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EFFECT OF ETHANOLIC EXTRACT OF LEAVES OF *PASSIFLORA INSARNATA* LINN. IN STREPTOZOTOCIN-INDUCED DIABETES IN RATS

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ABSTRACT: The worldwide prevalence of diabetes mellitus (DM) has risen dramatically over the past two decades; based on current trends, more than 360 million individuals will be having diabetes by year 2030. The present study was designed to investigate the hypoglycemic of effect of ethanolic extract of leaves of *Passiflora insarnata* linn. in streptozotocin-induced diabetes in rat. The pharmacological investigation suggested that the ethanolic extract of the leaves was beneficial in controlling the blood glucose levels, improves the lipid metabolism and prevents diabetic complications from lipid peroxidation in experimental diabetic rats. From this study, it can be concluded that the ethanolic extract of the plant exhibited protective and ameliorative effects against streptozotocin induced pancreatic cytotoxicity and severe hyperglycaemia by enhancing the peripheral utilization of glucose, correcting the impaired liver glycolysis and limiting gluconeogenic formation and also repairing and rejuvenating the residual beta cell population. These effects may be due to the presence of phenolic compounds, flavonoids, and other phytochemical constituents, which could act synergistically or independently in modulating the activities of glycolytic and gluconeogenic enzymes.

INTRODUCTION: The worldwide prevalence of diabetes mellitus (DM) has risen dramatically over the past two decades; based on current trends, more than 360 million individuals will be having diabetes by year 2030. Diabetes mellitus (DM) is the name given to a group of disorders characterized by chronic hyperglycaemia, polyurea, polydipsia, polyphagia, emaciation and weakness due to disturbance in carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion and / or insulin action.

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Diabetes mellitus is a metabolic disorder, characterized by chronic hyperglycemia, with disturbances of carbohydrate, fat and protein metabolism, resulting defects in insulin secretion, insulin action, or both [1].

Currently, many countries face large increases in the number of people suffering from diabetes. The World Health Organization estimated that about 30 million people suffered from diabetes in 1985 and the number increased to more than 171 million in 2000. It is estimated that the number will increase to over 366 million by 2030 and that large increases will occur in developing countries, especially in people aged between 45 and 64 years [2]. Diabetes mellitus still is the serious medical problem to human health due to rapid increase and lead the cause of death in the developed and developing countries.

There is increasing demand by patients to use natural products with antidiabetic activity due to side effects associated with the use of insulin and oral hypoglycaemic agents [3].

MATERIALS AND METHODS:

Collection and Identification of Plant Material:

In our country, more than 2000 medicinal plants have been recognized. *Passiflora insarnata* (Passifloraceae; Passion flower family) is an important medicinal plant of tropical and subtropical India. Its medicinal usage has been reported in the traditional systems of medicine such as Ayurveda, Siddha and Unani. *Passiflora insarnata* has been described as a Passion flower and has been used extensively for treatment of some diseases like as Anxiety, Insomnia, Convulsions, Sexual dysfunction, Cough and Cancer [4]. *Passiflora insarnata* aerial parts were procured from a commercial supplier who had cultivated *Passiflora insarnata* at village Khurrampur, district Saharanpur (Uttar Pradesh, India) in January and authenticated by Professor Gyanendra Tiwari, Department of Pharmacognosy, B.R. Nahata college of Pharmacy, Mandsaur (M.P.). A voucher specimen of plant (code no. BRNCP/BD/006/2009).

Extraction of Leaf Powder: The leaves of *Passiflora insarnata* were collected and dried in shade at room temperature. The dried leaves were powdered by using grinder to coarse powder after keeping them in an oven at 35^{o}C for 24 hours, packed into soxhlet apparatus and extracted with various solvents for 24 hrs. The excess of solvents were removed using rotatory flash evaporator. The obtained crude extract was stored in airtight container in refrigerator below 10^{0}C for further studies. The air dried plant material was coarsely powdered.

The powdered *Passiflora insarnata* leaf material (100 g) was packed into soxhlet apparatus and extracted successively with Petroleum ether (200 ml), Chloroform (200 ml), Ethylacetate (200 ml), Ethanol (200 ml), and Aqueous (200 ml). The filtrate was evaporated using rotary vacuum evaporator under reduced pressure ≤10 mmHg and extracts were stored in desiccators and used for subsequent experiments [5].

