Effect of *Cyclea peltata* Lam. roots aqueous extract on glucose levels, lipid profile, insulin, TNF-α and skeletal muscle glycogen in type 2 diabetic rats

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In view of multi-dimensional activity of plant drugs beneficial to complex disorders like diabetes, the present study has been undertaken to evaluate the effect of aqueous extract of *C. peltata* roots on serum glucose, lipid profile, insulin, inflammatory marker namely tumour necrosis factor (TNF)-α and muscle glycogen in type 2 diabetic rats. Aqueous extract of *C. peltata* at 40 and 60 mg/kg dose significantly decreased both the fasting and postprandial blood glucose of type 2 diabetic rats; 60 mg/kg dose having more pronounced effect on hyperglycemia. An enhanced insulin levels by the aqueous extract is primary for its glucose and lipid lowering activity. The extract significantly decreased the elevated TNF-α in type 2 diabetic rats. The extract at 40 and 60 mg/kg dose increased the glycogen levels in skeletal muscle by 58 and 60% respectively. Improved glycogen in peripheral tissue such as skeletal muscle indicates the ability of plant drug to combat insulin resistance of type 2 diabetes.

**Keywords:** *Cyclea peltata*, Glycogen, Insulin, TNF-α, Type 2 diabetes

The rising blood glucose in type 2 diabetes results due to a combination of unhealthy diet, physical inactivity and increasing abdominal adiposity in complex pathophysiologic process. Defect in insulin secretion in response to food leads to postprandial hyperglycemia an earliest metabolic abnormality to occur in type 2 diabetes. Reduced sensitivity of target tissues to the actions of insulin so called insulin resistance is a common feature of type 2 diabetes. Elevated circulating inflammatory markers such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6 are observed in patients with postprandial hyperglycemia. There is a recent notion that the activated innate immune system and chronic systemic inflammation are associated in pathogenesis of type 2 diabetes. Cytokine TNF-α stimulates the endothelial production of adhesion molecules such as E-selectin and vascular cell adhesion molecule-1 (VCAM-1) which accelerate atherosclerosis. Atherosclerosis delays the actions of insulin. In type 2 diabetes glycogen levels in peripheral tissue such as skeletal muscle are poor due to declined uptake of glucose.

*Cyclea peltata* Lam. (Menispermaceae) commonly known as paatha is a climbing shrub found throughout South and East India. Tuberous roots of the plant are used in the treatment of jaundice, stomachache, fever and asthma. The drug possesses anti-hypertensive, anti-inflammatory and immunomodulatory properties. In ayurveda decoction prepared from the roots is suggested for ‘Madhumeha’. Plant materials, their solvent extracts and purified compounds from them are useful in diabetes and associated hyperlipidemia in experimental animals and patients. Hence, the present study has been undertaken to evaluate the effect of aqueous extract of *C. peltata* roots on serum glucose, lipid profile, insulin, TNF-α and skeletal muscle glycogen in type 2 diabetic rats.

**Materials and Methods**

*Authentication of plant drug*—Roots of *C. peltata* was authenticated and deposited at Raw Materials Herbarium and Museum, National Institute of Science Communication And Information Resources (2005/Conslt/540/15), New Delhi.

*Aqueous extract and phytoconstituents*—One part of coarsely powdered (# 22: nominal mesh aperture of 710 µm) drug was boiled with 16 parts of water for 15 min and filtered hot through muslin cloth. Filtrate was then evaporated under reduced pressure in Rota-rod evaporator (Buchi RE 121, Japan). The dried
aqueous extract (5.4%) was packed in air tight container and stored in desiccators. Preliminary phytochemical analysis of the aqueous extract showed the presence of alkaloids, tannins and polysaccharides.

**Acute toxicity study**—Swiss albino mice (20-22 g) of either sex were divided into six groups of six animals each. Animals were fasted overnight but allowed free access to water prior to the experiment. Aqueous extract of *C. peltata* at different dose levels, i.e. 0.5, 1.0, 1.5, 2.0 and 2.5 g/kg body weight was administered once orally to respective experimental groups. Control group received 1 ml of distilled water. The mice were then observed for 48 h and mortality was recorded. Median lethal dose (LD₅₀) was determined according to Karber’s Method⁸.

