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Pharmacological Effects of *Pimpinella tirupatiensis* on Altered Urea Cycle and Liver Function Markers in Diabetic Rats

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Abstract: Recent studies are emphasizing on cure and or prevent the diabetes-associated complications by using herbal medicines, since complications are increasing around the world. The present study was aimed to investigate the pharmacological efficacies of *Pimpinella tirupatiensis* (Pt) extracts against diabetes-associated altered urea cycle and liver function markers. In this study, diabetes was induced by streptozotocin (STZ) injection (40 mg kg^{-1} b.wt.) in rats, and treated with *P. tirupatiensis* extracts for 30 days (750 mg kg^{-1} b.wt. orally). The significant ($p<0.01$) decrease in body weights, elevated serum glucose, urea, uric acid and creatinine levels along with elevated Aspartate amino transferase (AST) and Alanine amino transferase (ALT) activities were observed in diabetic rats. Interestingly, diabetic rats treated with *P. tirupatiensis* aqueous extracts showed significantly ($p<0.01$) lowered serum glucose levels and regained body weights compared to untreated diabetic rats. Elevated urea and uric acid contents were also significantly ($p<0.01$) controlled by *P. tirupatiensis*, which were similar to the standard anti-diabetic drug glibenclamide treatment. Furthermore, increased both AST and ALT activities were attenuated by *P. tirupatiensis*, which indicates diabetes-induced adverse effects on liver function markers were alleviated. These results clearly demonstrating that increased hostile milieu in urea cycle and liver function markers under diabetic condition could be reversed by *P. tirupatiensis* treatment. This study suggests that *P. tirupatiensis* aqueous extracts may be used as anti-diabetic remedy, however further confirmatory studies are necessary.

Key words: Herbal medicine, liver function markers, diabetes, uric acid, blood glucose

INTRODUCTION

Diabetes is the world's largest endocrine disease characterized by hyperglycemia that involved with several metabolic disorders and eventually cause organ failure and multi-organ dysfunction especially the eyes, kidneys, nerves, heart, and blood vessels (Rao *et al*., 2011; Sharma *et al*., 2008). It is a disease which effect the endocrine system involving metabolic disorders of carbohydrate, protein and lipids (Lawson-Evi *et al*., 2011). The IDDM is noticed both in adult and child hood (Urger and Foster, 1998; Muhlhauser *et al*., 2000).

Although, many drugs are available to manage diabetes, in most instances these are expensive for a developing country like India and they may also have adverse effects, e.g., hypoglycemia, obesity and other disorders after chronic treatment. In recent years, many research studies are focusing on natural herbal medicine to treat the diabetes-associated complication and also to avoid the side effects (Madinov *et al*., 2000; Pari and Umamaheswari, 2000; Rates, 2001; Hu *et al*., 2003; Haque *et al*., 2011). From the ancient times, herbal medicine has been widely used to treat many diseases including diabetes in India and all over the world. Among the natural herbs, *Pimpinella tirupatiensis* (Apiaceae) locally known as 'adavikothimeera' (forest coriander) is one of the important herbaceous medicinal plant used in Indian folk medicine to treat diabetes.

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P. tirupatiensis is an endemic species with seasonal occurrence with underground tubers, distributed on Tirumala Hills (1000 m above the sea level of Chittoor district, Andhra Pradesh, India (Madhava Chetty and Rao, 1990).

However, no scientific reports on the anti-diabetic properties of *P. tirupatiensis,* and its impact on urea cycle enzymes in diabetic rats. Therefore, the study was aimed to evaluate whether *P. tirupatiensis* extracts can control the blood glucose levels and revert the altered urea cycle in streptozotocin (STZ) induced diabetic rats. Furthermore, alterations in liver function markers also monitored in plant extract treated diabetic rats.

MATERIALS AND METHODS

Preparation of plant extracts: *Pimpinella tirupatiensis* was collected from Tirumala Hills of Chittoor district, Andhra Pradesh, India and the plant material was taxonomically identified and authenticated by the concerned herbarium officer, Department of Botany, S.V. University, Tirupati Andhra Pradesh. Voucher specimen 1533 was deposited in the Department of Botany. *Pimpinella tirupatiensis* tuberous roots were collected in the month of October, were dried and powdered. The powder was stored in airtight containers and was used for the extraction of the bioactive compounds in different solvents.

