

**EFFECT OF ETHANOL LEAF EXTRACT OF *Lantana camara* ON FASTING BLOOD GLUCOSE,
BODY WEIGHT AND KIDNEY FUNCTION INDICES OF ALLOXAN-INDUCED DIABETIC ALBINO
WISTAR RATS.**

ABSTRACT

The study was aimed at investigating the effect of ethanol leaf extract of *Lantana camara* on the fasting blood glucose, body weight and kidney function indices of alloxan-induced diabetic albino wistar rats. Thirty male albino wistar rats of weight 100-181 g were randomly assigned six groups of five rats each. Group 1 served as normal control. Group 2-6 animals were induced with 150 mg/kg body weight (B.W) of alloxan monohydrate intraperitoneally. Fasting blood glucose was checked 72hrs after induction and animals with blood glucose 200mg/dl and above were considered diabetic. Group 3 was treated with 50 mg/kg B.W of glibenclamide. Group 4,5 and 6 were orally treated with 600, 800 and 1000 mg/kg B.W of *Lantana camara* extract respectively for 21days. Blood sample was obtained by tail puncture to check glucose levels weekly with the use of glucometer. Animals treated with 600, 800 and 1000 mg/kg B.W of the extract showed significant decrease ($P<0.05$) in blood glucose, creatinine, urea and uric acid compared to untreated diabetic animals. Body weight of treated groups increased significantly ($P<0.05$) compared to the untreated group. The results obtained were synonymous to the glibenclamide treated group. This suggests that the extract possess antidiabetic potential and could be used in the treatment of diabetes. **and its complications.?**

Keywords: Diabetes, *Lantana camara*, Alloxan, Glucose, Kidney Function.

INTRODUCTION

Diabetes mellitus is a pancreatic metabolic disorder that results in the alteration of glucose metabolism due to irregularities in insulin secretion or inaction of insulin to glucose [1]. The condition is characterized by frequent urination, increased thirst, hyperglycemia. Diabetes mellitus has several aetiological progressions ranging from autoimmune destruction of β -cells and subsequent insulin deficiency (Type I) or decline in insulin activity (Type II) [2]. However, the two types of diabetes aggravate the alteration in carbohydrates, fat and proteins metabolism resulting in microvascular and macrovascular complications [3]. Other diabetes mellitus complications includes but not limited to nephropathy which culminates to dysfunction of renal function architecture, retinopathy with possible vision loss, non-alcohol fatty liver disease, cardiomyopathy,

neuropathy and hepatocellular carcinomas [4]. Diabetes Mellitus (DM) is recognized as one of the leading cause of morbidity and mortality. According to [5], the incidence of diabetes may globally rise by 48% between 2017 – 2045. Therefore, the regulation of blood glucose is a paramount strategy in abating hyperglycemia and its associated complications as well as improving the life of diabetic patients [6].

The administration of hypoglycemic agents is currently in practice to cushion the hyperglycemic effect but these chemotherapeutics possess adverse effects upon continuous usage [7]. Currently, scientific investigations and clinical evaluations have confirmed the potential use of some medicinal plants and herbal formulations in the restoration of normal glucose homeostasis. The World Health Organization (WHO) estimates that 80% of individuals in developing nations particularly Africa use traditional medicines for the treatment of different diseases [8].

Lantana camara is an important medicinal plant belonging to the family verbenaceae. Pharmacologically, *L. camara* is documented to possess hepatoprotective, antimicrobial, antioxidant, antidiabetic effects etc. [9]. It is an ornamental flowering plant with possible use in modern medicine. Phytochemical evaluation of this plant revealed the presence of bioactive compounds viz., flavonoids, phenolic compounds, alkaloids, saponins, glycosides tannins etc. [10]. Hence, the present study was aimed at investigating scientifically the effect of ethanol leaf extract of *L. camara* on the fasting blood glucose level, body weight and kidney function indices of alloxan-induced diabetic albino wistar rats.

