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ANTIDIABETIC ACTIVITY OF *LEONOTIS NEPTEFOLIA* LINN IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

The concerned study reveals the experimental investigation of the biological activity of *Leonotis nepetifolia* belong to Lamiaceae (mint) family, commonly known as annual lion's ear widely used in traditional system of medicine for the treatment of disease. The present study was designed to investigate the antidiabetic activity of ethanolic whole plant extract *of Leonotis nepetifolia* (EELN) in alloxan induced diabetic rats. EELN was administered at a dose of 250 and 500mg/kg(p.o) to alloxan induced diabetic rats. Glibenclamide used as a reference standard. Glibenclamide and results indicated the dose dependent effect, The antidiabetic activity produced by the extract may be due to increased uptake of glucose at the tissue level or by an increase in pancreatic beta cell function or due to inhibition of intestinal absorption of glucose. The effects of ethanol extracts on alloxan model were significant($p \leq 0.05$) and supports the use of this herbal drug as antidiabetic.

Key Words: Anti-diabetic, Alloxan model, EELN, Glibenclamide.

INTRODUCTION

Diabetes mellitus (DM) is a common metabolic disorder marked by elevated blood glucose concentration and excretion of glucose in urine, DM occurs either because of lack of insulin or the presence of factors that oppose the actions of insulin. The result of the insufficient action of insulin is an increase in blood glucose concentration higher than 160mg/dl which is above the normal value of 80-120mg/dl in humans. Statistics have shown that about 10% of the world's population suffers from DM. There are two major types of DM. Type 1 DM also known as Insulin Dependent Diabetes Mellitus (IDDM) is caused by massive loss of insulin secreting beta cells which could be as a result of viral or bacterial infection. Type 2 DM or Non-insulin Dependent Diabetes Mellitus (NIDDM) is caused by a combination of insulin resistance and altered insulin secretion, which disrupt the metabolism of glucose. Hyalinization and fibrosis are seen in the pancreatic islets of Langerhans, and the peripheral target cells are deficient in insulin receptors [1-5].

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Pathogenesis [6]

Type I diabetes mellitus (type 1 DM)

Type 1 DM is a chronic autoimmune disease associated with selective destruction of insulin-producing pancreatic beta cells. The onset of clinical disease represents the end stage of bcell destruction leading to type 1 DM. Several features characterize type 1 DM as an autoimmune disease:

[a] presence of immuno-competent and accessory cells in infiltrated pancreatic islets;
[b] association of susceptibility to disease with the class II (immune response) genes of the major histocompatibility complex (MHC; human leucocyte antigens HLA);
[c] Presence of islet cell specific autoantibodies;
[d] alterations of T cell mediated immunoregulation, in particular in CD4+ T cell compartment;
[e] the involvement of monokines and TH1 cells producing interleukins in the disease process.

[f] response to immunotherapy;
[g] frequent occurrence of other organ specific autoimmune diseases in affected individuals or in their family members. The mechanisms that cause the immune system to mount a response to this small population of highly specialized cells have been intensively studied.

Type 2(non-insulin-dependent diabetes mellitus (type 2 DM)

Type 2 DM has a greater genetic association than type 1 DM. The 100% concordance rate in identical twins is thought to be overestimated. The genetic factors are more important than environmental factors. Pancreatic abnormalities in islet secretory cells in type 2 DM are noted in beta, alpha and delta cells of the islets. Defects involving insulin secretion include relative decrease in basal secretion, decreased first and second phases of insulin response, glucose insensitivity and amino acid hypersensitivity of insulin release. Number and volume of beta cells are usually decreased to half of the normal, and, the alpha cell mass is increased leading to hyperglucagonemia. The islets exhibit hyalinization and amyloid deposition, containing islet amyloid polypeptide (IAPP) or amylin. This is a minor secretory peptide of the beta cells, released along with insulin and C-peptide, but its role in the pathogenesis of type 2 DM is not well understood [7]. This amylin is thought to produce insulin resistance [8]. IAAP is reduced with progression of type 2 DM [9]. Intimate contact between beta cells and amyloid deposit in type 2 DM is noted by electron microscopy [10]. Away from the islets in the exocrine pancreas, fatty infiltration and diffuse fibrosis are evident. Defective islet cell function is the primary event which may be due to an autoimmune reaction producing hyperglycemia in type 2 DM [11].

