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SCREENING OF ANTI-DIABETIC EFFECT OF POLYHERBAL AYURVEDIC FORMULATION - BILWADI CHURNA IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT: Herbal drugs obtained from numerous plants/herbs were extensively utilized in the prevention or treatment of various human ailments since ancient era. Ayurveda, the Indian traditional system of medicine means "science of life and longevity." The unbeaten heritage of this system is a treasure house of knowledge for both preventive and curative health care available to humankind. It has provided treatment of many diseases using herbs, metals, minerals and formulating them into potent dosage forms. It deals with both prevention and cures the disease of a human being most systematically. Ayurveda is one of the world's oldest alternative systems of medicines. Herbal products are of interest to many patients and health care practitioners because 70% of the world populations rely on herbal medicines for part of their primary health care system. This research work was designed to evaluate the anti-diabetic activity of Bilwadi Churna (a polyherbal formulation) in streptozotocin-induced diabetic rats and compare with standard drug glibenclamide. In conclusion, it demonstrated that polyherbal formulations have a significant and potent anti-diabetic potential. It is suggested to evaluate the mechanism of the anti-diabetic effect regarding polyherbal formulations by further research.

Keywords: Poly herbal formulations, Streptozotocin, Glibenclamide, Anti-diabetic and Bhiwadi- Churna

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INTRODUCTION: Ayurveda, the Indian traditional system of medicine means "science of life and longevity". The unbeaten heritage of this system is a treasure house of knowledge for both preventive and curative health care available to humankind. It has provided treatment of many diseases using herbs, metals, minerals and formulating them into potent dosage forms [1].

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Ayurveda is one of the world's oldest alternative systems of medicines. It deals with both prevention and cures the disease of the human being most systematically and present a close similarity to WHO'S the concept of health put forward in the modern era [2].

Ayurvedic herbal treatment is increasing by popular as the herbal preparation have no or least side effects [3]. Herbal medicines are the oldest form of health care to mankind. Ayurvedic herbal medicines use plants, seeds berries root, leaves, bark, or flower for medicinal purpose. The use of medicinal plants in the treatment of diseases was conceived by tribal people thousands of year ago [4]. Herbal products are of interest to many patients and

health care practitioners because 70% of the world populations rely on herbal medicines for part of their primary health care system. Due to several side effects of allopathic medicines in recent years, there has been an increase in the use of herbal medicines [5]. Diabetes Mellitus is a serious complex chronic condition characterized by hyperglycemia and a disturbance in metabolism. The incidence of diabetes mellitus is on the rise worldwide. Based on the World health organization (WHO) reports, the number of patients is expected to be increasing continuously [6].

Diabetes Mellitus is associated with impaired glucose metabolism that leads to an increase in free radical production and an increase in triglycerides and lipoproteins level with an increase in the risk of vascular and renal disease [7]. Despite the considerable progress in the management of Diabetes Mellitus, with insulin therapy, oral hypoglycemic agents, restricted diet, exercise either singly or in combination as there are remarkably good results have been reported with traditional medicines [8]. Diabetes gives rise to various secondary problems such as retinopathy peripheral vascular insufficiencies and neuropathy. This secondary problem takes place due to oxidative stress and DNA damage caused by the degradation of free radical in the cell. Diabetes mellitus is associated with increased oxidative stress [9].

Diabetes is a disease in which the blood glucose and blood sugar, levels are too high; glucose comes from the food we eat. Insulin is a hormone that helps the glucose gets into the cells to give them energy. Diabetes being a highly prevalent metabolic disorder requires making major changes in diet, exercise, and daily routine. Drug development is another most important aspect of the treatment of diabetes. So, the present study is going to evaluate the anti-diabetic potential of Bilwadi Churna in Streptozotocin-induced diabetic rats.

