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Evaluation of Antidiabetic Activity of the Polyherbal Formulation (PF-4) in the Management of Madhumeha (Diabetes Mellitus)

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***Abstract*: The antidiabetic activity of (PF-4) was evaluated by different scientific parameters after performing the pharmacognostic study, phytochemical study, toxicological study, experimental study on alloxan induced diabetic model. Dose selection was made on the basis of acute oral toxicity study as per OECD guidelines. In acute toxicity test, there was no such toxicity or no mortality found up to the dose of 1000 mg/kg body weight. In (OGTT) test at 30 min after oral glucose administration, the blood glucose level increased rapidly from the fasting value in all groups, and then subsequently decreased at 60 min and 90 min. In 60 min, (PF-4)-700mg /kg body weight and (PF-4)-500 mg/kg body weight both exhibited remarkable blood glucose lowering effect which was very close to the standard drug (Glibenclamide) and both groups (PF-4)700mg/k.g b.w and (PF-4)500 mg /k.g b.w) significantly($p<0.001$)lowered blood glucose level. In 90 min, (PF-4) also exhibited significant inhibition of blood glucose but (PF-4) 700 mg /kg b.w highly significant($p<0.01$) than (PF-4)500 mg/k.g b.w($p<0.05$). So the maximum hypoglycaemic effect of (PF-4) has been observed at 60 min, comparing with control and standard group. In alloxan induced diabetic model, both doses of (700 mg/kg body weight and 500 mg/kg body weight of PF-4 significantly ($P<0.001$) decreased the blood glucose in comparison with control group. The drug has the potential to act as an antidiabetic drug.**

***Keywords*: Polyherbal formulation, madhumeha.**

1. Introduction

Diabetes mellitus describes a metabolic disorder of multiple etiology characterized by chronic hyperglycaemia with disturbance of carbohydrate, protein and fat metabolism resulting from defect in insulin secretion, insulin action or both [1]. It is the most common endocrine disease and effects of diabetes mellitus include damage & dysfunction of multiple organs. It is one of the leading causes of death by disease. It silently kills the individual. So, recovery from it, really a great demandof society. The global impact of this disease is immense in terms of human suffering and economic burden. Therefore, the safe, cost effective, nontoxic, antidiabetic herbal drug is required for the management of the diabetic patient to control this silent killer disease. In Charak Samhita, Chapter 6th Maharsi Charak mentioned principles of management of madhumeha (Diabetes Mellitus) by describing pramehahara yoga. Among the lots of Pramehahara Yoga (formulation) the combination of Chitrak, *(Plumbago zeylanica Linn)* Chavya *(Piper chaba Hunte)*, Haritaki(*Terminalia chebula Retz*)and Saptaparni *(Alstonia scholaris R.BR*) was selected for this scientific study [2]. This research work was being carried out to detect the efficacy and affectivity of the above said drug through modern scientific parameters and establish a new therapeutic approach in Diabetes mellitus .

2. Materials and Method

A. Plant

Root of Chabya (*Piper chaba Hunter*), Fruit of Haritaki (*Terminalia chebula Retz)* Root of Chitrakmul *(Plumbago zeylanica Linn*), Bark of saptaparni (*Alstonia scholaris R. Br*).

Chabya belongs to piperaceae family having the synonyms of chai, Found throughout India in the warmer place. It is found in Malaysia, Singapore, Srilanka. It is a climber glabrous creeper that spreads on the ground or may take support of other trees. Leaves are (2-3) inch long, older leaves are dentate, dark in colour, younger leaf ovate. Flowers are monoceous, male and female flowers are born on different plants. Fruit is long when ripes it turns into red colour and when dries it attains black colour. Flowering time rainy season. It contains aromatic oil, piperine, piplartine, sitosterol and glycoside in the root of this plant. Root and fruit are used for medicinal preparation. Ayurvedic properties of Chabya has laghu, ruksha guna, katu rasa, katu vipak,usna virya kapha vata hara action. It has also the anti-inflammatory, analgesic, antipyretic Pharmacological action [3]. Haritaki belongs to combretaceae family, having the synonyms of abhaya, pathya, rohini, amrita, jivanti, found in deciduous forests of Indian subcontinent. It is medium to large tree up to 30m. Leaves are elliptic-oblong, acute tip, cordate at the base, glabrous. Flowers are monoceous, dull white to yellow colour strong unpleasant odour born in terminal spikes or short panicles. Fruits glabrous, ellipsoid to ovoid drupes, orange to brown colour. Haritaki contains glycoside, chebulin, chebulic acid, gallic acid, ethylgallate. Flower time rainy season. Haritaki has pancha rasa(except) lavan; laghu, ruksha guna, madhur vipak, usna virya. It is tridosa hara anuloman and rasayan.Fruit is the part Which is used maximum for medicinal preparation. It has rejuvenative, laxative, astringent, nervine appetite stimulant action [4].