The treatment of diabetes mainly involves a sustained reduction in hyperglycemia by the use of biguanides, thiazolidinediones, sulfonylureas D-phenylalanine and α-glucosidase inhibitors in addition to insulin. However, due to unwanted side effects the efficacies of these compounds are debatable and there is a demand for new compounds for the treatment of diabetes [12,13]. Hence plants have been suggested as a rich, as yet unexplored source of potentially useful antidiabetic drugs. Many traditional plants treatment for diabetes are used throughout the World. Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes but only a few have received scientific scrutiny. Among these plants is *Leonotis nepetifolia* which has been used in herbal medicine in many cultures. *Leonotis nepetifolia* belongs to the family *lamiaceae (mint family)* and has been described as a multipurpose plant which is used extensively for its medicinal properties. All parts of *Leonotis nepetifolia* possess valuable medicinal properties. It was originally native to tropical and subtropical Africa known as annual lion's ear and of common names such as: Klip Dagga, Lion's Tail, Christmas Candlestick, Candlestick and many others. The different alkaloids, flavonoids, diterpenoids, polyphenolics, iridoid glycosides and other constituents of leonotis may be involved in the observed anti-nociceptive, anti-inflammatory, arthritic effects of the plant's extract. It is also having anti-asthmatic and anti-diarrhea properties. In Trinidad's traditional medicine, an infusion is used against fever, coughs,womb prolapsed and malaria [14,15,16,]. This study was aimed at evaluating the hypoglycemic effects of ethanolic leaf extract of *Leonotis nepetifolia* on blood glucose levels in alloxan-induced diabetic on Wistar rats.

MATERIALS AND METHODS

Plant material

The whole plant of *Leonotis Nepetifolia* was collected from native species growing in deciduous forests of Tirumala region, Andhra Pradesh, India. The whole plant material has been identified taxonomically and authenticated by Dr.S.Madhava Chetty, Associate Professor department of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh.

Preparation of Ethanolic Extract

The whole plant of *Leonotis nepetifolia* was dried under shade and then made into a coarse powder. The powder of *Leonotis nepetifolia* was extracted with ethanol by Soxhlet apparatus i.e finely powdered *Leonotis nepetifolia* were packed in a thimble & inserted in the soxhlet apparatus. The extraction process with drug to ethanol ratio of 1:3 was maintained for 48 hours. After 48hrs the solvent became concentrated the alcohol content was filtered through cotton and then through filter paper (What man filter paper no.1) [17]. Then the solvent was allowed to evaporate using rotary evaporator at temperature 40-45^{o}C. Thus, the highly concentrated crude extract was obtained. The extract was then preserved in the freezer for the experimental uses [18].

Experimental Animals

Wistar rats of both sexes (170-250 g) were maintained under standard animal house conditions, at ambient temperature. They were fed with standard pellet diet and water ad libitum. Fasted animals were deprived of food for at least 16 h, but were allowed free access to water. All animals were carefully monitored and maintained in accordance with ethical recommendations. The prior approval for conducting the experiments in rats was obtained from our Institutional Animal Ethical Committee (IAEC Reg. No. 769/2010/CPCSEA).

Drug Administration

After seven days of alloxan induction, the diabetic rats were selected and ethanolic extract was administered

orally through intragastric tube at the following doses of 250 and 500 mg /kg body weight.

Oral Glucose Tolerance Test (OGTT)

The overnight fasted (18 hr) normal rats were divided into five groups and each group consists of six animals. They were provided with drinking water only. Normal saline solution was administered to group A and B animals. Ethanol extract (250 mg/kg and 500 mg/kg) was administered by oral route to group D and E. Group C animals were received glibenclamide (3 mg/kg, b.w.) as a standard. Glucose (2g/kg) load was fed 30 minutes after the administration of dose to group B, C, D, and E where group B as control. Blood was withdrawn from tail vein under mild ether anesthesia at initial of 0min, 60min, 120min, and 180 min after glucose (glucose load, 2g/kg) administration and glucose levels were estimated using glucose strips and a glucometer (Standard diagnostics Ltd). Blood glucose levels were noted and reported [19].