Mechanism of Action of Herbal Drugs: The mechanism of action of herbal antidiabetic can be grouped as follows-

1. Stimulation of insulin secretion from the beta cells of islets.
2. Reduction in insulin resistance.
3. Providing necessary elements such as calcium, magnesium, manganese, and copper for the beta cells.
4. Regenerating/or repairing pancreatic beta cells.
5. Increase in size and numbers of cells in islets of Langerhans.
6. Stimulation of glycogenesis and hepatic glycolytic.
7. Improvement in digestion along with a reduction in blood sugar and urea [10].

MATERIALS AND METHODS:

Experimental Animals: Adult albino rats (either sex) weighing from 200g to 250g (2 - 3 month) were used for the investigation. There was 12:12 hours light and dark cycle, and the temperature were maintained, the humidity was approx 40 - 60% and was fed with standard rats pellet diet and water *ad libitum* [2]. They were fasted for 18 hours before the experiment, with access to water. Animals were procured from the animal housing facility of I.P.S College of Pharmacy Gwalior. The study was performed according to the Animal Ethics Committee for control and supervision of an experimental animal.

Acute Toxicity Studies: The acute toxicity study was best carried out in mice using "fixed-dose combination method" by employing OECD Guidelines No 423. Swiss albino mice each weighing about 30-35 gm in weight were randomly selected, marked to permit individual identification with a marker and kept in their individual cages for toxicity studies, body weight was taken and kept for fasting for a period of 4 h before the dosing. Following the period of fasting, the animal was weighed, and test substance, churna (suspended in 5% v/v tween 80 solution) was administered. The animal was observed individually after dosing once in 30 minutes, with special attention during the first 4 hr and daily for 14 days, and body weight was taken [11].

Induction of Hyperglycemia: Adult albino rats (200 - 250 gm) of either sex was made diabetic with an intraperitoneal injection of 65 mg/kg body weight of streptozotocin dissolved in 0.1 M cold citrate buffer, pH 4.5, immediately before use. Streptozotocin-induced diabetic rats exhibited

massive glycosuria and hyperglycemia within few days. Diabetes was confirmed by measuring blood glucose levels more than 200 mg/dl was considered to be diabetic and used for experiment [12].

Experimental Design:

Group 1: Normal Group: In this group, all the animals were given normal saline and kept as a non-diabetic.

Group 2: Diabetic Group: In this group, all the animals were given streptozotocin.

Group 3: Standard Group: In this group, all the animals were treated with glibenclamide (10 mg/kg)

Group 4: (Test Group a): In this group, all the animals were treated with PHF (360 mg/kg).

Group 5: (Test Group b): In this group, all the animals were treated with PHF (900 mg/kg).

Biochemical Parameters:

Estimation of Blood Glucose Level: Blood samples were collected 1 hour after the last dose administration, on day 5, 10 15, 25, and 30 using a glucometer (One Touch Ultra). Thus the blood glucose level was estimated on Glucometer [13].

Estimation of Oral Glucose Tolerance Test (OGTT): Overnight fasted rats were divided into five groups of six animals each as mentioned as above and receive the respective treatments. After 30 minutes of drug administration, the rats of all the groups were orally administered with 2 g / kg of glucose. Blood samples were collected from the vein just before the drug administration and at 30, 60, 120, and 240 min after glucose loading. Blood glucose levels were measured immediately using glucometer with the same test strips [12].

Estimation of Lipid Profile (LDL, VLDL, HDL): The levels of total glycerides, triglycerides, low-density lipoprotein cholesterol (LDL-C), HDL was analyzed using a biochemical kit. The serum level of VLDLC was calculated using Friedwale formula[7].

Estimation of Serum Biomarkers (SGOT, SGPT): Blood was collected for estimation of serum biomarkers serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT). It was estimated using commercially available reagents kits (Span Diagnostics, Surat India) [14].

