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Research Article

MODULATORY EFFECT OF *LEUCAS ASPERA* ON OXIDATIVE STRESS AND GLUCOSE METABOLISM AGAINST DIABETIC COMPLICATIONS IN EXPERIMENTAL RATS

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ABSTRACT

Diabetes complications is associated with alterations in metabolic enzymes, hormones, lipid peroxidation leading to damage of liver and kidney. In the present study the traditional usage of *Leucas aspera* Wild against diabetes and its complications is explored experimentally by using *in-vivo* alloxan-induced diabetic rats. The results revealed that aqueous leaf extract at a dose of 200mg/kg b.w. exhibited significant decrease in ($P<0.05$) blood glucose levels and increase in body weight, insulin and C peptide levels. The changes in glucose haemostasis and metabolic alterations in the enzyme levels are due to lack of insulin and is reverted back to near normal level after the administration of *Leucas aspera* leaf extract. Meanwhile the storage of glycogen in the liver is significantly improved reducing the complications associated with diabetes. Similarly, the alterations in atherogenic index which is the main cause of cardiovascular diseases was inverted to near normal levels by improving the quantity of the HDL-C. Liver damage and renal dysfunction is the major complication during diabetes because of frequent lipid peroxidation, but treatment with *Leucas aspera* has a pronounce effect on these markers enzymes protecting the organs form further damage. Thus the results reveal that *Leucas aspera* has the potential and can be a candidate of choice without side effect.

Key words: Free radicles, diabetes, enzymes, lipid peroxidation, lipid profile.

INTRODUCTION

Diabetes mellitus (DM) is one of the common endocrine metabolic disorders with micro and macrovascular complications leading to mortality and morbidity all over the world [1]. The International Diabetes Federation recently reported that the number of people with diabetes will escalate from 246 million at present to 380 million by 2025 [2]. Changes in lifestyle, intake of energy-rich diets, and obesity are some of the factors causing the rise in the number of diabetics. It is evident that free radicals produced in the human system triggers oxidative damage by release of reactive oxygen species (ROS) and reactive nitrogen species (RNS) from activated neutrophil and macrophages. This over production leads to damage of various organs leading to various diseases like heart diseaseautism, cancer, diabetes, arthritis, Alzheimer's dementia, Parkinson's disease, cataracts and aging[3].

One third of diabetic patients (Type II) are treated with oral hypoglycaemic agents to stimulate the insulin production. The most common antidiabetic drugs are sulfonylurea, thiazolidinedione, biguanides, glinides and glibenclamide[4]. They produce adverse side effects like weight gain, gastrointestinal disorders, peripheral edema and liver disease[5]. Due to severe side effect of the chemically synthesized drugs, plants are widely used to treat diabetes. World Health Organization (WHO) also approves the use of plant drugs for different diseases including Diabetes mellitus[6]. In India, there are many plants used to treat diabetes mellitus. Some of the medicinal plants also used empirically in antidiabetic and antihyperlipidemic remedies, these plants contain phytochemicals like glycosides, terpenoids, alkaloids, flavonoids etc[7].

Leucas aspera was used to treat the diabetes mellitus. *Leucas aspera* (Willd.) commonly known as 'Thumbai'. It is distributed throughout India from the Himalayas down to Sri Lanka. The plant is used traditionally as an insecticide and antipyretic agent. Flowers are valued as stimulant, cough medicine, insecticide, aperient and diaphoretic. Leaves are frequently used for rheumatism, other skin diseases and used even during snake bites [8]. It is experimentally proved by many research that *Leucas aspera* is effective against diabetes [9]. Based on the above perception an attempt was made to determine the relationship between oxidative stress due to diabetes associated complication and a potential role of *Leucas aspera* to combat the complications.

MATERIALS AND METHODS

Collection and preparation of plant material

The leaves of the *Leucas aspera* plant were collected near to Rasipuram of Namakkal district and authenticated. The leaves were cleaned with distilled water, shade dried at room temperature. The dried leaves were coarsely powdered by using electric blender and stored separately in an air-tight container for further use[10].The powdered leaf sample was first macerated in water for 24 h for proper extraction, the residue was removed by filtration and the filtrate was concentrated under reduced pressure in a rotary evaporator at 60±10ºC to yield required quantity of crude extract and the resultant extract was stored below 10 ºC used for further studies[11].

