



EFFECT OF *DELONIX REGIA* SEED EXTRACT ON SOME LIVER ENZYME MARKERS AND ELECTROLYTES OF ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

The effect of seed extract of *Delonix regia* on some liver enzyme markers and electrolytes of alloxan-induced diabetic rats was evaluated. Dried seed powder (181 g) was soaked in a solution containing 400ml of ethanol and 1400ml of distilled water. Twenty-five albino rats of both sexes were randomised into five groups (A-E) based on their average weight. The animals fasted overnight. Diabetes was induced in Groups B - E by a single intraperitoneal injection of a freshly prepared alloxan monohydrate (100 mg/kg body weight). Group A rats were not induced with alloxan and served as normal control. Group B was diabetic and untreated and represent negative control, Group C - E rats were given varying concentrations (12%, 15% and 20%) of 20 mg/kg body weight of seed extract of *Delonix regia* respectively. Electrolyte (K^+ , Na^+ and Cl^-) levels as well as liver enzyme markers Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) were analysed after ten days of administration of extract. Results obtained showed that all alloxan-induced rats became diabetic after 96 hours with a blood glucose ranging from 9.35 to 10.3 mmol/L. The 20% dilution of the extract administered expressed a significantly higher anti-diabetic activity compared to the 12% and 15% dilutions. *Delonix regia* seed crude extracts significantly ($p < 0.05$) improved the levels of the electrolytes in diabetic treated rats when compared with the untreated rats and control. The extract significantly ($p < 0.05$) lowered the activities of AST, ALT and ALP in diabetic rats when compared to the untreated group.

Keywords: *Delonix regia*, serum enzyme activity, Alloxan monohydrate, diabetic rats.

INTRODUCTION

Diabetes mellitus is described as a chronic metabolic disorder of carbohydrates, proteins and fat due to absolute or relative deficiency of insulin secretion with/without varying degree of insulin resistance.¹ Currently over 150 million people are living with diabetes mellitus, a figure which is estimated to increase to 366 million people or more by the year 2030.² Type 1 diabetes mellitus can also be classified as immune-mediated. The majority of type 1 diabetes is that in which a T-cell mediated autoimmune attack leads to the loss of beta cells and thus

insulin.³ Complications of type 1 diabetes include ketosis, hypoglycaemia, infection, gastroparesis and endocrinopathies (e.g., Addison's disease).⁴ Type 2 diabetes mellitus is characterised by insulin resistance, which may be combined with relatively reduced insulin secretion. The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor.⁵ This is the most common type of diabetes mellitus diagnosed. In early stages of type 2 diabetes mellitus, the predominant abnormality is reduced insulin sensitivity. Hyperglycaemia can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glycogen

breakdown in the liver. Lifestyle changes or factors and genetics are highlighted to be the primary factors responsible for type 2 diabetes mellitus.⁶ Consumption of sugar-sweetened drinks in excess is associated with an increased risk.⁷ A lack of exercise is believed to cause 7% of type 2 diabetes cases.⁸ Gestational diabetes mellitus is somewhat similar to type 2 diabetes in several aspects including, a combination of relatively inadequate insulin secretion and responsiveness. This type of diabetes occurs in about 2-10% of all pregnancies and may improve or disappear after delivery.⁹ The basic symptoms of diabetes mellitus are weight loss, polyuria (increased urination), polydipsia (increased thirst) and polyphagia (increased hunger).¹⁰ These symptoms may develop rapidly in type 1 diabetes, while they usually develop much more slowly in type 2 diabetes. Therefore, deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus.¹¹ Alloxan-induced diabetes has been commonly employed as an experimental model of insulin dependent diabetes mellitus. The mechanism of action of alloxan has been thoroughly studied which currently can be characterised quite well. Alloxan-induced insulin release occurs for a short duration followed by the complete suppression of the islet response to glucose even when high concentrations of glucose were used.¹² The action of alloxan in the pancreas is preceded by its rapid uptake by pancreatic beta cells that have been proposed to be one of the important features determining alloxan diabetogenicity. Moreover, in pancreatic beta cells, the reduction process occurs in the presence of different reducing agents like reduced glutathione (GSH), cysteine, ascorbate and protein-bound sulfhydryl (-SH) groups.¹³