MATERIALS AND METHODS

COLLECTION OF PREPARATION OF PLANT EXTRACT

Lantana camara was collected from a farmland in Ikot Osurua, Ikot Ekpene Local Government Area, Akwa Ibom State and identified and authenticated by a Botanist in the Department of Biological Science, Akwa Ibom State Polytechnic, Ikot Osurua. The mature fresh leaves were detached from the stems, sorted, rinsed with distilled water, sliced and dried under shade. The dried leaves were ground into fine powder using an electric blender. Thereafter, 110 g of the powder was extracted in 70% ethanol by stirring intermittently, macerated for 72 hours and filtered through Whatman No. 4 filter paper. The filtrate was concentrated to paste by heating in a water bath at 40°C and refrigerated at 4 °C for further analysis.

INDUCTION OF DIABETES

30 albino wistar rats weighing 100 – 181 g were obtained from the animal house of Department of Biological Science, Akwa Ibom State Polytechnic, Ikot Osurua. The animals were fed *ad libitum* with commercial feed and clean drinking water; and acclimatized for two weeks before experiment. The rats were divided into non-diabetic control group and experimental groups.

Diabetes was induced in the experimental rats by intraperitoneal administration of 150 mg/kg body weight of alloxan monohydrates dissolved in 0.9% saline after overnight fast. After induction all rats were allowed

free access to feed and clean water. 72 hours after induction, blood sample obtained through the tail tip puncture of the rats were used to confirm diabetes in the rats by testing for hyperglycemia using glucometer. **Albino wistar rats** with fasting blood glucose concentration of 200mg/dl and above were considered diabetic and selected for the experiment. The use of **albino wistar rats** for the study was carried out in accordance with guidelines set by Institute for Laboratory Animal Research [11].

EXPERIMENTAL DESIGN

The 30 albino rats were divided into 6 groups of 5 rats each.

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|---------|---|---|
| Group 1 | - | Normal Control Group |
| Group 2 | - | Diabetic untreated group |
| Group 3 | - | Diabetic treated group (received 5mg/kg body weight glibenclamide). |
| Group 4 | - | Diabetic rats received 600mg/kg body weight of <i>L. camara</i> , |
| Group 5 | - | Diabetic rats received 800mg/kg body weight of <i>L. camara</i> |
| Group 6 | - | Diabetic rats received 1000mg/kg body weight of <i>L. camara</i> . |

Treatment was administered once a day orally for 21 days.

DETERMINATION OF BLOOD GLUCOSE CONCENTRATION

Fasting blood glucose was determined five times in the course of the study. Firstly, before the induction of diabetes by alloxan. Secondly, after 72 hours of induction of diabetes. The measurement was continued first week, second and third week of treatment. The blood glucose levels were taken by sterilizing the tails of the **albino wistar rats** with 10% alcohol, punctured with needles and the exuded blood was allowed to touch the test strip which was inserted into a calibrated glucometer (fine test glucometer). This gave direct reading after 9 seconds in mg/dL.

MEASUREMENT OF BODY WEIGHT

The body weight of the **albino wistar rats** was tabulated five times during the study (i.e. before alloxan injection (baseline values) 72 hours after alloxan injection, first week, second week and third week of the treatment period) with an ordinal weighing scale throughout the 21day treatment.

SAMPLE COLLECTION FOR ANALYSIS

At the end of 21days treatment, the rats were made to fast overnight and then euthanized under chloroform vapour and sacrificed. Whole blood was obtained by cardiac puncture into non-heparinized tubes and were allowed to clot for 1 hour 30 minutes. The sample was then centrifuged at 4000rpm for 30 minutes to recover the serum for the various biochemical assays.

BIOCHEMICAL ASSAYS

Creatinine, urea and uric acid were determined according to the methods described by [12, 13]

STATISTICAL ANALYSIS

Data analysis was performed using minitab statistical package. Values were expressed as mean \pm standard error of mean (SEM). Statistical significance of the results between groups were determined using ANOVA.

Differences between means were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Table 1: Effect of ethanol leaf extract of *Lantana camara* on the fasting blood glucose of alloxan-induced diabetic rats.

