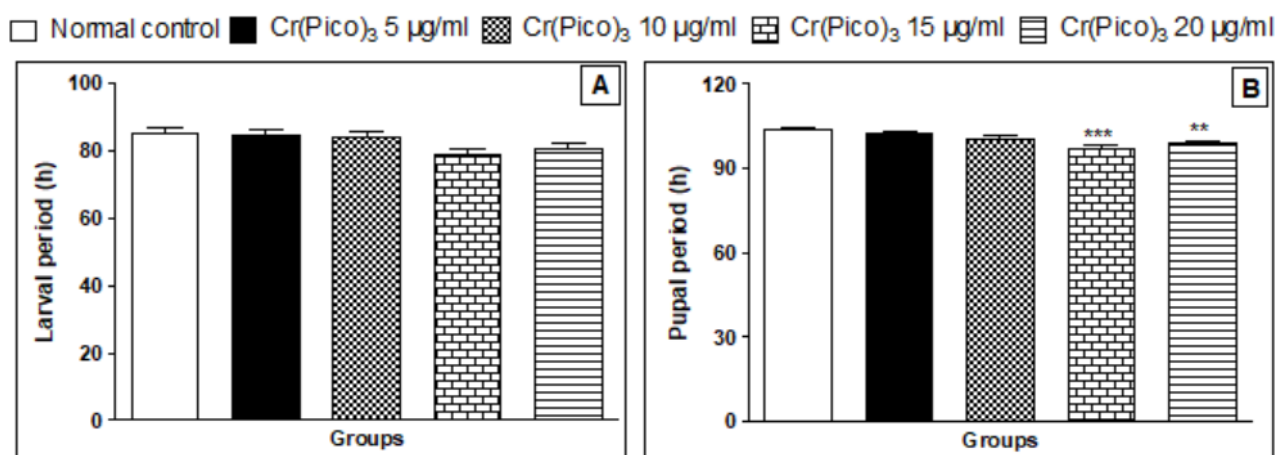
### 2.1. Effect of Cr(Pico)<sub>3</sub> on First generation (G1) flies

#### 2.1.1. Larval period

The normal control group exhibited a larval period of  $85.3 \pm 1.8$  hours. Supplementation with Cr(Pico)<sub>3</sub> at  $5 \mu\text{g/ml}$  resulted in a slight reduction to  $84.5 \pm 1.6$  hours, a decrease of 0.85%. At  $10 \mu\text{g/ml}$ , the larval period further decreased to  $82.6 \pm 1.9$  hours, representing a 3.09% reduction. A more pronounced reduction was observed at  $15 \mu\text{g/ml}$ , where the larval period significantly decreased to  $78.4 \pm 1.2$  hours, an 8.03% decrease. At the highest concentration of  $20 \mu\text{g/ml}$ , the larval period was  $79.7 \pm 1.5$  hours, showing a 6.47% reduction (Figure 1A).

#### 2.1.2. Pupal period

The pupal period in the normal control group was  $103.4 \pm 0.9$  hours. Treatment with Cr(Pico)<sub>3</sub> at  $5 \mu\text{g/ml}$  reduced the pupal period to  $102.5 \pm 0.5$  hours, a 0.87% decrease. At  $10 \mu\text{g/ml}$ , the pupal period further decreased to  $100.7 \pm 0.8$  hours, a reduction of 2.59%. A highly significant reduction was observed at  $15 \mu\text{g/ml}$ , with the pupal period decreasing to  $96.8 \pm 1.3$  hours, a 6.37% reduction ( $p < 0.001$ ). At  $20 \mu\text{g/ml}$ , the pupal period was  $99.1 \pm 0.9$  hours, showing a 4.18% reduction ( $p < 0.01$ ) (Figure 1B).



**Figure 1:** Effect of Cr(Pico)<sub>3</sub> on A] Larval period and B] Pupal period.

Values are expressed in mean  $\pm$  SEM, \*\* $p < 0.01$  and \*\*\* $p < 0.001$  compared with the normal control group.

#### 2.1.3. Percentage of larvae emergence

The percentage of larvae emergence in the normal control group was  $87.6 \pm 1.5\%$ . Supplementation with Cr(Pico)<sub>3</sub> at  $5 \mu\text{g/ml}$  resulted in a slight increase to  $87.7 \pm 1.4\%$ , an increase of 0.07%. At  $10 \mu\text{g/ml}$ , the larvae emergence rate further increased to  $88.3 \pm 1.5\%$ , representing a 0.73% increase. At  $15 \mu\text{g/ml}$ , the