Phytochemical Screening: The plant is a biosynthetic laboratory, not only for chemical compounds such as carbohydrates, proteins and lipids that are utilized as a food by man, but also for a multitude of compounds like glycosides, alkaloids, volatile oils, tannins etc., that exert a physiological and therapeutic effect. The compounds that are responsible for medicinal property of the drug are usually secondary metabolites. A systematic study of a crude drug embraces, through consideration of primary and secondary metabolites derived as a result of plant metabolism. The plant material is subjected to preliminary phytochemical screening for the detection of various plant constituents [6].

Preliminary phytochemical screening was carried out to find the presence of the active chemical constituents in various extracts such as alkaloids, flavonoids, tannins, phenolic compounds, saponins, steroids, fixed oils and fats [5]. In general, tests for the presence of phytochemical compounds involved the addition of appropriate chemical reagents to the extract in test tubes. The mixture was then shaken and/or heated as the case may be. The alkaloid was tested by using Dragendorff's, Mayer, Wagner's and Hager's test. The flavonoids were tested by alkaline reagent and Shinoda test. The tannins were tested by ferric chloride and gelatin test. The total phenolic content in extract was determined by Bromine water and Liebermann tests. Saponin content of *Passiflora insarnata* was determined by froth formation test. The steroids and triterpenoids were tested by Salkowski test. The presence of fats and fixed oils was determined by using 2,4-dinitrophenylhydrazine test. The phytochemical analysis of the *Passiflora insarnata* leaf extract shows the presence of Tannins, Alkaloids, Flavonoids and Phenolic compounds.

Pharmacological Evaluation of *Passiflora Insarnata:*

Selection of animals: Healthy young adult animals of commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant. Each animal, at the commencement of its dosing, should be between 8 and 12 weeks old and its weight should fall in an interval within + 20 % of the mean weight of any previously dosed animals.

The temperature in the experimental animal room should be 22ºC (+3ºC). Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be group-caged by dose but the number of animals per cage must not interfere with clear observations of each animal. Animal study was performed in the Division of Pharmacology, IPS, Gwalior, with due permission from the Institutional Animal Ethics Committee (No.1039/ac/07/CPCSEA).

The animals are randomly selected, marked to permit individual identification and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

Acute toxicity study: The acute toxicity study of the extract was determined according to the Organization for Economic Co-operation and Development (OECD) guidelines no. 420. Acute oral toxicity refers to those adverse effects occurring following oral administration of a single dose of a substance, or multiple doses given within 24 hours. Delayed death means that an animal does not die or appear moribund within 48 hours but dies later during the 14-day observation period. Dose is the amount of test substance administered. Dose is expressed as weight of test substance per unit weight of test animal (e.g. mg/kg).

Acute toxicity study was conducted to determine median lethal dose (LD_{50}) of the extracts. Acute toxicity study of extracts was carried out in adult albino rats of either sex by "up and down method" (OECD-425). Different dose levels of the extract were administered orally to the different groups of rats consisting of adequate number of animals. The animals were found safe up to 1000 mg/kg [7]**.** Doses for animal study were fixed as Experimental dose of extracts (mg/kg) = $LD_{50}/5$ [8].

Preparation of drug solution: The ethanolic extract (100 mg) and (200mg) of the leaves of *Passiflora insarnata* was dissolved in 10 ml of distilled water to prepare stock solution of 10 mg/ml. Appropriate dilution with distilled water were made to administered the desired doses according to the body weight of the rats in respected groups.

Streptozotocin-Induced Diabetes- Induction of experimental diabetes: Diabetes mellitus was induced in the albino rats by administering streptozotocin. Animals were allowed to fast for 24 hr and were injected a single dose of freshly prepared streptozotocin (50 mg/kg, i.p.) in sterile normal saline [9]. Before this the experimental animals were acclimatized for 7-14 days in an animal house. Blood glucose levels were measured by withdrawing a drop of blood from tip of the animal tail and placing it on the glucometer strip [10]. The accu-check glucometer with the help of strips gives the digital value of blood glucose level. Rats showing fasting serum glucose levels (>250 mg/dl) were selected for the study.

Oral glucose tolerance test (OGTT): Rats were divided in to four groups (n = 6) were administered with distilled water (10 ml/kg), Glibenclamide (standard drug) (10 mg/kg) [10]**,** and ethanolic extracts (PIELE) at dose of 100 and 200 mg/kg p.o. by help of oral gavages, respectively. OGTT was carried out after 15 days of treatment, during which the animals were fed with normal diets. Glucose (2.5 g/kg) was fed 30 min after the administration of extracts. On completion of 15 days of treatment, the rats were fasted over night and blood was withdrawn from tail-vein just prior to the drug administration (normal fasting) and at 0, 30, 60 and 120 min of glucose loading. Blood glucose levels were measured immediately by using a glucometer [11].