Groups	Blood glucose Before Induction (mg/dl)	Blood glucose 72 hours after Induction	Blood glucose After 1 Week treatment	Blood glucose After 2 Weeks treatment	Blood glucose After 3 Weeks treatment
1	93.60 \pm 2.66 ^{a,b}	80.20 \pm 3.58 ^b	86.40 \pm 3.96 ^c	85.80 \pm 3.10 ^c	88.80 \pm 6.41 ^b
2	86.80 \pm 5.14 ^{a,b}	247.80 \pm 13.90 ^a	225.40 \pm 16.50 ^a	213.20 \pm 3.43 ^a	211.20 \pm 2.71 ^a
3	102.60 \pm 3.31 ^a	213.80 \pm 5.50 ^a	153.40 \pm 17.40 ^b	150.60 \pm 11.6 ^b	82.20 \pm 2.75 ^b
4	79.60 \pm 2.01 ^b	215.80 \pm 4.44 ^a	174.20 \pm 11.40 ^{a,b}	160.80 \pm 10.3b ^a	109.40 \pm 10.00 ^b
5	91.80 \pm 4.80 ^{a,b}	206.80 \pm 14.7 ^a	165.60 \pm 16.40 ^b	148.20 \pm 3.43 ^b	100.60 \pm 7.63 ^b
6	81.20 \pm 3.12 ^b	251.80 \pm 20.7 ^a	174.80 \pm 8.89 ^{a,b}	133.00 \pm 3.39 ^b	90.40 \pm 6.48 ^b

Values with different superscripts are significantly different @ $P < 0.05$ and are expressed as Mean \pm SEM.

Table 2: Effect of ethanol leaf extract of *Lantana camara* on the Bodyweight (g) of alloxan-induced diabetic rats.

Groups	Body weight Before Induction (mg/dl)	Body weight 72 hours after Induction	Body weight After 1 Week treatment	Body weight After 2 Weeks treatment	Body weight After 3 Weeks treatment
1	100.00 ± 6.63 ^c	108.00 ± 5.91 ^b	116.6 ± 7.48 ^b	107.00 ± 2.61 ^{b,c}	135.6 ± 13.0 ^{a,b}
2	156.20 ± 4.85 ^{a,b}	142.80 ± 4.03	141.4 ± 8.02 ^{a,b}	143.02 ± 2.61 ^a	112.90 ± 1.95 ^{a,b}
3	181.80 ± 6.66 ^a	150.60 ± 12.2	149.20 ± 3.40 ^{a,b}	149.12 ± 4.31 ^a	164.16 ± 9.49 ^a
4	131.20 ± 5.42 ^{b,c}	122.00 ± 4.34 ^a	156.80 ± 9.26 ^a	122.59 ± 3.80 ^b	146.82 ± 1.35 ^{a,b}
5	145.20 ± 9.72 ^{a,b,c}	100.60 ± 7.63	139.2 ± 9.19 ^{a,b}	101.86 ± 4.17 ^c	156.10 ± 2.25 ^b
6	153.80 ± 6.22 ^{a,b}	98.40 ± 4.51	156.80 ± 8.97 ^a	100.20 ± 5.08 ^c	156.00 ± 1.66 ^{b,c}

Values with different superscript are statistically significant @ P<0.05 N = 5. Values are expressed as mean ± SEM.

Table 3: Effect of ethanol leaf extract of *Lantana camara* on kidney function indices of alloxan-induced diabetic rats.

Groups	Urea (mmol/L)	Creatinine (mmol/L)	Uric acid (mg/dL)
1	7.06 ± 0.29 ^b	46.40 ± 9.35 ^b	0.07 ± 0.01 ^b
2	9.57 ± 0.65 ^a	57.80 ± 3.02 ^a	0.13 ± 0.05 ^c
3	7.34 ± 0.98 ^b	51.00 ± 1.77 ^b	0.12 ± 0.01 ^b
4	8.08 ± 1.12 ^b	54.40 ± 2.91 ^{a,b}	0.14 ± 0.05 ^{a,b,c}
5	7.60 ± 0.65 ^b	51.80 ± 4.18 ^b	0.15 ± 0.01 ^{a,b,c}
6	7.48 ± 0.31 ^b	50.60 ± 2.48 ^b	0.07 ± 0.01 ^b

Values with different superscript are statistically significant at P<0.05 and are expressed as mean ± SEM.