Chemicals and solvents

All the chemicals and solvents were of analytical grade and obtained from Fischer Inorganic and Aromatic Limited, Chennai, India and Alloxan monohydrate was purchased from SD Fine Chem. Limited, Mumbai, India.

Experimental animal

Experiments were performed using sexually mature male albino wiatar rats weighing around 180-200g. Rats were provided with standard laboratory chow (Hindustan Lever Ltd., Bangalore. India) and had free access to water. The experiments were designed and conducted in accordance with the institutional guidelines (Reg. No: 1011/c/06/CPCSEA).

Acute toxicity studies

The acute oral toxicity study was carried out according to the guidelines set by OECD[12]. Minimum to maximum dose of 50mg/kg bw to 5000 mg/kg bw was evaluated for toxicity. The animals were maintained individually in the polypropylene cages and monitored continuously for two days for any changes like behavioral, neurological and autonomic profiles and for any lethality.

Induction of diabetes in rats

Diabetes was induced in overnight fasted male albino rats by a single intraperitoneal injection of freshly prepared alloxan monohydrate (150 mg/kg b.w.) dissolved in normal saline (0.9 % w/v NaCl in distilled water). Blood Glucose level was measured by using One-touch select glucometer and diabetes was confirmed after 72 h of induction. Rats with fasting blood glucose level more than 250 mg/dl were considered to be diabetic and they were selected for further studies[13].

Experimental design

Rats were divided in to six groups, six rats in each group. (i) Normal control rats, (ii) alloxan induced untreated control rats (iii) diabetic rats treated with *Leucas aspera* (50 mg/kg b.w.) (iv) diabetic rats treated with *Leucas aspera* (100 mg/kg b.w.) (v) diabetic rats treated with *Leucas aspera* (200 mg/kg b.w.) (vi) diabetic rats treated with standard drug glibenclamide (10 mg/kg b.w.)[10].
Group I and Il rats were fed distilled water alone, whereas extract and the drug treatment was given in aqueous solution daily using an intragastric tube for 25 days. Fasting blood glucose was monitored for every week throughout the experiment.

Sacrifice study

The animals were deprived overnight fast, sacrificed by decapitation and the blood was collected with and without anticoagulant. Tissue samples was instantly dissected out, washed, dried and weighed to measure their antioxidant status[13].

Biochemical profiling

The blood glucose level was monitored regularly using Gluco Chek glucose estimation kit (Aspen diagnostic (P) Ltd. Delhi, India) and the results were expressed in terms of milligrams per deciliter (mg/dl) of blood. Body weight of all experimental animals was monitored with a digital weighing scale. Insulin was estimated using Radio immuno assay (RIA) kit supplied by Linco research Inc, Stat diagnostic, Mumbai, India. C peptide was estimated following the method of Finlay and Dillard[14]. Lipid profile were estimated using standard kits purchased from Transasia Bio Medical Limited, Mumbai, India. Method of Friedwald *et al*., [15] was used to determination very low-density lipoprotein (VLDL-C) and low density lipoprotein (LDL- C).

Urea, Uric acid and creatinine were estimated using standard reagent kits purchased from Coral clinical systems, Goa, India. Alanine transaminase, alkaline phosphatase using standard kits purchased from Transasia Bio Medical Limited, Mumbai, India.

Estimation of antioxidant and metabolic enzymes

The activity of SOD was assayed by the method of Kakkar *et al*.,[16], Catalase activity was estimated according to Abei's[17], Lipid peroxidase by Fraga, *et al.*,[18], Glutathione per oxidase was assayed by the method of Tappel[19]. Hexokinase[20], glucose-6-phosphatase[21], fructose-1,6-bisphosphatase[22] and Glycogen content[23] were assayed.

STATISTICAL ANALYSIS

Values are presented as means ± SEM. The statistical significance was evaluated by one-way using the statistical software SPSS Version 17 (Origin Lab Corporation, USA). The data were analyzed by one way analysis of variance (ANOVA) followed by Tukey's test.