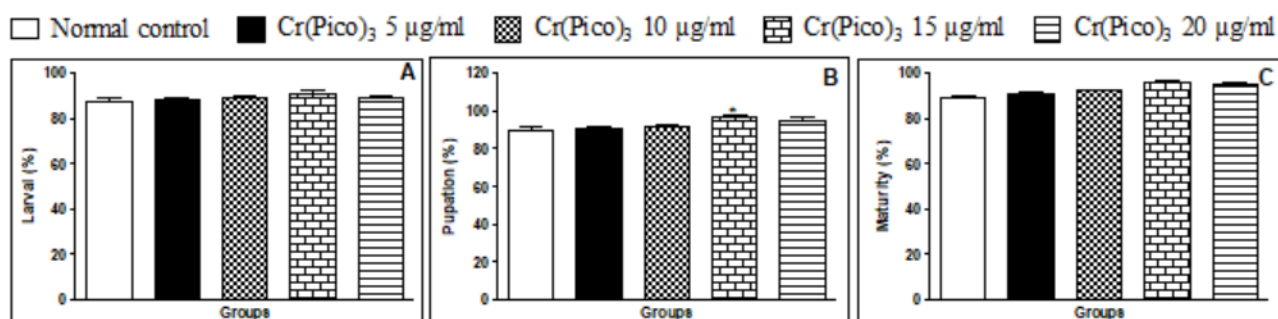
emergence rate increased more significantly to  $90.9 \pm 1.4\%$ , a 3.76% increase. At 20  $\mu\text{g/ml}$ , the percentage of larvae emergence was  $88.8 \pm 1.1\%$ , showing a 1.36% increase (Figure 2A).

#### 2.1.4. Percentage of pupae emergence

The percentage of pupae emergence in the normal control group was  $89.6 \pm 1.7\%$ . Cr(Pico)<sub>3</sub> supplementation at 5  $\mu\text{g/ml}$  increased the pupae emergence rate to  $91.0 \pm 0.8\%$ , a 1.56% increase. At 10  $\mu\text{g/ml}$ , the emergence rate further increased to  $91.4 \pm 1.3\%$ , representing a 2.01% increase. A significant increase was observed at 15  $\mu\text{g/ml}$ , with the pupae emergence rate reaching  $96.6 \pm 0.8\%$ , a 7.82% increase ( $p < 0.05$ ). At 20  $\mu\text{g/ml}$ , the pupae emergence rate was  $94.4 \pm 2.1\%$ , showing a 5.36% increase (Figure 2B).

#### 2.1.5. Percentage of adult fly emergence

In the normal control group, the percentage of adult fly emergence was  $89.2 \pm 2.4\%$ . Supplementation with Cr(Pico)<sub>3</sub> at 5  $\mu\text{g/ml}$  increased the emergence rate to  $90.8 \pm 1.9\%$ , a 1.79% increase. At 10  $\mu\text{g/ml}$ , the emergence rate further increased to  $92.2 \pm 1.9\%$ , representing a 3.36% increase. At 15  $\mu\text{g/ml}$ , the emergence rate increased more significantly to  $95.8 \pm 1.5\%$ , a 7.40% increase. At 20  $\mu\text{g/ml}$ , the percentage of adult fly emergence was  $95.4 \pm 1.5\%$ , showing a 6.95% increase (Figure 2C).



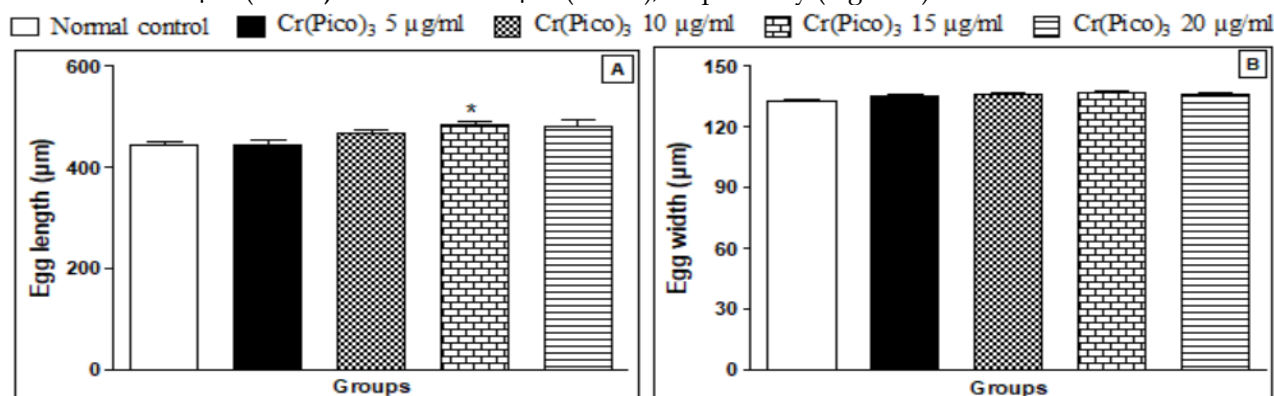
**Figure 2:** Effect of Cr(Pico)<sub>3</sub> on A] % of larval emergence, B] % of pupal emergence and C] % of adult flies emergence. Values are expressed in mean  $\pm$  SEM, \* $p < 0.05$  compared with the normal control group.

