Original Article

EFFECT OF TORILIS LEPTOPHYLLA WHOLE PLANT EXTRACT ON ORAL GLUCOSE TOLERANCE IN GLUCOSE INDUCED HYPERGLYCEMIC RATS

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ABSTRACT

Background: Diabetes mellitus is an endocrine disorder characterized by persistently raised blood glucose levels. Defects in insulin secretion and its peripheral actions lead to imbalance in glycemic homeostasis. This research is designed to determine the effect of Torillis leptophylla on glycemic changes after oral glucose challenge in rats in comparison with glibenclamide.

Material and Methods: This study was conducted at University of Health Sciences, Lahore from April, 2016 to September, 2016. Thirty adult albino Wistar rats of male sex, weighing 140-180 g were assigned to five groups (n=6) randomly. Animals were subjected to 12 hours fast. Fasting blood glucose levels were measured at zero hour, drawing blood samples from tail vein. Afterwards, distilled water was given to group A (negative control). Whole plant extract of Torillis leptophylla was administered orally to groups B, C and D at the doses of 100, 200 and 400 mg/kg respectively. Positive control was given glibenclamide by gavage as a reference drug at the dose of 5 mg/kg following the protocol described elsewhere.15 The rats were administered 4 g/kg oral glucose load ½ hour after the drug treatment. Repeated blood sampling was done at ½, 1, 1½ and 2 hours after glucose challenge. Changes in blood glucose level were noted. Data was analyzed with the help of SPSS, taking p value < 0.05 to be statistically significant.

Results: Torillis leptophylla and glibenclamide showed significant decline in blood glucose level (5.48 ±0.35 and 4.13 ±0.13 mmol/l respectively) as compared to negative control (7.67 ±0.13 mmol/l) after oral glucose load (p value < 0.0001).

Conclusion: Torillis leptophylla possesses glucose lowering activity comparable to glibenclamide. It could be used for developing new oral agents to treat diabetes mellitus.

Key Words: Blood Glucose Level, Glibenclamide, Oral Glucose Challenge, Torillis Leptophylla.

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INTRODUCTION:

Diabetes mellitus is a global health issue, affecting around 387 million people all over the world[1]. The prevalence of diabetes mellitus in Pakistan is alarmingly on rise with current value of 16.98%[2]. Poor glycemic control contributes to disease progression as atherosclerosis occurs at faster rate in diabetic patients. Chronic complications associated with micro and macro vascular systems add to the

increasing burden of morbidities and mortalities[3].

The therapeutic management of diabetes mellitus is based on the type of diabetes and specific needs of each patient. Insulin replacement strategy is mainstay of treatment in type I diabetes mellitus. Insulin sensitizers like metformin is prescribed as first line drugs in newly diagnosed type 2 diabetics. Insulin secretagogues, mainly presented by sulfonylureas, provide excellent glycemic control by augmenting insulin discharge from the pancreas. Drugs like sitagliptin prevent degradation of incretin and lower postprandial glycemic excursions. Glucose cotransporter inhibitors prevent hyperglycemia through inhibition of glucose absorption in the kidneys. Despite good glycemic response, severe hypoglycemia, lactic acidosis, fluid retention, systemic infections, insulin allergy and many other side effects are encountered with the use of these medications[4]. There is a growing interest in evaluating new compounds having better glycemic control and fewer side effects.

Oral glucose tolerance test provides a measure of physiological ability to control carbohydrate load and predicts the efficiency of a medication in acute hyperglycemia[5]. Hyperglycemia induced by oral glucose load in healthy rats offers a model for initial testing of any agent regarding its effects on post-meal glucose excursions. A drug that could minimize this post-meal blood glucose elevation can be investigated further for its potential anti-diabetic properties. Herbal medicines are in exercise since centuries. These are accessible and economical with negligible side effects making it imperative to study their effect of on improving glycemic changes[6].