RESULTS AND DISCUSSION
Acute toxicity studies

Results of the acute toxicity study represented non-toxic nature of the extracts with no lethality nor any toxic reactions observed at any of the doses selected until the end of the study period.

Effect of *Leucas aspera* aqueous leaf extract on body weight, blood glucose, insulin and C peptide

The blood glucose, body weight, insulin and C peptide levels in normal and experimental animal groups are summarized in Table 1. It is evident that diabetes induction causes significant increase in blood glucose; decrease in body weight, insulin and C peptide levels due to muscle wasting, loss of tissue protein and reduced secretion of the hormone, which might be due to the destruction of β-cells of pancreas there by inhibiting insulin release[11]. C-peptide and Insulin are the products of the enzymatic breakdown of proinsulin and released into the circulation in equimolar concentrations. The measurement of both C-peptide and insulin levels has been stated to be a valuable index of insulin secretion rather than insulin alone. Oral administration of aqueous leaf extract of *Leucas asperta* significantly increased the levels of plasma insulin and C-Peptide, reciprocally decreased levels of blood glucose was observed. The possible mechanism by which *Leucas aspera* bring about their hypoglycemic action may be by enhancing the insulin effects on plasma by increasing the secretion of insulin from the existing beta cells or by its release from the bound form.

Effect of *Leucas aspera* on Hexokinase, Glucose -6-phosphatase, Fructose-1.6-bisphospatase and Glycogen content

Hexokinase, Glucose-6-phosphatase and fructose-1,6-bisphophatase plays an important role in glucose homeostasis[24,25]. Imbalance in these enzymes may cause irregular metabolism increasing complications. During diabetic condition Hexokinase activity is impaired or inactivated since it is an insulin-dependent and insulin-sensitive enzyme[26]. The

altered glucose homeostasis is regulated by improving the action of Insulin and regularizing significantly the metabolic enzymes after treatment with *Leucas aspera* (Table 2). Lack of insulin effects and inactivates glycogen synthetase system and impairs liver to synthesize glycogen significantly. Administration of *Lucas aspera*, stimulated insulin synthesis, in turn activating glycogen synthetase promoting storage of hepatic glycogen (Table 2).

Effect of *Leucas aspera* aqueous leaf extract on lipid profile

Diabetes mellitus is associated with abnormal lipid metabolism leading to diabetic complication[27]. The levels of TC, LDL-C, VLDL-C and TG was significantly increased in alloxan induced diabetic rats, similarly there was a significant reduction in HDL-C (Figure 1). Treatment with *Leucas aspera* extract especially at the concentration of 200 mg/kg b.w. demonstrated significant raise in HDL-C, thereby decreasing the levels of TC, LDL-C, VLDL-C and TG. Action of Lipoprotein lipase is inhibited due to lack of insulin during diabetes leading to deranged lipoprotein[28]. Hence it is evident that treatment with *Leucas aspera* initiated production of insulin, in turn promotes the action of lipoprotein lipase to regulate the altered lipid profile which is a well-known risk factors of cardiovascular disease and reducing the risk. The results are in stream line with previously reports of other medicinal plants like *Solanum xanthocarpum*, *Cassia auriculata*, *Solanum trilobatum*[29,30].

Effect of *Leucas aspera* aqueous leaf extract on kidney function test

Diabetes mellitus is one of the major disorders which affect the kidneys; urea, uric acid and creatinine are markers of renal function. Table 3 describes the urea, uric acid and creatinine levels in normal and experimental animal groups. In alloxan induced diabetic rats, the urea, uric acid and creatinine level was significantly increased when compared to the normal rats. After treatment with 200 mg/kg b.w. of *Leucas aspera* leaf extract, the urea, uric acid and creatinine level in the diabetic rats was significantly decreased which was on par with the drug treated groups. Negative nitrogen balance with enhanced tissue proteolysis and decreased protein synthesis can contribute to increased urea levels, indicating impaired renal functions in diabetic animals. Treatment with folklore medicine has reported decline urea levels in experimental rats[31]. Hence, the results of the present study is on line the previous studies diminishing the side effects associated with diabetes.