## 2.2. Effect of Cr(Pico)<sub>3</sub> on second generation (G2) flies

### 2.2.1. Length and Width of Eggs

The normal control group exhibited an average egg length of  $443.8 \pm 7.7 \mu\text{m}$ . Supplementation with Cr(Pico)<sub>3</sub> at 5  $\mu\text{g/ml}$  slightly increased the egg length to  $446.0 \pm 7.8 \mu\text{m}$ , representing a 0.50% increase. At 10  $\mu\text{g/ml}$ , the egg length further increased to  $467.6 \pm 8.3 \mu\text{m}$ , showing a 5.36% increase. A more pronounced increase was observed at 15  $\mu\text{g/ml}$ , with the egg length reaching  $483.4 \pm 9.9 \mu\text{m}$ , an 8.92% increase. At 20  $\mu\text{g/ml}$ , egg length was  $482.0 \pm 11.5 \mu\text{m}$  (+8.61%).

The normal control group had an average egg width of  $133.1 \pm 0.6 \mu\text{m}$ . Supplementation with Cr(Pico)<sub>3</sub> at 5  $\mu\text{g/ml}$  increased the egg width to  $135.2 \pm 1.0 \mu\text{m}$ , a 1.58% increase. At 10  $\mu\text{g/ml}$ , the egg width further increased to  $135.5 \pm 0.8 \mu\text{m}$ , showing a 1.80% increase. At 15  $\mu\text{g/ml}$  and 20  $\mu\text{g/ml}$ , the egg widths were  $136.6 \pm 1.1 \mu\text{m}$  (2.63%) and  $135.9 \pm 1.1 \mu\text{m}$  (2.10%), respectively (Figure 3).



**Figure 3:** Effect of Cr(Pico)<sub>3</sub> on A] Egg length and B] Egg width.

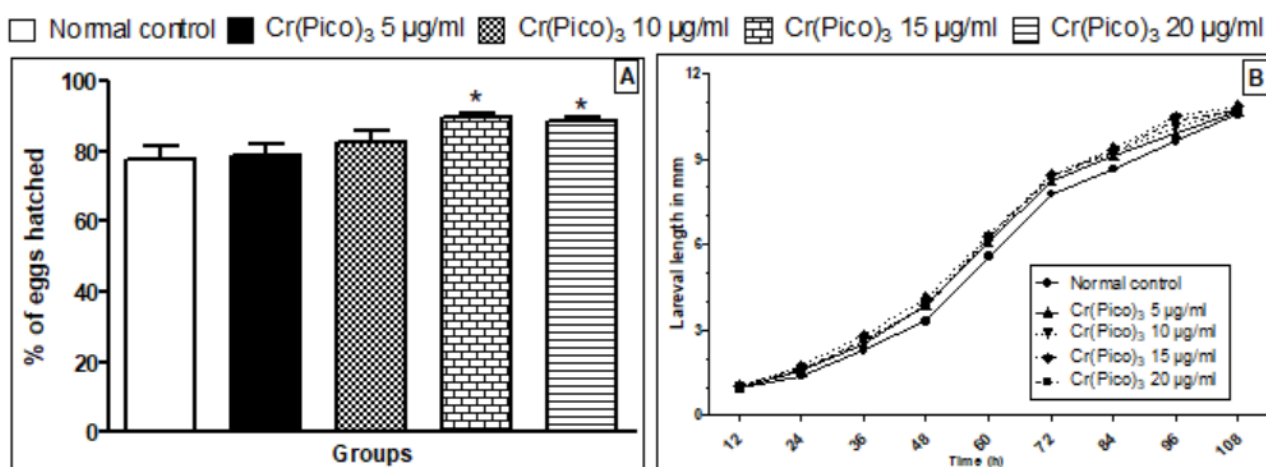
Values are expressed in mean  $\pm$  SEM, \* $p < 0.05$  and compared with the normal control group.

### 2.2.2. Percentage of eggs hatching

The normal control group exhibited a percentage of eggs hatch of  $77.7 \pm 3.6\%$ . Supplementation with  $\text{Cr}(\text{Pico})_3$  at  $5 \mu\text{g/ml}$  slightly increased the hatch rate to  $78.7 \pm 3.2\%$ , a 1.29% increase. At  $10 \mu\text{g/ml}$ , the hatch rate further increased to  $82.5 \pm 3.2\%$ , representing a 6.21% increase. Significant increases were observed at  $15 \mu\text{g/ml}$  and  $20 \mu\text{g/ml}$ , with hatch rates of  $89.8 \pm 1.1\%$  (+15.63%,  $p < 0.05$ ) and  $88.8 \pm 1.0\%$  (+14.36%,  $p < 0.05$ ), respectively (Figure 4A).

