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Evaluation of pyruvate kinase and glucose 6 phosphate dehydrogenase mRNA in *Drosophila melanogaster* administered aqueous *Datura stramonium* leaf extract

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ABSTRACT

Background: This study investigated the dose-dependent effects of aqueous *Datura stramonium* leaf extract on the expression of pyruvate kinase *(pyk)* a critical glycolytic enzyme and glucose-6-phosphate dehydrogenase *(G6PD)*.
Methods: The flies (50 flies/vial each performed in triplicates) were divided into four groups according to the different concentrations of *D. stramonium* extract. Adult flies were allocated to a control group (no extract) and three treatment groups (200, 400, and 800 mg/ 10g diet). The extract was administered to the flies *ad libitum*. Flies were collected into TRIzol reagent for preservation of mRNA and subsequently homogenized for analysis. Semi quantitative PCR was used to analyze the expression pattern of the genes and ImageJ was used to quantify the different bands on the PCR and GAPDH was used as the housekeeping gene. ANOVA and turkey post Hoc test was then used to determine the statistical significance between groups ($p<0.05$).
Results: Semi-quantitative analysis revealed that the extract significantly repressed the expression of both *pyk* and *G6PD* genes in all treated groups compared to controls. The repression of *pyk* was greater in the 800 mg/10g diet group (34.14±1.65) compared to the control (48.35±1.13, $p<0.05$). Similarly, *G6PD* expression was significantly reduced, particularly in the 200 mg/10g diet group (77.84±1.54) compared to the control (97.15±1.23, $p<0.01$)
Conclusion: These findings demonstrate that *D. stramonium* extract elicited significant, dose-dependent repression of both genes across all treatments relative to controls. Thereby possibly disrupting core metabolic pathways which play roles in glycolysis and antioxidant defense.

Keywords: *Drosophila melanogaster, Datura stramonium, G6PD, pyruvate kinase.*

INTRODUCTION

Datura stramonium, commonly known as Devil's trumpet or Jimson weed, belongs to the *Solanaceae* family and is renowned for its rich alkaloid content (1). The plant is most commonly used as an intoxicant and hallucinogen due to the presence of anticholinergic alkaloids such as scopolamine which is known to produce hallucination and delirium (2, 3). Widely cultivated across Asia, Africa, Europe, and tropical regions (3), it has been traditionally employed as an intoxicant and hallucinogen due to anticholinergic alkaloids such as scopolamine, which provoke hallucinations and delirium. In Ayurvedic medicine, it treats conditions like asthma, toothache, fever, infections, and rheumatism, demonstrating bronchodilatory, anti-obesity, antiviral, anti-inflammatory, neuromodulatory, antioxidant, and hypoglycemic activities (3, 4). Its bioactive constituents include major alkaloids (hyoscyamine, hyoscine, scopolamine, pseudotropine, atropine) (5), amino acids, and diverse secondary metabolites such as saponins, tannins, phenols, flavonoids, p-coumaric acid, 6-hydroxyhyoscyamine, anolide I, skimmianine, daturataturins A and B, quercetin, metelodine, caffeic acid, coumarins, ferulic acid, stigmasterol, campesterol, steroidal glycosides, and chrysin (4, 6, 7). This delicate balance between therapeutic potential and toxicity is evident in symptoms like hallucinations, dry skin, and severe side effects, which demands precise dosing to maximize benefits while minimizing risks. Nevertheless, traditional and ethnopharmacological applications across cultures indicate safe use at controlled low doses, with minimal adverse effects (2). This suggests toxicity is context-dependent, modulated by preparation methods, dosage, and individual variability rather than being inherently lethal. Previous studies have also documented the presence of phenolic and flavonoid content in *D. stramonium* ethyl acetate leaf extracts, correlating with notable antioxidant and protein kinase inhibitory potential (8). Given that pyruvate kinase activity is known to be significantly decreased in various tissues following oxidative stress (9), and *Datura stramonium* exhibits considerable antioxidant capabilities (10), it is hypothesized that components within the plant may exert modulatory effects on pyruvate kinase (PYK) and glucose-6-phosphate dehydrogenase (G6PD), thereby influencing cellular energy