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Chitrak belongs to plumbaginaceae family having the synonym of Dahana, Valya, Agnikar. It grows throughout IndIa, especially in Bengal, U.P, South India. Chitrak is perinial shrub. The plant is 0.5-1 m height. The leaves are simple, alternate, oblong. The flowers are white in colour and (10-25) cm long, inodorous, in terminal spikes. Flowering time September to November. Fruit is capsule. Chitrak has laghu ,ruksha, tiksna guna, katu ras, katu vipak, usna virjya. It has the vata-kaphahara property. Medicinal part of this plant is root. The main chemical composition of Chitrak is [i]plumbagine, plumbagin. Chitrak has also the hepato protective, antioxidant, anti-convulsant action [5].

Saptaparni (Alstonia scholaris R. Br): Saptaparni belongs in apocynaceae family. It is a evergreen, tropical tree native to Indian subcontinent having the synonyms of saptacchada, visal twak, sarad.It is large tree with whorled branches that grows up to 40 meter tall and stembark is greyish, leaves are in wroles of 5-7 times, glossy, in upperside, obovate, pedicles are usually long. Flowers are greenish white in colour, flowers bloom in the month of Decembe-March. The flowers are very fragrant. Fruits are slender follicles in pendulous clusters.Fruiting time from May –July. Bark of Saptaparni contains following alkaloIds-Ditamine, Echitammine. It has laghu, snighdha guna, kashay tikta rasa, usna virya, katu vipak., tridosa hara, dipan and hridya property. Saptaparni has anti-tussive, antioxidant, free radical scavenging action [6].

B. Animal

Adult albino rats of Wistar strain (150–200 g) of either sex was procured from local authorized licenced breeders with 12 hr light and 12 hr dark cycles. The control and experimental animals were provided food and drinking water *ad libitum*. All the animal experiments were conducted according to the ethical norms approved by IAEC. The experimental studies were done in the animal house of institute of post graduate Ayurvedic education & Research under Dept. of Dravyaguna (Reg No.1180/AC/08/CPCSEA, dated-27.03.08) according to the guidelines of CPCSEA after the approval of IAEC, Passed on 18/06/2012.

C. Chemicals & Instruments

Following is the list of chemicals used. Alloxan monohydrate (Sigma-Aldrich), Glibenclamide (Aventis Pharma Ltd., Verna, Goa), Dextrose (Emkay Labs, India), Normal Saline (Nirlife Healthcare), One touch Glucometer.

D. Preparation of the research Drug (PF-4)

All the plant materials were collected from reputed local supplier after proper authentication by dept. of Dravyaguna. After proper authentication, the entire dried herbs were cleaned properly and foreign materials are differentiated. Then collected samples were Washed and dried. After that the clean samples were crushed into course powder form and subjected to use for macroscopical and microscopical study. 380 gm of air-dried powder material (PF-4 1:1:1:1) was kept in soxhlet extractor successively with the following solvents according to order of polarity) Petroleum ether, Chloroform, Ethyl acetate, Acetone, Ethanol, Water. Each time before extracting with the solvent, the powdered materials were dried in air oven below 50 degrees centigrade. Finally, it was macerated the mace with chloroform water for 24 hours to obtain aqueous extract. Each extract by distilling off the solvent it was concentrated and then evaporating to dryness on the water bath. The yield of the extracts was 1.13%, 0.42%, and 15.20%,3.49%,4.89%w/w for petroleum ether, chloroform, acetone ethanol and water, respectively. All the extracts were preserved in a refrigerator till further use. Preliminary phytochemical analysis was carried out in all extracts by different methods of phytochemical screening [7].