### 2.2.3. Mean larval length

The mean larval length was assessed in each group by randomly selecting ten larvae from each bottle at 12-hour intervals. The results of mean larval length revealed that supplemented with  $\text{Cr}(\text{Pico})_3$  had no statistically significant variation compared to the normal control (Figure 4B).



**Figure 4:** Effect of  $\text{Cr}(\text{Pico})_3$  on A) Egg hatching and B) Mean larval length.

Values are expressed in mean  $\pm$  SEM, \* $p < 0.05$  compared with normal control group.

### 2.2.4. Length and width of the pupae

The normal control group had a mean pupae length of  $3.4 \pm 0.3$  mm. Supplementation with  $\text{Cr}(\text{Pico})_3$  at concentrations of  $5 \mu\text{g/ml}$ ,  $10 \mu\text{g/ml}$ ,  $15 \mu\text{g/ml}$ , and  $20 \mu\text{g/ml}$  resulted in pupae lengths of  $3.6 \pm 0.5$  mm (+3.78%),  $3.6 \pm 0.4$  mm (+4.65%),  $3.6 \pm 0.4$  mm (+5.23%), and  $3.6 \pm 0.2$  mm (+4.94%), respectively.

The mean pupae width in the normal control group was  $1.1 \pm 0.5$  mm. Supplementation with  $\text{Cr}(\text{Pico})_3$  at concentrations of  $5 \mu\text{g/ml}$  and  $10 \mu\text{g/ml}$  increased the pupae width to  $1.1 \pm 0.5$  mm (+5.11%) and  $1.2 \pm 0.7$  mm (+9.85%), respectively. At  $15 \mu\text{g/ml}$  and  $20 \mu\text{g/ml}$ , the pupae widths were  $1.2 \pm 0.6$  mm (+11.94%,  $p < 0.01$ ) and  $1.2 \pm 0.6$  mm (+10.04%,  $p < 0.05$ ), respectively (Table 1).

**Table 1:** Effect of  $\text{Cr}(\text{Pico})_3$  on length and width of pupae and Adults fly thorax-width

Groups	Pupal length (mm)	Pupal width (mm)	Adults fly thorax-width ( $\mu\text{m}$ )	
			Male	Female
Normal control	$3.4 \pm 0.3$	$1.1 \pm 0.5$	$865.4 \pm 10.8$	$924.2 \pm 8.6$
$\text{Cr}(\text{Pico})_3$ 5 $\mu\text{g/ml}$	$3.6 \pm 0.5$	$1.1 \pm 0.5$	$874.2 \pm 6.7$	$954.8 \pm 5.5$
$\text{Cr}(\text{Pico})_3$ 10 $\mu\text{g/ml}$	$3.6 \pm 0.4$	$1.2 \pm 0.7$	$893.6 \pm 9.2$	$962.6 \pm 7.7$
$\text{Cr}(\text{Pico})_3$ 15 $\mu\text{g/ml}$	$3.6 \pm 0.4$	$1.2 \pm 0.6$	$932.4 \pm 5.5^*$	$1050.2 \pm 6.4^*$
$\text{Cr}(\text{Pico})_3$ 20 $\mu\text{g/ml}$	$3.6 \pm 0.2$	$1.2 \pm 0.6$	$901.2 \pm 9.6$	$986.6 \pm 9.4$

Values are expressed in mean  $\pm$  SEM, \* $p < 0.05$  compared with normal control

### 2.2.5. Adults fly thorax-width

For adult male flies, the normal control group had a thorax width of  $865.4 \pm 10.8 \mu\text{m}$ . Supplementation with  $\text{Cr}(\text{Pico})_3$  at  $5 \mu\text{g/ml}$  resulted in a slight increase to  $874.2 \pm 6.7 \mu\text{m}$  (+1.02%), whereas at  $10 \mu\text{g/ml}$ , the