Anti-diabetic study

Induction of Diabetes in rats by alloxan [20]

Rats were made diabetic by a single I.P. injection of 150mg/kg of Alloxan monohydrate dissolved in saline to overnight fasted animals. It is followed by 0.5 ml of 25% Dextrose after 2 hours of Alloxan and 5% Dextrose solution ad libitum for next 24hours. After 7 days of Alloxan, blood samples were withdrawn from rat tail vein and blood glucose levels were estimated in all animals. Animals with blood glucose level ≥ 250mg/dl (Diabetic) were selected for study.

Experimental Design

Experimental rats were divided into five groups of six animals each and treated for 21 days as follows.

Group A - Normal control

Group B - Diabetic control (receive vehicle)

Group C - Diabetic+ glibenclamide (10mg/kg b.wt)

Group D - Diabetic+ Ethanol Extract of *Leonotis neptifolia* (500 mg/kg b.wt)

Group E - Diabetic+ Ethanol Extract of *Leonotis neptifolia* (250 mg/kg b.wt)

Blood samples were collected from rat tail vein and blood glucose levels are estimated using one touch glucometer on 1^{st}, 7^{th},14^{th} and 21^{st} days.

RESULTS

The pharmacognostical studies were made on the whole plant of *Leonotis nepetifolia*, these observations will help in the botanical identification and standardization of the crude drug form and also to distinguish the drug from adultrants. Pharmacognostical evaluation designed to detect and check adultration and exhausted drug, absence of other parts of plant, presence of abnormal proportion of extrenious matter**.** Preliminary tests were carried out for the presence or absence of phytoconstituents like Glycosides, Flavanoids, Saponins, Alkaloids, Carbohydrates, Sterols, Proteins, Phenolic compounds and reducing compounds.

Phytochemical evaluation

The effects of extracts of *Leonotis nepetifolia* (500 mg/kg and 250 mg/kg) on glucose tolerance test are shown. The supplementation of *Leonotis nepetifolia* improved the glucose tolerance in the fasted normal rats after 180 minutes of treatment.

The purpose of choosing alloxan monohydrate as the diabetes-inducing agent was known to produce diabetes mellitus irreversibly with a single dose administration by selective nectrotic action on the beta cells of pancrease leading to insulin deficiency. Insulin deficiency leads to various metabolic aberrations in animals i.e., increased blood glucose level was reported.

The ethanolic extract of whole plant *Leonotis nepetifolia* against alloxan induced diabetic rat studies, Showed reduction in blood sugar level more over improvement of body weight of the extract treated animal further supports the anti-diabetic effect as diabetic condition is associated with loss of body weight.

Table 1. Phytochemical Evaluation of Extract of *Leonotis Neptefolia* (Whole Plant)

Type of phytoconstituent	Water extract	Ethanol Extract
Alkaloids	-	+
Carbohydrates	+	+
Flavanoids	+	+
Glycosides	+	+
Saponins	+	-
Steroids & Terpinoids	-	+
Proteins and amino acids	+	+
Fixed oil and volatile oil	-	-
Phenolics and Tannins	+	+

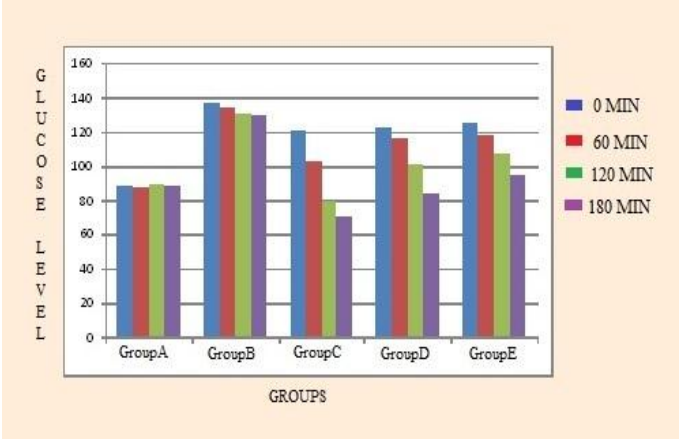
Table 2. Effect of Ethanolic Extract of *Leonotis Neptefolia* on Blood Glucose Level in Oral Glucose Tolerance Test