Preparation of extract: The *Pimpinella tirupatiensis* tuberous root was dried in the shade, powdered and the powder was used for the extraction of potential antidiabetic principles into water solvent. *Pimpinella tirupatiensis* tuberous root powder was soaked in water in different glass jars for 2 days at room temperature and the solvent was filtered. This was repeated three to four times until the extract give no coloration. The extract was distilled and concentrated under reduced pressure in the Rotary Evaporator (Model no-HS-2005V) and finally freeze dried by lyophilizer (Lyodel). The yield of the aqueous extract is 8.25% (w/w in terms of dried starting material).

Selection of animals: Wistar strain male albino rats (n = 30), weighing 130±10 g and six months age were used in this study. All the rats were maintained in the polypropylene cages (six rats per cage), at an ambient temperature of 25±2°C with 12-h-light/12-h-dark cycle. Rats allowed free access to standard chow (Hindustan Lever Ltd., Bangalore, India) and water *ad libitum* during the study. All the experiments in this study were performed according to the regulations for the care and use of laboratory animals and this study was approved by the Institutional Animal Ethical Committee and its resolution number; 09 (ii) /a/CPCSCA/IAEC/07-08/SVU/Zool/ dated 26/6/08.

Induction of diabetes: Streptozotocin (STZ) solution was freshly prepared in 0.1 M citrate buffer (pH 4.5) and 40 mg kg^{-1} b.wt. was injected intraperitoneally in a volume of 1 mL kg^{-1} b.wt. (Rakieten *et al*., 1963). Because STZ is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose (5-10 mL) orally after 6 h of injection for the next 48 h to prevent hypoglycemia. Neither death nor any other adverse effect was observed at the tested concentration throughout the study. After one week, rats with diabetes (i.e., high blood glucose levels, 200-300 mg dL^{-1}) that exhibited glycosuria and hyperglycemia were selected for the experiment.

Experimental design: Thirty rats were divided into five equal groups and treated as follows:

- **Normal control (NC):** Six rats in this group received normal saline (0.9%) everyday for 30 days by orogastric tube
- **Diabetic control (DC):** Rats were made diabetic by a single intraperitoneal injection of streptozotocin (40 mg kg^{-1} b.wt.) as described in 'induction of diabetic' section and 6 rats considered as diabetic control for this group
- **Normal plus *Pimpinella tirupatiensis* treatment (Pt):** Rats (n = 6) were fed with aqueous extract of *Pimpinella tirupatiensis* at the dose of 750 mg kg^{-1} b.wt. per day for 30 days by an orogastric tube
- **Diabetic plus *Pimpinella tirupatiensis* treatment (D+Pt):** Another set of diabetic rats (n = 6) received *Pimpinella tirupatiensis* aqueous extracts at the dose of 750 mg kg^{-1} b.wt. for the same period of 30 days orally
- **Diabetic plus glibenclamide treatment (D+Gl):** Diabetic rats (n = 6) in this group were orally administered with a standard anti-diabetic drug glibenclamide at the dose of 20 mg kg^{-1} b.wt. daily for 30 days by an orogastric tube

Blood sample collection: After completion of 30 days treatment blood samples were collected from the retro-orbitally of the eye under moderate ether anesthesia by using capillary tubes (Micro Hematocrit Capillaries, Mucaps). Blood samples from all the rats were collected in to separate fresh vials containing sodium fluoride and sodium oxalate as anti-coagulant/anti-glycolytic agents, and plasma was separated in a electric centrifuge (Remi Udyog, New Delhi) at 2000 rpm for 2 min. Samples collection was done in the morning at 8 to 9 am stored at -20°C serum used for the biochemical analysis.