Antioxidants like superoxide dismutase, catalase and the non-enzymatic scavengers of hydroxyl radicals have been found to protect against alloxan toxicity.¹⁴ Alloxan elevates systolic free Ca^{2+} concentration in the beta cells of pancreatic islets. The calcium influx results from the ability of alloxan to depolarise pancreatic beta cells that further opens voltage-dependent calcium channels and enhances calcium entry into pancreatic cells. The increased concentration of Ca^{2+} ion further contributes to supraphysiological insulin release that along with ROS has been noted to ultimately cause damage in beta cells of pancreatic islets cells.¹⁵ The nutritional performance of rats fed diets containing seeds was achieved without expensive pre-treatment of the seeds or for supplementation of the diets with individual amino acids. The seeds of *Delonix regia* contained

only low levels of essentially non-toxic lectin and they have great potential for development as a source of dietary protein for man and animals.¹⁶ *Delonix regia* is a flamboyant tree native to Madagascar. This study was designed to evaluate the effect of *Delonix regia* seed extract on some liver enzyme markers and electrolytes of alloxan-induced diabetic rats.

MATERIALS AND METHOD

Seed Sample

Delonix regia seeds were harvested from the University Park, University of Port Harcourt, Nigeria and were authenticated by a biotechnologist, Dr. Ekeke Chimezie of the Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt, Rivers State, Nigeria. Its voucher specimen Number is UPH /V/1269. Clean seeds were selected and dried for 5 days at 37°C (room temperature) for easy milling. The seeds were however milled by means of a machine grinder and a blender and the flour obtained was then transferred into a clean polyethylene bag and stored properly.

Chemical Reagents

Alloxan monohydrate was obtained from Sigma-Aldrich Chemical Company, St Louis, U.S.A. All the other chemicals used were obtained commercially and were of analytical grade.

Experimental Animals

Twenty-five mature albino rats of the Wistar strain of both sexes weighing between 100-175g were obtained from the animal house of the Department of Biochemistry, University of Port Harcourt, Nigeria. The animals were housed in a well ventilated experimental animal house under constant environmental and adequate nutritional conditions throughout the period of the experiment. They were fed on standard rat chow (grower mash) and water was given liberally, *ad libitum*. The animals were acclimatised for one week prior to the commencement of the experiment.

Preparation of Seed Extract

One hundred and eighty-one grammes of the dried seed powder was soaked in a solution containing 400ml of ethanol (Annular

grade, 99-100%) and 1400ml of distilled water. This mixture was shaken for 5 minutes after which it was left to stand for 24 hours at 37°C before filtering with a clean filter paper (Whatman no. 1). The filtrate was stored in the refrigerator and then 12%, 15% and 20% dilution were prepared from stock.

Preparation of Alloxan and Induction of Diabetes

One thousand five hundred milligrammes of alloxan was dissolved in 150ml of normal saline. The animals fasted overnight. Thereafter diabetes was induced by a single intraperitoneal injection of a freshly prepared alloxan monohydrate (100 mg/kg body weight). Control (normal) rats were not injected with alloxan. The fasting blood glucose level of blood samples drawn from the tail vein puncture was determined using One-touch ultraeasy glucometer. A drop of blood was placed on the test strip inserted into the glucometer which automatically displayed the level of glucose in the blood. Only rats with blood glucose level higher than 9.0mmol/L were considered diabetic and used for the experiment. Feeding was stopped 12 hours before blood collection.

Experimental Design

The study was conducted in compliance with NIH guide for the care and use of laboratory animals (pub No: 85-23 Revised 1985). Animal ethical clearance number (DPGCAE: BCH 057) for this study was obtained from the department of Biochemistry, University of Port Harcourt. The experimental period was 10 days and the extract was given orally once per day using cannula. The rats were acclimatised to the animal house conditions. Twenty-five albino rats of both sexes were randomised into five groups (A-E) such that;

Group A (Normal control): Control rats were fed with grower mash and water only, *ad libitum*. Parameters from this group served as baseline data.

Group B (Diabetic control): This consisted of diabetic untreated rats fed with grower mash and water only, *ad libitum*.

Group C: The rats in this group were maintained on the same diet as group A and B. They were also diabetic but were treated using 12% dilution of the extract at a dose of 20mg/kg daily, all through the experiment duration.