Diabetes is a metabolic alteration in blood glucose homeostasis due to insulin insufficiency, insensitivity or both. The control of glucose levels within the physiological milieu is necessary for effective functioning of the biological system. However, alteration of this biomolecule as a result of metabolic defects can lead to metabolic dysfunction. Alloxan is a diabetogenic agent that its mechanism of action is based on the destruction of the pancreatic beta cells through the generation of reactive oxygen species [14]. Uncontrolled diabetes is one of the predisposing factors to kidney dysfunction [15].

The study showed a non-significant difference in the fasting blood glucose of all the animals prior to induction of diabetes. However, post-diabetic induction indicated a significant increase in the fasting blood glucose of all the groups when compared to the normal control. Following 21 days treatment with leaf extract, the fasting blood glucose level decreased significant (P<0.05) when compared to the untreated

diabetic group. These observations are in line with the report of [16]. The report also corroborates with the report of [17] who studied the antidiabetic activity of *Lantana camara* fruit in normal and streptozotocin-induced diabetic rats. The increased in the glucose levels after diabetic induction suggest the destruction of pancreatic β -cells resulting in the loss of the efficacy of the pancreas to secrete insulin required for glucose absorption [18].

The reduction in blood glucose level of the animals treated with ethanol leaf extract of *Lantana camara* may be due to renewed β -cells and or increased sensitivity to insulin, activated by the constituent(s) of the plant extract [19]. The mechanism of hypoglycemic effect of the extract may be attributed to increased insulin sensitivity and upregulation of insulin receptors or short activation of the beta-cells of the pancreas resulting to insulin release [20]. Furthermore, the hypoglycemic activity of this plant could be a resultant effect of the inhibition of hepatic glucose synthesis or activation of glucose utilization by peripheral tissues as well as inhibitors to tubular renal glucose re-absorption [21]. It was observed that 1000 mg/kg bw of *L. camara* extract administration reduced blood glucose comparable to glibenclamide.

Bodyweight evaluation is one of the general indicators employed in assessing the metabolic regulation for diabetes mellitus. The study indicated a significant reduction in bodyweight of untreated diabetic group when compared to the normal. The observation is similar to the report of [22]. The decrease in bodyweight may follow the fact that diabetes mellitus is associated with increased glycogenolysis, lipolysis and gluconeogenesis which result in muscle wasting and excessive breakdown of tissue proteins [23].

Treatment of the diabetic rats significantly increased the bodyweight back to normal as also seen in glibenclamide treated group when compared to the untreated group. This finding aligns with the previous study conducted by [13]. However, the increase in bodyweight of the treated groups may be attributed to its shielding property in monitoring muscle wasting by reversing gluconeogenesis, lipolysis, glycogenolysis as well as appropriate control of glycemic status [24]. Furthermore, it could be suggested that there was a shift to carbohydrate as a source of energy with the preservation of proteins and fats which resulted in prevention of bodyweight decrease in diabetic rats treated with *L. camara* and glibenclamide. Serum creatinine, urea and uric acid of the untreated group 2 increased significantly when compared to the control group. This may be due to the deficiency or insensitivity of the insulin and subsequent inability of glucose to enter the extrahepatic tissues, thereby activating gluconeogenesis as an alternative route of glucose supply which further resulted to the generation of free glucogenic amino acids into the plasma [25]. These amino acids are then deaminated in the liver and then increase blood urea [26].

Creatinine is a metabolic intermediate of muscle creatine and its concentrations in blood is a factor of body muscle mass [27]. The increased level of creatinine in untreated diabetic group could be due to decreased body weight caused by muscle wasting as a result of the stimulation of lipolysis and proteolysis due to insulin defect [28]. The increase in uric acid concentrations observed in untreated group may follow the assertion of protein glycation which may also stimulate muscle wasting and increase the release of purine, the major source of uric acid. Administration of *L. camara* leaf extract decreased the levels of urea,

creatinine and uric acid, regulated homeostatically glucose level and subsequently protect against renal impairment due to diabetes complications.

CONCLUSION

The study revealed that ethanol leaf extract of *L. camara* at a dose of 1000 mg/kg body weight possesses effective hypoglycemic and nephroprotective effects comparable to the chemotherapeutic antidiabetic drug (glibenclamide). However, pharmacological investigations are recommended to exactly elucidate the mechanism of the observed hypoglycemic and nephroprotective potentials.

