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Research Article

# EFFECTS OF *LEUCAS CEPHALOTES* EXTRACT ON INSULIN RESISTANCE AND ASSOCIATED METABOLIC SYNDROME IN TYPE II DIABETIC RATS

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## ABSTRACT

***Background:*** Type II diabetes is the most common metabolic disorder caused by inadequate insulin secretion by pancreatic β-cells to compensate for insulin resistance in peripheral tissues, which leads microvascular and macrovascular complications. Novel treatment strategies for the management of insulin resistance and associated metabolic syndromes, plant based medicines contributing much with minimal side effects like lactic acidosis.

***Objective*:** As Insulin resistance and associated metabolic syndrome contributes to the pathogenesis of type 2 diabetes mellitus, we investigated whether methanolic extract of whole plant *Leucas cephalotes* (MELC) plays any role in preventing type-2 diabetes mellitus and associated metabolic syndrome in streptozotocin (STZ) induced diabetic rats.

***Materials and Methods*:** The MELC was prepared by solvent extraction procedure. Rat pups of age 5 days were divided into 4 groups, with 6 animals in each. **Group-I** pups served as vehicle control and received 1% sodium carboxy methyl cellulose (CMC), **Group-II** served as diabetic control and was treated only with single dose of streptozotocin (80mg/kg, *i.p*), **Group-III** & **Group-IV** served as test groups were treated with MELC at doses of 150mg/kg and 300mg/kg respectively for about 45 days after single dose administration of STZ (80mg/kg, *i.p*). On 30$^{th}$ and 45th day, blood samples were collected from all animals and levels of fasting glucose, fasting insulin, uric acid, Hydrogen Sulphide ($H_2S$), triglycerides and total cholesterol levels in plasma were estimated.

***Results*:** The plasma fasting glucose levels were found to be significantly ($p<0.001$) elevated in single dose streptozotocin treated diabetic control than vehicle control. However, in low dose and high dose test groups, fasting glucose levels, fasting insulin levels, insulin resistance, all the biochemical parameters associated with metabolic syndrome such as fasting insulin values, Triglyceride, Total cholesterol, $H_2S$ and Uric acid levels were significantly ($p<0.01$and $p<0.001$) decreased after chronic administration of MELC for 45 days and the decrease was more prominent with higher dose.

***Conclusions*:** The present investigation reveals that MELC administration shows significant effect on insulin resistance and associated metabolic syndrome in a dose dependent manner. So, hereby the beneficial action of MELC on human type-II diabetes may be suggested.

**Keywords:** *Leucas cephalotes;* Insulin resistance; metabolic syndrome; Streptozotocin; Type-2 Diabetes mellitus.

## 1. Introduction

Type-2 diabetes is the most common disease affecting over 50 million people in the world [1]. Type 2 diabetes mellitus ($T_2DM$) is characterized by the emergence of postprandial (post-meal) and subsequently fasting hyperglycemia (fasting plasma glucose concentrations >125 mg/dL). Hyperglycemia results from a failure of pancreatic β-cells to secrete adequate insulin to compensate for insulin resistance in peripheral tissues.

The long term consequences of type 2 diabetes make it imperative to focus on the development of novel treatment strategies for the management of insulin resistance and the metabolic syndrome. Hyperglycemia mostly associated with the pathogenesis of diabetic dyslipidaemia, micro-and macrovascular complications [2]. Hence there is a prerequisite to focus on better prevention and treatment of the devastating disease with many complications. Due to several limitations of currently available drugs including side effects like weight gain, hypoglycemia, lactic acidosis and failure of response after prolonged use, plant based medicines are gaining prominence in treatment of metabolic diseases like diabetes [3-4]. It is reported that many medicinal plants had pancreatic beta cells re-generating, insulin releasing activities to work against insulin resistance [5]. *Leucas cephalotes* (synonym: *L. Capitata)* is a herb of the family Labiatae and has been used for the treatment of asthma, paralysis, scabies, fever, coughs and colds, edema, urinary complications and diabetes [6-7]. *L.cephalotes* extract contains terpenes, sterols and flavones [7] besides glycosides and alkaloids [6]. All these active principles are known to possess hypoglycemic properties. Polyphenolic compounds, especially flavonoids, having the antidiabetic properties [8]. In this view, we have studied the antidiabetic activity of methanolic extract of *L.cephalotes* (MELC) and also effect on insulin resistance & insulin sensitivity and associated metabolic syndrome in diabetic rats by using the previously validated homeostasis models.