Torilis leptophylla is member of Apiaceae family. Its local name is "charikanger"[7]. It is found in Hazara, Chitral, Kashmir and Margalla hills from April to July. Presence of alkaloids, glycosides, flavonoids, tannins and terpenoids with significantly high total flavonoid content has been established via the phytochemical screening[8,9,10]. Torilis leptophylla have shown remarkable antibacterial, anti-inflammatory and neuroprotective activity[9,11]. Limited work is done regarding effect of plant on blood glucose level (BGL) as per literature review. In the light of composition and therapeutic safety in folk medicine, the present research model is designed to examine the effect of Torilis leptophylla on oral glucose tolerance in glucose loaded hyperglycemic rats.

MATERIAL AND METHODS

It was an experimental study conducted after thorough consideration and registered consent by Ethical Review Committee and Advanced Studies & Research Board, University of Health Sciences (UHS), Lahore dated 4th February, 2016. The work was carried out in Pharmacology Department, UHS, Lahore from April to September, 2016. Adult albino Wistar rats of male sex with weight around 140-180 g were procured from the Experimental Research Laboratory, UHS, Lahore. They were kept in polycarbonate cages with room temperature set at 22-24°C and humidity kept around 45-46% under the light/dark cycle of 12/12 hours (h). Standard Rat diet and water were supplied to the animal's ad libitum[7]. Guidelines provided by the Ethical Review Committee for Medical and Biomedical Research, UHS, Lahore, were strictly followed throughout the research.

Fresh flowering shoots of the plant were collected from the National Agriculture Research Centre Islamabad, shade-dried, crushed and soaked in 2 L methanol at 25°C for 48 h[8,12]. A Rotary evaporator was used to concentrate the filtrate under decreased pressure, keeping the temperature around 40°C. The concentrated extract was then freeze-dried with the help of a lyophilizer. The obtained sample was stored at 4°C[7,12]. Fresh samples were used, keeping the dose around 1 ml, with the final strengths of whole plant extract prepared as 100, 200 and 400 mg/kg body weight (b.w.) according to the groups. Aqueous solutions were vortexed scrupulously at 10 × 100 rpm to create well-homogenized mixtures.

Thirty adult male animals were allocated into 5 groups named A, B, C, D and E (n=6) randomly[13]. Rats were subjected to 12 h fast. At 0 h, blood samples were drawn from the tail vein, and fasting BGL was measured using an Accu chek active glucometer (Roche Diagnostics, SR0033182)[14]. Group A (Negative Control) was given a single dose of distilled water (d.w). Whole plant extract of Torillis leptophylla was administered orally to groups B, C and D at the doses of 100, 200 and 400 mg/kg, respectively. Group E was

administered glibenclamide 5 mg/kg b.w. of pharmaceutical grade by gavage. Glibenclamide, a sulfonylurea, is used as a reference drug in oral glucose tolerance tests to standardize the findings[15]. Half an hour after the treatment, animals were challenged with 4 g/kg oral glucose load[14]. Tail vein was punctured at regular intervals after oral glucose challenge (½, 1, 1½ and 2 h) and BGL was monitored with glucometer to assess the extent of glycemic changes[13].

Values were presented as Means ± Standard Deviation (SD). One-way analysis of variance (ANOVA) was applied to measure any statistical difference between the groups treated with whole plant extract of Torillis leptophylla, positive control group and negative control group, followed by the post hoc Tukey's test. Analysis was conducted using the SPSS 20. A difference in the Mean values of $p < 0.05$ was noted as statistically significant difference. Graphs were drawn with the help of Microsoft Office Excel 2007[7].

RESULTS

At zero-hour, blood glucose level of all animals was 5 to 5.5 mmol/l (Table 1) with no significant difference between the groups (Figure 1). Half hour after glucose challenge, group A (negative control) showed highest BGL of 9.2 ±0.44 mmol/l in comparison to group B, (Torilis leptophylla 100 mg/kg) 7.86 ±0.13 mmol/l, group C (Torilis leptophylla 200 mg/kg) 6.51 ±0.19 mmol/l, group D (Torilis leptophylla 400 mg/kg) 6.46 ±0.15 mmol/l and group E (glibenclamide 5 mg/kg; reference drug) 5.45 ±0.24 mmol/l (Table 1) with significant difference from all groups ($P < 0.001$) (Table 1). At 2 h BGL of group A was still notably higher with significant difference from all treated groups ($P < 0.001$) (Table 1).

