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مقاله پژوهشی

اثر وابسته به دوز عصاره آبی برگ تاتوره (Datura stramonium) بر بیان mRNA ژن‌های Eiger، Wingless، Glob1 و Dpp و میزان بقای مگس سرکه (Drosophila melanogaster)

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چکیده

مقدمه: *تاتوره (Datura stramonium) گیاهی دارویی و روان فعال حاوی آلکالوئیدهای تروپانی است که با وجود سمیت شناخته شده، اثرات مولکولی آن بر مسیرهای رشد و تنش به‌خوبی بررسی نشده است. این مطالعه با هدف بررسی اثرات عصاره آبی برگ تاتوره بر بیان ژن‌های تنظیمی کلیدی (Eiger، Wingless، Glob1 و Dpp) در مگس سرکه (Drosophila melanogaster) انجام شد.*

مواد و روش‌ها: *مگس‌های سرکه از نوع وحشی (سویه Harwich) به چهار گروه تقسیم شدند و با رژیم‌های غذایی حاوی ۰، ۲۰۰۰، ۴۰۰۰ یا ۸۰۰۰ میلی‌گرم بر کیلوگرم از عصاره تغذیه شدند. آزمون بقا طی یک دوره ۲۱ روزه انجام شد و سطوح بیان ژن با استفاده از روش PCR نیمه کمی و با به‌کارگیری نرم‌افزار ImageJ برای اندازه‌گیری شدت باندها تعیین شد.*

نتایج: *با افزایش غلظت عصاره، میزان بقا به‌طور تدریجی کاهش یافت، به‌گونه‌ای که در غلظت ۸۰۰۰ میلی‌گرم بر کیلوگرم، تا روز ۲۱ مرگ‌ومیر کامل مشاهده شد. تجزیه‌وتحلیل بیان ژن نشان داد که بیان Wingless ($P<۰/۰۵$) و Eiger ($P<۰/۰۱$) در غلظت‌های ۴۰۰۰ و ۸۰۰۰ میلی‌گرم بر کیلوگرم، به‌صورت معنی‌دار و وابسته به دوز افزایش یافت. همچنین، افزایش بیان Glob1 در هر دو غلظت مذکور مشاهده شد ($P<۰/۰۵$). در مقابل، بیان Dpp در غلظت‌های ۲۰۰۰ و ۸۰۰۰ میلی‌گرم بر کیلوگرم به‌طور معنی‌داری کاهش یافت ($P<۰/۰۱$).*

بحث: *مواجهه با تاتوره موجب اختلال در مسیرهای مولکولی کلیدی در مگس سرکه شده و با فعال‌سازی پاسخ‌های تنشی، هم‌زمان مسیرهای پیام‌رسانی مرتبط با رشد و تمایز سلولی را مهار می‌کند. تغییرات مولکولی مشاهده شده، همراه با کاهش میزان بقا، نشان‌دهنده سمیت قابل‌توجه و وابسته به دوز این گیاه بوده و بر اهمیت استفاده کنترل شده از آن تأکید دارد.*

واژه‌های کلیدی: تاتوره، Eiger، Wingless، Glob1، Dpp، مگس سرکه، سمیت، Gegemu.

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ارجاع: آرومارن آستین ایروغاما، اجیایلیا فستوس ایدیاکه، ایماهه نیسی انوسامودیانا. اثر وابسته به دوز عصاره آبی برگ تاتوره (Datura stramonium) بر بیان mRNA ژن‌های Eiger، Wingless، Glob1 و Dpp و میزان بقای مگس سرکه (Drosophila melanogaster). مجله دانش و تندرستی در علوم پایه پزشکی ۱۴۰۵؛۲۱(۱):۸۲-۷۳.

Introduction

Datura stramonium L., commonly known as thorn apple, Angel's trumpet, or jimson weed, is a medicinal plant belonging to the family Solanaceae. Although it is believed to have originated in the Americas, it is now widely distributed across tropical and temperate regions of the world, including Nigeria, India, Australia, China, and Ethiopia (1). The plant grows naturally along roadsides, pastures, wastelands, and cultivated lands, often without the need for cultivation (2). D. stramonium is rich in tropane alkaloids particularly atropine, hyoscyamine, and scopolamine which are responsible for both its medicinal and toxic properties (2). Traditionally, the plant has been used in the treatment of asthma, bronchitis, hemorrhoids, eczema, wounds, and other inflammatory conditions (2, 3). Modern studies have further demonstrated its antioxidant, antimicrobial, anti-inflammatory, antifungal, anticancer, and antiasthmatic activities, highlighting its therapeutic potential (1).