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metabolism and redox homeostasis. Furthermore, the identification of compounds like neophytadiene within *D. stramonium* extracts, which has demonstrated potent cytotoxic effects against MCF-7 cell lines, suggests a plausible link between specific phytoconstituents and their influence on metabolic pathways, potentially through enzyme modulation (11). In *Drosophila melanogaster*, a premier model for toxicological research owing to its conserved metabolic pathways and genetic tractability (12). *D. stramonium* alkaloids disrupt pivotal glycolytic (*pyk*) and pentose phosphate pathway (*G6PD*) enzymes (13). This impairment hampers energy production, NADPH generation, and antioxidant defenses, hallmarks of toxicity reflected in dose-dependent gene repression and lethality. This investigation delves into the biochemical ramifications of *Datura stramonium* leaf extracts on key metabolic enzymes: pyruvate kinase (pyk) and glucose-6-phosphate dehydrogenase (G6PD), given their critical roles in glycolysis and the pentose phosphate pathway, respectively.

MATERIALS AND METHODS

Area of Study

The research was carried out at the University of Benin, located in Benin City. Edo State, Nigeria. Founded in 1970. The University of Benin (UNIBEN) is a renowned public research institution and one of Nigeria's foremost institutions for higher learning.

Plant Collection and Identification

Datura stramonium leaves were collected from Arigidi community in Akoko North Local Government Area, Ondo State, Nigeria. The plant material was authenticated at the University of Benin's Herbarium, Department of Plant Biology and Biotechnology, with voucher number UBH-D315.

Preparation of Plant Extract

Fresh leaves of *Datura stramonium* were separated from the stems and washed thoroughly under running tap water. The cleaned leaves were air-dried at room temperature (20°C) until brittle to ensure proper preservation. The dried leaves were pulverized into a fine powder using a commercial blender. The resulting powder was weighed using an electronic sensitive balance, yielding 1398 g. The powdered material was soaked in distilled water in a transparent container for 24 hours at ambient temperature. After soaking, the mixture was stirred thoroughly to enhance extraction. The solution was then subjected to repeated decantation and filtration using double-layered cheesecloth and cotton wool. This process was repeated six times until the plant residue became colorless, indicating complete extraction. The combined filtrates were transferred to Tetfund Research in *Irvingia gabonensis* anti-diabetic study (TRIGAS) research laboratory for freeze-drying. After freeze-drying, 94 g of extract was obtained and stored in an airtight container at freezing temperature (-20^{0}C) for future use. The aqueous extract then gave a final yield of 6.72%.

$$\text{Percentage yield (\%)} = \frac{Final\ weight}{Initail\ weight} \times 100$$

Percentage yield (%) = 6.72%

Study Population and Design

Oregon strain of *Drosophila melanogaster* (1–3 days old) were obtained from the Drosophila Laboratory, Department of Biochemistry University of Ibadan, Oyo state, Nigeria. Breeding started immediately to have more flies for the experimental studies, the study was conducted in a dedicated laboratory facility equipped with appropriate infrastructure and equipment for rearing and manipulating *Drosophila melanogaster*. The laboratory, Biomedical Toxicology and Chemical Safety Research Laboratory, Central Biomedical Research, located at University of Benin. Fifty (50) flies were contained in each vial. The flies where grouped into 4 groups which comprised of a control (group 1), group 2 (200mg/10g diet), group 3 (400mg/10g diet) and group 4 (800mg/10g diet) and fed for 21 days *ad libitum*. The flies were fed with standard diet of cornmeal, maize meal, agar-agar, glucose, nipagin, ethanol and distilled water. The flies were sub cultured every 3 days to maintain the population of the flies with each vial.

Laboratory Assays

RNA extraction and semi-quantitative RT-PCR with gel densitometry

pyk and *g6pd* mRNA Assay

RNA was isolated from the adult *Drosophila melanogaster* with TRizol Reagent (ThermoFisher Scientific) and converted to cDNA using ProtoScript First Strand cDNA Synthesis Kit (NEB). PCR amplification of (pyk and g6pd) was done using OneTaq® 2X Master Mix (NEB).

After the experimental phase, 5 flies were randomly selected from each vial and imbedded in 0.3mL of TRIzol (Invitrogen Life Technologies, Inc., Carlsbad, CA) reagent inside an eppendorf tube for proper preservation. The flies were then homogenized using a plastic pestle. RNA lyase buffer was then added to the homogenate, after which it was spun at 10000 rpm for 10 minutes. The supernatant which contain the RNA was carefully removed and placed in a separate eppendorf tube. Then RNA precipitating buffer was then and centrifuged at 10000 rpm for 30 minutes. The supernatant was carefully removed remaining the RNA precipitant at the bottom of the tube. RNA wash buffer was then added and centrifuge again at 10000 rpm for 5 minutes. This step was repeated 3 times and nuclease free water was then added to break down the RNA into a pellet form. The RNA was then quantified using a UV spectrophotometer at 260nm.