Group D: The rats in this group were maintained on the same diet as group A and B. They were also diabetic but were treated

using 15% dilution of the extract at a dose of 20mg/kg daily, all through the experiment duration.

Group E: The rats in this group were maintained on the same diet as group A and B. They were also diabetic but were treated using 20% dilution of the extract at a dose of 20mg/kg daily, all through the experiment duration.

Collection of Blood Sample

After ten days of the treatment, the fasting blood glucose level of blood samples drawn from the tail vein puncture was determined using One-touch ultraeasy glucometer. A drop of blood was placed on the test strip inserted into the glucometer which automatically displayed the level of glucose in the blood. The animals to be sacrificed were first anaesthetized with chloroform (inhalational anaesthesia) followed by cervical dislocation. Each animal was then placed on a dissecting slab and then cut along the thorax down the abdominal region; blood was collected via cardiac puncture and dispensed into Heparin bottle for liver enzymes and electrolyte assays.

Determination of liver enzymes

Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) were analysed by kinetic methods kits from Randox (United Kingdom) using a double-beam spectrophotometer.

Serum Electrolytes

The blood samples collected into the lithium heparin sample bottles were centrifuged at 1500rpm for 5 minutes and the plasma was obtained. The ISE 4000 Analyser was used to determine the various electrolytes.

STATISTICAL ANALYSIS

Data were expressed as the mean \pm standard deviation (SD). Comparison of the data from test control groups of animals was analysed by One-Way Analysis of Variance (ANOVA) at the confidence limit of 95% and where applicable, Least Significant Difference (LSD) was used to determine significant results, differences between groups were considered statistically significant at $p < 0.05$.

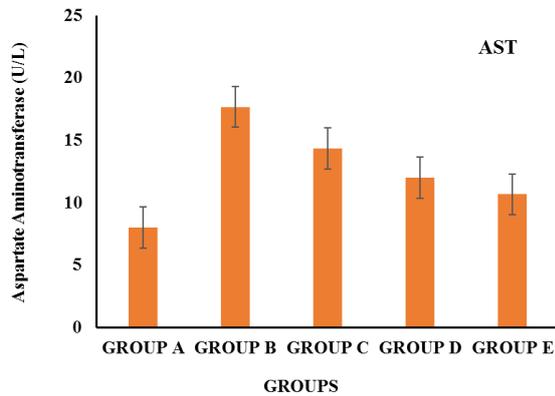


Figure 1: Effect of administration of crude *Delonix regia* seed extract on AST activity of alloxan-induced diabetic rat

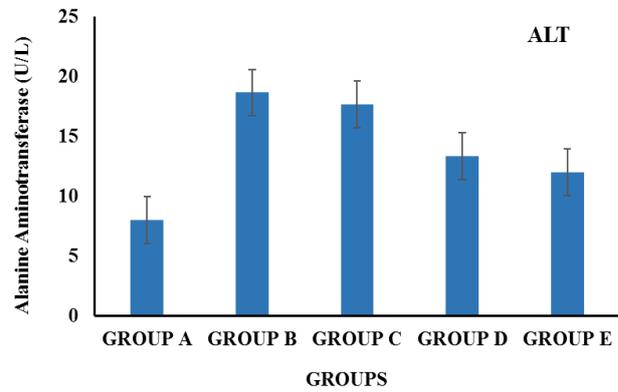


Figure 2: Effect of administration of crude *Delonix regia* seed extract on ALT activity of alloxan-induced diabetic rat

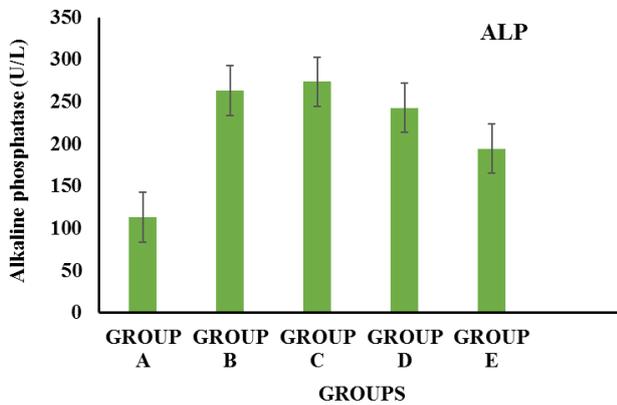


Figure 3: Effect of administration of crude *Delonix regia* seed extract on ALP activity of alloxan-induced diabetic rat