Despite these beneficial effects, D. stramonium is considered highly toxic due to its tropane alkaloid content. Poisoning can occur following accidental or intentional ingestion and is characterized by anticholinergic symptoms such as mydriasis, dry mouth, tachycardia, flushing, urinary retention, agitation, hallucinations, and delirium (2, 3). Severe intoxication may result in seizures, coma, respiratory failure, and death, making D. stramonium an important public health concern (4). Therefore, while the plant possesses significant medicinal value, its use requires strict caution due to its potentially serious toxic effects.

To safely and effectively investigate these toxicological effects, this study utilizes Drosophila melanogaster, a well-established model organism in genetics and toxicology. Approximately 75% of genes linked to human diseases have functional counterparts in Drosophila, making this species particularly valuable for medical research (5). Furthermore, its short life cycle and well-mapped genome make Drosophila an effective system for exploring how substances like D. stramonium influence gene expression and physiological outcomes. This study focuses on four key genes: Eiger, wingless (wg), glob1, and decapentaplegic (dpp), which are associated with apoptosis, developmental signaling, oxidative stress response, and morphogenesis, respectively.

Eiger (Egr) is the sole tumor necrosis factor (TNF) homolog in Drosophila melanogaster and is structurally related to mammalian TNF superfamily ligands, particularly ectodysplasin-A2 (EDA-A2) (6, 7). It encodes a type II transmembrane protein that can be cleaved and secreted to bind its receptors, Wengen (Wgn) and Grindelwald (Grnd), thereby activating intracellular signaling pathways, especially the c-Jun N-terminal kinase (JNK) pathway (6). Egr plays diverse roles in regulating apoptosis, immune responses, tissue growth and regeneration, wound healing, nutrient responses, neuroprotection, sleep regulation, and tumor surveillance, highlighting its importance in maintaining tissue homeostasis and organismal health (7, 8).

Wingless (wg) encodes a secreted signaling protein in Drosophila melanogaster and represents the founding member of the Wnt gene family (9, 10). The discovery of the wg gene led to the identification of Wnt signaling, a highly conserved cellular communication pathway that regulates cell proliferation, differentiation, and developmental processes across a wide range of organisms (9). As a morphogen, the Wingless protein mediates cell-to-cell communication by providing positional information that guides embryonic patterning and tissue development (10). In Drosophila, Wingless plays vital roles in embryogenesis, wing development, stem cell maintenance, tissue regeneration, and the regulation of cell growth and differentiation (10, 11). Beyond its developmental functions, Wingless contributes to the formation and maintenance of the nervous system, including the regulation of neuromuscular junction development and neuronal communication (12).

The glob1 gene of Drosophila melanogaster encodes Globin1, a heme-containing globin protein that plays multiple physiological and developmental roles beyond the traditional function of globins in oxygen transport (13). Glob1 is predominantly expressed in the tracheal system, fat body, and nervous tissues, where it contributes to the maintenance of cellular oxygen homeostasis and supports aerobic metabolism (13). In addition, Glob1 helps regulate intracellular levels of reactive oxygen species (ROS), thereby protecting cells from oxidative stress and maintaining normal cellular function (14). Studies have shown that adequate expression of glob1 is essential for the proper development and maintenance of the nervous system and preserving the integrity of the F-actin cytoskeleton (14, 15). Emerging evidence also suggests that Glob1 has neuroprotective functions by modulating oxidative stress pathways, thereby reducing neuronal damage (16).

The decapentaplegic (dpp) gene encodes Dpp, a secreted morphogen belonging to the Bone Morphogenetic Protein (BMP) family of the TGF-β superfamily (17). Dpp functions as a key signaling molecule that regulates embryonic dorsal-ventral patterning, tissue growth, cell proliferation, differentiation, and organ development throughout the life cycle of the fly (17, 18). In the wing imaginal disc, Dpp forms a concentration gradient that controls wing patterning and growth (19). Beyond developmental roles, Dpp contributes to developmental timing by modulating ecdysone production, suppressing premature autophagy, and maintaining lipid homeostasis (20, 21).

