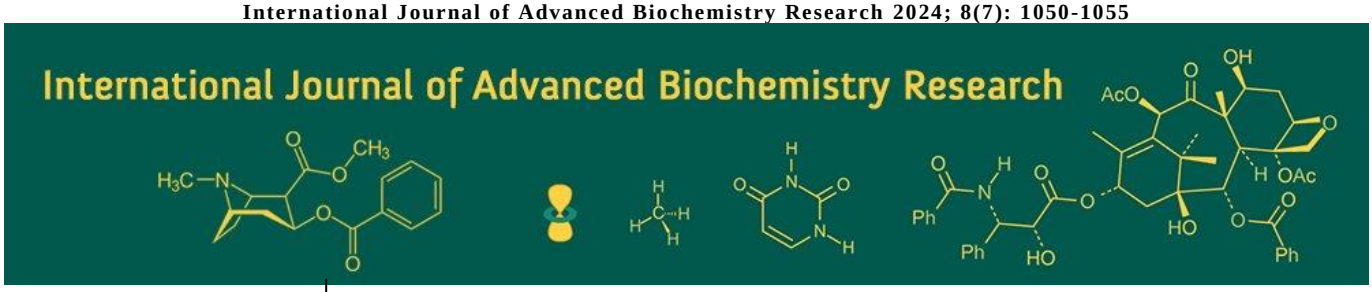

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(7): 1050-1055
www.biochemjournal.com
Received: 05-05-2024
Accepted: 11-06-2024

Owolabi Olutunmise Victoria
Department of Medical Biochemistry, Afe Babalola University, Ado-Ekiti, Nigeria

Omiyale Benjamin Olusola
Department of Medical Biochemistry, Afe Babalola University, Ado-Ekiti, Nigeria

ONI James Olukayode
Department of Pharmacology and Therapeutics, Afe Babalola University, Ado-Ekiti, Nigeria

Alli Smith Yemisi Rufina
Department of Biochemistry, Ekiti State University, Ado-Ekiti, Nigeria

Corresponding Author:
Owolabi Olutunmise Victoria
Department of Medical Biochemistry, Afe Babalola University, Ado-Ekiti, Nigeria

Effects of *Datura stramonium* on some key glycolytic enzymes in male albino rats

Owolabi Olutunmise Victoria, Omiyale Benjamin Olusola, ONI James Olukayode and Alli Smith Yemisi Rufina

DOI: https://doi.org/10.33545/26174693.2024.v8.i7m.1663

Abstract
This study investigated the effect of aqueous extract of *Datura stramonium (DS)* on the activities of the enzymes involved in carbohydrate metabolism. Twenty five animals were distributed into 5 groups of 5 animals each, Doses (50, 100, 200 and 400 mg/kg bodyweight) of aqueous extract of DS were given orally for 28 days to groups II, III, IV and V respectively while group I served as the control and were given distlilled water only. The animals were sacrificed at the expiration of the experiment after an overnight fast, brain, spleen and lungs were excised and the activities of hexokinase, aldolase, pyruvate kinase and lactate dehydrogenase were determined. Significant Increase in the activities of of all enzymes except lactate dehydrogenase were observed in a dose dependent manner in the animals when compared with the control ($p< 0.05$). In conclusion, aqueous extract of DS exhibit anti-hyperglycemic effects and might be a the mechanism behind its anti diabetic properties.

Keywords: *Datura stramonium*, aqueous extract, hexokinase, aldolase, pyruvate kinase, dose-dependent