E. Acute oral toxicity studies

The acute toxicity of aqueous extract of PF-4 on different doses (100 mg/kg. ,500mg/kg ,700mg/kg,1000mg/kg) was done following the OECD guideline. It has been observed that no mortality found up to the dose of 1000mg/kg body weight and there was not found any behavioral changes and toxic effect up to 24 hrs. Finally, the animals were observed up to 14 days [8], [9].

F. Oral glucose tolerance test

Albino rats of either sex were divided into four groups (n=6) and fasted overnight. Then fasting blood glucose level was determined of each rat of particular group through the one touch glucometer. After that Gr-1 was administered Distilled water served as control group, Gr-2 was given PF-4 (500mg /kg Body weight extract), Gr-3 was given PF-4(700mg/kg body weight), Gr-4 was given Glibenclamide (600µg/kg body weight). 30 min after the Drug administration, Glucose (2gm/kg body weight) was orally administered all rats of each group. Blood levels were determined in blood samples collected at 0 min (just prior glucose administration),30min,60min,90 min after glucose administration [10].

G. Alloxan-induced diabetic model

Alloxan monohydrate (Sigma Alrich) was first weighed individually for each animal according to its weight and then solubilized with 0.2 ml saline just prior to injection. Diabetes was induced in the wister rats of either sex by injecting it at a dose of 150 mg/kg b. wt. intraperitoniall, after overnight fast (access to only water). After 1 hr of alloxan administration, the animals were given feed *ad libitum* and 5% dextrose solution was also given in feeding bottle for a day to overcome the early hypoglycemic phase. The animals were kept under observation, and after 72 hr, blood glucose was measured by glucometer. The wister rats of either sex (glucose level > 250 mg/dl) were separated and were included in the study. They were divided into four different groups for this experiment, with each group containing six animals(n=6) Group I –served as control received distilled water. Group-II received(PF-4) at a dose of 500mg/Kg body weight.Group-III received (PF-4) at a dose of 700 mg/kg body weight. Group-IV rats were treated with Glibenclamide at a dose of 600 µg/kg body weight. The treatment duration was 14days.During the experiment blood glucose was measured by one touch glucometer on 3rd day, 7th day and 14 th day [10].

H. Statistical analysis

The results of the study were subjected to one-way analysis of variance (ANOVA) followed by Dunnett's t-test for multiple comparisons. Values with P <0.05 were considered significant.

3. Results

A. Standardization and phytochemical screening

In pharmacognostic study, it has been observed that powder form of (PF-4) having whitish brown in colour, astringent in taste without any specific odour and microscopically, lots of alkaloid containing cell, stone cell,calcium oxalate crystals, pitted vessel, parenchymatous cells were found. Through the phytochemical screening, alkaloids, Sapponin, glycosides, triterpenoids and tannin were found in (PF-4). It has been exhibited that Glycoside,tannin and triterpenoids. The TLC of the aqueous extract exhibited the presence of three compounds which were obtained using the solvent system Toluene: ethyl acetate: Formic acid: Methyl alcohol (3:3:0.8:0.2). This study has been confirmed by UV spectrophoto metric analysis and HPLC analysis on the basis of presence of peaks. HPLC analysis had given three peaks (7.798 min,7.726 min and 18.910 min) which reflected the three major compounds isolation.

B. Toxicity study

The acute toxicity of aqueous extract of PF-4 on different doses (100 mg/kg, 500mg/kg, 700mg/kg, 1000mg/kg) was done following the OECD guideline. It has been observed that no mortality found up to the dose of 1000mg/kg body weight and there was not found any behavioral changes and toxic effect up to 24 hrs. Finally, the animals were observed up to 14 days, but at higher dose 1000/kg body weight there was some changes occurred for 1-2 hrs, like less motor activity, letherginess, salivation etc., were observed in three animals. These activities became normal after 2 hrs. That's why the therapeutic dose of (PF-4) was selected 700mg /kg as highest dose and 500mg /kg as lowest dose.