Evaluation of anti-diabetic activity: The animals were allowed to acclimatize to the laboratory environment for a week and then randomly divided in to five groups (n = 6) mentioned as follows [12]:

Group I – Untreated (Normal control) group

Group II – Streptozotocin treated (Diabetic Control) group.

Group III – Streptozotocin induced diabetic group of rats treated with 10 mg/kg/day Glibenclamide (standard).

Group IV – Streptozotocin induced diabetic rats treated with ethanolic extract of leaves

of *Passiflora insarnata* 100 mg/kg p.o. for 15 days (treated).

Group V – Streptozotocin induced diabetic rats treated with ethanolic extract of leaves of *Passiflora insarnata* 200 mg/kg p.o. for 15 days (treated).

Blood glucose levels were measured on day 1, 4, 7, 10 and 15 of the study by glucometer [13]. The effects of the ethanolic extract of *Passiflora insarnata* on diabetic rats were estimated on the 15th day after sacrificing the animals by decapitation. Serum lipid profiles [14, 15] and liver glycogen levels [16] and changes in body weight were assessed in the diabetic animals treated with extracts and compared with diabetic control and normal animals.

Changes in body weight: At the end of 15 days treatment the body weight of diabetic control group decreased whereas treatment with PIELE (200 mg/kg, p.o.) and Glibenclamide (10 mg/kg, p.o.) significantly recovered the body weight towards normal level.

Biochemical Study: Serum lipid profile including the level of TG, TC, VLDL, LDL, HDL, SGOT, SGPT, ALP were estimated and recorded as per standard method.

Statistical Analysis: Statistical difference in the mean analyzed using one-way ANOVA followed by Turkey's multiple comparison tests P<0.001 was considered as statistically more significant.

RESULTS AND DISCUSSION:

Phytochemical Analysis:

The Plant *Passiflora insarnata* was evaluated for phytochemical investigation and assessment of its pharmacological activity. The various observation and results obtained from evaluations are discussed in this chapter. The percentage yield of the petroleum ether extract, chloroform extract, ethyl acetate extract, ethanolic extract, and aqueous extract was found to be 3.40, 2.66, 2.90, 10.53, 9.17% w/w respectively. The qualitative identification test revealed the presence of sugars, saponins, flavanoids, alkaloids and glycosides in ethanolic extract.

Pharmacological Evaluation: The acute toxicity study indicates that all the extracts were safe up to 1000 mg/kg body weight. Therefore 1000 mg/kg dose was considered as a safe dose, so 1/5th of the LD_{50} was considered as the working dose. In the case of oral glucose tolerance test, the animals were divided in to four groups having 6 animals in each groups. Group A was kept normal, while group B,C,D, received Glibenclamide (10mg/kg), *Passiflora insarnata* ethanolic leaf extract (100 mg/kg), (200 mg/kg) respectively. Ethanolic extract at a dose level of (200 mg/kg) was effective in the OGTT. There was a significant reduction in blood glucose level observed at a dose level of (200 mg/kg) of the ethanolic extract as compared to glucose loaded control group. Blood glucose level reached a peak of 363.66 mg/dl at 30 min after oral glucose loading in control rats. The ethanolic extract of *Passiflora insarnata* at a dose level of (200 mg/kg) produced a significant decrease of blood glucose level at 60 and 120 min (P< 0.001) after glucose loading and the effect persisted until 120 min. Shown in **Fig. 1**.