Introduction
Datura stramonium is a medicinal plant commonly referred to as Devil's trumpet and Jimson weed. It belongs in the Solanaceae family, which are known to be rich in alkaloids (Babiker *et al*., 2017) [4]. 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 (Mukhtar *et al*., 2019, Sharma *et al*., 2021) [21, 28]. *Datura stramonium* is widely cultivated in Asia, Africa, Europe and other tropical regions (Sharma *et al*., 2021) [28]. Despite having hallucinogenic and poisonous potentials, *D. stramonium* has incredibly high medicinal property which has been deployed in ayurdevic medicine for the treatment of multiple medical conditions such as asthma, toothache, fever, infections and rheumatism. Studies and reports have indicated that the plant has bronchodilator, anti-obesity, antiviral, anti-inflammatory, neuromodulatory, antioxidant and hypoglycaemic activities (Sharma *et al*., 2021, Alum *et al*., 2023) [28, 3]. Owolabi *et al* 2023 [23] also reported significant changes in sperm analysis and testicular histomorphology following the administration of *Datura stramonium* extract
Alkaloids in this plant include but are not limited to hyoscyamine, hyoscine, scopolamine, pseudotropine and the highly medicinal atropine (Korkmaz *et al*., 2019) [18]. *D. stramonium* also contains amino acids such as glutamate, tyrosine, alanine and phenylalanine (Melaku and Amare, 2020) [8]. Saponins, tannins, phenols, alkaloids and flavonoids have been implicated in the pharmacological effect of the plant. Some of the identified ones include p-coumaric acid, 6-hydroxyhyoscymine, anolide I, skimmianine, daturataturins A and B, quercetin, metelodine, caffeic acid, coumarins, ferulic acid, stigmasterol, campesterol, steroidal glycosides and chrysins (Singh *et al*., 2013, Alum *et al*., 2023) [29, 3]. The contest between the toxic and the therapeutic effect of this plant is highly recognized. Hence, the pharmacological and therapeutic potentials are to be deployed with precise knowledge of its possible toxicological outcomes, so as to be able to evade its severe side effects which may include the most common hallucination, dry skin and other possible side effects (Mukhtar *et al*., 2019) [21].

D. stramonium has been employed for a number of studies such as Alum *et al*. (2023) [3] reported the possible antidiabetic effect of the methanolic seed extract in diabetic rats.

Materials and methods

Chemicals and reagents: Tris-HCl, magnesium chloride, hydrazine sulfate, magnesium sulfate, glucose-6-phosphate dehydrogenase, and adenosine diphosphate were obtained from Sigma Aldrich, Missouri, USA.), reduced adenine dinucleotide (NADH), glucose, fructose-1,6-bisphosphate, phosphoenol pyruvate, and lactate dehydrogenase were obtained from Carl Roth, Germany. The kit used for the determination of lactate dehydrogenase was purchased from Cypress diagnostics, Belgium.

Plant collection and extraction

Fresh samples of *Datura stramonium* were collected from Ureje quarters, Ado-Ekiti, Ekiti State, Nigeria. The fresh leaves and seeds were rinsed with distilled water and air-dried at room temperature until it was completely dry. The dried sample was homogenized using an electrical grinder. Extraction was carried out using a modified method of Ogunmoyele *et al*., (2019) [22]. Three hundred grams (300g) of the pulverized *Datura stramonium* was weighed and soaked in distilled water for 24 hours after which it was decanted and filtered using Whatman's filter paper, the filtrate was kept secured in a container and placed in a fridge. This process was repeated until a clear filtrate was obtained indicating that most of the active component in *Datura stramonium* has been extracted. The resulting filtrate was pooled and evaporated using a water bath at 40 ºC. The extract was dissolved in distilled water at a concentration of 50 mg/ml, 100 mg/ml, 2000 mg/ml, and 400 mg/ml.

Experimental animals

Twenty-five (25) male albino rats weighing between 180g and 200g were used for this experiment. They were randomly assigned into five groups of five animals each and housed in different cages at the animal house of the College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti. Access to standard rat pellets and clean water were given *ad libitum*. They were allowed 14 days to acclimatize. Approval for this study was obtained from Afe Babalola University Health Research Ethical Committee (ABUADHREC) with the number ABUADHREC/08/03/2023/2024. The principles of laboratory animal care were followed during the animal research (NIH publication #85–23, revised in 1985).

Study design: Experimental animals were randomly distributed into five groups of five animals each. Each group was exposed to graded doses of aqueous extract of *Datura stramonium* for twenty-eight days while the control received distilled water for the duration of the experiment. At the expiration of the experiment, animals were sacrificed under light ether anaesthesia. Brain, lungs and spleen were harvested, rinsed in normal saline to remove blood stains, blotted dry, and then kept in the freezer until further analysed.