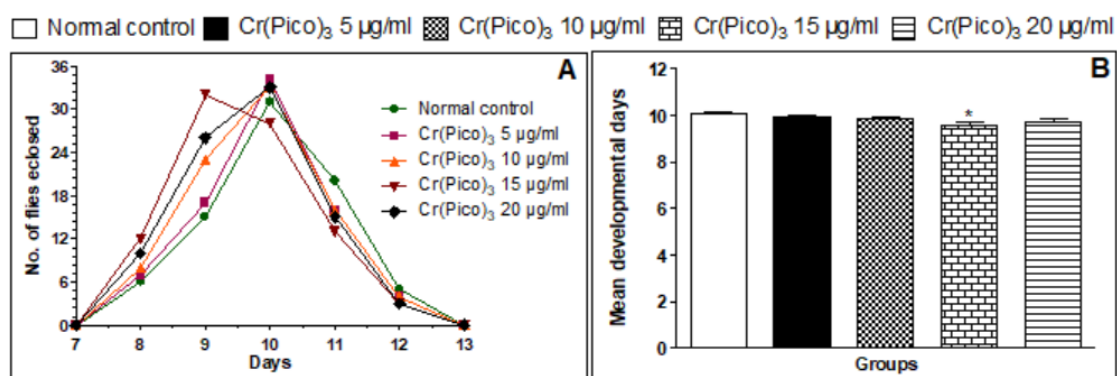
thorax width increased further to  $893.6 \pm 9.2 \mu\text{m}$  (+3.26%). A significant increase was observed at  $15 \mu\text{g/ml}$ , with a thorax width of  $932.4 \pm 5.5 \mu\text{m}$  (+7.74%,  $p < 0.05$ ). At  $20 \mu\text{g/ml}$ , it was  $901.2 \pm 9.6 \mu\text{m}$  (+4.14%) (Table 1).

For adult female flies, the normal control group had a thorax width of  $924.2 \pm 8.6 \mu\text{m}$ . Supplementation with  $\text{Cr(Pico)}_3$  at  $5 \mu\text{g/ml}$  and  $10 \mu\text{g/ml}$  increased the thorax width to  $954.8 \pm 5.5 \mu\text{m}$  (+3.31%) and  $962.6 \pm 7.7 \mu\text{m}$  (+4.16%), respectively. At  $15 \mu\text{g/ml}$ , a significant increase was observed with a thorax width of  $1050.2 \pm 6.4 \mu\text{m}$  (+13.63%,  $p < 0.05$ ). At  $20 \mu\text{g/ml}$ , the thorax width was  $986.6 \pm 9.4 \mu\text{m}$  (+6.75%) (Table 1).

#### 2.2.6. Dynamics of egg eclosion and mean developmental time

In all experimental groups, flies emerged between day 8 and day 12 of the study. Notably, the highest number of flies emerged on day 10 in all groups except for the  $\text{Cr(Pico)}_3$   $15 \mu\text{g/ml}$  supplemented group. In this group, the peak emergence occurred on day 9, indicating a slightly accelerated development compared to the other groups (Figure 5A).

The mean developmental time in the normal control group was  $10.0 \pm 0.1$  days. Supplementation with  $\text{Cr(Pico)}_3$  at  $5 \mu\text{g/ml}$ ,  $10 \mu\text{g/ml}$ , and  $20 \mu\text{g/ml}$  showed slight reductions in developmental time to  $9.9 \pm 0.1$  days (-1.29%),  $9.8 \pm 0.1$  days (-2.19%), and  $9.7 \pm 0.1$  days (-3.39%), respectively. At  $15 \mu\text{g/ml}$ , the developmental time was significantly reduced to  $9.6 \pm 0.1$  days (-4.58%,  $p < 0.05$ ). These findings suggest that  $\text{Cr(Pico)}_3$  can slightly accelerate developmental time, with the most pronounced effect observed at  $15 \mu\text{g/ml}$  (Figure 5B).



**Figure 5:** Effect of  $\text{Cr(Pico)}_3$  on A) Fly eclosion and B) Mean developmental time.

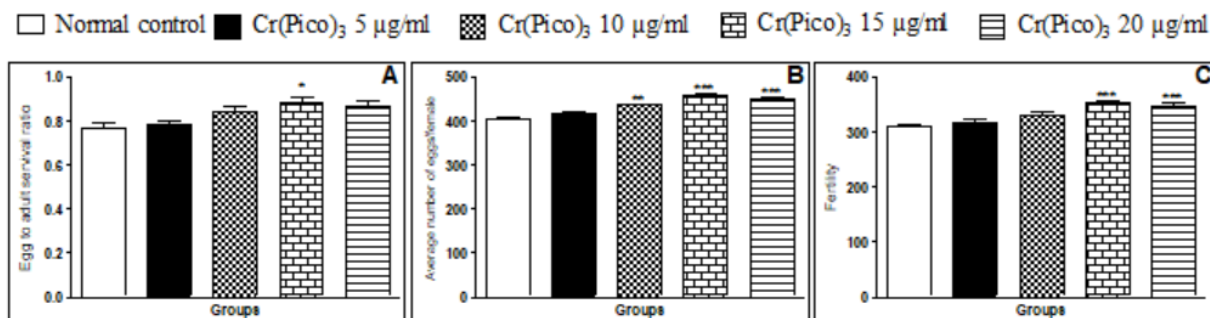
Values are expressed in mean  $\pm$  SEM, \* $p < 0.05$  compared with the normal control group.