In early phase of diabetic nephropathy, hyper filtration and increase in creatinine clearance resulting in no changes in creatinine levels. But in the later stages the creatinine level starts increasing[32]. Similar results were observed during the study but after treatment with *Leucas aspera* leaf extract creatinine levels represented decline in the treated groups, proving the ability of the plant to combat diabetic complications.

Effect of *Leucas aspera* aqueous leaf extract on liver function test

Table 4 represents the effect of *Leucas aspera* leaf extract on AST, ALT and ALP. The liver marker enzymes was significantly increased in alloxan induced diabetic rats reflecting the damage in the liver. Raise in the levels of ALT and AST under insulin deficiency is associated with irregular gluconeogenesis and ketogenesis during diabetes. Similarly, alterations in levels of ALP and Acid phosphatase (ACP) enzymes is also associated with liver dysfunction and reflecting into blood stream during diabetes[33]. Treatment with *Leucas aspera* aqueous leaf extract, especially at 200 mg/kg b.w. showed significantly decrease in the AST, ALT and ALP enzyme levels to near levels.

Table 1: Effect of *Leucas aspera* aqueous leaf extract on body weight, blood glucose, insulin and C peptide levels of control and experimental group of animals

Groups	Body weight (gm)	Blood glucose (mg/dl)	Insulin (µU/ml)	C Peptide (ng/ml)
Group I	199.17±10.68	125.17±3.71	16.37±3.71	7±0.27
Group II	130±8.37**	475.83±8.33**	6.95±0.22**	2.61±.11**
% of Change (I Vs II)	**-34.72**	**-73.69**	**-57.54**	**-77**
Group III	163.5±3.33	267.67±8.52*	9.02±0.18*	2.72±0.03*
% of Change (III Vs II)	**+25.769**	**-43.74**	**+29.78**	**+3.83**
Group IV	180.83±3.76	177±2.68*	11.37±0.09*	3.87±.16*
% of Change (IV Vs II)	**+39.1**	**-62.8**	**+63.59**	**+48.27**
Group V	189.5±6.53***	133.83±3.87**	14.08±0.11**	5.86±0.11**
% of Change (V Vs II)	**+45.769**	**-71.87**	**+102.58**	**+124.6**
Group VI	195±10.49**	100.17±0.89**	14.83±0.27**	6.21±0.19**
% of Change (VI Vs II)	**+50**	**-78.98**	**+113.38**	**+137.93**

The data were expressed as mean ± SEM and each value represents six individual observations, evaluated by one- way ANOVA followed by Tukey's test. 'P' denotes the statistical significance, * P<0.05, ** P<0.005. Diabetic control was compared with normal control and treated groups were compared with diabetic control. + & - indicates the percentage of change over the diabetic control and treated groups

Table 2: Effect of *Leucas aspera* on Hexokinase, Glucose -6-phosphatase, Fructose-1.6-bisphospatase and Glycogen of control and alloxan-induced experimental diabetes in rats

Groups	Hexokinase µmoles of glucose phosphorylated/ min /g protein	Glucose- 6-phosphatase (µmol of Pi liberated/ min/ mg protein)	Fructose-1,6-bisphospatase (µmol of Pi liberated/h/ mg protein)	Glycogen (mg/100 g tissue)
Group I	12.2±0.3	0.11±0.11	0.31±0.01	12.4±0.18
Group II	7.9±0.3**	0.3±0.04**	0.46±0.03**	7.4±0.32**
% of Change (I Vs II)	**+2.53**	**-63.33**	**+48.38**	**-40.32**
Group III	8.1±0.1	0.25±0.01	0.42±0.02	8.9±0.13
% of Change (III Vs II)	**+7.59**	**-16.66**	**-8.69**	**+20.27**
Group IV	8.5±0.1*	0.21±0.03**	0.38±0.09**	9.5±0.9*
% of Change (IV Vs II)	**+36.7**	**-30**	**-17.39**	**+28.37**
Group V	10.8±0.3**	0.18±0.02**	0.35±0.12**	11.4±1.2**
% of Change (V Vs II)	**+41.77**	**-40**	**-23.91**	**+54.05**
Group VI	11.2±0.1**	0.18±0.01**	0.33±0.41	11.2±1.4**
% of Change (VI Vs II)	**+41.77**	**-40**	**-28.91**	**+54.05**