C. Oral glucose tolerance test model

Oral glucose tolerance test (OGTT) was performed to evaluate the peripheral glucose utilization. 30 after the oral glucose administration, the blood glucose level increased rapidly from the fasting value in all groups, and then subsequently decreased at 60 min and 90 min. But in control group the blood glucose level increased more than standard group and research drug group. After 30 min the blood glucose of control group increased from (72.66±1.145) mg/dl to (137.83±2.548) mg/dl. But in 60min, the blood glucose level of standard and (PF-4) -700 group and (PF-4)- 500 group was decreased from (126.33± 1.406) mg/dl to (84.33± 1.351) mg/dl, 121.83± 2.072 mg/dl to 84.33±1.855 mg/dl and (116.33± 3.527) mg/dl to (95.83± 1.796) mg/dl respectively. In 90 min the blood glucose level of standard and (PF-4) -700 group and(PF-4)- 500 group was reached at 64.83± 1.701 mg/dl, 72± 2.113 mg/dl and 89 ± 2.556 mg /dl respectively from the initial blood glucose level. In 60 min, (PF-4)-700mg /kg body weight and (PF-4)-500 mg/kg body weight both exhibited remarkable blood glucose lowering effect which was very close to the standard drug and both groups (PF-4) 700mg/k.g b.w and (PF-4) 500 mg/k.g b.w) significantly ($p<0.001$) lowered blood glucose level. In 90 min, (PF-4) also exhibited significant inhibition of blood glucose but (PF-4) 700 mg /kg b.w highly significant($p<0.01$) than (PF-4)500 mg/k.g b.w ($p<0.05$).So the maximum hypoglycaemic effect of (PF-4) has been observed at 60 min, comparing with control and standard group.

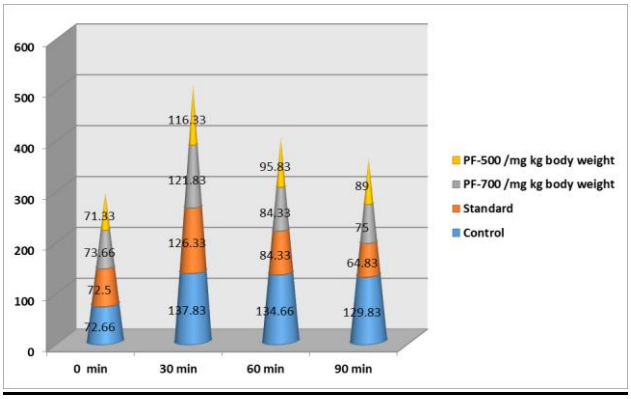

Fig. 1. Oral glucose tolerance test

Fig. 1 showing the effect of (PF-4) in different doses on glucose tolerance test in normal rats. At 30 min after glucose administration, the peak of blood glucose level increased rapidly from the fasting value in all groups, and then subsequently decreased at 60 min and 90 min. In 60 min, (PF-4)-700mg /kg body weight exhibited remarkable blood glucose lowering effect which was very close to the standard drug. But in 90 min, standard drug exhibited more effective result than (PF-700mg/kg body weight), and (PF-500mg/kg body weight).

D. Alloxan-induced diabetic model

In alloxan induced diabetic model, alloxan was used as a diabetogen. It induced diabetes by destroying beta cells of the pancreas partially, through production of reactive oxygen species (ROS) [11]. But administration of PF-4 in different doses on diabetic rats showed a sustained hypoglycaemic activity. Both doses of (700 mg/kg body weight and 500 mg/kg

Table 1
Result of oral glucose tolerance test in rats

Group	0 min	30 min	60 min	90 min
	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM
Control	72.66± 1.145	137.83± 2.548	134.66± 1.686	129.83± 1.351
Standard (Glibenclamide-600µg/kg body weight	72.5± 0.763	126.33± 1.406	84.33± 1.351***	64.83± 1.701**
(PF-4)-700mg/kg Body weight)	73.66 ± 1.115	121.83± 2.072	84.33±1.855***	72± 2.113**
(PF-4)- 500mg/kg body weight	71.33± 0.843	116.33± 3.527	95.83± 1.796***	89 ± 2.556*

The value of result was expressed in Mean ±SEM. n=6, *p<0.05, **p<0.01.***p<0.001 as compared with control group (Annova followed by Dunnett's t test.)

body weight of PF-4 significantly (P<0.001) decreased the blood glucose in comparison with control group. Serum glucose was highly increased (23.99%) in control group, in respect of standard and research group. In 14th day the percentage of inhibition of blood glucose in (PF-4)700mg/kg body weight), and (PF-4)500mg/kg body weight) and standard drug were 69.52%, 62.66% and 79.29% respectively.