Statistical Analysis: Statistical analysis was carried out using one way ANOVA as primary test followed by Dunnet's t-test using graph pad software. All the results were expressed as mean S.D for 6 animals in each group. Data are expressed as mean S.D Significant at $P<0.05$*, $P<0.01$**, and $P<0.001$***.

RESULTS AND DISCUSSION:

Physicochemical Parameters: The formulation was also standardized studying the various parameters like ash value, total ash value, water-soluble ash value, acid insoluble ash value, moisture contents, water-soluble extractive value, alcohol soluble extractive value, bulk density, tapped density, carr's index, Hausner ratio. Results are tabulated in previous chapters, as shown above.

Physicochemical parameters of the herbal formulation are tabulated in **Table 1** below. Degradation and deterioration by fungus depend upon the amount of water present in plant material. The loss on drying (moisture content) at 105 ºC was found to be 6%. Total ash value indicated the amount of mineral and earthy material present in plant material. Analytical results showed total ash value of 9.5%. The water-soluble ash value was found to be 4.1%. The insoluble acid value was found to be 2.1%. The water-soluble extractive value indicates the presence of sugar, acid, and inorganic compounds. The less or more extractive value indicates the addition of exhausted material.

TABLE 1: PHYSICOCHEMICAL PARAMETERS

S. no.	Parameter	Result
1	Water soluble extractive value	12.14 ± 0.18
2	Alcohol soluble extractive value	11.31 ± 0.21
3	Moisture content	6%
4	Total ash value	9.5%
5	Acid-insoluble ash value	2.1%
6	Water soluble ash value	4.1%
7	Bulk density	0.45 m/v
8	Tapped density	0.64 m/v
9	Carr's index	19.6%
10	Hausner ratio	1.42

The water-soluble extractive value was found to be 12.14. The alcohol soluble extractive value was found to be 11.31. Tapped density gives information on the consolidation of powder. The tapped density was found to be 0.64 m/v, and the

bulk density was 0.45 m/v. The Hauser's ratio and Carr's index both are a measure of the flow property of powder. Smaller Carr's index betters the flow property. Carr's index and Hauser's ratio was 19.6% and 1.42%.

Glucose Tolerance Test: The Herbal formulation seems to be safe up to 4 gm/kg because even at high doses no toxic or deleterious effects were seen immediately or during 3-14 days of the observation period. In the present research, the herbal formulation showed hypoglycemic effect 360, and 900 mg/kg in a dose-related manner both in normal as well as glucose loaded normal fasted rats, as shown in **Table 2**.

Effect on Blood Glucose Level: It is to be studied whether polyherbal formulation brought about these changes by acting through a pancreatic mechanism similar to that of glibenclamide as synthetic drug or by inhibition of glucose absorption through gastrointestinal tract like other famous herbal drugs reported. Before the *in-vivo* studies of polyherbal formulation, oral glucose tolerance test was performed in Rodents. The minimum dose of Polyherbal Formulation that is 360 mg/kg showed the lesser antihyperglycemic effect in comparison to the maximum dose that is 900 mg/kg as calculated from the dose calculation chart based on the surface area of some common laboratory species and man.

Effect on Body Weight: PHF exhibited a positive effect on the body weight of rats. **Table 4** depicts the effects on body weight as follows-

TABLE 2: EFFECT OF HERBAL FORMULATION ON ORAL GLUCOSE TOLERANCE TEST

S. no.	Treatment	Blood Glucose Concentration (mg/dl)				
		Normal fasting	0 min	30 min	60 min	120 min
1	Control (vehicle)	126.66 ± 6.08	237.83 ± 3.13	349.66 ± 4.08	250.33 ± 4.50	180.50 ± 4.20
2	Standard (10 mg/kg)	97.83 ± 0.31*	191.83 ± 5.07*	239.50 ± 2.73*	171.16 ± 3.31*	135.00 ± 2.82*
3	PHF (360mg/kg)	117.18 ± 6.19	241.87 ± 4.89	341.76 ± 4.46	237.34 ± 4.14	189.58 ± 4.18
4	PHF (900mg/kg)	110.33 ± 7.55*	164.66 ± 7.06*	241.16 ± 6.36*	170.66 ± 6.40*	151.83 ± 4.87*

Each value represents mean ± S.E.M. n = 5.*Represents statistical significance *vs.* control (P<0.001).

