FORMULATION AND EVALUATION OF ANTIDIABETIC ACTIVITY IN POLYHERBAL FORMULATION CONTAINING EXTRACTS OF INDIGENOUS MEDICINAL PLANTS

Jha Santosh Kumar [1*], Pawar Aakash Singh [1]

[1]Institute of Pharmaceutical Sciences, SAGE University, Indore (M.P.), India
*Corresponding Author: E-mail: santoshjha4u@gmail.com
Mob.: +918770942014

ABSTRACT

Objective: The main moto is formulation and evaluation of the anti-diabetic activity of the polyherbal formulation containing extract of *Ficus glomerata* and *Swertia chirayita*. Conventional medicine system offers a valuable resource for exploring new therapeutics, and polyherbal formulations are particularly promising due to their synergistic effects and reduced adverse effects.

Method: The extraction of leaves of *Swertia chirayita* and fruits of *Ficus glomerata* were obtained after drying and crushing the plant parts by Soxhlet apparatus. The extraction has been carried out using "Petroleum ether" then "Hydroalcohol" (Ethanol: Water; 75:25) for powders of the plants. Every time before removing with the next dissolvable, powdered material was air dried beneath 100^0C. The extracted solvent was evaporated using the water bath at 100^0C. The obtained extract was made into granules and filled into the capsule.

Conclusion: The formulation obtained was evaluated for various quality assessment test and in vivo study for anti-diabetic activity. The observation obtained from the in-vivo studies showed sudden fall in the blood glucose level in standard (Glibenclamide treated) animal group from 3rd day, whereas blood glucose remains raised till 7th day in the animal treated with polyherbal formulation (250gm, 500mg) but eventually reduced to subsequent level till the end of 21 days as compared to animals treated with the standard Glibenclamide drug.

Keywords: Anti-diabetic activity, Glibenclamide, Polyherbal formulation, *Ficus glomerata*, *Swertia chirayita*.

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INTRODUCTION

The diabetes mellitus is a chronic metabolic disorder that results in the increased blood glucose level along with abnormalities in protein, lipids and carbohydrate metabolism. The rise in the levels of blood glucose occur due to reduce in the insulin production or due to development of resistance in cells towards insulin. In India, diabetes has become a significant healthcare issue. According to a nationwide urban study conducted in 2005, 15.1% of individuals in urban India have diabetes.[1] Recent statistics have shown how rural India's socioeconomic change has an influence. In the past 15 years, the shift has taken place, and the prevalence has increased from 2.4% to 6.4%. It is expected that more than 100 million individuals are globally impacted by this condition, which will be elevated to 366 million people by 2030. Noninsulin dependent

diabetes mellitus (NIDDM Type 2), which is linked to higher postprandial hyperglycaemia, is the most common form in both the global and Indian contexts (WHO, 2006).[2]

The Acarbose, Miglitol, and Voglibose are a few examples of hypoglycemic medications that have drawbacks and are known to cause harmful side effects. As a result, research into safer, more focused and more effective, hypoglycemic medications has continued to be crucial, and natural extracts from traditionally used medicinal plants that are easily accessible hold considerable promise for the development of novel antidiabetic drugs. Therefore, plants are a possible source of antidiabetic medications (and other drugs as well), but the scientific community hasn't given this fact enough attention.[3] Some of the herbal extracts have been studied for the antidiabetic activity under the current work.

Since the time of Charaka and Sushruta, the indigenous treatments were employed in India to treat diabetes mellitus. Many of the drugs that are currently on the market have either been directly or indirectly obtained from plants, which have historically been an excellent source of pharmaceuticals. According to ethnobotanical data, 800 plants may have anti-diabetic properties. When tested using currently known experimental procedures, a number of these plants shown anti-diabetic efficacy. The extracts obtained from plants have shown remarkable affect in the treatment of diabetes mellitus. The active constituents responsible for anti- diabetic affect are alkaloids, glycosides, galactomannan, polysaccharides, peptidoglycans, hypoglycans, guanidin, steroids, carbohydrates, glycopeptides, terpenoids, amino acids, and inorganic ions are a few of these substances.[3]

MATERIAL AND METHOD

Streptozotocin and Nicotinamide were purchased from “Hi Media Laboratories Pvt. Ltd.”, Mumbai, India. The Glibenclamide was received as a gift sample from “The Tirupati”, Ponta Sahib, Himachal Pradesh. The preliminary phytochemical screening of extract was conducted using different chemicals and reagents like Tannic acid, Lead acetate, Sodium Hydoxide, Hydrochloric acid, Ammonium hydroxide, Mayer’s reagent, Hager’s reagent, Millon’s reagent and many more. All these chemicals and reagents were purchased from “Loba Chemie Pvt. Ltd.”, Mumbai, India.

Collection and authentication of plants

The leaves of plant *Swertia chirayita* and the fruits of *Ficus glomerata* were collected and authenticated by Dr. Jaswinder Mehta, Department of Botany, Carrier College, Bhopal, India. The plant materials were dried under shade, pulverised and kept in the air tight container.