#### 2.2.7. Egg to adult ratio

The egg-to-adult ratio in the normal control group was  $0.77 \pm 0.0$ . Supplementation with  $\text{Cr(Pico)}_3$  at  $5 \mu\text{g/ml}$  and  $10 \mu\text{g/ml}$  slightly increased the ratio to  $0.78 \pm 0.0$  (+1.30%) and  $0.84 \pm 0.0$  (+9.09%), respectively. A significant increase was observed at  $15 \mu\text{g/ml}$ , with the ratio rising to  $0.88 \pm 0.0$  (+14.29%,  $p < 0.05$ ). At  $20 \mu\text{g/ml}$ , the ratio was  $0.87 \pm 0.0$  (+12.99%). These results suggest that  $\text{Cr(Pico)}_3$  supplementation improves the egg-to-adult ratio, particularly at higher concentrations (Figure 6A).

#### 2.2.8. Fecundity and Fertility

The normal control group had an average fecundity of  $403.2 \pm 4.1$ . Supplementation with  $\text{Cr(Pico)}_3$  at  $5 \mu\text{g/ml}$  and  $10 \mu\text{g/ml}$  increased fecundity to  $418.6 \pm 2.4$  (+3.82%) and  $437.4 \pm 2.2$  (+8.47%,  $p < 0.01$ ), respectively. A significant increase was observed at  $15 \mu\text{g/ml}$ , with fecundity reaching  $459.3 \pm 3.0$  (+13.91%,  $p < 0.001$ ). At  $20 \mu\text{g/ml}$ , fecundity was  $451.7 \pm 2.7$  (+12.03%,  $p < 0.001$ ). These findings indicate that  $\text{Cr(Pico)}_3$  supplementation enhances fecundity, with the most substantial effect at  $15 \mu\text{g/ml}$  (Figure 6B).



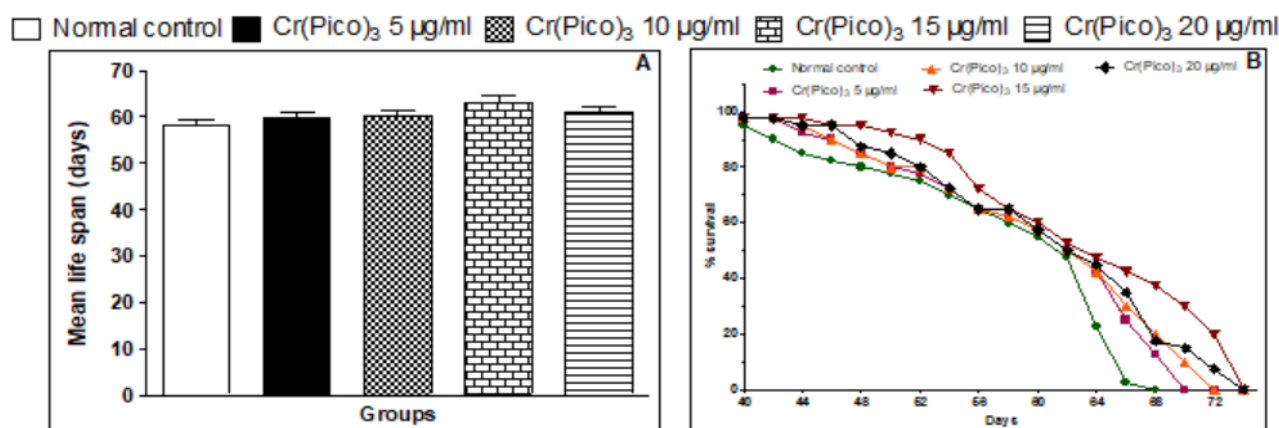
**Figure 6:** Effect of Cr(Pico)<sub>3</sub> on A] Egg to adult survival ratio, B] Fertility and C] Fecundity.

Values are expressed in mean ± SEM, \**p*<0.05, \*\**p*<0.01 and \*\*\**p*<0.001 compared with the normal control group.

Supplementation with Cr(Pico)<sub>3</sub> at 5 µg/ml resulted in a slight increase in fertility to 318.6 ± 5.5 (+2.37%). Significant increases were observed at 10 µg/ml, 15 µg/ml, and 20 µg/ml, with fertility rising to 331.4 ± 5.2 (+6.50%), 353.6 ± 4.7 (+13.64%, *p* < 0.001), and 348.4 ± 6.0 (+11.93%, *p* < 0.001), respectively. These results suggest a dose-dependent enhancement of fertility with Cr(Pico)<sub>3</sub> supplementation (Figure 6C).