ETHICAL APPROVAL

The use of animals for the study was carried out in accordance with guidelines set by Institute for Laboratory Animal Research (ILAR) (2000).

REFERENCES

- [1] Deepthi, B., Sowjanya, K., Lidiya, B., Bhargavi, R.S. and Babu, P.S. (2017). A modern review on Diabetes mellitus: an inhibitory metabolic disorder. *Journal in silico & In Vitro Pharmacology*, 3:1-14.
- [2] Khazeem, M. M. (2011). Study the effect of *Teucrium polium* L. Aerial Parts Extract on Normal and Alloxan-Induced Diabetic mice. Iraq: College of Science, Al-Nahrain University. 101 p. M. Sc. Thesis.
- [3] Edem, G. D., Okon, K. A., Essien, S. I. and Bassey, E. I., (2021). *Lantana camara*: a potent influential factor in improving the gastric mucosa of wistar rats ravaged by ulcer. *Biological and Clinical Sciences Research Journal*,1: 17.
- [4] Piero, M. N., Njagi, J. M., Kibiti, C. M., Ngeranwa, J. N. and Njagi, E. N. M. (2012). Metabolic complications of diabetes mellitus: a review. *South Asian Journal of Biological Sciences*, 2: 37-49.
- [5] International Diabetes Federation (2017). IDF Diabetes Atlas, 8th edn. Brussels, Belgium: *International Diabetes Federation*.
- [6] Chang, M. S., Oh, M. S., Kim, D. R., Jung, K. J., Park, S., Choi, S. B., Ko, B. S. and Park, S. K. (2006). Effects of *okchun san*, herbal formulation, on blood glucose levels and body weight in a model of type 2 diabetes. *Journal of Ethnopharmacology* 103:491-5.
- [7] Westphal, S. A. and Palumbo, P. J. (2003). Insulin and oral hypoglycemic agents should not be used in combination in the treatment of type 2 diabetes. *Archieve International Medicine*, 163:1783-5.

- [8] Oyeboode, O., Kandala, N., Chilton, P. J. and Lilford, R. J. (2016). Use of traditional medicine in middle-income countries: a WHO-SAGE study. *Oxford Journal of Health Policy and Planning*. 31 (8): 984-991
- [9] Kalita, S., Kumar, G., Karthik, L., Venkata, K. and Rao, B. (2012). A Review on Medicinal Properties of *Lantana camara* Linn. *Research Journal Pharmacology and Technology* 5(6):1-6
- [10] Kensa, V. M. (2011). Studies on phytochemical screening and antibacterial activities of *Lantana camara* Linn. *Plant Sciences Feed*. 1 (5): 74-79
- [11] Institute for Laboratory Animal Research, National Research Council (2000). Humane endpoints for animals used in Biomedical Research and testing. *ILAR journal*, 41:59-123.
- [12] Weatherburn, M.W. (1967) Urease-Berthelot Colorimetric Method for in Vitro Determination of Urea. *Analytical Chemistry*, 39, 971-974.
- [13] Bartels, H. and Bohmer, M. (1972) Quantitative Determination of Creatinine. *Clinica Chimica Acta*, 37, 193.
- [14] Lucchesi, A. N., Cassettari, L. L. and Spadella, C. T. (2015). Alloxan-induced diabetes causes morphological and ultrastructural changes in rat liver that resemble the natural history of chronic fatty Liver disease in humans. *Journal of Diabetes Research*, 2: 1-11.
- [15] Gounden, V. and Jialal, I. (2019). Renal function tests. In: stat pearls. Treasure Island (fc): stat pearls publishing.
- [16] Ganesh, T., Saikat, S., Thilagam, E., Thamotharan, G., Loganathan, T. and Raja, C. (2010). Pharmacognostic and anti-hyperglycemic evaluation of *Lantana camara* (L.) var. aculeate leaves in alloxan-induced hyperglycemic rats, *International Journal of Research and Pharmaceutical Sciences* 1(3): 247-252
- [17] Venkatachalam, T. (2009). Physicochemical and preliminary phytochemical studies on the *Lantana Camara* (L.) fruits. *International Journal of Pharmacy and Pharmaceutical Sciences*. 3 (1): 52-54.
- [18] Meigs, J. B., Muller, D. C., Nathan, D. N., Blake, D. R. and Andres, R. (2003). The natural history of progression from normal glucose tolerance to type 2 diabetes in the Baltimore longitudinal study of Aging. *Diabetes* 52(6): 1475-1484.
- [19] Sharma, S. B., Nasir, A., Prabhu, K. M. And Murthy, P. S., (2006). Antihyperglycemic effect of the fruit-pulp of *Eugenia jambolana* in experimental diabetes mellitus. *Journal of Ethnopharmacology*, 104(3):367-373.
- [20] Ayodhya, S., Kusum, S. and Anjali, S. (2010). Hypoglycaemic activity of different extracts of various herbal plants. *International Journal of Research in Ayurveda and Pharmacy*, 1(1):212-224.