Group 1: Control group
Group 2: Aqueous extract of *D. stramonium* 50 mg/kg
Group 3: Aqueous extract of *D. stramonium* 100 mg/kg
Group 4: Aqueous extract of *D. stramonium* 200 mg/kg
Group 5: Aqueous extract of *D. stramonium* 400 mg/kg

Preparation of homogenate for hexokinase, aldolase, pyruvate kinase and lactate dehydrogenase activities

Homogenates were prepared according to the method of Abdel-Hamid (2013). Ten (10) percent of the homogenate was prepared by placing 0.2g of organs in 1.8ml of 0.1 M Tris–HCl buffer (pH 7.4), this was done by 6 up and 6 down strokes at 1100rpm using a power-driven Teflon pestle in potter-Elvehjem glass homogenizing cup maintained at 4ºC. The homogenate was then centrifuged at 4000rpm at 4ºC for 10 minutes. The supernatant was carefully pipetted into an Eppendorf tube for the determination of hexokinase, aldolase, pyruvate kinase, and lactate dehydrogenase activities.

Enzyme assays

Hexokinase activity was determined by the method of Colowick (1973) [9] while aldolase was evaluated based on Boyer's modification of the hydrazine assay (Jagannathan *et al.*, 1956) [16]. Activities of pyruvate kinase were determined according to the method of Buchner and Pfleiderer (1955) [6], Lactate dehydrogenase activity was determined spectrophotometrically according to the method of Borgmann *et al*. (1974) [5] as described in Cypress Diagnostic kit manual.

Statistical analyses

Results were expressed as mean ± standard error of mean (SEM). Data was analysed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. $p<0.05$ was considered statistically significant.

Results

Effect of *Datura stramonium* on brain, spleen and lung hexokinase in male Wistar rats

Administration of *Datura stramonium significantly* increased hexokinase in the brain, spleen and lung of all the experimental animals when compared with control group.

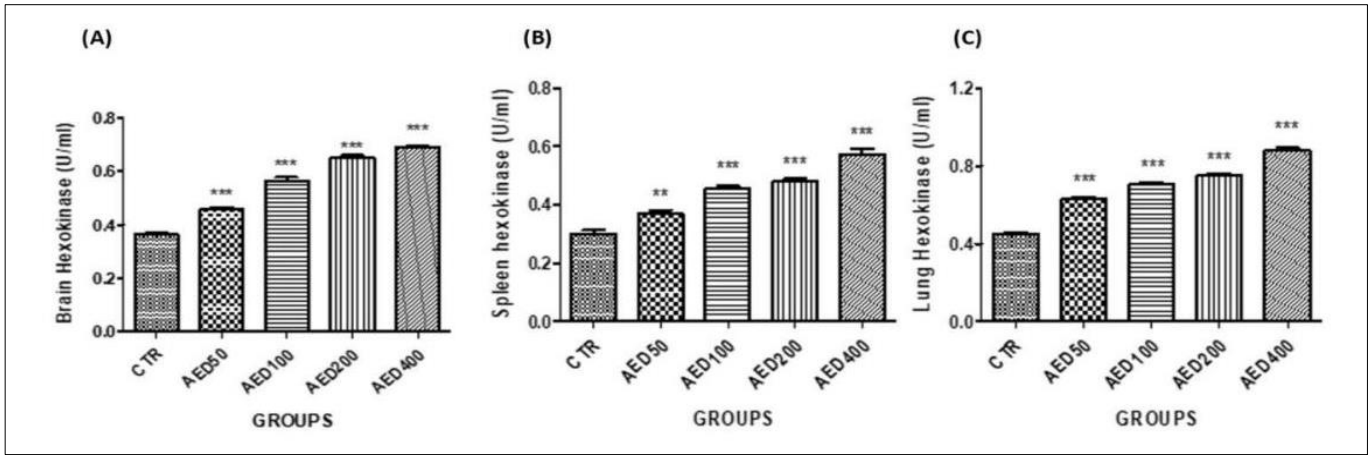

Fig 1: Effect of *Datura stramonium*on brain hexokinase (a), spleen hexokinase (b) and lung hexokinase (C) in male Wtstar rats. Data are represented as mean ± SEM. n-5 and analyzed by one -way ANOVA, followed by Turkey post hoc test. (***P<0.0001, **P<0.001 vs Control)

Effect of *Datura stramonium* on brain, spleen and lung aldolase in male Wistar rats: Administration of *Datura stramonium significantly* increased aldolase in the brain, spleen and lung of all the experimental animals when compared with control group.