Table 1. Changes in glycemic profile of rats after oral glucose challenge (n=6).

Group	Blood Sugar Level (mmol/l)				
Time	0 h	½ h	1 h	1½ h	2 h
Group A (Negative Control)	5.19 ±0.12	9.2 ±0.44	8.71 ±0.32	7.88 ±0.16	7.67 ±0.13
Group B (Torilis leptophylla 100 mg/kg)	5.2 ±0.14	7.86 * ±0.13	7.19 * ± 0.26	6.81 * ±0.33	6.53 * ±0.3
Group C (Torilis leptophylla 200 mg/kg)	5.25 ±0.19	6.51 * ±0.19	6.46 * ± 0.18	6.25 * ±0.26	6.15 * ±0.18
Group D (Torilis leptophylla 400 mg/kg)	5.23 ±0.2	6.46 * ±0.15	6.31 * ± 0.28	6.06 * ±0.32	5.48 * ±0.35
Group E (glibenclamide 5 mg/kg) (Positive control)	5.2 ±0.19	5.45 * ±0.24	4.82 * ±0.27	4.59 * ±0.28	4.13 * ±0.13

Results are written as Mean ±SD. * denotes p value < 0.001, indicating significant difference as compared to control group.

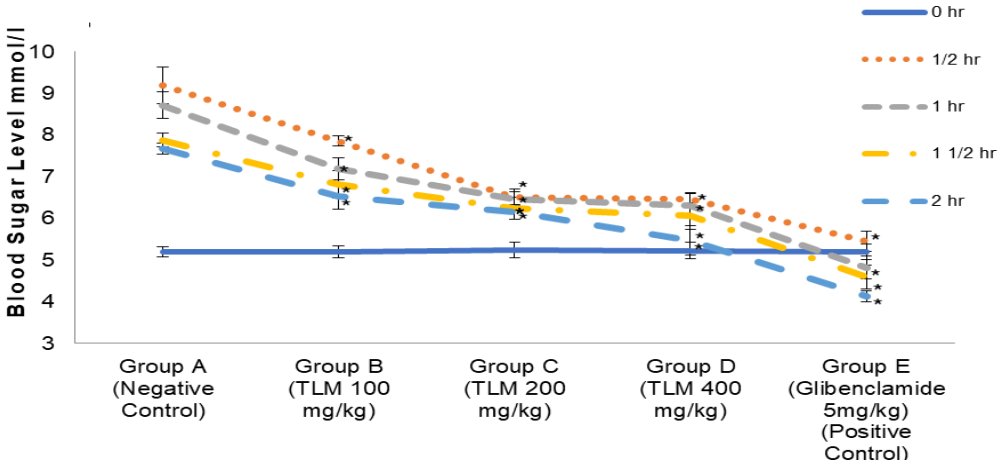

Figure 1. Changes in glycemic profile of rats after oral glucose challenge (n=6).

Results are written as Mean ±SD. * denotes p value < 0.001, indicating significant difference as compared to control group.

DISCUSSION

Diabetes mellitus is an endocrine disorder represented by gradual decline in functioning β cells of pancreas[4]. Loss of β cell function over time is best explained by individuals who progress from states of impaired glucose metabolism to advanced disease stage labeled as diabetes mellitus[16]. Suppressed insulin sensitivity further deteriorates the glycemic control in type 2 diabetic patients[4,17]. Oral glucose load is considered as highly efficient tool to assess any abnormality in glycemic homeostasis at initial stages of metabolic dysfunction[14,15].