### 2.2.9. Mean lifespan

The mean lifespan in the normal control group was 58.3 ± 1.4 days. Supplementation with Cr(Pico)<sub>3</sub> at 5 µg/ml and 10 µg/ml increased the mean lifespan to 59.6 ± 1.4 days (+2.30%) and 60.3 ± 1.4 days (+3.47%), respectively. At 15 µg/ml and 20 µg/ml, the mean lifespan increased to 63.5 ± 1.4 days (+9.02%, *p* < 0.05) and 61.4 ± 1.4 days (+5.26%), respectively. These findings suggest that Cr(Pico)<sub>3</sub> supplementation may contribute to a modest increase in lifespan, particularly at higher concentrations (Figure 7).



**Figure 7:** Effect of Cr(Pico)<sub>3</sub> on A] Mean lifespan, and B] Survival of flies.

Values are expressed in mean ± SEM.

### 2.2.10. Antioxidant enzymes

In the normal control group, the levels of antioxidant enzymes were recorded as follows: 28.6 ± 1.6 (CAT), 76.8 ± 1.3 (GST), and 71.7 ± 3.6 (SOD). Upon treatment with 5 µg/ml and 10 µg/ml of Cr(Pico)<sub>3</sub>, modest increases in the levels of antioxidant enzymes were observed across all parameters. Specifically, at 5 µg/ml, the levels of CAT, GST, and SOD were 30.7 ± 1.5, 77.3 ± 1.5, and 72.0 ± 2.12, respectively. At 10 µg/ml, these levels were further elevated to 31.33 ± 0.76 (CAT), 78.17 ± 1.77 (GST), and 76.2 ± 1.8 (SOD). At 15 µg/ml, the levels of CAT (35.2 ± 0.8, *p* < 0.01) and GST (84.3 ± 2.1, *p* < 0.05), as well as SOD (84.3 ± 3.5, *p* < 0.05), compared to the control group. At 20 µg/ml, the levels of CAT, GST, and SOD were 33.3 ± 0.9, 80.5 ± 2.0, and 79.5 ± 3.1, respectively (Table 2).

**Table 2:** Effect of Cr(Pico)<sub>3</sub> on levels of antioxidant enzymes

Groups	Catalase (U/mg protein)	GST (nmol CDNB conjugated/min/mg protein)	SOD (U/min/mg protein)
Normal control	28.7 ± 1.6	76.8 ± 1.3	71.7 ± 3.6
Cr(Pico) <sub>3</sub> 5 µg/ml	30.7 ± 1.5	77.3 ± 1.5	72.0 ± 2.1
Cr(Pico) <sub>3</sub> 10 µg/ml	31.3 ± 0.8	78.2 ± 1.8	76.2 ± 1.8
Cr(Pico) <sub>3</sub> 15 µg/ml	35.2 ± 0.8**	84.3 ± 2.1*	84.3 ± 3.5*
Cr(Pico) <sub>3</sub> 20 µg/ml	33.3 ± 0.9	80.5 ± 2.0	79.5 ± 3.1

Values are expressed in mean ± SEM, \*p<0.05 and \*\*p<0.01 compared with the normal control.

### 3. DISCUSSION

The findings of this study demonstrate that chromium picolinate (Cr(Pico)<sub>3</sub>) supplementation significantly improves reproduction and development in *Drosophila melanogaster*, likely through its effects on oxidative stress modulation and metabolic enhancement. The dose-dependent response observed in the study indicates that the most optimal benefits were seen at 15 µg/ml, where Cr(Pico)<sub>3</sub> reduced both larval and pupal periods and significantly enhanced pupal and adult emergence rates. These results suggest that Cr(Pico)<sub>3</sub> enhances energy metabolism and reduces oxidative damage, promoting more efficient developmental transitions, which are crucial for reproductive success [10,11].

The improvement in fecundity and fertility at the optimal concentration further supports the hypothesis that Cr(Pico)<sub>3</sub> plays a key role in enhancing reproductive health by modulating oxidative stress. The significant increase in antioxidant enzyme activities- CAT, SOD, and GST- observed in the treated groups highlights the ability of the compound to strengthen the defence against ROS [12]. These enzymes are essential in detoxifying ROS and preventing oxidative damage to cellular structures, particularly in sensitive reproductive tissues [13,14]. The upregulation of antioxidant enzymes in response to Cr(Pico)<sub>3</sub> supplementation suggests that the compound not only prevents oxidative damage but also actively enhances the body's ability to cope with oxidative stress [13,14].

The observed plateau in benefits at higher concentrations of Cr(Pico)<sub>3</sub> (20 µg/ml) indicates that excessive supplementation may not provide additional advantages and could even reduce efficacy. This dose-dependent response is consistent with previous studies, where optimal dosages of chromium supplements yielded the most beneficial outcomes, and excessive intake either resulted in no further improvement or a decline in effects [16]. This highlights the importance of careful dose determination when using Cr(Pico)<sub>3</sub> or other chromium supplements to avoid potential toxicity or diminished efficacy [17].