While Datura stramonium is widely recognized for its toxic and psychoactive properties due to tropane alkaloids, the specific molecular mechanisms through which D. stramonium influences these key developmental and stress-response pathways remain poorly understood. In particular, there is a critical knowledge gap regarding its precise effects on the expression of genes involved in apoptosis (Eiger), Wnt signaling (wg), oxidative stress regulation (glob1), and morphogenetic signaling (dpp) in Drosophila melanogaster. Addressing this gap is essential for understanding the molecular basis of D. stramonium-induced toxicity and its broader impact on cellular homeostasis. Therefore, this study aimed to investigate the dose-dependent effects of aqueous Datura stramonium leaf extract on the mRNA expression of Eiger, wingless, glob1, and dpp, and to evaluate associated survival outcomes in Drosophila melanogaster using semi-quantitative PCR analysis.

Methods

Study Design and Location

This experiment and Drosophila husbandry was conducted at the Biomedical Toxicology and Chemical Safety (BIOTOXCS) Research Laboratory, Central Biomedical Research, University of Benin, Nigeria. Drosophila melanogaster flies were obtained from the Department of Biochemistry, University of Ibadan, Nigeria. The flies were maintained under standard laboratory conditions (12-hour light/dark cycle, 25°C temperature).

Drosophila melanogaster Culturing

The wild type Drosophila melanogaster (Harwich strain) obtained from the Drosophila laboratory Department of Biochemistry, University of Ibadan, were maintained and reared on the Biomedical Toxicology and Chemical Safety (BIOTOXCS) Research Laboratory, Central Biomedical Research, University of Benin, Nigeria on a corn meal medium containing 1% w/v brewer`s yeast 2% powered milk. 1% w/v agar, and 0.8% v/w nipargin at constant temperarure and humidity (22-24 oC, 60-70% relative humidity) under 12 h dark/light cycle conditions. A standard cornmeal diet, formulated with: cornmeal (52g), brewer`s yeast (5g), glucose (3.5g), agar agar (7.9g), nipargin (1g), and ethanol (2ml). Distilled water was used in the preparation of the cornmeal diet (33).

Ethical Approval

The animal study protocol was approved with the research ethics committee College of Medical Sciences, University of Benin, Nigeria (research ethics committee approval number: CMS/REC/2024/695)

Procedure for Feed Preparation

850 mL of distilled water was boiled; 150 mL of the boiled water was used to mixed 52g of cornmeal separately, a small portion of the boiling water was used to dissolved 5g of yeast. 7.9g of agar was added to the boiling water and was stirred continuously until it was fully dissolved. The dissolved cornmeal was added and was stirred thoroughly, 3.5g of glucose was added into the meal and it was stirred properly, the dissolved yeast was added while stirring continuously. 1g of niargin was dissolved in ethanol and was mixed with the meal. The meal was removed from heat and was transferred into vials for cooling (33)

Experimental Design

The experimental design used for this study was a randomized controlled trial. Drosophila melanogaster were randomly assigned to different treatment groups, each exposed to a specific concentration of the Datura stramonium leaf extract. Each group consisted of 50 adult flies and a control group was included, consisting of flies not exposed to the extract. This design allowed for a comparison between the effects of different concentrations of Datura stramonium aqueous leaf extract on the Eiger, Wingless, Glob1, and Dpp mRNA in Drosophila melanogaster (34). To maintain consistency, flies within each treatment group were reared under the same environmental conditions, including temperature, humidity, and lighting. The duration of exposure to Datura stramonium aqueous leaf extract was standardized across all treatment groups to ensure comparability of results.

Flies were randomly selected from vials that had been prepared less than 17 days prior and anaesthetized by cooling in the freezer for 5 minutes at 4 °C. To minimize handling stress during the counting process, care was taken, and a soft-bristled brush was used.

Plant Collection and Extract Preparation

Datura stramonium leaves were collected from Arigidi community in Ondo State, Nigeria, and authenticated at the Department of Plant Biology and Biotechnology, University of Benin (Voucher number: UBH-D315). Leaves were air-dried, powdered (1398g), and soaked in distilled water. The extract was filtered multiple times and freeze-dried, yielding 94 g of aqueous extract.

Experimental Groups

Adult Drosophila melanogaster (1–3 days old) were randomly assigned into four groups: group A (control group 0 mg/kg), group B (2000mg/kg of Datura stramonium leaf extract), group C (4000mg/kg of Datura stramonium leaf extract), and group D (8000mg/kg of Datura stramonium leaf extract). The experiments were repeated in 3 repeats to ensure precision of data (each groups had 3 vials containing 50 flies). All groups received a standard cornmeal diet consisting of maize meal, yeast, glucose, agar, nipagin, ethanol, and distilled water.