Biochemical assays: Serum glucose levels were estimated by oxidase method (Barham and Trinder, 1972). Urea and uric acid levels were estimated in the serum by the method (Fossati *et al*., 1980; Thomas, 1998a, b), respectively. Serum creatinine was estimated by the method of Thomas (1998a, b); Serum aspartate amino transferase (AST) and alanine amino transferase (ALT) activities were assayed by the method of Moss and Handerson (1999).

Statistical analysis: The results were expressed as mean±SEM of six rats per group and the statistical significance was evaluated by one-way analysis of variance (ANOVA) using the SPSS (version 15.0) program followed by LSD. Values were considered statistically significant when $p<0.01$.

RESULTS

In the present study, the significant ($p<0.01$) decrease in body weight was observed in diabetic rats during the course of experiment. However, diabetic rats treated with *P. tirupatiensis* aqueous extracts for 30 days didn't showed the decreased body weights and this group revealed similar body weight changes with standard anti-diabetic drug glibenclamide treatment. Increased body weights against diabetes was significant on day-15 and day-30 in diabetic treated groups compared to diabetic untreated group on respective days (Table 1). Values are significant compared to normal control ($p<0.01$) and diabetic control ($p<0.01$).

Effect of *P. tirupatiensis* treatment on serum glucose levels in normal and diabetic rats were presented in Table 2. The striking characteristic feature of diabetic was expressed in this study with high serum glucose levels in diabetic rats after STZ injection, and this was continued on day-15 and also on day-30. Although,

Table 1: Effect of oral administration of *Pimpinella tirupatiensis* aqueous extracts on changes in body weights in normal control and diabetic rats

	Body weight (g)		
Groups	Day 1	Day 15	Day 30
Normal control	187±1.5	198±4.1	214±3.3
Diabetic control	163±0.97*	133±1.67*	109±2.97*
P. tirupatiensis treatment	187±1.19	199±3.9	228±2.19
Diabetic plus *P. tirupatiensis*	175±1.72*#	185±2.65*#	196±5.92*#
Diabetic plus *glibenclamide*	181±2.55*#	187±2.62*#	199±1.35*#

All the values are Mean±SEM of six individual observations, Values are significant compared to normal control (*$p<0.01$) and diabetic control (#$p<0.01$)

Table 2: Effect of oral administration of *Pimpinella tirupatiensis* aqueous extracts on serum glucose levels in normal control and diabetic rats

	Serum glucose (mg dL^{-1})		
Groups	Day 1	Day 15	Day 30
Normal control	87±0.9	89±1.66	88±2.33
Diabetic control	363±1.07*	385±2.89*	390±3.97*
P. tirupatiensis treatment	87±0.12	89±2.29	85±2.99
Diabetic plus *P. tirupatiensis*	375±1.92*#	175±3.62*#	104±2.28*#
Diabetic plus *glibenclamide*	347±1.65*#	196±2.65*#	101±0.55*#

All the values are Mean±SEM of six individual observations, Values are significant compared to normal control (*$p<0.01$) and diabetic control (#$p<0.01$)

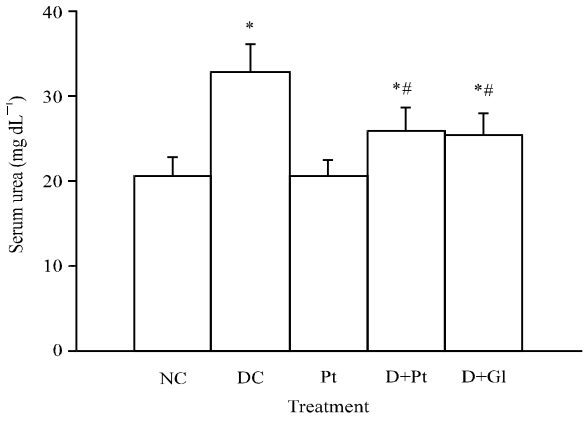

Fig. 1: Effect of oral administration of *Pimpinella tirupatiensis* aqueous extracts on serum urea levels in normal and diabetic rats, Values are significant compared to normal control (NC, *$p<0.01$) and diabetic control (DC, #$p<0.01$)

P. tirupatiensis alone didn't showed any effect on serum glucose levels, surprisingly diabetic rats received *P. tirupatiensis* aqueous extracts showed significantly decreased serum glucose levels on day 15 (175±3.62 mg dL^{-1}) and day-30 (104±2.28 mg dL^{-1}) compared to day-1 (375±1.92 mg dL^{-1}). Decreased serum glucose levels were similar with glibenclamide treatment in this study.