Complementary DNA (cDNA)

The extracted RNA was utilized to synthesize cDNA by combining all components of the cDNA synthesis kit according to the manufacturer's instructions. These components included random primers, oligonucleotides, deoxynucleotides, and reverse transcriptase buffers. The reaction mixture was incubated in a thermocycler at 42°C for 1 hour to facilitate reverse transcription, followed by heating to 75°C to deactivate the reverse transcriptase enzyme. This process ensured the complete conversion of RNA into cDNA. For semi quantitative PCR, gene expression levels were normalized to the glyceraldehyde-3-phosphate dehydrogenase *(GAPDH)* housekeeping gene.

The primer sequences used were as follows:

G6PD	
FORWARD PRIMER	CGAATCGAAAGGCTTGCGTA
REVERSE PRIMER	CTTCTTCTTGGCCAGATCGC
pyk	
REVERSE PRIMER	CGCATGAACTTCTCTCACGG
FORWARD PRIMER	CGCCCTTCTTCAGCTCAAT

Gene Amplification and Electrophoresis

The quantitative RT-PCR amplification of the *pyk* and *g6pd* genes were carried out using a semi quantitative real-time PCR system under optimized thermal cycling conditions. The reaction began with an initial denaturation and polymerase activation step at 95 °C for 5 minutes. The amplification proceeded through 40 cycles of denaturation, annealing, and extension. Each cycle included a denaturation step at 95 °C for 15 seconds to separate the DNA strands. This was followed by an annealing step at 55 °C for 30 seconds to allow specific primer binding to the *pyk and g6pd* genes. An extension step was then performed at 72 °C for 30 seconds to enable DNA synthesis by the polymerase enzyme. Fluorescence data were collected at the end of each extension step during every cycle to monitor amplification in real time. A melt curve analysis ranging from 65 °C to 95 °C was conducted to confirm amplification specificity and detect non-specific products. In addition, template controls (NTC) and reverse transcriptase-negative (RT–) controls were included and showed no amplification, confirming the absence of contamination and genomic DNA interference, and the reaction was finally held at 4 °C to preserve the amplified products until further analysis. After PCR amplification, DNA gel loading dye was added to the amplified products. A 1% agarose gel was prepared with TBE buffer, allowed to solidify, and the samples were loaded for electrophoresis. Upon completion of the run, an image of the gel was captured and analyzed using ImageJ software. The band intensities were quantified densitometrically using ImageJ for further analysis.

Statistical Analysis

The data from this research was presented and analyzed using the GraphPad Prism 8.0 (California, USA). Analysis of variance (ANOVA) was used to compare the treatment groups for continuous variables. A *Tukey HSD post hoc* test was applied when a significant difference was observed in the ANOVA results. Bar charts and error bars used to illustrate the mRNA gene expression patterns were from biological triplicates from the study. Statistical significance represented by $p<0.05$.

RESULTS

To determine the effect of *Datura stramonium* on glycolysis and carbohydrate metabolism in *Drosophila melanogaster*, *pyk* and *G6PD* genes were analyzed. *pyk* gene is orthologous to human pyruvate kinase and it is a gene that codes for PYK enzyme important in the glycolytic pathway while The *G6PD* gene codes for glucose-6-phosphate dehydrogenase. This enzyme, which is active in virtually all types of cells, is involved in the normal processing of carbohydrates. Figure 1 showed the semi quantitative PCR and agarose gel analysis of *pyk* mRNA from adult *Drosophilia melanogaster* administered varying concentration of *Datura stramonium* aqueous leaf extract. The Data showed that there was a significantly lower expression levels of *pyk* gene in the groups administered 200mg/10g diet (44.37±0.87), 400mg/10g diet (44.16±1.67) and 800mg/10g diet (34.14±1.65) ($p<0.01$) aqueous leaf extracts of *Datura stramonium* when compared to control group (48.35±1.13). Similarly, 800mg/10g diet aqueous leaf extracts of *Datura stramonium* also had a significantly lower ($p<0.05$) expression of *pyk* gene when compared to the groups administered 200mg/10g diet and 400mg/10g diet *Datura stramonium* leaf extract. Figure 2 reveal the Semi quantitative PCR and agarose gel analysis of *g6pd* mRNA in *Drosophila melanogaster* administered different concentration of *Datura stramonium.* The results showed that there was a significant lower expression ($p<0.01$) of *g6pd* gene in the groups administered 200mg/10g diet (77.84±1.54), 400mg/10g diet (86.02±1.63) and 800mg/10g diet (79.48±0.65) aqueous leaf extracts of *Datura stramonium* respectively when compared to control group (97.15±1.23). Furthermore, when comparing 200mg/10g diet administered group to 400mg/10 diet administered group, there was a significant repression of *g6pd* in the 2000mg/10g diet administered group as shown in figure 2.