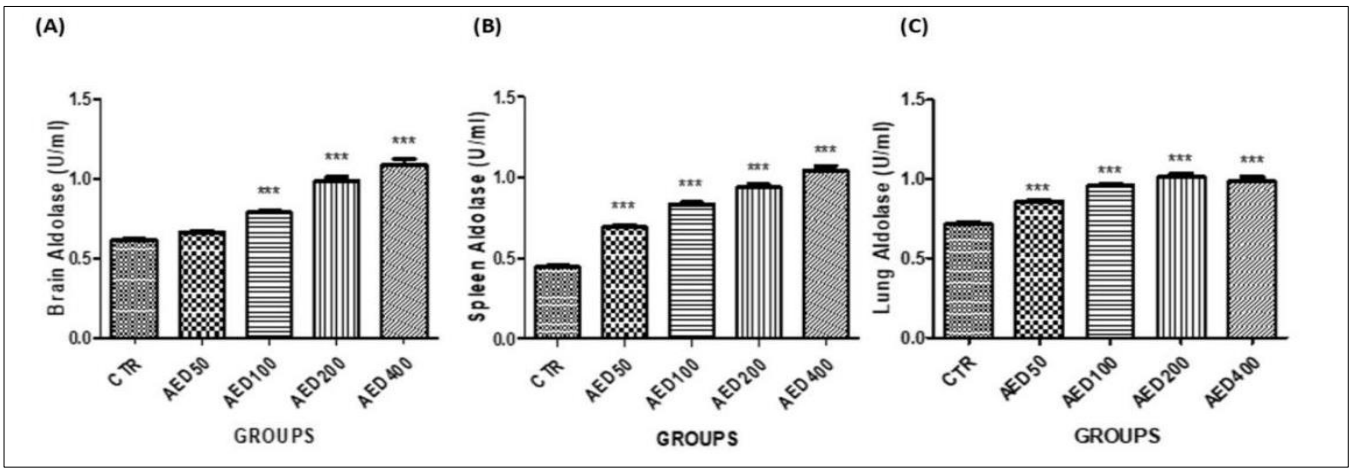

Fig 2: Effect of *Datura stramonium*on brain aldolase (a) spleen aldolase (b) and aldolase (c) in male wistar rats.
Data are represented as mean ±SEM. n=5 and analyzed by one- way Anova, Followed by Turkey post hoc test. (***P<0.001vs Control).

Effect of *Datura stramonium* on brain, spleen and lung pyruvate kinase in male Wistar rats

Administration of *Datura stramonium significantly* increased pyruvate kinase activity in the brain, spleen and lung of all the experimental animals when compared with control group.

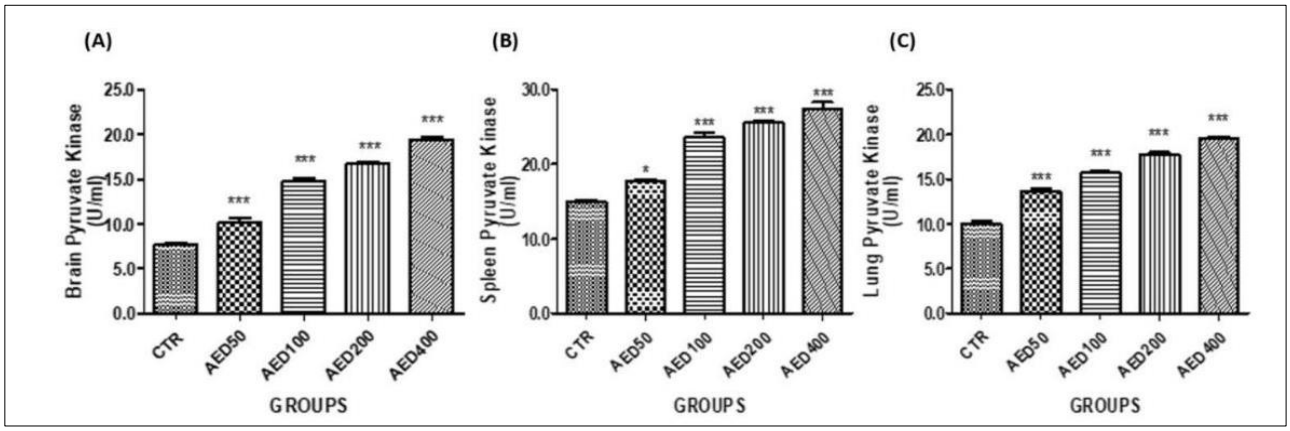

Fig 3: Effect of *Datura stramonium*on brain pyruvate kinase (a) Spleen pyruvate kinase (b) and lung pyruvate kinase (C) in male wistar rats. Data are represented as mean ±SEM n=5 and analyzed one – way anova followed by turkey post hoc test. (***p<0.0001,*p<0.05vs Control).