Figure 1 represents the changes in urea levels in control and treated rats. Here, the increased ($p<0.01$) urea

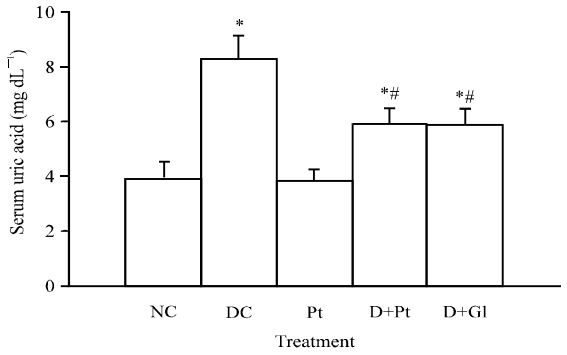

Fig. 2: Effect of oral administration of *Pimpinella tirupatiensis* aqueous extracts on serum uric acid levels in normal and diabetic rats, Values are significant compared to normal control (NC, *p<0.01) and diabetic control (DC, #p<0.01)

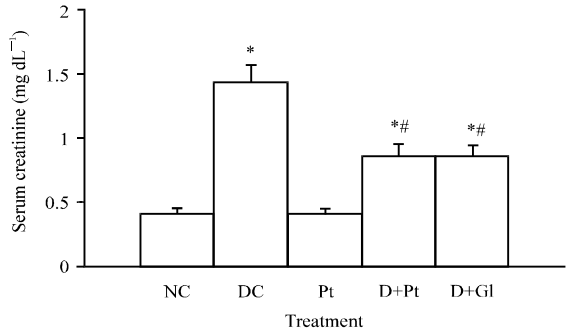

Fig. 3: Effect of oral administration of *Pimpinella tirupatiensis* aqueous extracts on serum creatinine levels in normal and diabetic rats. Values are significant compared to normal control (NC, *p<0.01) and diabetic control (DC, #p<0.01)

levels were observed in diabetic rats was significantly decreased by *P. tirupatiensis* treatment. The beneficial effect of *P. tirupatiensis* treatment to the diabetic rats was evidenced by decreased serum urea values in group IV, which results were parallel with standard anti-diabetic drug treatment.

Estimated serum uric acid levels in this study were significantly (p<0.001) elevated in diabetic rats, which indicates increased purine degradation during diabetic condition (Fig. 2). This elevation was 2-fold higher in diabetic rats compared to normal control rats. However, elevated uric acid levels were significantly controlled by *P. tirupatiensis* treatment in diabetic treated rats.

Figure 3 shows significantly increased serum creatinine levels in diabetic rats compared to normal control rats. However, the results state that *Pimpinella* treatment to diabetic rats controlled the further elevation

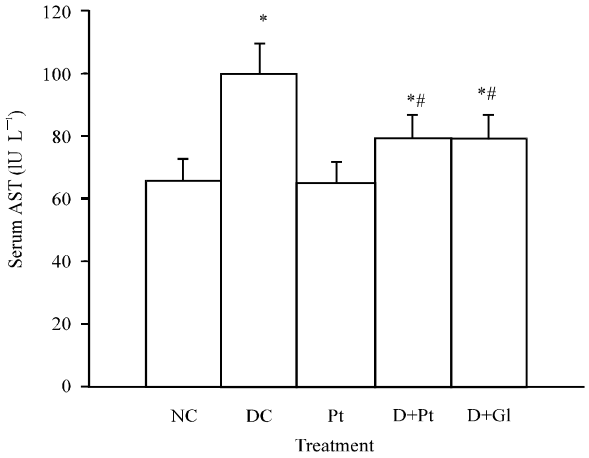

Fig. 4: Effect of oral administration of *Pimpinella tirupatiensis* aqueous extracts on AST activity in normal and diabetic rats, Values are significant compared to normal control (NC, *p<0.01) and diabetic control (DC, #p<0.01)

