

## Biomarker Changes In Diabetic Rats Treated with Ethanolic Plant Extract of *Passiflora Foetida* Linn

Ravi Babu Birudu

Department of Zoology Aquaculture, Acharya Nagarjuna University, Guntur, A.P, India.

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The objective of this study was to evaluate the antidiabetic activity of *Passiflora foetida* in a model of dextrose -induced experimental diabetes in rats. Diabetes was induced in albino rats by administration of dextrose through feed. The ethanol extracts of *Passiflora foetida* at a dose of 500 and 250 mg/kg of body weight were administered at a single dose per day to diabetes induced rats for a period of 14 days. The effect of ethanol extract of *Passiflora foetida* leaf extract on blood glucose, plasma insulin, creatinine, glycosylated haemoglobin, serum protein, , serum enzymes (serum glutamate pyruvate transaminases) , serum glutamate oxaloacetate transaminases, and alkaline phosphatase) were measured in the diabetic rats. Acute toxicity studies were conducted. The increased blood glucose, glycosylated haemoglobin and other biochemical parameters level were observed in diabetic rats treated with both doses of ethanol extract of leaf *Passiflora foetida* compared to diabetic control rats. In diabetic rats, ethanol extract of *Passiflora foetida* leaf administration, altered the biochemical parameters were in diabetic control rats. Ethanol extract of *Passiflora foetida* leaf possesses significant antidiabetic activity in diabetic rats.

**Keywords:** Biomarker, Diabetes, Dextrose, SGOT, SGPT, *Passiflora foetida*.

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Diabetes mellitus is an epidemic occurring in adults throughout the world .Diabetes is the leading cause of many diseases like heart attack, blindness and kidney diseases.. It is the fourth main cause of death in most developed countries. The number of patients of diabetes is estimated to reach 330 million by the year 2025, according to International Diabetes Federation. This numerical increase will occur in developing countries. By the year 2025, over 75% of people with diabetes will reside in developing countries, as compared to 62% in 1995<sup>1</sup>. Currently available therapies for diabetes include insulin and various

oral antidiabetic drugs like as sulfonylureas, biguanides and glinides. Many of them have a number of serious adverse effects; therefore, the search for more effective and safer hypoglycemic agents is one of the important areas of investigations<sup>2</sup>. Many plant extracts have been possessing hypoglycemic activity when taken orally<sup>3</sup>. According to the World Health Organization, there are more than 1 200 plant species have been used in the treatment of diabetes mellitus and many number of plant showed hypoglycemic activity in laboratory testing<sup>4</sup>.

Some medicinal plants have been reported to be useful in diabetes. Medicinal plants have been used in antidiabetic and antihyperlipidemic remedies. Antihyperglycemic activity of the plants is mainly due to their ability to restore the function of pancreatic tissues by causing an increase in

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\* To whom all correspondence should be addressed.  
E-mail: ravibabubiotech@gmail.com

insulin output or inhibiting the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. Searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which demonstrate alternative and safe effects on diabetes mellitus. Most of the plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids etc. that are frequently implicated as having antidiabetic effect<sup>5</sup>. The study of plant for hypoglycemic, antioxidant and hypolipidemic activities may give new pharmacological approaches in the treatment of diabetes mellitus<sup>6</sup>.

The genus *Passiflora* belongs to Passifloraceae family and widely spread in tropical region. Passion fruit is an important fruit crop in many tropical and subtropical countries due to its edible fruits, ornamental use and medicinal properties. Some species (*P. edulis*, *P. quadrangularis*, *P. ligularis*) are chiefly cultivated into the production of fruit juice. Eleven species, including *P. foetida* and *P. tripartita* (= *P. mollissima*) are recorded as weeds in variable parts around the world<sup>7</sup>. Both *P. foetida* and *P. tripartita* are closely related taxonomically. *Passiflora foetida* is native to the southwestern United states (southern Texas and Arizona), Mexico, the Caribbean, Central America, and much of South America. *Passiflora foetida* has been introducing to the tropical region in the world, such as Southeast Asia and Hawaii. It is a creeping vine like other members of the genus, and yields an edible fruit. The specific name, foetida, means "stinking" in Latin and refers to the strong aroma emitted by the damaged foliage<sup>8</sup>. *Passiflora foetida* L. known as passion fruit is an exotic fast-growing perennial vine occurring in USA. the fresh or dried whole plants and their preparations are accepted for medicinal use in Europe for the treatment of nervous anxiety<sup>9,10</sup>. *P. foetida* belongs to the same family and are indigenous to many countries, west tropical Africa and in India. Leaves of the plant utilized as folk medicine for treatment of anti-anxiety, stress and insomnia. It is are also useful for the treatment of hysteria, skin inflammation, cough and fever. Chemical constituents in *P. foetida* include hydrocyanic acid, groups of flavonoids and Harman alkaloids.