Effect of *Datura stramonium* on brain, spleen and lung lactate dehydrogenase activity in male Wistar rats

Administration of *Datura stramonium significantly* increased LDH activity in the brain of all the experimental animals except AED100 that was significantly decreased when compared with control group. LDH activities of AED50 and AED100 but not AED200 animals were significantly increased in the spleen when compared with control. LDH activity of AED400 animals was significantly decreased when compared with control animals. However, a significant decrease was observed in the LDH activities of all the experimental animals in the lung, when compared with control group.

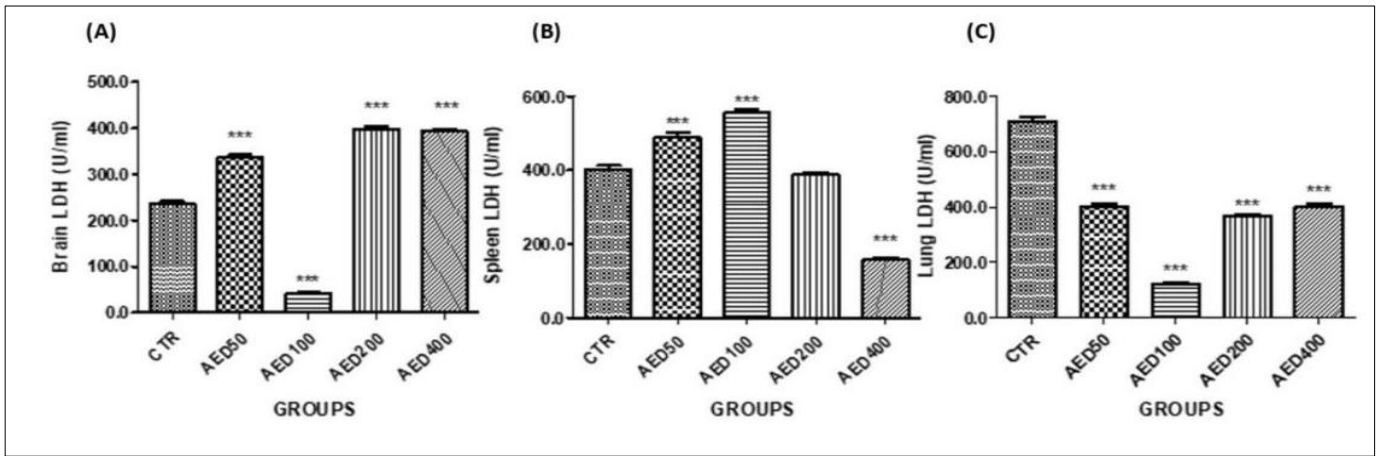

Fig 4: Effect of *Datura stramonium*on brain LDH (a) Spleen LDH (b) and lung LDH (c) in male wistar rats. Data are represented as mean ± SEM n=5 and analyzed by one – way Anova, followed by turkey post hoc test. (***p<0.001 vs Control)

Discussion

Glucose is undoubtedly known to be the most preferred source of energy in the mammalian system, and as such the cells require a constant supply of glucose. Chronic and acute disturbances in glucose availability, usage and storage have been discovered to lead to diseases conditions (Szablewski, 2017) [30]. Studies involving glucose metabolism and included enzymes have generated even more interest recently as impaired glucose metabolism has been identified in diseased conditions which are often difficult to manage such as obesity, diabetes and neurological conditions such as Alzheimer's disease (Rebelos *et al*., 2021) [27]. Hence, the ability of *D. stramonium* to initiate changes in metabolic enzymes involved in glucose metabolism as reported in this study may prove to be beneficial in glucose homeostasis and management of related conditions.

Hexokinase is an isoenzyme of glucokinase that catalyzes the phosphorylation of glucose to glucose-6-phosphate and plays a pivotal role in the maintenance of glucose homeostasis. It is the first enzyme in glycogen systhesis, pentose phosphate pathway and glycolytic pathway (Prasath and Subramanian, 2011) [25]. In this study, the administration of D stramonium resulted in a dose dependent increase in the level of hexokinase in the brain, spleen and lung hexokinase levels.