Laboratory Analysis

Survival Assay

Both sexes of D. melanogaster (Harwich strain) 1 to 3 days old, were divided into different groups (50 flies /replicate, n = 4), and treated separately with aqueous Datura stramonium leaf extract (0 mg/kg, 2000 mg/kg, 4000 mg/kg, 8000 mg/kg) respectively. The flies were changed at least once per week into similar concentration of Datura stramonium leaf extract. Mortality rates were recorded daily in the flies and were used to plot a survival curve of a 21 days period.

Gene Expression Analysis

At the end of the administration period, the flies were collected and RNA preserved using TRIzol reagent. cDNA was synthesized with ProtoScript First Strand cDNA Synthesis Kit. Semi-quantitative PCR was performed using OneTaq 2X Master Mix, and amplification was done for Eiger, Wingless, Glob1, and Dpp genes. GAPDH was used as the housekeeping gene. Final PCR products were analyzed on 1% agarose gels stained with ethidium bromide. Band intensities were measured using ImageJ software (34). Data were analyzed using GraphPad Prism 8.0. Significance was considered at $P<0.05$. Error bars represent mean ± SEM.

Primer Sequences:

Rna	Forward primer	Reverse primer
Eiger	TCGATAATCTCCAGCAGCGT	CGCCAACATCATCCACAGAG
Wingless	CAGTTAGTCCGAATGCAGCC	GTTCGGGTGATGGATCTTGC
Glob1	GTTAGTCACATTCCGCGGAC	CCTCATCTACTTGGCGTTGC
Dpp	ACGGTGGTGCTGAAGAACTA	CATTGACGGCTTGGAACCAA

Source: Hu et al., 2013

Statistical analysis was done with graph pad prism 8.0 (Graph pad prism software, Buston USA) The data were expressed as mean ± SEM followed by Turkey post hoc test which calculated the significant differences among different treatment groups. Statistically significant difference was sets at $P<0.05$

Results

Table 1 shows the 21-day survival analysis revealed a dose-dependent decline in the survival rates of Drosophila melanogaster exposed to Datura stramonium aqueous leaf extract. The control group maintained a high survival rate of 94.74% throughout the study period. In contrast, treatment groups exhibited progressive mortality corresponding to increasing extract concentrations. By day 21, the survival rate in the 2000 mg/kg group had dropped to 48.87%, while the 4000 mg/kg group recorded 20.13%, and the 8000 mg/kg group showed complete mortality (0.00%). Statistical analysis confirmed a significant reduction in survival across treated groups compared to the control ($P = 0.0151$) (Figure 1).

Semi-quantitative PCR analysis revealed increased Wingless mRNA expression across all treated groups relative to the control. The 4000 mg/kg group showed the most pronounced elevation, with a mean expression of 78.54 ± 1.05, which was statistically significant ($P<0.05$) when compared to the control. Although the 2000 mg/kg and 8000 mg/kg groups also exhibited upregulated Wingless expression, these changes were not statistically significant.

The expression of Eiger mRNA demonstrated a clear dose-dependent increase. The 4000 mg/kg and 8000 mg/kg treatment groups showed significantly elevated levels (66.23 ± 1.05 and 70.12 ± 1.85, respectively) compared to the control group (47.21 ± 0.94), with $P<0.01$. A moderate increase was also observed in the 2000 mg/kg group (51.64 ± 1.63), although this was not statistically significant (figure 3).

Treatment with D. stramonium extract led to significant upregulation of Glob1 mRNA expression in all test groups. Compared to the control (39.56 ± 1.99), the 2000 mg/kg group showed increased expression (43.0 ± 1.42), while the 4000 mg/kg and 8000 mg/kg groups demonstrated statistically significant upregulation (48.4 ± 1.14 and 48.7 ± 1.41, respectively; $P<0.05$). The pattern was dose-dependent (figure 4).

In contrast to the other genes, Dpp mRNA expression was significantly repressed in all groups treated with D. stramonium extract. The most marked reductions were observed in the 2000 mg/kg (50.7 ± 0.65) and 8000 mg/kg (50.1 ± 1.67) groups, both of which were statistically significant when compared to the control (65.1 ± 1.64; $P<0.01$). The 4000 mg/kg group showed an intermediate repression level (58.5 ± 1.08;$P<0.05$) (figure 5).