**Dose and drug solution**—In ayurveda 5 to 10 g of the powdered root in the form of decoction is used in treatment of diabetes⁵. In considering the extractive value (5.4%) and rat metabolic rate (7 times higher than humans), 40 and 60 mg/kg/day doses of aqueous extract were selected for screening the activity⁹. The drug solution was prepared by dissolving the required quantity of aqueous extract in distilled water.

**Animals**—Wistar albino rats of either sex weighing 140-160 g were housed under standard laboratory conditions (25°C±2°C, 55 ± 5% RH with a regular 12:12 h L: D cycle). Animals were given standard rat pellet (Golden feeds, Mehrauli, New Delhi) and tap water *ad libitum*. The study protocol (Protocol number: 06/DIPSAR/ IAEC/ 2004) was approved by Institutional Animal Ethical Committee (IAEC), Delhi Institute of Pharmaceutical Sciences and Research (DIPSAR), New Delhi.

**Streptozotocin induced neonatal rat model for type 2 diabetes**—Type 2 diabetes was induced by administering streptozotocin at a dose of 90 mg/kg intraperitoneal injection in two-day-old neonatal rats. After six weeks of streptozotocin injection, rats showing the fasting blood glucose more than 160 mg/dl were considered as type 2 diabetes positive¹⁰.

**Experimental groups**—Wistar albino rats of either sex were randomly allotted into five groups of six animals each. Group I served as normal and received distilled water. Group II served as type 2 diabetic untreated control and received distilled water. Group III was type 2 diabetic treated with 40 mg/kg of aqueous extract of *C. peltata*. Group IV was type 2 diabetic treated with 60 mg/kg of aqueous extract of *C. peltata*. Group V was type 2 diabetic treated with a combination of glibenclamide (2 mg/kg) and metformin hydrochloride (175 mg/kg) prepared in 0.5% CMC suspension. Drug treatment was given on every day morning with the help of oral catheter for six weeks. Body weight was determined at the end of every week. After six weeks of drug treatment parameters such as serum fasting glucose¹¹, postprandial glucose¹², insulin¹³, lipid profile¹⁴,¹⁵ and TNF-α¹⁶ were analyzed using respective kits. Tissue glycogen was determined by colorimetric method¹⁷.

**Statistical analysis**—Data are expressed as mean ± SE. Statistical comparison between different groups was done using One-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. *P*<0.05 was considered as statistically significant.

**Results**

**Effect on blood glucose**—Aqueous extract of *C. peltata* at both doses, i.e. 40 and 60 mg/kg showed significant (*P*<0.01) decrease in fasting blood glucose of type 2 diabetic rats. Difference inbetween 40 and 60 mg/kg dose was found to be significant (*P*<0.05) when analyzed for inter-group comparison indicating that the drug at higher dose had better effect. Postprandial blood glucose was significantly (*P*<0.01) decreased with the aqueous extract. However the extract at both dose levels was unable to bring down the postprandial blood glucose to normal but was higher at 140 mg/dl (Table 1).

**Effect on lipid profile, insulin and TNF-α**—Aqueous extract of *C. peltata* at both doses, i.e. 40 and 60 mg/kg decreased the elevated serum triglycerides, total cholesterol and TNF-α in type 2 diabetic rats. The extract was able to enhance the insulin levels in diabetic rats. Total cholesterol was reduced and HDL-cholesterol was increased. Difference inbetween 40 and 60 mg/kg dose was insignificant (*P*>0.05) when analyzed for inter-group comparison with respect to lipid profile, insulin and TNF-α (Table 1).

**Effect on body weight**—Two weeks of drug treatment did not improve the body weight of diabetic rats. By the end of third week, both the doses of aqueous extract of *C. peltata* significantly (*P*<0.05) increased the body weight of diabetic rats as compared to untreated diabetic control group (Fig. 1). Progress in weight gain of drug treated animals was continued for further weeks.
Effect on muscle glycogen—Aqueous extract of *C. peltata* at 40 and 60 mg/kg doses and glibenclamide with metformin combination increased the glycogen levels in skeletal muscle by 58, 60 and 70.7% respectively as compared to untreated diabetic control group (Table 1).

Acute toxicity—The aqueous extract at various doses, i.e. 0.5, 1.0, 1.5, 2.0 and 2.5 g/kg indicated no mortality and behavioral changes in mice upto 48 h after the treatment.