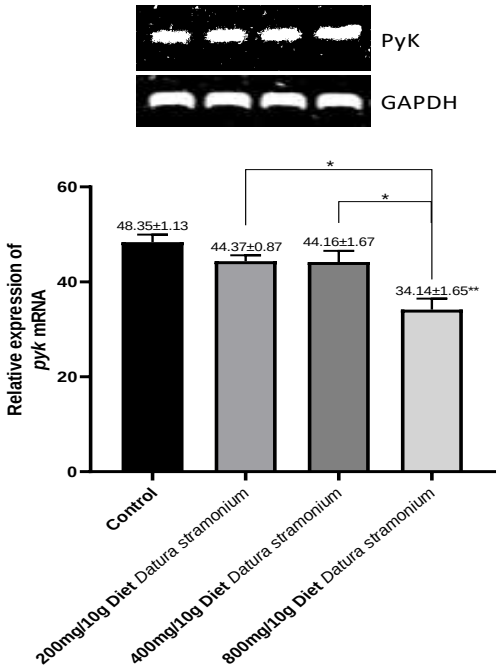


Figure 1. Relative expression of *pyk* mRNA in adult *Drosophila melanogaster* following exposure to aqueous *Datura stramonium* leaf extract. Representative gel bands and densitometric quantification normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are shown. Data are presented as mean ± SEM. P < 0.05, P < 0.01 versus control.

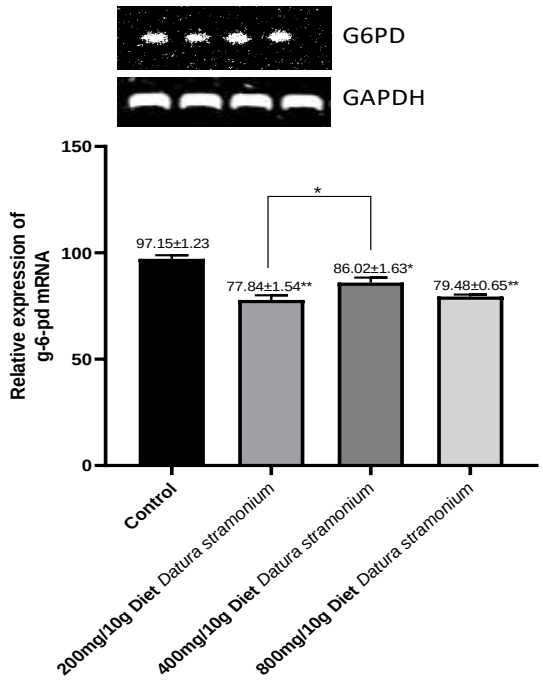


Figure 2. Relative expression of *g6pd* mRNA in adult *Drosophila melanogaster* following exposure to aqueous *Datura stramonium* leaf extract. Representative gel bands and densitometric quantification normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are shown. Data are presented as mean ± SEM. P < 0.05, P < 0.01 versus control.