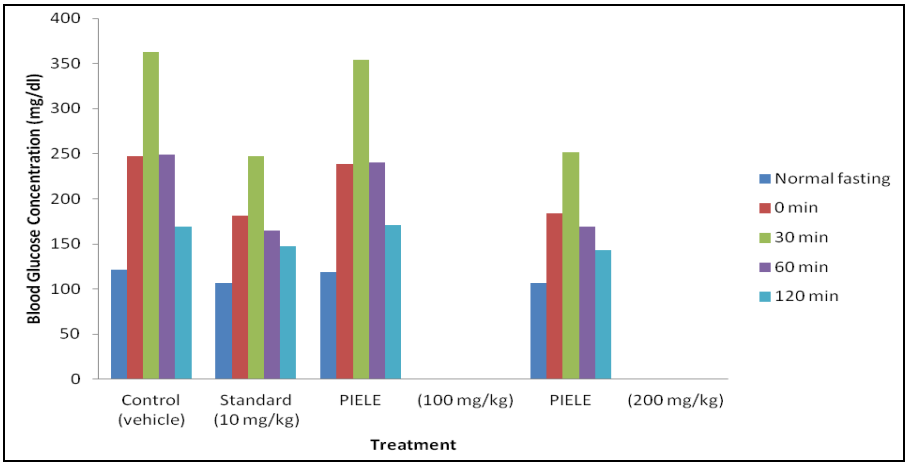

FIG.1: EFFECT OF ETHANOLIC EXTRACT OF LEAVES OF *PASSIFLORA INSARNATA* ON ORAL GLUCOSE TOLERANCE TEST

The antihyperglycaemic effect of ethanolic extract of *Passiflora insarnata* in streptozotocin induced diabetic rats after prolonged treatment is depicted in **Table 1**. In streptozotocin induced diabetic rats, the ethanolic extract at a dose level of (100 mg/kg) and (200 mg/kg) showed the significant reduction in fasting blood glucose level when compared to diabetic control group at the end of 15 days experimental period. The dose of (200 mg/kg) was found better as compared to (100 mg/kg). It indicates that the ethanolic extract at a dose level of (200 mg/kg) exhibited similar effect as that of standard drug. Shown in **Table 1**.

TABLE1: EFFECT OF ETHANOLIC EXTRACT OF LEAVES OF *PASSIFLORA INSARNATA* ON FASTING BLOOD GLUCOSE LEVELS IN DIABETIC RATS.

S.no.	Treatment	Fasting blood glucose concentration (mg/dl)				
		Day 1	Day 4	Day 7	Day 10	Day 15
1.	Normal control	127.86 ± 12.00	112.16 ± 9.19	107.00 ± 15.53	114.83 ± 16.26	119.83 ± 14.02
2.	Diabetic control	313.67 ± 34.46	324.17 ± 40.93	327.40 ± 37.48	307.50 ± 51.86	304.00 ± 47.00
3.	Standard (10 mg/kg)	360.50 ± 35.55*	214.00 ± 30.96*	167.17 ± 40.65*	153.50 ± 27.04*	131.33 ± 19.21*
4.	PIELE (100 mg/kg)	312.70± 15.42	293.86 ± 33.27	288.57 ± 23.75	273.87 ± 25.34	258.56 ± 21.70
5.	PIELE (200 mg/kg)	299.67 ± 14.22	246.68 ± 43.47*	187.83 ± 27.04*	175.17 ± 21.45*	147.83 ± 19.67*

Each value represents mean ± S.E.M. $n = 6$. **Represents statistical significance vs. control ($P < 0.01$) *Represents statistical significance vs. control ($P < 0.001$)

The body weight of the streptozotocin induced diabetic rats decreases significantly as compared to other groups while ethanolic extract of *Passiflora insarnata* at a dose of (100 mg/kg) and (200 mg/kg) causes significant increase in body weight towards normal level. The effect of the dose at (200 mg/kg) showed better results as compared to (100 mg/kg) **(Table 2**).

TABLE 2: EFFECT OF ETHANOLIC EXTRACT OF LEAVES OF *PASSIFLORA INSARNATA* ON BODY WEIGHT OF STREPTOZOTOCIN INDUCED DIABETIC RATS.

S.no.	Groups	Body weight (g)	
		Day 1	Day 15
1.	Normal control	159.83 ± 0.98	157.00 ± 1.22
2.	Diabetic control	156.91 ± 0.66	150.50 ± 0.50
3.	PIELE(100 mg/kg)	158.65 ± 1.03	156.24 ± 1.13
4.	PIELE (200 mg/kg)	159.83 ± 1.29	158.56 ± 1.63*
5.	Glibenclamide(10mg/kg)	160.54 ± 1.30	159.46 ± 1.24

Each value represents mean ± S.E.M. $n = 6$.
*Represents statistical significance vs. diabetic control ($P < 0.05$).