GROUPS	0 min	60 min	120 min	180 min
Group A	89±2.82	88±3.29	90±3.77	89±4.24
Group B	137±4.71	134±7.54	131±8.01	130±8.48
Group C	121±8.95	103±9.42	80±5.18	71±5.65
Group D	123±6.12	116±6.59	101±7.07	84±0.47
Group E	125±0.94	118±1.41	107±1.88	95±2.35

Table 3. ***Effect of Ethanolic extract of Leonotis neptifolia on blood glucose level in alloxan induced diabetes***

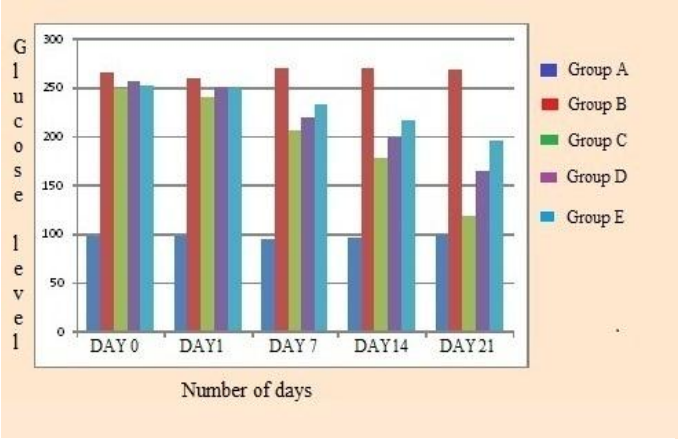
GROUPS	0 DAY	1ST DAY	7th DAY	14th DAY	21st DAY
Group A	98±1.88	97±4.24	95±1.41	96±0.94	99±2.35
Group B	266±0.47	260±3.77	269±3.29	270±1.41	268±3.77
Group C	249±4.24	240±1.88	206±2.82	177±3.29	119±3.09
Group D	256±3.77	251±0.94	219±4.24	198±2.35	164±2.82
Group E	252±2.35	248±4.71	232±6.12	216±5.65	196±5.18

Figure 1. ***Effect of different groups on blood glucose(mg/dl) level in Oral Glucose tolerance test***



Values are given as mean ± S.E.M for groups of six animals each. Group'D' EELN (500mg/kg) treated diabetic rats were compared with Group 'C' glibenclamide *treated diabetic rats at 60, 120, and 180min. The decreased blood glucose level of the test animals (Group'D' & 'E') shows that the extract exhibit significant antidiabetic activity when compared to standard* glibenclamide *and results indicated the dose dependent effect.*

Figure 2. ***Effect of different groups on blood glucose(mg/dl) level in alloxan-induced diabetic***



*Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at *p<0.05. Group'D'&'E'-EELN (250mg/kg, 500mg/kg) treated diabetic rats were compared with Group'C'-* glibenclamide *treated diabetic rats. The decreased blood glucose level of the test animals (Group'D' & 'E') shows that the extract exhibit significant antidiabetic activity when compared to standard* glibenclamide *and results indicated the dose dependent effect.*

From the above results Crude ethanol extracts exhibited antidiabetic property in Alloxan – induced diabetic rats as evident from the findings. At the 21st day of post administration of ethanol extracts, Reduction of blood glucose in the treated group appear to be higher than that in the untreated group. The decreased blood glucose level of the test animals shows that the extract exhibit significant antidiabetic activity when compared to standard glibenclamide and results indicated the dose dependent effect, The antidiabetic activity produced by the extract may be due to increased uptake of glucose at the tissue level or by an increase in pancreatic beta cell function or due to inhibition of intestinal absorption of glucose. The effects of ethanol extracts on alloxan model were significant ($p \leq 0.05$) and supports the use of this herbal drug as antidiabetic.

Statistical-Analysis

Data were expressed as means ± standard error mean. Statistical comparisons were made by using one-way ANOVA followed by TUKEY'S multiple comparison test. The level of significance was set at 0.05.

CONCLUSION

Our preliminary phytochemical analysis has indicated that flavonoids and alkaloids have been reported to exert potent hypoglycemiceffect, and that ethanolic whole plant extracts of *Leonotis neptefolia* at high dose

(500mg/kg) exhibited significant anti-diabetic activity than ethanolic whole plant extracts at low dose(250mg/kg) in alloxan induced diabetic rats. Further investigation is in necessary to determine the exact phytoconstituents responsible for anti-diabetic activity.