- [21] Eddouks, M., Jouad, H., Maghrani, M., Lemhadri, A. and Burcelin, R. (2003). Inhibition of endogenous glucose production accounts for hypoglycaemic effect of *Spergularia purpurea* in diabetic mice. *Phytomedicine* 10: 594-599.
- [22] Ewenighi, C., Dimkpa, U., Onyeausi, J., Onoh, L., Onoh, G., Ezeugwu, U., Ilo, C. and Agbapuonwu, N. (2015). Estimation of glucose level and body weight in alloxan induced diabetic rat treated with aqueous extract of *Garcinia kola* seed. *Ulutas Medical Journal*, 1(2):26-30.
- [23] Swanston-Flatt, S. K., Day, C., Bailey, C. J. and Flatt, P. R. (1990). Traditional plant treatments for diabetes. Studies in normal and streptozotocin diabetic mice. *Diabetologia* 33:462–4.
- [24] Badawneh, M., Aljamal, J., Alsehli, B. R. and Daradka, M. H. (2017). Antidiabetic Effect of Ethanolic Extract of *Traganum nudatum* On Alloxan Induced Diabetics Wistar Rats, *International Journal of Biology, Pharmacy and Allied Sciences*, 6(5): 918-930.
- [25] Middleton (Jr), E., Kandaswami, C. and Theoharides, T.C. (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacological Reviews*, 52(4):673-751.
- [26] Min, B. S., Kim, Y. H., Lee, S. M., Jung, H. J., Lee, J. S., Na, M. K., Lee, C. O., Lee, J. P. and Bae, K., (2000). Cytotoxic triterpenes from *Crataegus pinnatifida*. *Archives of Pharmacal Research*, 23(2):155-158.
- [27] Ani, O .N., Udedi, S. C., Akpata, E. I., Ezeigwe, O. C., Oguazu, C. E., Onyishi, C. K. and Nwakaudu, E. N. (2020). Effects of Ethanol Leaf Extract of *Justicia carnea* on Biochemical Indices of Alloxan-Induced Diabetic Rats. *Journal of Biotechnology and Biochemistry (IOSRJBB)*, 6(2): 39-46.
- [28] Blann, A. (2014). Routine blood tests 1: why do we test for urea and electrolytes? *Nursing Times*, 110(5): 19-21.

These references to be referred by the author and to be incorporated

Kazmi, I., Rahman, M., Afzal, M., Gupta, G., Saleem, S., Afzal, O., & Anwar, F. (2012). Anti-diabetic potential of ursolic acid stearyl glucoside: A new triterpenic glycosidic ester from *Lantana camara*. *Fitoterapia*, 83(1), 142-146.

Kazmi, I. (2012). *Characterization and anti-diabetic evaluation of Lantana camara*. LAP Lambert Academic Publishing.

Balti, T., Charradi, K., Mahmoudi, M., Oueslati, N., Limam, F., & Aouani, E. (2022). Paradoxical anti-diabetic effect of *lantana camara* leaf extract and pancreatic oxidative stress relieved by grape seed and skin extract. *Pharmaceutical chemistry journal*, 55(11), 1219-1228.

Jawonisi, I. O., & Adoga, G. I. (2015). Hypoglycaemic and hypolipidaemic effect of extract of *Lantana camara* Linn. leaf on alloxan diabetic rats. *J Nat Sci Res*, 5, 6-10.