A correlation has been established between insulin efficiency and hexokinase levels. An increase in hexokinase level as reported in this study is linked to an increase in insulin efficiency and enhanced glucose metabolism, which suggests that the enzyme is an insulin dependent enzyme (Gothandam *et al*., 2019) [15]. Hence, an increase in hexokinase levels is indicative of an increase in insulin function and efficiency and consequentially improved glucose metabolism, controlled levels of glucose availability in the blood and increased ATP production (Gothandam *et al*., 2019) [15]. An increase in hexokinase function as reported in this study may be indicative of the plant's ability to stimulate effective function of the spleen which has been reported to harbour stem cells that act as precussor to insulin-producing pancreatic cells (Ley *et al*., 2012) [20], prevent anomalies related to impaired glucose metabolism in the lungs such as interstitial fibrosis, mucus overproduction in the airway and alveolar capillary microangiography (Khateeb *et al*., 2019) [17] and in the brain Alzheimer's disease (Kuehn, 2020) [19].

Aldolase converts a sixcarbon product, fructose 1,6-bisphosphate (F1,6BP), into two three-carbon products: G3P and DHAP. They are in all human tissues and impaired expression and availability has been a subject of much study because it can be linked with a number of human diseases which includes but is not limited to diabetes, schizophrenia, ischemia, Alzheimer's disease, traumatic brain injury and cancer (Chang *et al*., 2018) [7].

The administration of *D. stramonium* significantly increased the level of aldolase in the brain, spleen and lung in a dose dependent manner. High level of aldolase has been implicated in a number of diseased conditions such as diabetes and traumatic brain injury (El-desoky *et al*., 2013, Gerst *et al*., 2018, Chang *et al*., 2018) [10, 14, 7]. However, high level of aldolase has yet been identified with improved glucose metabolism (Pirovich *et al*., 2021) [24]. These variations in results may be associated to different isoforms of aldolase, tissue of interest, diseased condition of interest and the fact that aldolase is a proficient sensor for glucose availability at low or high levels. When intracellular levels of fructose 1,6 bisphosphate is low, aldolase activates the AMP-activated protein kinase (AMPK) which stimulates alternative pathway to energy generation in the cell, especially catabolic mechanisms (Pirovich *et al*., 2021) [24]. The increased expression of aldolase by *D. stramonium* may be believed to signify an increase in glucose metabolism and usage in correlation with the activity of related enzymes which are also reported in this study.

Pyruvate kinase is a ubiquitously expressed key glycolytic enzyme that catalyzes the conversion of phosphoenol pyruvate to pyruvate with the generation of ATP and the

altered expression could be expected to impair the glucose metabolism and energy production (Prasath and Subramanian, 2011, Gothandam *et al*., 2019) [25, 15]. In diabetic conditions, there is always an observable and significant decrease in the activity of pyruvate kinase in all tissues, and this is indicative of impaired glucose metabolism. Therapeutic compounds and plant molecules which have has a form of recognized or suggested protective effects against hyperglycemic identified in diabetes have always been found to significantly increase the activity of pyruvate kinase (Agius *et al*., 2020) [2]. Under homeostatic conditions, *D. stramonium* significantly increased the activity of pyruvate kinase in a dose dependent concentration in this study. This is indicative of a significant glucose regulatory effect which can be accounted for by increased glycolysis, and energy production as well as decreased gluconeogenetic production of glucose. This is also suggestive of the ability of the plant extract to significantly regulate the enzyme pyruvate kinase to ensure improved energy supply to the brain (Feksa *et al*., 2003) [12], possible role in the management of lung cancer which has been associated with a form of pyruvate kinase (Su *et al*., 2019, Wang *et al*., 2020) and the management of spleen assocated pyruvate kinase deficiency.

Lactate dehydrogenase catalytically converts pyruvate to lactate in anaerobic glycolysis, which can be converted to glucose during gluconeogenesis (Prasath and Subramanian, 2011, Frenkel *et al*., 2023) [25, 13]. It is an important glucose metabolizing enzyme as increase levels of the enzyme has been implicated in the impairment of insulin secretion (Gothandam *et al*., 2019) [15].