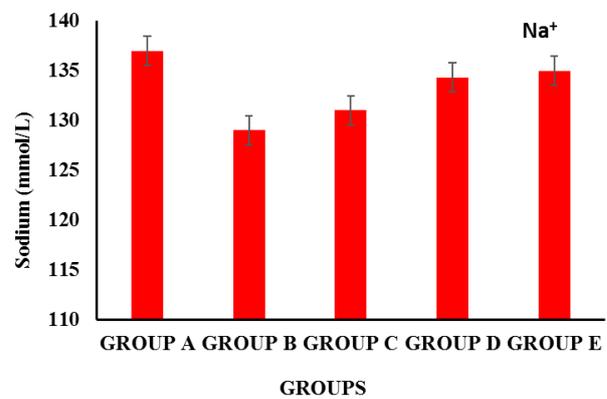


Figure 4: Effect of administration of crude *Delonix regia* seed extract on Sodium level of the alloxan-induced diabetic rat

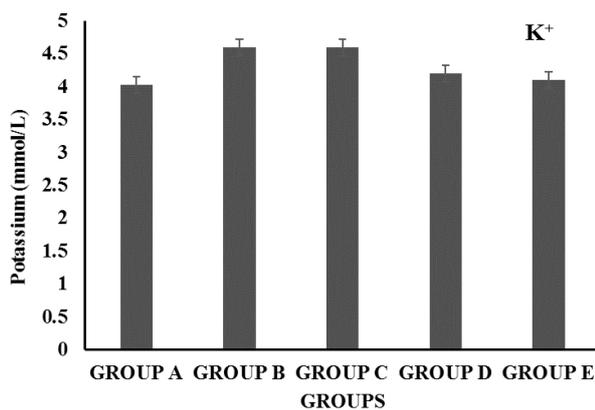


Figure 5: Effect of administration of crude *Delonix regia* seed extract on Potassium level of the alloxan-induced diabetic rat

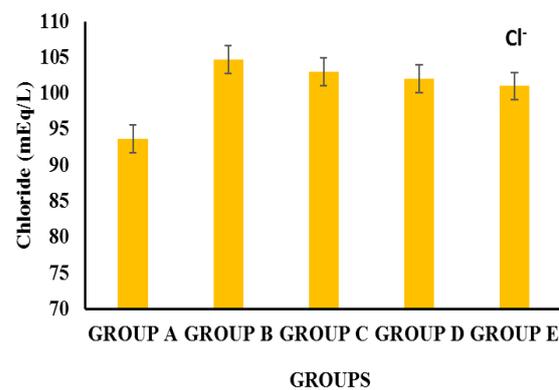


Figure 6: Effect of administration of crude *Delonix regia* seed extract on Chloride level of the alloxan-induced diabetic rat

RESULTS

Results obtained after administration of alloxan showed that all the alloxan-induced rats became diabetic after 96 hours with blood glucose which ranged between 9.35 and 10.3 mmol/L. No statistical difference was observed among the diabetic groups before the treatment. The blood glucose obtained 10 days after administration of varying dose of the extract was however significantly reduced ($p < 0.05$) in the diabetic rats treated with extract.

Compared with the control, the activities of ALT, AST and ALP in the serum were significantly ($p < 0.05$) elevated in the untreated diabetic rats (Figure 1-3). Elevations were reverted back that can be compared favourably ($p > 0.05$) with their respective control in the extract treated animals.

There was a significant ($p < 0.05$) increase in the levels of the Sodium (Na^+), Potassium (K^+) and Chloride (Cl^-) ions in the untreated diabetic rats when compared with the control and the treated rats (Figure 4-6). The administration of the crude extract of *Delonix regia* seeds significantly ($p < 0.05$) decreased the level of this parameter in the diabetic treated rats.