Table 1- Percentage survival analysis and log-rank test for survival analysis trend in Drosophila melanogaster administered different concentrations of aqueous leaf extract of Datura stramonium

Days	Control (%)	2000mg/kg D. stramonium (%)	4000mg/kg D. stramonium (%)	8000mg/kg D. stramonium (%)	Logrank test for trend	P value
0.00	100.00	100.00	100.00	100.00	5.907	0.0151
1.00	100.00	100.00	100.00	100.00		
2.00	100.00	100.00	95.00	95.65		
3.00	94.74	94.74	95.00	91.30		
4.00	94.74	89.47	89.72	91.30		
5.00	94.74	84.21	89.72	86.74		
6.00	94.74	78.95	89.72	86.74		
7.00	94.74	78.95	89.72	86.74		
8.00	94.74	73.31	89.72	86.74		
9.00	94.74	73.31	82.82	86.74		
10.00	94.74	73.31	75.92	86.74		
11.00	94.74	73.31	69.02	80.54		
12.00	94.74	73.31	69.02	68.15		
13.00	94.74	73.31	69.02	68.15		
14.00	94.74	73.31	60.39	68.15		
15.00	94.74	73.31	60.39	60.58		
16.00	94.74	61.09	60.39	60.58		
17.00	94.74	48.87	60.39	51.93		

18.00	94.74	48.87	60.39	51.93
19.00	94.74	48.87	40.26	20.77
20.00	94.74	48.87	20.13	20.77
21.00	94.74	48.87	20.13	0.00

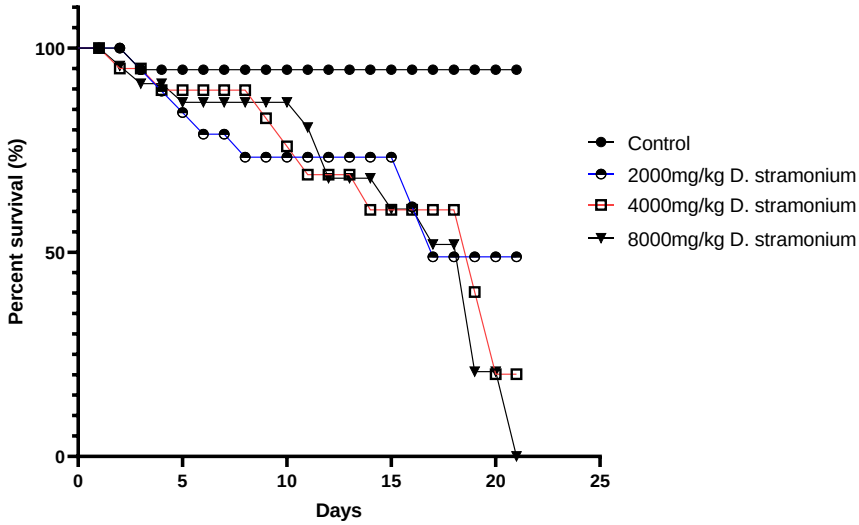

Figure 1: Survival curve analysis showing the percentage survival in Drosophila melanogaster administered different concentrations of Datura stramonium aqueous leaf extract

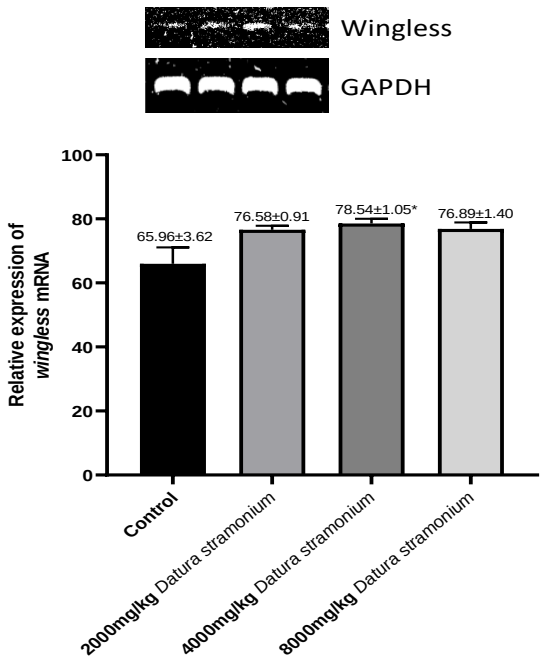

Figure 2: PCR and agarose gel analysis of wingless mRNA from adult Drosophila melanogaster administered varying concentrations of Datura stramonium aqueous leaf extract. Error bar represents mean±SEM. Statistical significance is represented by (*P<0.05, **P<0.01, ***P<0.001).