## 1. Materials and Methods

The process of collection & extraction of whole plant of *L.cephalotes* was reported earlier [9]. Methonolic extract of *L.cephalotes* (MELC) was used for this study.

### *2.1. Animals*

4 Pregnant Female wistar rats (300-350g) obtained from Mahaveer Enterprises, Hyderabad and housed according to the ethics of animals. These rats were delivered within 1-2 days. The experiment was initiated after getting the approval from the Institutional Animal Ethical Committee (No. VCP/2012/10/6/13). Acute oral toxicity study was performed (as per OECD-423 guidelines) by using various doses of MELC upto 2000mg/kg.

### *2.2. Experimental design*

Rat pups [neonates] were divided in to 4 groups with 6 in each:

**Group-I** – (Vehicle control group) treated with 1% Sodium CMC.

**Group-II**- (Diabetic control) treated only with Streptozotocin [80mg/kg, I.P]

**Group-III**-(low dose treated group) received MELC (150mg/kg) for 45 days after streptozotocin single dose (80mg/kg, I.P) administration.

**Group-IV**-(High dose treated group) received MELC (300mg/kg) for 45 days after streptozotocin single dose (80mg/kg, I.P) administration. [10]

### *2.3.Method of induction of type 2 diabetes*

The rat pups ($5^{th}$ day old) treated with a single 80 mg/kg (i.p) injection of streptozotocin (Sigma, St. Louis) in 0.1 M sodium citrate buffer, pH 4.5. After 45days, animals with blood glucose levels greater than 150 mg/dl were considered diabetic [11].

### *2.4. Biochemical estimations*

Blood samples collected were analyzed for few biomarkers that represent insulin resistance and metabolic syndrome. Fasting glucose, fasting insulin were estimated to calculate insulin resistance (IR) and insulin sensitivity (IS) using homeostasis model. Triglyceride (TG), total cholesterol (TC), $H_2S$ and uric acid levels were measured in plasma samples using diagnosing kits in order to assess the effect of MELC extract on metabolic syndrome.

#### *2.4.1. Insulin estimation*

Fasting insulin levels used to assess the degree of insulin resistance and also for selection of most appropriate antidiabetic drug in patients with type 2 diabetes. Elevated fasting insulin is a hallmark of the metabolic syndrome [12].

#### *2.4.2. Assessment of insulin resistance and insulin sensitivity*

Insulin resistance and insulin sensitivity is tightly associated with major metabolic problems including obesity, hypertension, coronary artery disease, dyslipidemias, and a cluster of metabolic and cardiovascular abnormalities that define the metabolic syndrome. Elevated insulin resistance and decreased insulin sensitivity depict the prevalence of type-2 diabetes mellitus.

#### *2.4.3.Hydrogen sulphide ($H_2S$) estimation*

$H_2S$ is considered to be an important vasodilator, inducing endothelium dependent and K+ATP channel-dependent vasorelaxation *in vivo* and *in vitro* [13]. The decreased or increased $H_2S$ values depict the increased or decreased

blood pressure in the body and hence reflect one of the factors of metabolic syndrome, i.e. blood pressure.

### *2.5. Statistical analysis*

All variables are expressed as mean ± SD; (n=6). One-way ANOVA followed by Dunnett's Multiple Comparison Test was used for statistical evaluation. P<0.05 was considered as statistically significant.

## 3. Results

In this study, streptozotocin (80mg/kg) successfully induces type-2 diabetes in rat pups by elevating fasting glucose levels >150 mg/dL. Results of acute toxicity studies revealed no mortality with 2000mg/kg dose of MELC.

### *3.1. Insulin resistance (IR) and insulin sensitivity (IS):*

IS and IS was calculated from fasting blood glucose levels and fasting insulin levels using homeostasis model of assessment. The estimated fasting glucose levels were on $30^{th,}$ $45^{th}$ day were reported in table 1.

**Table 1: Fasting glucose levels (mg/dL) in diabetic and MELC test groups**

| Groups/days | Vehicle control | Diabetic control | Diabetic + Low dose (150mg/kg) | Diabetic + High dose (300mg/kg) |
|---|---|---|---|---|
| **$30^{th}$ day** | 77.83 ± 9.58 | 185±12.6# | 162.66 ± 10.8* | 135 ± 11.6** |
| **$45^{th}$ day** | 74.71 ± 4.31 | 148.59±7.87# | 129.33 ± 8.70 ** | 93.51±8.65*** |

*All the values are expressed as mean ± SD (n=6); # P<0.001 VS Vehicle control; *p<0.05 ;**p<0.01,***p<0.001 VS Diabetic control*

In $30^{th}$ day samples, significant (p<0.05 and p<0.01) decrease of fasting glucose levels was observed in low dose and high dose test group respectively, but more significant (p < 0.01 and p< 0.001) decrease of fasting glucose was observed with 45 days.