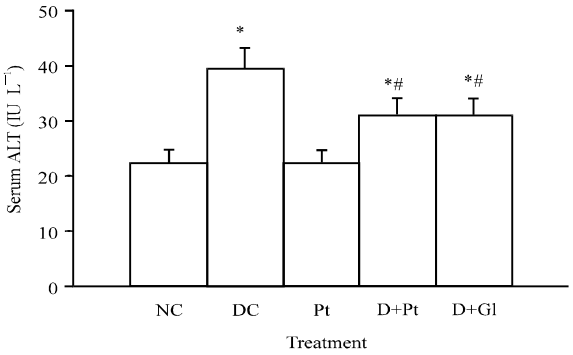

Fig. 5: Effect of oral administration of *Pimpinella tirupatiensis* aqueous extracts on ALT activity in normal and diabetic rats, Values are significant compared to normal control (NC, *p<0.01) and diabetic control (DC, #p<0.01)

of creatinine levels. Although creatinine levels still higher than normal control even after 30 days of *P. tirupatiensis* treatment, but the values were significantly lower than the diabetic untreated group.

Effect of *P. tirupatiensis* treatment on liver function markers enzymes including, AST and ALT were showed in Fig. 4 and 5. The activity of AST was significantly (p<0.01) increased id diabetic rats compared to normal rats. Nevertheless, diabetic rats treated for 30 days with *P. tirupatiensis* showed lower AST activity, which was parallel to the glibenclamide treatment. In this study, similar trend of results were also observed in ALT activity (Fig. 5), which indicates diabetes-induced adverse effects on liver function marker enzymes were significantly

($p<0.01$) attenuated by *P. tirupatiensis* treatment. This was firmly evidenced by attenuated both AST and ALT enzyme activities.

DISCUSSION

The present study demonstrated the beneficial effects of *Pimpinella tirupatiensis* aqueous extracts on streptozotocin (STZ)-induced diabetic rats. Oral administration of *P. tirupatiensis* aqueous extracts for 30 days to diabetic rats showed a significant drop in serum glucose levels and regained the reduced body weights. Elevated urea, uric acid and creatinine levels in diabetic rats were observed (Eidi *et al*., 2011). These were significantly attenuated by oral administration of *P. tirupatiensis* treatment. Furthermore, liver function enzymes AST and ALT which were elevated in diabetic rats were significantly controlled in *P. tirupatiensis* treated diabetic rats. This result indicates that the strength of antidiabetogenic action of the herbal extract. Theoretically, hypoglycemic plants act through a variety of mechanisms (Jasmine and Daisy, 2007). As a first report in this field, at present juncture, this study was unable to explain the pin point mechanism behind the antidiabetogenic effects. However, the present results trust worthy and providing a fundamental aspects for further studies.

In accordance to this hypothesis, the beneficial effect of *P. tirupatiensis* aqueous extracts was evidenced by decreased blood glucose levels in diabetic rats. Elevated serum glucose levels in STZ-induced diabetic rats confirming the abnormalities of glucose levels, which might be due to destruction of pancreatic β-cells by STZ injection. *P. tirupatiensis* extracts may play a role in regulating the β-cells' function thus maintain the lower glucose levels. The exact mechanism behind the control of serum glucose levels by *P. tirupatiensis* treatment needs to be elaborated in future studies. Another diabetic feature was evidenced by reduced whole body weights during diabetes and this was countered by *P. tirupatiensis* supplementation in diabetic treated rats. Both controlling the serum glucose levels and maintains the body weights with *P. tirupatiensis* treatment in diabetic rats were similar with the results of standard anti-diabetic drug glibenclamide treatment.