## MATERIALS AND METHODS

### Plant material

The fresh leaves of *Passiflora foetida* were collected from the area of botanical garden, Acharya Nagarjuna University, Guntur. The plant material was identified and authenticated by a taxonomist in the University. The collected leaves were dried under shade and then ground into fine powder using laboratory mortar and pestle. The powder (100 gm) of leaves was macerated in solvent containing 70% ethanol and 30% distilled water at room temperature for 72 h. This was then filtered using a filter paper and the filtrate was evaporated to dryness on water bath at 60 °C and dried brown extract was kept in an air tight bottle until used. All chemicals and drugs used were obtained commercially and were analytical grade. Dextrose was purchased from National Scientific Laboratory, PVT Ltd, Vijayawada.

### Acute toxicity studies of the plant extract

The index of the acute toxicity was the LD50. In the initial phase, rats were divided into 3 groups of 6 rats each and were treated with *P. foetida* extract at doses of 100 mg, 250 mg, 500 mg and 1000 mg/ kg body weight orally. The animals were observed for 24 h for signs of toxicity including death. Based on the results the next experiment was designed<sup>11</sup>.

### Induction of experimental diabetes

Normal healthy male Wistar albino rats, 9-12 weeks old with an average weight of 200-250 gm were procured from the Mahaveer Enterprises, Bagh Amberpet, Hyderabad. They were housed in polypropylene cages and fed with a standard chow diet and water ad libitum. The animals were acclimatized to the conditions by maintaining them at a temperature 25±2°C and relative humidity 55±10 at 12 hour each at dark and light cycle for about 7 days prior to dosing and during the commencement of experiment. All experimental procedures involving animals were conducted in accordance with the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). Dextrose was used to induce diabetes mellitus in male albino wistar rats. A freshly prepared dextrose solution was given orally at 6.6 gm/rat/5ml. After 15 days, rats with moderate diabetes having glycosuria and hyperglycemia were selected for the experiment.

**Experimental design**

Group 1: Normal rats .

Group 2: Diet induced diabetes diseased rats (diet induced controlled).

Group 3: Diet induced diabetes rats treated with plant extract (PSF) (100 mg/kg bw).

Group 4: Diet induced diabetes rats treated with plant extract (PSF) (250 mg/kg bw).

Group 5: Diet induced diabetes rats treated with plant extract (PSF) (500 mg/kg bw).

Group 6: Diet induced diabetic rats treated with standard glipizide(4mg/kg bw).

The animals were sacrificed at the end of experimental period of 14 d by decapitation. Blood was collected, seraseparated by centrifugation at 3 000 g for 10 min.

**Estimation of insulin, glucose, creatinine and glycosylated haemoglobin**

Serum glucose was measured by the o-tolidinemethod<sup>12</sup>. Insulin level was assayed by ELISA kit<sup>13</sup>. Serum creatinine was estimated by the method of Owen et al[14].Glycosylated haemoglobin (HbA1c) estimation was carried out by a modified colorimetric method of Karunanayake and Chandrasekharan<sup>15</sup>.

**Estimation of protein, albumin, globulin, SGPT, SGOT,ALP**

Serum protein<sup>16</sup> and serum albumins were determined by quantitative colorimetrically method by using bromocresol green. The total protein minus the albumin gives the globulin, serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) was measured spectrophotometrically by utilizing the method of Reitman and Frankel<sup>17</sup>. Serum alkaline phosphatase (ALP) was measured by the method of King and Armstrong<sup>18</sup>.