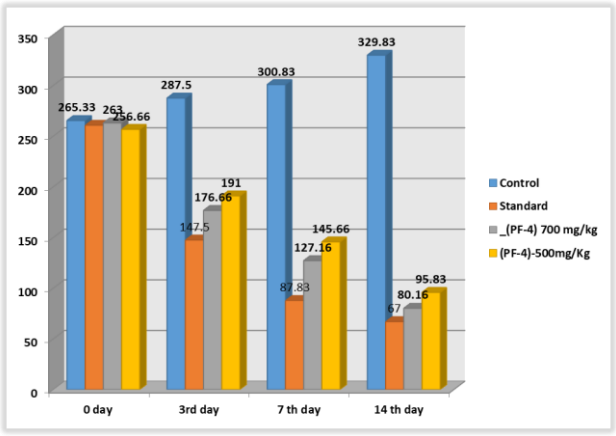

Fig. 2. Observation of alloxan induced diabetes mellitus in rats

Fig. 2, showing the hypoglycaemic activity of (PF-4) in different doses in alloxan induced diabetic rats. Here It was observed that in 14th day blood glucose level significantly decreased in comparison with control group. Serum glucose was highly increased (23.99%) in control group, in respect of standard and research group. (PF-4) 700 mg/kg body weight was shown more remarkable hypoglycaemic activity than (PF-4)500 mg/kg body weight at 14th day. In 14th day Inhibition of percentage of blood glucose in PF-700mg/kg body weight), and (PF-500 mg/kg body weight) and standard drug were (69.52%, 62.66% and 79.29%) respectively.

E. Discussion

From the ancient time to modern era Diabetes mellitus is great threatening to mankind. Besides the miraculous achievement of modern medical science, humanity is passing through the horror of this silent killing disease due to rapid urbanization, sedentary life style, dietary habits and stressful life. The global impact of this disease is immense in terms of human suffering and economic burden. In the present study, the hypoglycaemic effect of (PF-4) may be due to the presence of glycoside isolated from the aqueous extract of (PF-4).It exhibited significant hypoglycemic, and serum insulin raising effects in moderately diabetic rats with close similarities to the effects of a minimal dose of glibenclamide. Polyherbal formulation-4 (PF-4) having the component of root of Chitrak *(Plumbago zeylanica Linn*), root of Chabya *(Piper chaba Hunter)*, fruit of Haritaki (*Terminalia chebula Retz*), bark of Saptaparni (*Alstonia scholaris R.BR*) performed remarkable hypoglycaemic activity in the management of Diabetes mellitus. Through this study, the antidiabetic activity of (PF-4) was evaluated by various scientific parameter after performing the pharmacognostic study, phytochemical study, toxicological screening, animal experimental study. In oral glucose tolerance test and alloxan induced diabetic model the drug (PF-4) proved its sustained hypoglycaemic activity due to presence of Phenolic compound & Glycosides using the TLC, UV-spectroscopy and HPLC chemical analysis [11].

4. Conclusion

Alloxan, a β-cytotoxin, causes a massive destruction of β-cells of the islets of Langherhans, resulting in reduced synthesis and release of insulin. The function of the insulin system is suppressed, which leads to high level of hyperglycemia and eventually to death, but (PF-4) showed potent antidiabetic effect in alloxan-induced diabetic rats and reduced the mortality rate significantly. During the study, the pharmacological action of (PF-4) may be due to the presence of active constituent which increase the insulin level by activating insulin receptor and protect β cell by synergistic properties of antioxidants and helping in reduction of excess fat by hot potency of research drug.

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