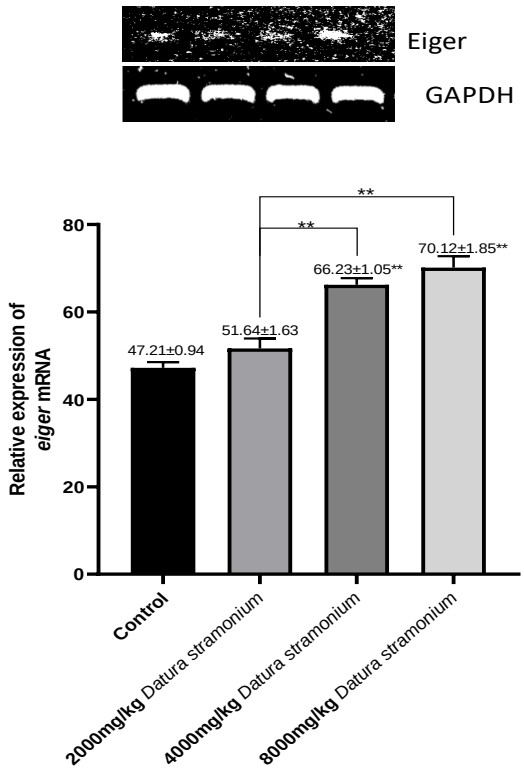

Figure 3: PCR and agarose gel analysis of eiger mRNA from adult Drosophila melanogaster administered varying concentrations of Datura stramonium aqueous leaf extract. Error bar represents mean±SEM. Statistical significance is represented by (*P<0.05, **P<0.01, ***P<0.001).

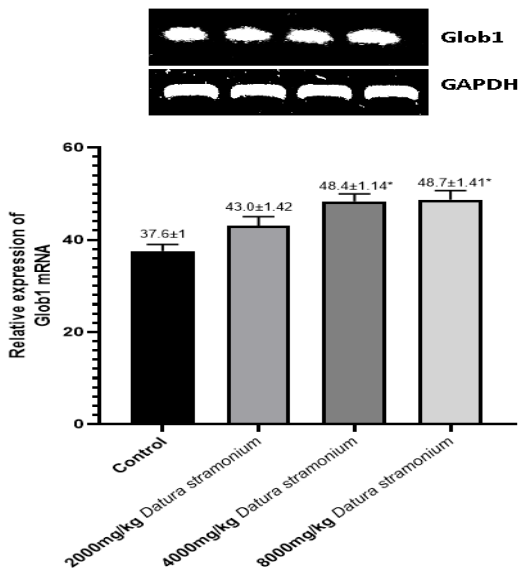

Figure 4: PCR and agarose gel analysis of glob1 mRNA from adult Drosophilia melanogaster administered varying concentration of Datura stramonium aqueous leaf extract. Error bar represents mean ± SEM. Statistical significance represented by (*P<0.05, **P<0.01).

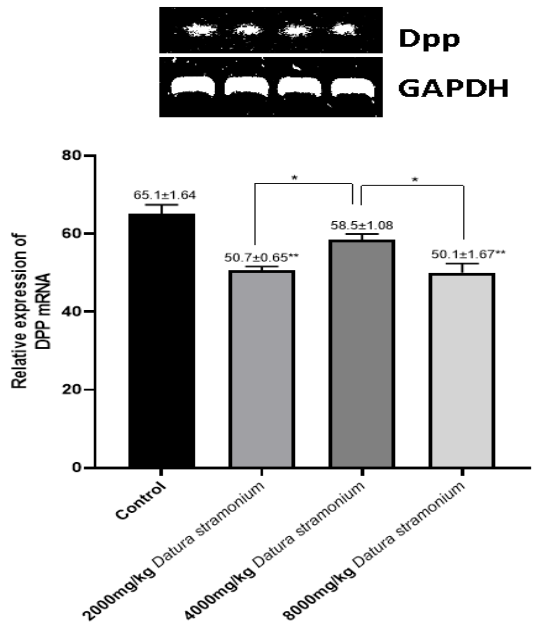

Figure 5: PCR and agarose gel analysis of dpp mRNA from adult Drosophilia melanogaster administered varying concentration of Datura stramonium aqueous leaf extract. Error bar represents mean ± SEM. Statistical significance represented by (*P<0.05, **P<0.01).