DISCUSSION

The findings from this study provide insights into the effects of *Datura stramonium* poisoning on pyruvate kinase (PYK) and glucose-6-phosphate dehydrogenase (G6PD) enzyme activity in *Drosophila melanogaster*. The results showed that *Datura stramonium* exposure significantly altered PYK and G6PD gene expression, indicating disrupted glycolytic and pentose phosphate pathways. The *pyk* gene in *Drosophila melanogaster* encodes pyruvate kinase, a key enzyme in glycolysis, and its disruption by *Datura stramonium* extract indicates a potential compromise in ATP generation and overall energy metabolism. The repression of *pyk* may mirror effects observed in human pyruvate kinase isoforms (PKLR/PKM), which are critical for energy production and red blood cell function (14). The downregulation of *pyk* gene expression in response to *Datura stramonium* exposure suggests that the plant's toxic compounds may interfere with the glycolytic pathway, leading to impaired energy metabolism. This finding aligns with prior studies reporting the inhibitory effects of *Datura stramonium* on enzyme activity (15). Research suggests that tropane alkaloids, such as atropine and scopolamine, the primary bioactive compounds in *Datura stramonium*, influence cellular pathways, including glycolysis, by interacting with key enzymes and regulatory proteins (16). Secondary metabolic compounds in *D. stramonium* have been reported to inhibit protein kinase activities, including tyrosine kinases, which are critical for regulating glycolytic enzymes like pyruvate kinase, leading to downstream effects such as *pyk* gene repression (17). Furthermore, scopolamine and related alkaloids can alter gene expression through epigenetic mechanisms, such as histone acetylation or methylation, contributing to *pyk* suppression (18). Together, these findings highlight the potential of *Datura stramonium* to disrupt energy metabolism through its toxic effects on glycolysis.

The suppression of glucose-6-phosphate dehydrogenase (G6PD) by *Datura stramonium* constituents, particularly tropane alkaloids such as atropine and scopolamine may significantly impacts the pentose phosphate pathway (PPP), which is essential for maintaining redox balance and NADPH production. This suppression disrupts the detoxification of reactive oxygen species (ROS), exacerbating oxidative stress and cellular damage (19). Furthermore, the relative lower expression of *g6pd* gene may also suggest impaired activity in the pentose phosphate pathway, further disrupting redox homeostasis and antioxidant defense mechanisms. The suppression of this pathway limits nucleotide synthesis and NADPH availability, increasing oxidative vulnerability, particularly in erythrocytes, and heightening the risk of hemolytic anemia in G6PD-deficient individuals

The inhibition of G6PD activity may result from structural changes in the enzyme or epigenetic modifications affecting gene expression, as reported by (20). In *Drosophila* models,

tropane alkaloids have been shown to disrupt conserved metabolic pathways, highlighting their relevance to human systems and metabolic disorders. This is supported by simulations of secondary metabolite-induced gene expression changes, which validate the impact of *Datura stramonium* on G6PD expression and broader metabolic regulation (21, 22). The altered gene expression patterns observed may also be attributed to the neurotoxic and cytotoxic effects of compounds like scopolamine and hyoscyamine (23).There is an intricate interplay between these enzymatic modulations and the documented neurotoxic effects of *Datura stramonium*, particularly its impact on oxidative stress and neuro-degeneration (24). A previous study have shown that the central anticholinergic effects and rapid onset of action observed with *D. stramonium* extracts, which can lead to severe symptoms including hallucinations and respiratory failure, may be linked to profound metabolic disturbances initiated by altered PK and G6PD activities (25). These metabolic perturbations, potentially mediated by the plant's diverse secondary metabolites like alkaloids and flavonoids, could disrupt cellular energetics and redox balance, contributing to the systemic toxicity observed in Datura stramonium poisoning (4, 26). The results of this study have implications for understanding the mechanisms of *Datura stramonium* toxicity and may contribute to the development of strategies for mitigating its harmful effects. Furthermore, the findings of this study highlight the importance of considering the potential risks associated with exposure to *Datura stramonium*, particularly in agricultural and environmental settings. In comparison to other studies which similarly analyzed the role of plant secondary metabolites in oxidative stress and metabolic pathway regulation, with therapeutic insights focused on oxidative damage and enzyme modulation (20, 22). While previous studies have focused on the plant's neurotoxic and cytotoxic effects, this study provides evidence for the disruption of glycolytic and pentose phosphate pathways by mRNA modulation.

Conclusion: This study demonstrates that *Datura stramonium* poisoning alters PYK and G6PD gene expression, possibly leading to disrupted glycolytic and pentose phosphate pathways in *Drosophila melanogaster*. The dose-dependent toxicity of *Datura stramonium* observed in this study highlights the importance of considering the potential risks associated with exposure to this plant.

Limitations: The main limitation of this study was not able to isolate and identify the specific compounds and phytochemical constituents responsible for the observed enzymatic modulations. And couldn't validate these effects at the protein and functional levels.

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Conflicts of Interest: None declared

Data availability: Available upon reasonable request from the corresponding author

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