**Statistical analysis**

Results were expressed as mean  $\pm$  SD for six rats in each experimentalgroup.Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) 9.05 software. The data were analyzed using one way analysis of variance (ANOVA) and group means were compared with Dunnett's Test P values < 0.05 were considered as significant.

**RESULT**

**Phytochemical constituents:**The phytochemical screening of ethanol extract of *Passiflora foetida* leaf revealed the presence of alkaloid,flavonoid, phenol, saponin, steroid, tannin, terpenoid, sugarand glycoside.

**Blood glucose level and other parameters**

Table 1 shows the levels of blood glucose, serum insulin, creatinine and glycosylated hemoglobin of normal, diabetic control and drug treated rats. There was a significant (P<0.01) increase blood glucose level in induced diabetic rats (Group II) when compared with normal rats (Group I).A dministration of leaf extract of P.foetida (Groups III IV & V) and glipizide(Group III) tended to bring the parameters significantly (P <0.05, P<0.01) towards the normal. Serum insulin level of diabetic control group was significantly ( P<0.01 ) decreased when compared to normal control group (Group I). The extract and Glipizide group of diabetic rats significantly (P<0.01) increased the serum insulin. A significant (P<0.05) elevation in creatinine was observed in induced diabetic rats (Group II ) when compared to control rats. The *P.foetida* extracts were administrated orally to diabetic rats for 14 d reversed the creatinine level

**Table 1.** *P.foetida* Plant extract on Biochemical parameters in experimental rats

	Insulin (mIU/MI)	Glucose	Creatinine	Glycosylated haemoglobin
Normal	21.710	134.50 $\pm$ 5.75	0.58 $\pm$ 0.23	5.62
Diabetic controlled	8.190	428.67 $\pm$ 25.97*	1.17 $\pm$ 0.21*	9.41
Standard Glipizide-4 mg/kg body weight	19.200	144.00 $\pm$ 12.02	1.00 $\pm$ 0.14*	5.22
Plant extract low dose-100mg/kg body weight	14.810	275.17 $\pm$ 24.65*	1.10 $\pm$ 0.15*	6.80
Plant extract Medium dose-250mg/kg body weight	15.621	254.00 $\pm$ 21.12*	0.93 $\pm$ 0.12*	6.35
Plant extract High dose-500mg/kg body weight	18.930	133.83 $\pm$ 4.02	0.55 $\pm$ 0.19	5.90

to near normal. Administration of ethanol extract of *P.foetida* leaf (500 mg/kg) and glipizide significantly ( $P<0.05$ ) reduced HbA1c level compared to diabetic control rats.

#### Biochemical parameters

The level of total protein and liver marker enzymes such as SGPT, SGOT and ALP in the serum of diabetic rats are presented in Table 2. Significant reductions in serum protein was observed in induced diabetic rats (Group II) when compared to control rats (Group I). On administration of ethanol extract of *P.foetida* leaf to the diabetic rats, protein level were found to be restored in normal. Also, the SGPT, SGOT and ALP levels were elevated significantly in induced diabetic rats compared to control rats. Both the doses of *P.foetida* leaf extracts and glipizide treatment significantly reduced above parameters compared to diabetic control rats

### DISCUSSION

Diabetes mellitus is one of the most familiar chronic diseases associated with carbohydrate metabolism. Diabetes mellitus has high prevalence, morbidity, and mortality globally; it is regarded as a non curable but controllable disease. Different synthetic drugs, plant remedies and dietary modification play an efficient role in the reduction of the suffering that it causes. The potential role of medicinal plants as hypoglycemic agents has been reviewed by several researchers<sup>19,20</sup>.

In diabetic condition, elevated blood glucose, polyuria, polydipsia and polyphagia are commonly observed. The increase in blood glucose level after dextrose administration may be due to insulin deficiency or resistance state in diabetic rats. Administration of ethanol extract of *P.foetida*

leaf significantly reduced blood glucose level in diabetic rats which represents reversal of insulin resistance or increasing insulin secretion possibly by regeneration of damaged pancreatic  $\beta$ -cells in diabetic rats<sup>21</sup>. Earlier, many plants have been studied for their hypoglycemic and insulin release stimulatory effects<sup>22-23</sup>.