In this study, the all doses of *D. stramonium* significantly reduced the activity of lactate dehydrogenase in the lungs, some of the doses in the spleen and brain of rats. However there was significant reduction in the lactate dehydrogenase levels in all reported organs, which may be a testament to the potentials of the plant to support insulin function, regulation of NADP/NADH ratio, improved activity of pyruvate kinase and pyruvate metabolism for mitochondrial energy production and by that improve proper metabolic processes involved in the physiological use of glucose (Gothandam *et al*., 2019) [15]. The regulatory effect of the plant on the considered enzyme in three different organs may also be indicative of the plants potential in the management of brain tumour, cancer, anaemia, muscle trauma, liver conditions, encephalitis and meningitis, all of which have irregular metabolism of lactate dehydrogenase in their pathogenesis (Farhana and Lappin, 2023) [11].

Conclusion

In conclusion, the present study indicated that oral administration of *D. stramonium* exhibits anti-hyperglycemic and glucose regulatory effect as a result of the modulatory effect it exerted on enzymes responsible for glucose metabolism. Based on the result, *D. stramonium* has glucose regulatory and insulin enhancing effect hence has potentials in the management of diabetes and Alzheimer's disease. Further research is recommended to delineate its mechanism of action for antidiabetic and neuroprotective function

Author Contributions

Owolabi O. V. designed the study, Omiyale B.O. and Owolabi O.V. carried out most of the bench work: Oni J.O and Alli Smith Y.R. analyzed the data: Owolabi O.V prepared the first draft of the manuscript, Alli Smith Y. R reviewed the final draft of the manuscript. All authors read and approved the final manuscript.

Funding: Personal funding

Data availability: Data available on request

Consent for publication: Authors approved the submission of this research work for publication

Conflict of Interest: The authors declare no conflict of interest

References

1. Abdel-Hamid NM, Abdel-Ghany MI, Nazmy MH, Amgad SW. Can methanolic extract of Nigella sativa seed affect glyco-regulatory enzymes in experimental hepatocellular carcinoma? Environmental Health and Preventive Medicine. 2013;18(1):49-56.
2. Agius L, Ford BE, Chachra SS. The metformin mechanism on gluconeogenesis and AMPK activation: the metabolite perspective. International Journal of Molecular Sciences. 2020;21(9):3240.
3. Alum EU, Aja W, Ugwu OP, Obeagu EI, Okon MB. Assessment of vitamin composition of ethanol leaf and seed extracts of *Datura stramonium*. Avicenna Journal of Medical Biochemistry. 2023;11(1):92-97.
4. Babiker F, Jamal P, Mirghani MES, Ansari AH. Characterization, purification and identification of some alkaloids in *Datura stramonium*. International Food Research Journal. 2017;24:1667-1675.
5. Borgmann U, Moon TW, Laidler KJ. Molecular kinetics of beef heart lactate dehydrogenase. Biochemistry. 1974;13(25):5152-5158.
6. Buecher T, Pfleiderer G. Pyruvate kinase. Methods in Enzymology. 1955;1:435-440.
7. Chang YC, Yang YC, Tien CP, Yang CJ, Hsiao M. Roles of aldolase family genes in human cancers and diseases. Trends in Endocrinology & Metabolism. 2018;29(8):549-559.
8. Melaku BC, Amare GG. Evaluation of antidiabetic and antioxidant potential of hydromethanolic seed extract of *Datura stramonium* Linn (Solanaceae). Journal of Experimental Pharmacology. 2020;12:181-189.
9. Colowick SP. 1 The Hexokinases. In: Boyer PD, Lardy H, Myrback K, editors. The Enzymes. Volume 9. 3rd ed. New York: Academic Press; c1973. p. 1-48.
10. El-desoky MA, Amin AE, El-refai MO, Farid RJ, Labib AA. Evaluation of some biochemical changes in diabetic patients. The Egyptian Journal of Hospital Medicine. 2013;50(1):150-155.
11. Farhana A, Lappin SL. Biochemistry, Lactate Dehydrogenase. [Updated 2023 May 1]. In: Stat Pearls. Treasure Island (FL): StatPearls Publishing; c2024 Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557536/
12. Feksa LR, Cornelio AR, Dutra-Filho CS, de Souza Wyse AT, Wajner M, Wannmacher CMD, *et al*. Characterization of the inhibition of pyruvate kinase caused by phenylalanine and phenylpyruvate in rat brain cortex. Brain Research. 2003;968(2):199-205.