Fasting insulin levels reflect the insulin resistance intensity and type 2 diabetes prevalence. The fasting insulin levels of $30^{th}$ day and $45^{th}$ day samples were estimated in all the groups and are shown in tables 2.

**Table 2: Fasting insulin levels (mU/L) diabetic and MELC test groups.**

| Groups/days | Vehicle control | Diabetic control | Diabetic + Low dose (150mg/kg) | Diabetic + High dose (300mg/kg) |
|---|---|---|---|---|
| **$30^{th}$ day** | 0.151± 0.025 | 0.698 ± 0.139# | 0.525 ± 0.094* | 0.436 ±0.115** |
| **$45^{th}$ day** | 0.16± 0.019 | 0.475± 0.073 # | 0.381± 0.075 ** | 0.215± 0.022 *** |

*All the values are expressed as mean ± SD (n=6); # P<0.001 VS Vehicle control; *P<0.05,**p<0.01, ***p<0.001 VS Diabetic control*

The insulin resistance (IR) values were calculated based on previously validated homeostasis model assessment based on formula. HOMA-IR = {Fasting Insulin in mU/L} X {Fasting Blood Glucose in mg/dl}/405. Accordingly, insulin resistance values were estimated in all the group of rats and represented in the table 3.

**Table 3: Insulin resistance levels in diabetic and MELC test groups.**

| Groups/days | Vehicle control | Diabetic control | Diabetic + Low dose (150mg/kg) | Diabetic + High dose (300mg/kg) |
|---|---|---|---|---|
| **$30^{th}$ day** | 0.030 ± 0.008 | 0. 318± 0.034# | 0.269 ± 0.022* | 0.213 ± 0.036** |
| **$45^{th}$ day** | 0.036± 0.003 | 0.174 ± 0.028 # | 0.122 ± 0.012** | 0.039 ± 0.006*** |

*All the values are expressed as mean ± SD (n=6); # P<0.001 VS Vehicle control; *p<0.05,**p<0.01,***p<0.001 VS Diabetic control.*

IS, is one of the important characteristics of type 2 diabetes mellitus and diminished values represent the prevalence of type 2 diabetes and the IS values were calculated based on previously validated homeostasis model assessment using formula, HOMA-S = 1/HOMA-Insulin Resistance and the calculated values were represented in the table 4.

**Table 4: Insulin sensitivity levels in diabetic and MELC test groups.**

| Groups/days | Vehicle control | Diabetic control | Diabetic + Low dose (150mg/kg) | Diabetic + High dose (300mg/kg) |
|---|---|---|---|---|
| **30th day** | 34.08 ± 4.35 | 3.27 ± 0.716# | 4.80 ± 0.925* | 7.62 ± 1.346** |
| **45th day** | 27.86 ± 2.81 | 5.88 ± 1.055# | 8.29 ± 0.861* | 25.55 ± 3.312*** |

*All the values are expressed as mean ± SD (n=6); # P<0.001 VS Vehicle control; *P<0.05,**p<0.01, ***p<0.001 VS Diabetic control*

Elevated triglyceride levels depict the metabolic disturbance associated with type 2 diabetes mellitus. The estimated triglyceride values were presented in table 5.

**Table 5: Triglyceride (mg%) levels in diabetic and MELC test groups.**

| Groups/days | Vehicle control | Diabetic control | Diabetic + Low dose (150mg/kg) | Diabetic + High dose (300mg/kg) |
|---|---|---|---|---|
| **30th day** | 55.27 ± 7.42 | 153.5 ± 11.04# | 122.14 ± 11.36* | 110.23 ± 9.28* |
| **45th day** | 49.33 ± 6.91 | 149.33 ± 6.97 # | 97.73 ± 8.04 ** | 74.8 ± 5.67 *** |

*All the values are expressed as mean ± SD (n=6); # P<0.001 VS Vehicle control; *p<0.05, **p<0.01,***p<0.001 VS Diabetic control.*

Elevated total cholesterol & uric acid values levels depict the metabolic syndrome of type 2 diabetes and the estimated values in all the groups of rats on 30th and 45th day were presented in table 6 & 7.