The broader implications of these findings suggest that Cr(Pico)<sub>3</sub> could be a valuable dietary supplement for improving reproductive outcomes in both model organisms and potentially humans, particularly in cases where oxidative stress is a major contributing factor to reproductive dysfunction. Given the conservation of metabolic and antioxidant pathways across species, the beneficial effects observed in *Drosophila* could translate to higher organisms, although further research is needed to confirm this. Studies in mammalian models, especially those involving reproductive health and oxidative stress, would be necessary to fully understand the therapeutic potential of Cr(Pico)<sub>3</sub> in clinical settings [18].

### 4. CONCLUSION

The Cr(Pico)<sub>3</sub> supplementation is beneficial for both reproductive health and development in *Drosophila melanogaster*. By enhancing antioxidant defences and supporting energy metabolism, Cr(Pico)<sub>3</sub> has the potential to mitigate the negative effects of oxidative stress on reproductive outcomes.

Future studies should focus on elucidating the molecular mechanisms through which Cr(Pico)<sub>3</sub> modulates oxidative stress and reproductive physiology. Additionally, exploring its effects in other model organisms, including mammals, will be crucial in determining its translatability and potential therapeutic applications in improving reproductive health and managing stress-related reproductive challenges.

## 5. MATERIALS AND METHODS

### 5.1. Chemicals

Chromium picolinate was obtained from Oceanic Laboratories (P) Ltd, Tarapur, India. Thiobarbituric acid was sourced from Hi-Media Laboratories Pvt. Ltd, Mumbai, India, and 5,5-dithiobis-2-nitrobenzoic acid from Sigma-Aldrich, St. Louis, USA. All other chemicals utilized were of analytical grade.

### 5.2. *Drosophila melanogaster*

The National Centre for Biological Sciences (TIFR), Bangalore, provided wild strains of *D. melanogaster* (W1118). The normal corn flour cream agar medium was used for their cultivation, and they were maintained at  $25 \pm 1^\circ\text{C}$  in a low-temperature incubator (REMI) with 65% humidity [13,14]. The procedure for the preparation of corn flour cream agar medium is given in our previous publication [14].

### 5.3. Exploring effects of $\text{Cr}(\text{Pico})_3$ on reproduction and development

Five distinct treatment groups were established as follows, Group I (Normal control) - basal diet without any additional supplementation; Group II ( $\text{Cr}(\text{Pico})_3$  5  $\mu\text{g}/\text{ml}$ ) - basal diet + 5  $\mu\text{g}/\text{ml}$  of  $\text{Cr}(\text{Pico})_3$ ; Group III ( $\text{Cr}(\text{Pico})_3$  10  $\mu\text{g}/\text{ml}$ ) - basal diet + 10  $\mu\text{g}/\text{ml}$  of  $\text{Cr}(\text{Pico})_3$ ; Group IV ( $\text{Cr}(\text{Pico})_3$  15  $\mu\text{g}/\text{ml}$ ) - basal diet + 15  $\mu\text{g}/\text{ml}$  of  $\text{Cr}(\text{Pico})_3$ ; Group V ( $\text{Cr}(\text{Pico})_3$  20  $\mu\text{g}/\text{ml}$ ) - basal diet + 20  $\mu\text{g}/\text{ml}$  of  $\text{Cr}(\text{Pico})_3$ .

The experiment employed five replicates for each treatment group to ensure statistical reliability. The effect of  $\text{Cr}(\text{Pico})_3$  was assessed over two generations of flies. Each vial contained five pairs of 6-day-old flies, which were allowed to lay eggs for 10 hours before removal. The vials were then maintained at  $25^\circ\text{C}$  until egg hatching and larval emergence, leading to the development of adult flies. In these first-generation flies (G1), parameters of pupation and maturity percentage (larval period, pupal period, percentage of larvae emergence, percentage of pupae emergence, and percentage of adult flies emergence) were evaluated.

Following this, five pairs of 6-day-old G1 flies were introduced into each vial containing the same concentration of  $\text{Cr}(\text{Pico})_3$  as mentioned above to develop second-generation flies (G2). The flies were allowed to lay eggs for 10 hours before being removed.

Subsequent parameters, including morphometric analysis of eggs, larvae, pupae, and adults, fitness (dynamics of egg exclusion, mean developmental time, egg to adult ratio), fertility, fecundity, and lifespan, were evaluated in the G2 flies according to the procedures outlined in our recent publications [14, 15].

Then, G2 flies were crushed in an ice-cold buffer, and levels of antioxidant enzymes such as catalase [16], GST [17, 18], and SOD [19,20] were estimated in the supernatant.

### 5.4. Statistical analysis

The data are presented as mean  $\pm$  SEM. Five replicates per group were used to ensure sufficient statistical power. One-way ANOVA followed by Tukey's post-hoc test was used to assess the statistical significance between treatment groups. At  $p < 0.05$ , differences were deemed statistically significant.

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