In the present experimental study, the whole plant extract of Torilis leptophylla has shown remarkable improvement in oral glucose tolerance of rats. After the oral glucose challenge, Negative control rats initially developed a state of hyperglycemia with their BGL rising up to 90% of 0 h BGL. This is in accordance with the previous studies conducted by Zambrana et al. who exhibited around a 100% rise in BGL after the oral glucose load[13]. Torilis leptophylla minimized blood glucose excursions after glucose load and kept the BGL near to the fasting BGL value (5.48 ±0.35 mmol/l at 2 h vs 5.23 ±0.2 mmol/l at 0 h, Table1) with significant difference ($P < 0.0001$) from Negative control group (Table: 1, Fig: 1). Mnif and Aifa have reported a noticeable decrease in area under the glucose tolerance curve with Cuminum cyminum L, producing 11% decline in 2 h post load glucose level as compared to control which is significantly less than Torilis leptophylla.[18] In another study 36% decrease in 2 h post load glucose level was observed with Ammodaucus leucotrichus which is significantly more pronounced than Torilis leptophylla[19].

Glibenclamide, as a reference drug, exhibited significant increase ($P < 0.0001$) in glucose tolerance when comparison with negative control (Table: 1, Fig: 1). Similar results were earlier shown by Burnett et al. and Singh et al. They noted that glibenclamide prevented BGL from rising beyond 5 mmol/lit after oral glucose challenge[20,21]. Glibenclamide enhances insulin secretion from β cells of pancreas. The drug inhibits ATP-sensitive K^+ channels present on cell membrane leading to depolarization that ultimately open up voltage gated calcium channels. Elevated plasma calcium level then triggers the release of insulin from storage vesicles. The raised serum insulin levels prevent a steep rise in postprandial BGL after oral glucose challenge and increases glucose tolerance[22].

The whole plant extract of Torilis leptophylla produced comparable results with those of glibenclamide with 29 % decline in 2 h post load BGL. The insulin-mimetic and insulin-secretagogue activity of plant extracts contribute to their blood glucose lowering function[7,8]. Phytochemical components of plants, categorized into terpenoids, alkaloids, flavonoids, phenolics, etc. possess these antidiabetic properties as shown by many previous researches[5,8,12]. The statistically significant reduction in BGL observed with Torilis leptophylla and glibenclamide after oral glucose challenge indirectly proves insulin secretagogue activity of Torilis leptophylla. This effect may explain the plant's underlying mechanism of increasing glucose tolerance by stimulating insulin release like glibenclamide.

Many similar studies conducted on plants belonging to the Apiaceae family have shown their beneficial effects on glucose metabolism such as Anethum graveolens, Trachyspermum ammi, Foeniculum vulgare, Carum carvi, Coriandrum sativum and Pimpinella anisu[19]. The glucose lowering effect observed with Torilis leptophylla can be attributed to the significant amount of flavonoids present in it as reported by many studies[6,7,8]. It is confirmed by Saeed et al. that Torilis leptophylla possesses remarkable antioxidant properties as evidenced by a significantly reduced thiobarbituric acid reactive substance (TBARS) content of the liver ($P < 0.05$)[7]. Many previous studies have reported strong association between TBARS content in the liver and serum HbA1c levels[12,15,24].

Diabetes mellitus is associated with multiple complications such as neuropathies, ischemic heart disease, atherosclerotic degenerative changes, interstitial nephritis and retinopathies.

Polyphenolic compounds such as flavonoids naturally occur in plants and vegetables. Research conducted on these plant constituents has established clearly their hypoglycemic potential by stimulating insulin release and enhancing insulin sensitivity[25]. Flavonoids also reduce inflammation and oxidative stress thus providing a cheap and effective remedy for curing diabetes as well as its co-morbidities[26]. Further advances in the production of flavonoid-based drugs may have a significant impact on diabetes mellitus and its complications.

CONCLUSION

It can be concluded from this study that Torilis leptophylla significantly improved tolerance for oral glucose load most likely via stimulating insulin secretion like glibenclamide and can be used to develop new anti-diabetic agents. Incidence of diabetes mellitus is on rapid rise, alarmingly increasing morbidity and mortality day by day. Traditionally used medicinal plants should be explored to discover new remedies for diabetes mellitus with higher efficiency, stronger potency, lesser toxicity and long-term benefits to curtail this global pandemic.

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CONFLICT OF INTEREST:
None.

AUTHOR'S CONTRIBUTION:
TM: Conceptualization & Manuscript Writing
SB: Data Collection
NSA: Data Analysis
BS: Manuscript Writeup
SN: Literature Review
JF: Critical Review

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