TABLE 3: EFFECT OF PHF 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	127.83 ± 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	325.17 ± 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*	168.17 ± 40.65*	153.50 ± 27.04*	131.33 ± 19.21*
4	PHF (360 mg/kg)	310.57 ± 15.42	293.86 ± 33.27	289.57 ± 23.75	273.87 ± 25.34	258.56 ± 21.70
5	PHF (900 mg/kg)	299.67 ± 14.22	245.67 ± 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).

TABLE 4: EFFECT OF PHF 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	PHF (360 mg/kg)	158.65 ± 1.03	156.24 ± 1.13
4	PHF (900 mg/kg)	159.83 ± 1.29	158.56 ± 1.63*
5	Standard drug (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).

Effect on Lipid Profile: Increase lipid peroxidation seen in the diabetic condition is attributed to increased oxidative stress in the cells as a result of the depletion of the antioxidant system. In support of this view, levels of both enzymatic anti-oxidant decreased and lysosomal enzymes increased in diabetic rats. It is interesting that in polyherbal formulation treated animals, there was a decrease in lipid peroxidation, increases levels of antioxidants enzymes, and decrease in lysosomal enzymes.

Effect on Serum Biomarkers: Serum biomarkers include SGOT and SGPT. The effect was tabulated in **Table 6**.

TABLE 5: EFFECT OF PHF ON LIPID PROFILE IN STREPTOZOTOCIN INDUCED DIABETIC RATS

S. no.	Treatment	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
1	Normal control	38.87 ± 1.02	25.33 ± 2.27	18.13 ± 0.90
2	Diabetic control	78.67 ± 1.52*	141.80 ± 6.60*	26.53 ± 0.83*
3	PHF (mg/kg)	23.00 ± 1.29	51.00 ± 5.29	25.43 ± 1.06
4	PHF (mg/kg)	32.33 ± 3.21***	56.53 ± 0.70***	23.13 ± 0.30**
5	Glibenclamide (10mg/kg)	34.4* ± 2.8	29.8** ± 1.2	16.74 ± 0.98

Each value represents mean ± S.E.M. $n = 6$. *Represents statistical significance *vs*. normal control ($P<0.05$). **Represents statistical significance *vs.* diabetic control ($P<0.01$). ***Represents statistical significance *vs.* diabetic control ($P<0.001$).

TABLE 6: EFFECT OF ON SERUM BIOMARKERS IN STREPTOZOTOCIN INDUCED DIABETIC RATS

S. no.	Groups	SGOT	SGPT
1	Untreated control	56.07 ± 4.10	60.15 ± 1.01
2	Diabetic control	150.72 ± 5.40	132.06 ± 3.93*
3	Glibenclamide (10 mg/kg)	62.40 ± 6.05	74.60 ± 10.41#
4	PHF (360 mg/kg)	90.40 ± 2.70	84.76 ± 3.8
5	PHF (900 mg/kg)	70.84 ± 2.68	76.925 ± 3.79*

All values are expressed as mean ± S.E.M. ($n = 6$) diabetic control was compared with untreated control, and extracts were compared with the diabetic control. *$P<0.05$, **$P<0.01$, #$P<0.001$.

CONCLUSION: From the present research work it can be concluded that the polyherbal formulation is a potential anti-diabetic herbal formulation and hence it is important for further research to investigate its underline mechanism of action and also long term toxicity studies in rodents. After performing the preclinical studies in animals, the herbal product needs to be tried on human diabetic patients to ensure its therapeutic efficacy and safety parameters.

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CONFLICT OF INTEREST: Nil

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