Discussion

This study investigated the dose-dependent effects of aqueous Datura stramonium leaf extract on survival and the expression of genes associated with apoptosis (Eiger), developmental signaling (wingless), oxidative stress regulation (glob1), and morphogenesis (dpp) in Drosophila melanogaster. The findings demonstrated that exposure to the extract significantly reduced survival while altering the expression of all four target genes, indicating that D. stramonium exerts both toxicological and molecular effects on key pathways involved in cellular homeostasis and development.

The survival assay revealed a clear dose-dependent decline in survival, with complete mortality occurring in flies exposed to 8000 mg/kg of the extract by day 21. This observation is consistent with previous reports describing the toxic effects of D. stramonium, which have largely been attributed to its high concentrations of tropane alkaloids, including atropine, hyoscyamine, and scopolamine (2, 22, 23). These alkaloids are known to interfere with cholinergic neurotransmission and induce severe neurological and systemic disturbances that may culminate in respiratory failure, organ dysfunction, and death (3, 4). Experimental studies have also demonstrated that Datura species can induce oxidative stress, neurotoxicity, and behavioral abnormalities through disruption of cellular redox balance (24, 25). The progressive mortality observed in the present study therefore suggests that prolonged exposure to the extract overwhelms physiological defense mechanisms, resulting in cumulative toxicity and reduced survival.

Semi-quantitative PCR analysis showed increased wingless expression in all treatment groups, with significant upregulation observed at 4000 mg/kg. The wingless gene encodes a secreted signaling protein that functions as the founding member of the Wnt signaling pathway, a highly conserved regulatory system involved in embryonic patterning, tissue regeneration, stem cell maintenance, and cellular differentiation (9, 10, 11). Wnt signaling pathways are known to respond to cellular stress and tissue injury by promoting survival and regenerative processes. Consequently, the increased wingless expression observed in this study may represent a compensatory response aimed at maintaining tissue integrity and developmental homeostasis in the presence of toxic insult. Similar activation of developmental signaling pathways has been reported following exposure to environmental stressors and xenobiotics that disrupt normal cellular function (1). The observed upregulation therefore suggests that D. stramonium exposure stimulates adaptive signaling mechanisms intended to counteract phytochemical-induced cellular damage.

The expression of Eiger increased significantly in a dose-dependent manner, with the highest levels observed in the 4000 mg/kg and 8000 mg/kg groups. Eiger is the sole tumor necrosis factor (TNF) homolog in Drosophila and plays a central role in apoptosis, inflammation, immune regulation, and stress signaling through activation of the JNK pathway (6, 7). Increased Eiger expression is generally associated with cellular stress and programmed cell death, particularly under conditions of tissue injury or toxic exposure (26). The significant induction of Eiger observed in this study therefore suggests activation of apoptotic pathways in response to D. stramonium toxicity. This interpretation is supported by previous reports indicating that plant-derived alkaloids can trigger oxidative stress and inflammatory responses that ultimately lead to apoptosis (27). The simultaneous increase in Eiger expression and decline in survival supports the hypothesis that activation of apoptosis contributes to the toxic

effects of the extract and may represent an important mechanism underlying the observed mortality.

Similarly, glob1 expression was significantly elevated in response to D. stramonium administration. The glob1 gene encodes Globin1, a heme-containing protein involved in oxygen homeostasis, oxidative stress regulation, and protection against reactive oxygen species (ROS) (13, 28). Previous studies have demonstrated that glob1 expression increases under stressful environmental conditions as part of an adaptive response designed to preserve cellular redox balance and protect tissues from oxidative damage (14). The upregulation observed in the present study suggests that exposure to D. stramonium generates oxidative stress, thereby stimulating antioxidant defense mechanisms. This interpretation is supported by reports indicating that alkaloids, flavonoids, and other phytochemicals present in D. stramonium can alter cellular redox status and induce oxidative stress responses (24, 29). Therefore, increased glob1 expression likely represents a protective response aimed at limiting oxidative damage and maintaining cellular homeostasis during toxic exposure.