13. Frenkel A, Shiloh A, Azulay B, Novack V, Klein M, Dreiher J, *et al*. The role of lactate dehydrogenase in hospitalized patients, comparing those with pulmonary versus non-pulmonary infections: A nationwide study. PLoS ONE, 2023, 18(3).
14. Gerst F, Jaghutriz BA, Staiger H, Schulte AM, Lorza-Gil E, Kaiser G, *et al*. The expression of aldolase B in islets is negatively associated with insulin secretion in humans. The Journal of Clinical Endocrinology & Metabolism. 2018;103(12):4373-4383.
15. Gothandam K, Ganesan VS, Ayyasamy T, Ramalingam S. Antioxidant potential of theaflavin ameliorates the activities of key enzymes of glucose metabolism in high fat diet and streptozotocin–induced diabetic rats. Redox Report. 2019;24(1):41-50.
16. Jagannathan V, Singh K, Damodaran M. Carbohydrate metabolism in citric acid fermentation. 4. Purification and properties of aldolase from *Aspergillus niger*. Biochemical Journal. 1956;63(1):94-100.
17. Khateeb J, Fuchs E, Khamaisi M. Diabetes and lung disease: an underestimated relationship. Review of Diabetic Studies. 2019;15(1):1-15.
18. Korkmaz MF, Bostanci M, Onur H, Çağan E. *Datura stramonium* poisoning: A case report and review of the literature. The European Research Journal. 2019;5(1):186-188.
19. Kuehn BM. In Alzheimer research, glucose metabolism moves to center stage. JAMA. 2020;323(4):297-299.
20. Ley EJ, Singer MB, Clond MA, Johnson T, Bukur M, Chung R, *et al*. Long-term effect of trauma splenectomy on blood glucose. Journal of Surgical Research. 2012;177(1):152-156.
21. Mukhtar Y, Tukur S, Bashir RA. An overview on *Datura stramonium* L. (Jimson weed): A notable psychoactive drug plant. American Journal of Natural Sciences. 2019;2(1):1-9.
22. Ogunmoyole T, Adeyeye RI, Olatilu BO, Akande OA, Agunbiade OJ. Multiple organ toxicity of *Datura stramonium* seed extracts. Toxicology Reports. 2019;6:983-989.
23. Owolabi OV, Enye LA, Omiyale BO, Saka OS, Akintayo CO. Testicular Histomorphology Following *Datura stramonium* Administration in Adult Male Wistar Rats. Agricultural Science Digest; c2023. DOI: 10.18805/ag.DF-502.
24. Pirovich DB, Da'dara AA, Skelly PJ. Multifunctional fructose 1, 6-bisphosphate aldolase as a therapeutic target. Frontiers in Molecular Biosciences. 2021;8:719678.
25. Prasath GS, Subramanian SP. Modulatory effects of fisetin, a bioflavonoid, on hyperglycemia by attenuating the key enzymes of carbohydrate metabolism in hepatic and renal tissues in streptozotocin-induced diabetic rats. European Journal of Pharmacology. 2011;668(3):492-496.
26. Ramachandran V, Saravanan R. Efficacy of asiatic acid, a pentacyclic triterpene on attenuating the key enzymes activities of carbohydrate metabolism in streptozotocin-induced diabetic rats. Phytomedicine. 2013;20(3-4):230-236.
27. Rebelos E, Rinne JO, Nuutila P, Ekblad LL. Brain glucose metabolism in health, obesity, and cognitive decline-does insulin have anything to do with it? A narrative review. Journal of Clinical Medicine. 2021;10(7):1532.
28. Sharma M, Dhaliwal I, Rana K, Delta AK, Kaushik P. Phytochemistry, pharmacology, and toxicology of Datura species - A review. Antioxidants. 2021;10(8):1291.
29. Singh LR, Singh OM. *Datura stramonium*: An overview of its phytochemistry and pharmacognosy. Research Journal of Pharmacognosy and Phytochemistry. 2013;5(3):143-148.
30. Szablewski L. Glucose transporters in brain: in health and in Alzheimer's disease. Journal of Alzheimer's Disease. 2017;55(4):1307-1320.