DISCUSSION

Plants have always been a good source of drugs. Numerous studies have revealed that a wide variety of plant extracts are effective in lowering glucose level in alloxan-induced diabetic animals.¹⁷ It has been reported that the levels of plasma enzymes such as transaminases increased significantly in conditions of tissue damage especially liver and heart due to heart disease conditions or administration of chemical agents. Diabetes mellitus (DM) is a metabolic disorder which occurs when the pancreas produces insufficient amounts of insulin, or when the individual's system fails to respond appropriately to insulin due to defects in reactive oxygen species scavenging enzymes and high oxidative stress impairing pancreatic beta cells. Characteristic symptoms of DM include increases in blood glucose, presence of glucose in urine, excessive urination, thirst, hunger, unexplained weight loss and problems with carbohydrate, fat and protein metabolism.^{18, 19}

The activities of AST, ALT and ALP have been reported to increase in alloxan-induced diabetic rats.²⁰ In this study, a significant ($p < 0.05$) increase in the activities of serum AST, ALT

and ALP in the diabetic untreated rats was observed when compared with the control. Measurement of the activities of serum enzyme markers in body fluids can be used to assess the degree of assault and the toxicity of a chemical compound on organs/tissues.²¹ Such measurements can also be used to indicate tissue cellular damage caused by a chemical compound long before it is revealed by histological techniques.¹ The seed extract was effective in significantly lowering the activities of AST, ALT and ALP when compared with the untreated diabetic rats. It has been suggested that one of the contributing factors in the complications of diabetes and other endocrine disorder is electrolytes imbalance resulting from kidney failure, dehydration and fever and vomiting.²² The increased volume and metabolites excretions via the kidneys, usually in excess of normal thresholds give rise to imbalance in homeostasis with respect to electrolytes.²³ It imperative that the increased electrolytes and water levels usually observed in diabetes could lead to depletion of the extracellular fluid electrolyte and thus lead to the excretion of the electrolyte by parietal and non-parietal cells,²⁴ which may account for the observed significant decrease in the serum Na^+ and K^+ of the diabetic untreated rat when compared with control. On the other hand, the significant increase in serum Cl^- may be due to renal tubular acidosis or metabolic acidosis. However, the reversal of the electrolytes following oral administration of extract suggests that the extract could effectively attenuate the altered extracellular fluid electrolytes levels of the diabetic treated rats. The significant reduction of the elevated blood glucose in the diabetic rats to values comparable with the control by the crude ethanol extract of *Delonix regia* in this study shows the hypoglycemic activity of the extract. This study has also revealed that the crude ethanol extract of *Delonix regia* effectively ameliorates some complications and metabolic imbalance associated with diabetes. The 20% dilution of the extract administered expressed a higher anti-diabetic activity compared to the other dilutions. This report complements an early findings^{25, 26} on its anti-diabetic activities and justifies its safe use traditionally as an herbal supplement in the management of diabetes mellitus.

CONCLUSION

The present study suggests that seed extract of *Delonix regia* possess hypoglycaemic activity and may have the potential to reduced elevated serum enzyme activities caused by alloxan-induced diabetes. Further work is needed to isolate and

characterise the anti-diabetic bioactive compound(s) present in the extract, elucidation of its possible mode of action(s) and its toxic implications in the organs of experimental animals.