In contrast to the stress-associated genes examined, dpp expression was significantly repressed in all treatment groups. The decapentaplegic (dpp) gene encodes a Bone Morphogenetic Protein (BMP) homolog that functions as a morphogen regulating embryonic patterning, tissue growth, cell proliferation, differentiation, and organogenesis in Drosophila (17, 18). Dpp signaling is essential for coordinating developmental processes and maintaining tissue integrity throughout the life cycle of the organism (19, 20). The suppression of dpp expression observed in this study suggests that D. stramonium interferes with morphogenetic signaling pathways required for normal growth and development. Similar disruptions in BMP signaling have been associated with developmental abnormalities, impaired tissue maintenance, and increased susceptibility to cellular dysfunction (30, 31). Furthermore, reduced dpp expression has been linked to inflammation-mediated neurodegeneration and altered tissue homeostasis in Drosophila (32). Consequently, the downregulation of dpp observed in the present study may indicate compromised developmental integrity and disruption of normal morphogenetic processes resulting from phytochemical toxicity.

Collectively, the observed upregulation of wingless, Eiger, and glob1, coupled with the repression of dpp, suggests that exposure to D. stramonium activates multiple cellular defense pathways while simultaneously suppressing critical developmental signaling mechanisms. The induction of genes involved in stress adaptation, oxidative stress management, and apoptosis likely represents a protective response to toxic exposure, whereas suppression of dpp indicates disruption of pathways required for growth and morphogenesis. These findings provide important mechanistic insight into the molecular basis of D. stramonium-induced toxicity and demonstrate that the biological effects of the plant extend beyond physiological toxicity to include significant alterations in conserved genetic pathways responsible for maintaining cellular and developmental homeostasis.

Conclusion

The mRNA expression of wingless, Eiger, and glob1 was significantly upregulated in response to aqueous Datura stramonium leaf extract exposure, while dpp expression was significantly downregulated in a dose-dependent manner. These transcriptional alterations indicate that the extract aggressively triggers apoptotic and oxidative stress responses while simultaneously suppressing essential morphogenetic signaling pathways. Coupled with the profound reduction in survival rates, these molecular findings highlight the plant's potent toxicity. Given the increasing incidence of D. stramonium abuse, this study provides critical mechanistic evidence of its cellular destructiveness, underscoring the urgent public health need to discourage its recreational misuse and unregulated consumption.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

All authors collaborated on, critically reviewed, and approved the study, taking full responsibility for its intellectual content, accuracy, and integrity. A.A.I. conceptualized and designed the study, and performed the statistical data analysis. E.F.I. and I.N.E. secured funding. A.A.I., E.F.I., and I.N.E. developed the experimental protocol. All authors participated in the literature search, conducted the laboratory experiments, and contributed to the discussion and interpretation of the data. E.F.I. and I.N.E. drafted the initial manuscript. All authors read and approved the final manuscript.

Data Availability Statement

The datasets generated and analyzed during the study are openly available in the Science Data Bank repository at https://doi.org/10.57760/sciencedb.31189 (Reference number: 31189).

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Original Article

Dose-Dependent Modulation of Eiger, Wingless, Glob1, and Dpp mRNA Expression and Survival Outcomes in Drosophila melanogaster Exposed to Aqueous Datura stramonium Leaf Extract

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Abstract:

Introduction: *Datura stramonium is a widely used medicinal and psychoactive plant known for its tropane alkaloids and other bioactive compounds. While its toxicological effects are well recognized, its molecular impact on developmental and stress-related pathways remains underexplored. This study investigated the effects of aqueous D. stramonium leaf extract on the expression of key regulatory genes (Eiger, Wingless, Glob1, and Dpp) in Drosophila melanogaster.*

Methods: *Wild-type flies (Harwich strain) were divided into four groups and fed diets containing 0 mg/kg, 2000 mg/kg, 4000 mg/kg, or 8000 mg/kg of the extract. A 21-day survival assay was conducted, and gene expression levels were determined using semi-quantitative PCR with ImageJ for band quantification.*

Results: *Survival decreased progressively with increasing extract concentration, reaching complete mortality at 8000 mg/kg by day 21. Gene expression analysis revealed a significant, dose-dependent upregulation of Wingless ($P<0.05$) and Eiger ($P<0.01$) at 4000 and 8000 mg/kg, and increased Glob1 expression at both doses ($P<0.05$). In contrast, Dpp expression was significantly repressed at 2000 mg/kg and 8000 mg/kg ($P<0.01$).*

Conclusion: *These findings indicate that D. stramonium exposure disrupts key molecular pathways in D. melanogaster, triggering stress and defence responses while suppressing morphogenetic signalling. The observed molecular alterations, coupled with reduced survival, highlight the plant's potent dose-dependent toxicity and the importance of its controlled use.*

Keywords: Datura, Eiger, Wingless, Glob1, Dpp, Drosophila, Toxicity, Gegemu.

Conflict of Interest: No

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