Urea is the main end product of protein catabolism in the body, which was found to increase in diabetic rats. Accumulation of urea in experimental diabetes may due to the enhanced breakdown of both liver and plasma proteins (Green and Miller, 1960). Alterations in nitrogen homeostasis may leads to increased hepatic elimination of urea nitrogen and increase peripheral release of nitrogenous substances. Thus, the observed negative nitrogen balance may partly because of changes occurring within the hepatocytes (Almdal *et al*., 1986). The oral administration of *P. tirupatiensis* to diabetic rats significantly decreased the serum urea levels, which indicates the prophylactic role of *P. tirupatiensis* extracts in the protein metabolism.

Uric acid, one of the major endogenous water-soluble antioxidants in the body, has been thought to be a metabolically inert end product of purine metabolism (Facchini *et al*., 1991). The present study results showed that uric acid levels were significantly increased in diabetic rats. This may be due to metabolic disturbance in diabetic rats, which was well established by previous studies through elevated xanthine oxidase activity, lipid peroxidation, triglycerides and cholesterol levels (Madinov *et al*., 2000; Anwar and Meki, 2003). Elevated levels of serum uric acid may be due to either an increase in uric acid production or a decrease in its excretion (Modan *et al*., 1987). Accumulating evidences indicates that increased uric acid levels in diabetic rats are associated with increased free radical production that apparently leads to increase oxidative stress (Baynes, 1991). Thus, the elevated levels of circulating uric acid levels may be an indicator of the body that trying to protect itself from the deleterious effects of free radicals by maintaining the stable antioxidant status. Interestingly, uric acid prevents oxidative modification of endothelial enzymes and preserves the ability of endothelium to mediate vascular dilatation in the face of oxidative stress (Becker, 1993) On the other hand, increased uric acid reflects increased xanthine oxidase activity, which is a main source for free radical production that can cause deleterious effects in diabetic condition. In the present study, the increased levels of serum uric acid observed in diabetic rats were maintained to near normalcy by the administration of *P. tirupatiensis,* which also indicates the free radical scavenging activity of *P. tirupatiensis.*

Creatinine is a byproduct of the breakdown of creatine and phosphocreatine, which are considered as an energy storage compounds in muscle. The serum creatinine concentration may vary based on a number of factors including diet composition ability of the kidney to excrete creatinine. An elevation in creatinine usually occurs simultaneously with an increase in blood urea nitrogen. Creatinine concentration is often used as a variable not only to assess impairment of kidney function but also as clinical end point to detect treatment related toxic effects of compounds on the kidney in experimental animals (Travlos *et al*., 1996). In this study, elevated urea levels are paralleled with elevated creatinine levels. The oral feeding of *P. tirupatiensis* for one month significantly reduced the serum creatinine level. From this results, the early renal changes occurred in the diabetic rats may be improved by the oral administration of *P. tirupatiensis* aqueous extracts supplementation.

Aminotransferases, such as ALT and AST were measured as the concentration of intracellular hepatic

enzymes that have leaked into the circulation and serve as a marker of hepatocyte injury. It is hypothesized that elevation in ALT, AST are considered as predictors of diabetes (Harris, 2005). Further, the elevation in the levels of these gluconeogenic enzymes, whose gene transcription was suppressed by insulin, could indicate impairment in insulin signaling rather than purely hepatocyte injury (O'Brien and Granner, 1991). In accordance with these findings, streptozotocin treatments has a significant role in the alteration of liver functions since the activity of AST and ALT were significantly higher than those of normal values. However, diabetic rats treated with *P. tirupatiensis* for 30 days showed diminished these enzyme activities to the basal levels. Controlled hepatic enzymes by plant extract treatment in diabetic rats reflect the improved hepatic functions.

CONCLUSION

The data of the present study suggests that *Pimpinella tirupatiensis* may have beneficial effects against diabetic complications that hold the hope of new generation of antidiabetogenic drugs. The altered liver marker levels were regained to the normal levels with an oral administration of *P. tirupatiensis* for a period of 30 days. However, comprehensive chemical and pharmacological researches are required to find out the exact mechanism behind the anti-diabetogenic effect and to identify the active constituent(s) responsible for this effect.

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