The data were expressed as mean ± SEM and each value represents six individual observations, evaluated by one- way ANOVA followed by Tukey's test. 'P' denotes the statistical significance, ** P < 0.05 * P < 0.005, **. Diabetic control was compared with normal control and treated groups were compared with diabetic control. + & - indicates the percentage of change over the diabetic control and treated groups

Table 3: Effect of *Leucas aspera* leaf extract on urea, uric acid and creatinine levels of control and experimental group of animals

Groups	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
Group I	17.12±0.35	1.47±0.01	0.72±0.06
Group II	54.38±1.20**	2.00±0.07**	2.55±0.08**
% of Change I Vs II)	**+217.64**	**+36.05**	**+254.16**
Group III	28.47±0.59	1.80±0.03	1.70±0.03
% of Change (III Vs II)	**-47.64**	**-10**	**-33.33**
Group IV	26.08±0.29	1.66±0.03*	1.49±0.06
% of Change (IV Vs II)	**-52.04**	**-17**	**-41.56**
Group V	22.75±0.64**	1.50±0.02**	1.22±0.05**
% of Change (V Vs II)	**-58.16**	**-25**	**-52.15**
Group VI	19.88±0.42**	1.46±0.02**	1.00±0.05**
% of Change (VI Vs II)	**-63.442**	**-27**	**-60.78**

The data were expressed as mean ± SEM and each value represents six individual observations, evaluated by one- way ANOVA followed by Tukey's test. 'P' denotes the statistical significance, ** P < 0.05 * P < 0.005. Diabetic control was compared with normal control and treated groups were compared with diabetic control. + & - indicates the percentage of change over the diabetic control and treated groups

Table 4: Effect of *Leucas aspera* leaf extract on AST, ALT and ALP levels of control and experimental group of animals

Groups	AST (U/dl)	ALT (U/dl)	ALP (U/l)
Group I	41.43±1.42	59.58±1.03	51.52±0.87
Group II	62.29±0.64**	91.36±1.10**	83.54±0.95**
% of Change (I Vs II)	**+49.65**	**+53.34**	**+62.15**
Group III	59.15±1.72	85.83±1.33	79.37±1.15
% of Change (III Vs II)	**-5.04**	**-6.25**	**-4.99**
Group IV	50.34±0.92*	76.08±0.71*	71.81±1.34*
% of Change (IV Vs II)	**-19.18**	**-16.72**	**-14.04**
Group V	47.48±0.89**	64.20±1.42**	62.18±1.40**
% of Change (V Vs II)	**-23.77**	**-29.72**	**-25.56**
Group VI	43.37±1.32**	61.88±0.85**	58.54±0.94**
% of Change (VI Vs II)	**-30.37**	**-32.26**	**-29.92**

The data were expressed as mean ± SD and each value represents six individual observations evaluated by one-way ANOVA followed by Duncan's Test. P denotes the statistical significance at ** P < 0.05 * P < 0.005. Diabetic control rats were compared with normal group and treated groups were compared with diabetic control rats.

Table 5: Effect of *Leucas aspera* leaf extract on SOD, CAT, LPO and GPx levels of control and experimental group of animals

Groups	SOD (U/mg of protein)	CAT (µmol/min/mg of protein)	LPO (U/ mg of protein)	GPx µmol/min/mg of protein
Group I	48.51±0.79	38.39±0.73	0.18±0.03	5.65±0.05
Group II	15.95±8.90**	12.76±0.69**	0.42±0.03**	2.71±.02**
% of Change (I Vs II)	**-67.12**	**-66.7**	**+133.33**	**-52.03**
Group III	18.78±1.04	28.43±0.89	0.26±0.02	3.08±0.10
% of Change (III Vs II)	**+17.74**	**+122.80**	**-38.09**	**+13.65**
Group IV	28.59±0.60*	32.48±1.46*	0.24±0.02*	3.68±0.14*
% of Change (IV Vs II)	**+79.24**	**+154.54**	**-42.85**	**+35.79**
Group V	39.87±1.79**	35.46±0.47**	0.22±0.02**	4.64±0.08**
% of Change (V Vs II)	**+149.96**	**+177.89**	**-47.61**	**+71.21**
Group VI	40.63±3.20**	37.80±1.04**	0.19±0.02**	5.17±0.12**
% of Change (VI Vs II)	**+154.73**	**+196.23**	**-54.76**	**+90.77**