The streptozotocin induced diabetic rats developed a state of hypercholesterolemia and hypertriglycerodemia. The abnormalities in lipid metabolism in diabetes generally leads to elevation in the levels of serum lipids and lipoproteins that in turn plays an crucial role in the development of premature and severe atherosclerosis. Treatment of diabetic rats with ethanolic extracts of *Passiflora insarnata* at a dose of (100 mg/kg) and (200 mg/kg) normalized the hyperlipidemia which was occurred due to induction of diabetes with streptozotocin. The effect of the ethanolic extract at the dose of (200 mg/kg) was found better as compared to the (100 mg/kg). Significant difference was observed in serum lipid profile (TG, TC, VLDL, LDL, HDL) and ratios in the extract treated animals, when compared with diabetic control animals ($P < 0.01$). Serum Total cholesterol, Triglycerides, LDL, VLDL were significantly reduced as compared to the diabetic control group, while HDL level increases as compare to the diabetic control group. **(Fig. 2)**

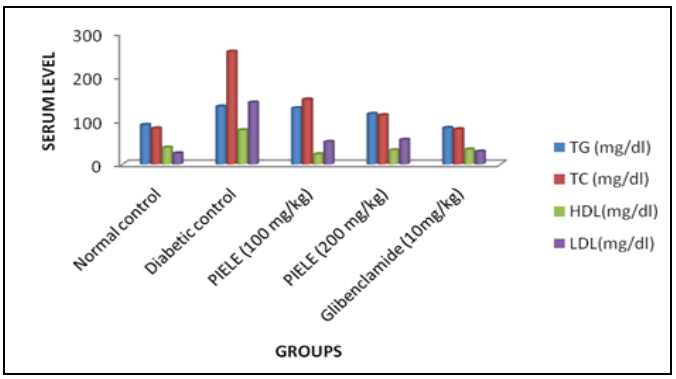

FIG. 2: EFFECT OF ETHANOLIC EXTRACT OF LEAVES OF *PASSIFLORA INSARNATA* ON LIPID PROFILE IN STREPTOZOTOCIN INDUCED DIABETIC RATS

The elevated levels of SGOT, SGPT, ALP in the diabetic control group reflected the significant alteration of liver function by streptozotocin induction. Treatment of different groups with ethanolic extract of *Passiflora insarnata* at a dose level of (100 mg/kg) and (200 mg/kg) significantly lowered the SGOT, SGPT, ALP level as compared to diabetic control rats. The effect of the drug was found significant at the dose of (200 mg/kg) as compare to (100 mg/kg). **(**Shown in **Fig.3.)**

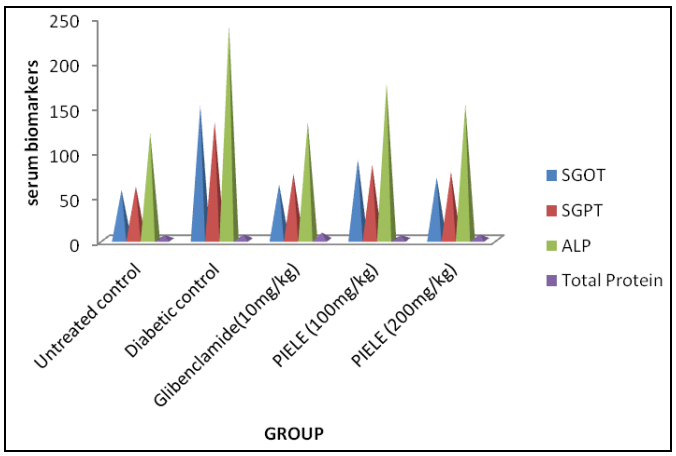

FIG. 3: EFFECT OF ETHANOLIC EXTRACT OF *PASSIFLORA INSARNATA* ON SERUM BIOMARKERS IN STREPTOZOTOCIN INDUCED DIABETIC RATS.

CONCLUSION: The results of the present study clearly indicated that the ethanolic extract exhibited significant hypoglycaemic activity in streptozotocin induced diabetic rats, comparable to the effect exhibited by standard drug Glibenclamide. It can be concluded that the ethanolic extract of the plant exhibited protective and ameliorative effects against streptozotocin induced pancreatic cytotoxicity and severe hyperglycaemia by enhancing the peripheral utilization of glucose, correcting the impaired liver glycolysis and limiting gluconeogenic formation and also repairing and rejuvenating the residual beta cell population.

These effects may be due to the presence of phenolic compounds, flavonoids, and other phytochemical constituents, which could act synergistically or independently in modulating the activities of glycolytic and gluconeogenic enzymes. However, further experimental investigations are needed to identify and explore the lead molecule and to elucidate exact mechanism of action for anti-diabetic effect.

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CONFLICT OF INTEREST: Nil

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