**Table 6: Total cholesterol levels (mg%) in diabetic and MELC test groups.**

| Groups/days | Vehicle control | Diabetic control | Diabetic + Low dose (150mg/kg) | Diabetic + High dose (300mg/kg) |
|---|---|---|---|---|
| **30th day** | 59.44 ± 4.91 | 133.80 ± 15.17# | 131.16 ±8.08 | 123.56 ± 7.174* |
| **45th day** | 66.87± 8.14 | 109.80± 4.31# | 93.87±5.67** | 81.21± 6.32*** |

*All the values are expressed as mean ± SD (n=6); # P<0.001 VS Vehicle control; *p<0.05, **p<0.01,***p<0.001 VS Diabetic control*

**Table 7: Uric acid levels (mg/dL) in diabetic and MELC test groups.**

| Groups/days | Vehicle control | Diabetic control | Diabetic + Low dose (150mg/kg) | Diabetic + High dose (300mg/kg) |
|---|---|---|---|---|
| **30th day** | 2.63±0.51 | 11.96 ±1.08# | 9.28 ± 1.27* | 8.92 ±1.07* |
| **45th day** | 2.57 ± 0.68 | 9.56 ± 0.86 # | 7.18 ± 0.97 ** | 4.58 ± 0.86*** |

*All the values are expressed as mean ± SD (n=6); #P<0.001 VS Vehicle control; *p<0.05, **p<0.01 ,***p<0.001 VS Diabetic control*

$H_2S$ levels are considered to be one of the important biomarker depicting metabolic syndrome as the increase or decrease in $H_2S$ values depict the blood pressure in test groups. On 30$^{th}$ and 45$^{th}$ days, the $H_2S$ values were estimated and results were represented in table 8.

**Table 8: $H_2S$ levels (μ mol/L) in diabetic and MELC test groups.**

| Groups/days | Vehicle control | Diabetic control | Diabetic + Low dose (150mg/kg) | Diabetic + High dose (300mg/kg) |
|---|---|---|---|---|
| **30$^{th}$ day** | 56.08 ± 7.85 | 12.8 ± 2.95# | 25.48 ± 3.983** | 29.23 ± 5.58** |
| **45$^{th}$ day** | 57.5 ± 6.9 | 13.2 ± 4.92 # | 24.58 ± 5.79** | 42.92± 5.34*** |

*All the values are expressed as mean ± SD (n=6); # P<0.001 VS Vehicle control; **P<0.01, ***p<0.001 VS Diabetic control;*

## 4. Discussion

In view of antidiabetic [14-15] and antioxidant reports of *L.cephalotes,* the present study investigated the effect of MELC on insulin resistance and associated metabolic syndrome type2 diabetic rats. Treatment with MELC produced a significant (p<0.05) dose and time dependent decrease in plasma fasting glucose values (table-1), indicating its antidiabetic activity. However, the hyper-insulinemia associated with type 2 diabetes, was significantly (p<0.01 and p<0.001) decreased in test groups treated with MELC than diabetic control, reflected the dose dependent and time dependent ameliorating properties of MELC on type-2 diabetic condition.

Insulin resistance, an important pathogenic mechanism in type 2 diabetes and cause of all metabolic complications, was observed to be increased in diabetic pups. However, insulin resistance was found to be significantly decreased and insulin sensitivity was significantly increased in MELC treated groups evidencing the ameliorating capability of MELC in type-2 diabetic conditions (table 3 &4).

As shown in table 5-8, the parameters represent the metabolic syndrome such as TG, total cholesterol, uric acid and $H_2S$ levels were altered in diabetic controls than vehicle control confirming the prevalence of metabolic syndrome in this model of diabetes induced by STZ in neonatal rats. However, treatment with MELC for 30-45 days to these diabetic rat pups causes significant reduction of TG, TC, uric acid and significant elevation of $H_2S$ levels indicating the beneficial effect against the metabolic disturbance in diabetes. Chronic administration of MELC normalized the gaseous molecule in the present study indicating the improvement of insulin resistance and associated metabolic syndrome. This may be due to the presence of active principles like terpenes, flavanoids, alkaloids and glycosides which have both antioxidant and anti-hyperglycemic properties [8,16]. All the results obtained were correlating with that of the results reported by Sanjay *et al*. [17] in normalizing the elevated levels of fasting glucose, fasting insulin, triglycerides, total cholesterol, $H_2S$ and uric acid levels.

## 5. Conclusion

Based on the results of the study, it was concluded that MELC was proved as antidiabetic activity and producing beneficial effects against the metabolic syndromes associated with type II diabetes in neonatal rats. Further studies are to be conducted to extrapolate these data results in humans to establish the role of extract of Leucas cephalotes in controlling type II Diabetes mellitus and its complications.

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