In diabetes, elevated level of creatinine is observed which may be due to renal damage caused by abnormal glucose regulation or elevated glucose and glycosylated protein tissue levels<sup>24</sup>. In present study, significant increase in creatinine level were observed in diabetic rats compared to normal control rats which indicate impaired renal function in diabetic rats. The treatment with ethanol extract of *P.foetida* lowered the above parameters significantly compared to diabetic control rats and it showed protective effect of ethanol extract of *P.foetida* on the kidneys.

In diabetes, HbA1c is considered as a diagnostic marker and helps to know about degree of protein glycation, long-term blood sugar level and correlation of diabetes associated complications<sup>25,26</sup>. HbA1c has been found to be increased over a long period of time in diabetes. During diabetes, the excess of glucose present in blood reacts with haemoglobin to form glycosylated haemoglobin<sup>27</sup>. The rate of glycation is proportional to the concentration of blood glucose. In present study, diabetic rats showed significant increase ( $P<0.01$ ) H bA1c level compared with normal rats. The ethanol extract of *P.foetida* whole plant treated rats showed a significant decrease ( $P<0.05$ ) in the content of glycosylated haemoglobin that could be due to an improvement in glycaemic status.

In diabetic condition, occurrence of reduction in protein may be due to proteinuria, albuminuria or increased protein catabolism, which

**Table 2.** *P.foetida* Plant extract on Biochemical parameters in experimental rats

	Protein	SGPT	SGOT	ALP
Normal	5.95±0.19	45.80±2.31	19.96±2.33	113.19±4.91
Diabetic controlled	9.75±0.27*	78.25±2.22*	69.46±8.99*	261.05±1.98*
Standard Glipizide-4 mg/kg body weight	7.83±0.22*	64.05±2.22*	22.53±7.51	153.38±2.3*
Plant extract low dose-100mg/kg body weight	8.37±0.22*	73.11±2.95*	26.60±3.48	248.83±2.40*
Plant extract Medium dose-250mg/kg body weight	7.68±0.15*	57.61±2.13*	24.35±1.36	186.27±2.89*
Plant extract High dose-500mg/kg body weight	6.07±0.22	45.16±2.15	18.70±0.75	114.60±2.6

are clinical markers in diabetic nephropathy<sup>28</sup>. The protein level was reduced after the induction of diabetes and treatment of ethanol extract of *P.foetida* increased both levels considerably in diabetic rats towards normal level. This action possibly is through increase in the insulin mediated amino acid uptake, enhancement of protein synthesis and/ or inhibition of protein degradation<sup>29</sup>. Also, increased serum SGOT, SGPT and ALP levels were reported in diabetes and it may be due to liver dysfunction<sup>30</sup>. In this study, increased level of SGOT, SGPT and ALP was observed in diabetic rats which may have occurred by leakage of enzymes from the liver cytosol into the blood stream. Diabetic rats treated with ethanol extract of *P.foetida* leaf significantly reduced both enzyme levels which represents the protective action of ethanol extract of *P.foetida* leaf in diabetic condition

### CONCLUSION

In the present study, the administration of *P.foetida* leaf extracts to dextrose induced hyperglycemic rats demonstrated prominent reduction in blood sugar level, normalization of serum biochemical profile compared to dextrose control rats. The phytochemical analysis has shown the presence of potent phytochemicals such as flavonoids, terpenoids, glycosides, steroids, saponin and phenols. Several authors reported that flavonoids, steroids/ terpenoids, phenolic acids are known to be bioactive antidiabetic principles<sup>31,32</sup>. Flavonoids are known to regenerate the damaged beta cells in the alloxan induced diabetic rats and acts as insulin secretagogues<sup>33</sup>. In the present study, the phytochemical analysis of ethanol extract of *Passiflora foetida* leaf clearly prints out the presence of above said active principles. The preliminary investigation on the antihyperglycemic, of ethanol extract of *P.foetida* leaf will be significant to proceed further in this path for the isolation of active principles responsible for the antidiabetic activity.

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