REFERENCES

1. Joel EB, Lenka JL, Luka CD. Effect of Aqueous Leaf Extract of *Murraya Koenigii* on Some Biochemical and Haematological Indices of Normal and Alloxan-Induced Diabetic Rats. *Journal of Biological Science and Bioconservation*. 2014; 6(2):72 – 87.
2. Wild SG, Roglic A, Green R, Sicree R, King H. Global prevalence of diabetes: estimated for the year 2000 and projection for 2030. *Diabetes Care*. 2004; 27(5):1047-1053.
3. Rother KI. Diabetes treatment - bridging the divide. *The New England Journal of Medicine*. 2007; 356 (15): 501-1499.
4. Merck Manual Professional, *Diabetes Mellitus: Diabetes Mellitus and Disorders of Carbohydrate Metabolism*. Merck Publishing. 2010.
5. Shoback D. Greenspan's basic & clinical endocrinology (9th ed.). New york: McGraw-Hill Medical. 2011
6. Riserus U, Willett WC, Hu FB. Dietary fats and prevention of type 2 diabetes. *Progress in Lipid Research*, 2009; 48(1): 44-51.
7. Malik VS, Popkin BM, Bray GA, Despres JP, Hu FB. Sugar-sweetened beverages, Obesity, Type 2 Diabetes and cardiovascular disease risk. *Circulation*, 2010; 121(11): 64-1356.
8. Lee IM, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT. Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of Burden of disease and life expectancy. *The Lancet*, 2012; 380 (9838):219-229.
9. National Diabetes Clearinghouse, National Diabetes Statistics. U. S. Department of Health and Human Services, 2011.
10. Cooke DW, Plotnick L. Type 1 diabetes mellitus in paediatrics. *Paediatrics Review*, 2008; 29 (11):84-374.
11. American Diabetes Association. Insulin Basics. Reviewed 26 July, 2015.
12. Kliber A, Szkudelski T, Chichlowska J. Alloxan stimulation and subsequent inhibition of insulin release from in situ perfused rat pancreas. *Journal of Physiology and Pharmacology*, 1996; 47:8-321.
13. Zhang H, Zdolsek JM, Brunk UT. Alloxan cytotoxicity involves lysosomal damage. *Acta pathologica, microbiologica, et immunologica Scandinavica*, 1992; 100:309-16.
14. Ebel H, Peschke D, Bromme HJ, Morke W, Blume R, Pescke E. Influence of melatonin on free radical-induced changes in rat pancreatic beta-cells in vitro. *Journal of Pineal Research*, 2000; 28:65-72.
15. Etuk EU. Animal models for studying diabetes mellitus. *Agriculture and Biology Journal of North America*, 2010; 1:14-130.
16. Jameel A, Nirmal SA, Rub RA, Budhavale SK, Pattan SR. An Overview Of *Delonix Regia*: Chemistry and pharmacological profile. *Pharmacologyonline*, 2009; 2: 544-549.
17. Sikarwar MS, Patil MB. Antidiabetic activity of *Crateva nurvala* stem bark extracts in alloxan-induced diabetic rats. *Journal of Pharmacology and Bioavailability Science*. 2010; 2:18-21.
18. Adesokan AA, Oyewole OI, Turay BMS. Kidney and Liver Function Parameters in Alloxan-Induced Diabetic Rats Treated with Aloe *Barbadensis* Juice Extract. *Sierra Leone Journal of Biomedical Research*. 2009; 1 (1): 33-37.
19. Aloulou A, Hamden K, Elloumi D, Ali MB, Hargafi K, Jaouadi B, Ammar E. Hypoglycemic and antilipidemic properties of kombucha tea in alloxan-induced diabetic rats. *BMC Complementary and Alternative Medicine*. 2012; 12:63.
20. Nwanjo HU. Studies on the effect of aqueous extract of *Phyllanthus niruri* on plasma glucose level and some hepatospecific markers in diabetic Wistar rats. *International Journal of Laboratory Medicine*. 2007; 2 (2):1-18.
21. Yakubu MT, Bilbis LS, Lawal M, Akanji MA. Effect of repeated administration of sildenafil citrate on selected enzyme activities of liver and kidney of male albino rats. *Nigerian Journal of Pure and Applied Sciences*. 2003; 18: 156-305.
22. Rao GM. Serum electrolytes and Osmolarity in Diabetes mellitus. *Indian Journal of Medical Science*. 1992; 46: 301-303.
23. Tanko Y, Sada NH, Mohammed KA, Jimoh MY, Mohammed A. Effect of ethanolic extract of *Caralluma daizielli* on serum electrolytes levels of fructose-induced diabetes in Wistar rats. *Annual Biochemistry Research*. 2013; 4:157-166.

24. Onunogbo CC, Ohaeri OC, Eleazu CO. Effect of mistletoe (*Viscum album*) extract on the blood glucose, liver enzyme and electrolyte balance in alloxan induced diabetic rats. *American Journal of Biochemistry and Molecular Biology*. 2012; 2150-4210.
25. Mahafuzur R, Nazmul H, Asish KD, Tozammal H, Rownak J, AK, Mohammed R. Effect of *Delonix Regia* Leaf Extract on Glucose Tolerance in Glucose-induced Hyperglycemic Mice. *African Journal Traditional, Complementary and Alternative Medicine*, 2011; 8(1): 34–36.
26. Shantha SN, Muthusamy P, Radha R. Evaluation of anti-diabetic activity on ethanolic extract of *Delonix regia* seeds *International Journal of Advanced Research and Development*, 2016; 1(4): 83-86.

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