Discussion

Postprandial hyperglycemia is an independent risk factor for cardiovascular diseases⁷. Most of the currently available anti-diabetic therapies reduce the fasting blood glucose but have a little impact on postprandial hyperglycemia. Aqueous extract of *C. peltata* had significant ($P<0.01$) effect on both fasting and postprandial hyperglycemia of type 2 diabetic rats. Although unable to maintain the normal levels in case of postprandial hyperglycemia, the extract had a marked effect on elevated levels. Accelerated lipolysis and decreased uptake of free fatty acids from circulation due to insulin deficiency impair the lipid profile in diabetes. Body weight of type 2 diabetic rats was found to be less during the course of development due to accelerated lipolysis. Aqueous extract of *C. peltata* decreased the triglycerides, total cholesterol and increased HDL-cholesterol in type 2 diabetic rats. Increased HDL-cholesterol is significant in preventing the atherosclerosis¹⁸. The extract showed significant ($P<0.01$) increase in serum insulin levels of type 2 diabetic rats. An enhanced insulin levels by the extract is a primary factor for its glucose and lipid lowering activity¹⁹. Roots of *C. peltata* are reported to contain tetrandrine and phaeanthine as major alkaloids (Bisbenzyl-isoquinoline type)²⁰. The drug also contains water soluble polysaccharides in high proportion⁴. Biological activity of *C. peltata* may be related to the alkaloids and polysaccharides present in it. Alkaloids of *Eugenia jambolana*²¹, *Sida cordifolia*²² and *Vinca rosea*²³ showed significant anti-diabetic activity by enhancing the secretion of insulin. Inflammatory cytokine TNF-α has direct inhibitory effect on tyrosine kinase and phosphorylation cascade of insulin signaling pathway. TNF-α mediate insulin resistance also through increasing the free fatty acids in

<table>
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<th>Parameter</th>
<th>Normal control</th>
<th>Diabetic control</th>
<th>Diabetic treated with</th>
<th>Glibenclamide &amp; metformin</th>
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| FBG (mg/dl)                | 89.2 ± 2.7     | 184.7 ± 3.6     | 135.1 ± 3.4ᵇᵇ       | 118.7 ± 3.4ᵇᵇᶜᵇᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜᵇᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜᶜ$bᶜaddComponent(1).  

The extract showed significant ($P<0.01$) increase in serum insulin levels of type 2 diabetic rats. An enhanced insulin levels by the extract is a primary factor for its glucose and lipid lowering activity¹⁹. Roots of *C. peltata* are reported to contain tetrandrine and phaeanthine as major alkaloids (Bisbenzyl-isoquinoline type)²⁰. The drug also contains water soluble polysaccharides in high proportion⁴. Biological activity of *C. peltata* may be related to the alkaloids and polysaccharides present in it. Alkaloids of *Eugenia jambolana*²¹, *Sida cordifolia*²² and *Vinca rosea*²³ showed significant anti-diabetic activity by enhancing the secretion of insulin. Inflammatory cytokine TNF-α has direct inhibitory effect on tyrosine kinase and phosphorylation cascade of insulin signaling pathway. TNF-α mediate insulin resistance also through increasing the free fatty acids in
circulation, stimulation of insulin counter-regulatory hormones or by inhibiting the glucose-stimulated insulin release by pancreatic β-cells. Chronic systemic inflammation disrupts the body’s ability to process insulin. TNF-α was found to be elevated in type 2 diabetic rats. A variety of stressors such as infection, tissue injury and food cause macrophages, adipocytes, endothelial cells etc., to secrete TNF-α. C. peltata decreased the elevated levels of TNF-α in type 2 diabetic rats. Effect on TNF-α indicate the anti-inflammatory and immunomodulatory property of C. peltata is related with its glucose and lipid lowering activity. In type 2 diabetic rats, glycogen levels of peripheral tissue such as skeletal muscle are poor. Improved glycogen levels of treated animals indicated the uptake of glucose by the peripheral tissue and thereby reduced insulin resistance of type 2 diabetes. The effect may be mediated through the up-regulation of peroxisome proliferator activated receptors (PPARs). Up-regulation of PPARs enhances glucose transporters (GLUT-4) activity which intern facilitates the uptake of glucose by the peripheral tissue. PPARs agonists promote anti-inflammatory and lipid lowering effects. Interrelated multi-dimensional activity of the plant drug C. peltata is responsible for its potent effect on type 2 diabetes where the cause and consequence are multi-factored.

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