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REFERENCES

1. Steiner DF, Tager HS, Chang SJ, Nanjo K, Sanke T, Rubenstein AH. Lessons learned from molecular biology of insulin-gene Mutations. *Diabetes care*, 13, 1990, 600-609.
2. Peter JW. Diabetes, In ABC of diabetes. 3rd ed. BMJ Pub 1993, 1-3.
3. Ghosh R, Sharatchandra KH, Rita S and Thokchom IS. Hypoglycemic activity of *Ficus hispida* (bark) in normal and diabetic albino rats. *Indian Journal of pharmacology*, 36, 2004, 222-225.
4. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. *Nature*, 414, 2001, 414, 782-787.
5. Akindele O Adeyi, Babatunde A Idowu, et al. Rat model of food-induced non-obese-type 2 diabetes mellitus, comparative pathophysiology and histopathology.
6. M.F. Al Homsi and ML Lukic, et al. An Update on the pathogenesis of Diabetes Mellitus, *Department of Pathology and Medical Microbiology (Immunology Unit*)Faculty of Medicine and Health Sciences, UAE University, Al Ain, United Arab Emirates.*
7. Steiner DF, Ohagi S, Nagamatsu S, et al., Is Islet Amyloid Polypeptide a Significant Factor in Pathogenesis or Pathphysiology of Diabetes? *Diabetes*, 40(3), 1991, 305-9.
8. Molina JM, Cooper GJS, Leighton B and Olefsky JM. Induction of insulin resistance in vivo by amylin and calcitonin gene-related peptide. *Diabetes*, 39, 1990, 2605.
9. Enoki S, Mitsukawa T, Takemura J, et al. Plasma islet amyloid polypeptide levels in obesity, impaired glucose tolerance and non-insulin-dependent diabetes mellitus. *Diabetes Research & Clinical Practice*, 15(l), 1992, 97-102.
10. Westermark P. Fine Structure of Islets of Langerhans in Insular Amyloidosis. *Vichows Arch. Path. Anat*, 359, 1973, 1-18.
11. Zavala AV, Fabiano DE Bruno LE, Cardoso AI, et al. Cellular and humoural autoimmunity markers in Type 2 (non-insulin-dependent.) diabetic patients with secondary drug failure. *Diabetologia*, 35, 1992, 1159-1164.
12. U.K Prospective Diabetes Study Group, Overview of six years therapy of type 2 diabetes, a progressive disease. *Diabetes*, 44, 1995, 1249-1258.
13. Moller DE. New drug targets for type 2 diabetes and the metabolic syndrome. *Nature*, 414, 2001, 821-827.
14. Mendes, John. Cote ce Cote la, Trinidad & Tobago Dictionary, Arima, Trinidad, 1986, 135.
15. http,//www.tropilab.com/lionsear.html (tropilab, exporter and wholesaler of medicinal plants, herbs and tropical seeds.
16. http,//amazoniaexotics.co.uk/index.php?p=2_25 (Amazonia exotics, rare plants and seeds)
17. Hossain MS, Ahmed M, Islam A. Hypolipidemic and hepatoprotective effects of different fractions of ethanolic extract of immature leaves of *Mangifera indica* (Linn.) in alloxan induced diabetic rats. *International Journal of Pharmaceutical Sciences and Research*, 1(11), 2010, 132-138.
18. M. Asadujjaman, M.S. Hossain Islam. Antihyperglycemic and glycogenesis effects of different fractions of *brassica oleracea* in alloxan induced diabetic rats. *International Journal of Pharmaceutical Sciences and Research*, 2(6), 2011.
19. G. Jagatha and N. Senthilkumar. Evalution of anti-diabetic activity of methanol extract of *digera muricata* (l) mart in alloxan induced diabetic rats. *International Journal of Pharmaceutical Sciences and Research*, 2(6), 2011.
20. Ravinder Sangala, Devender Rao Kodati. Evaluation of Antidiabetic Activity of *Annona Squamosa Linn Seed* in Alloxan – Induced Diabetic Rats. *International journal of preclinical research*, 2, 2011.