The data were expressed as mean ± SD and each value represents six individual observations evaluated by one-way ANOVA followed by Duncan's Test. P denotes the statistical significance at ** P < 0.05 * P < 0.005. Diabetic control rats were compared with normal group and treated groups were compared with diabetic control rats.

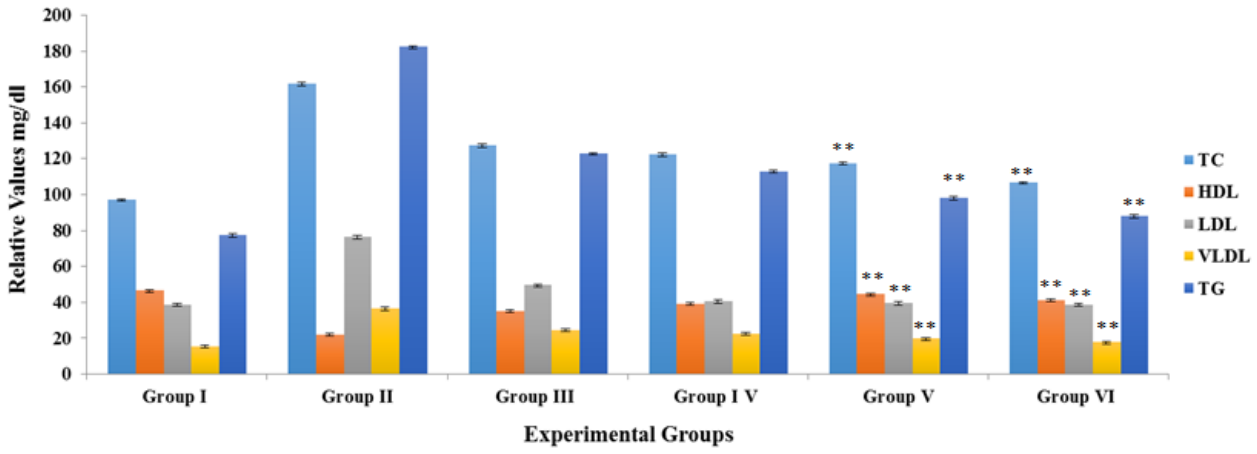

Figure 1: Effect of aqueous leaf extract of *Leucas aspera* on lipid profile of the normal alloxan induced diabetic rats

The data were expressed as mean ± SD and each value represents six individual observations evaluated by one-way ANOVA followed by Duncan's Test. P denotes the statistical significance at ** P < 0.05 * P < 0.005. Diabetic control rats were compared with normal group and treated groups were compared with diabetic control rats.

Effect of *Leucas aspera* aqueous leaf extract on antioxidant enzymes

It is evident that in diabetic condition tissue damage is considered to be mediated by free radicals by attacking membrane through peroxidation of unsaturated fatty acids leading to changes in the enzymes levels[34]. In the present study the SOD and LPO levels were increased significantly, whereas CAT and GPx were observed to be decreased when compared to the normal rats. Treatment with *Leucas aspera* leaf extract, especially with 200 mg/kg b.w. concentration showed significantly changes in all the antioxidant levels (Table 5). Decreased lipid peroxidation and increased antioxidant status may be one of the mechanisms by which drug treatment could contribute to prevent diabetic complications[35]. Therefore, administration of *Leucas aspera* has made a balance in maintaining lipid peroxidation and antioxidant status in experimental rats.

CONCLUSION

Folklore medicine usage was vanished but has regained its importance after the usage of allopathic drugs with tremendous side effects. Hence the community is looking back for folklore medications for various ailment. The current research supports the traditional usage of *Leucas aspera* against diabetes and also helps to improve the antioxidant status by diminishing the production of free radicles the culprit instigating the complications associated with diabetes.

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