Extraction of Plant Material

The leaves of the plant *Swertia chirayita* and fruits of *Ficus glomerata* were cutted into little pieces using sterile scissor and washed under running tap water to remove all the dust impurities, afterward the plant leaves were dried at room temperature (under shade). The dried plant parts were powdered using the motor and pestle.

Around 100 gm of air-dried powdered plant material was placed in Soxhlet apparatus, the solvent with “Petroleum ether” and then “Hydroalcohol (Ethanol: Water; 75:25)” for powders

of the plants. Every time before removing with the next dissolvable, powdered material was air dried beneath 100^0C. The extracted solvent was evaporated using the water bath at 100^0C. After the evaporation the extracted samples were stored in cold for further analysis.[4]

Preparation of Polyherbal Formulation (PHF)

The polyherbal formulation (capsules) contained the hydroalcoholic extracts of leaves of *Swertia chirayita* and fruits of *Ficus glomerata* were prepared in the different ratio (as mentioned in Table 1). The purity of the polyherbal formulation was tested as per the WHO guidelines for the quality control of herbal materials.[5, 6]

The formulation was prepared with trials having a different ratio of binders and selecting the quantity of lubricants and preservatives, and finally the procedure was optimized. The hydroalcoholic extracts of leaves of *Swertia chirayita* and fruits of *Ficus glomerata* were mixed in the ratio as mentioned in Table 1 and then taken for the preparation of capsules by filling with granules made through wet granulation method using lactose solution as a binder. The wet mass was passed through sieve number 22 to obtain granules. The granules were dried at 45°C in a tray dryer. The dried granules were then lubricated with Magnesium Stearate. The lubricated granules are mixed with diluents and preservatives. The formed granules were optimized and filled in capsules coloured yellow-red of size "0" in a capsule filling machine. The capsules were then deducted and transferred into labelled container and then samples were evaluated as per testing requirements. Each 500 mg of a capsule contained the extracts of *Swertia chirayita* and *Ficus glomerata* along with lactose and excipients in quantity sufficient (q.s.).

Table 1: Preparation of Poly Herbal Formulation (Capsule)

Ingredients	Formulation							
	F1	F2	F3	F4	F5	F6	F7	F8
SCE	75	50	100	100	85	80	100	85
FGE	75	100	50	100	65	80	75	75
Lactose	200	100	150	100	100	120	150	200
Magnesium stearate	100	200	150	150	200	170	125	90
Methyl paraben	25	25	25	25	25	25	25	25

Propyl paraben	25	25	25	25	25	25	25	25
Net weight (mg)	500	500	500	500	500	500	500	500

Evaluation of PHF (Capsule)

The capsules containing herbal extracts were evaluated for their description, uniformity of dosage units, weight variation, disintegration time, moisture content etc. are compared with Indian pharmacopoeia standards.

The different formulation were evaluated for its organoleptic properties like color, odor and taste, moisture content, pH.

Weight variation

The weight of twenty capsules were individually weighed and the average weight of the capsule were calculated. The individual weight of each capsule should be within the limits of 90% and 110% of the average weight.

Disintegration time

The disintegration test was performed using the instrument digital microprocessor-based disintegration test apparatus (Electro lab, Mumbai, India). One capsule was introduced into each tube and a disk was added to each tube. The assembly was placed in water in a 1000 ml beaker. The volume of water at its highest point was at least 25 mm below the surface of the water and at its lowest point was at least 25 mm above the bottom of the beaker. The apparatus was operated and maintained at a temperature of 37 ± 2°C.[7]

Anti-Diabetic Activity

In-vivo study for anti-diabetic activity was carried out on Wistar albino rats of 4 months, of both sexes, weighing between 110 to 160 gm. They were provided from the MLR Institute of Pharmacy, Dundigal, Hyderabad-43, Medchal, Telangana. The animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at temperature 25±2°C relative humidity 44 –56% and light and dark cycles of 12:12 hours, fed with standard pallet diet and water *ad libitum* during the experiment. The experiment was approved by the

institutional ethics committee and as per CPCSEA guidelines (approval no. IAEC/2021-22/RP-03).[8]

Acute oral toxicity studies

The oral acute toxicity study was performed as per OECD guidelines (425) on Wistar albino rats. Three animals were selected for maximum tolerable dose (2000mg/kg) of each polyherbal preparation. The animals were examined individually for any toxicity sign like convulsion, tremor, circling, depression and mortality after dosing for 24 hours. All observations were recorded animal wise systemically. The findings of study included till 7th day, there was no toxic signs noticed in animals. Hence administered dose was concluded to be tolerable.

Diabetes Model

The diabetes mellitus was induced in animals by a single dose of freshly prepared Streptozotocin administered through intraperitoneal route. Streptozotocin solution of 10 mg/ ml was prepared in ice-cold citrate buffer 0.1 M, pH 4.5 kept in ice and was administered at a dose of 60mg/kg body weight on day 1st. Treatment was given after diabetes induction (day 3rd) for 21 days.[9]

Grouping and dosing:

Animals were divided into five groups containing six animals in each.

Group	Dosing and treatment
I	Normal control (vehicle only, 1ml/100gm)
II	Diabetic control, Streptozotocin 60g/kg, i.p.
III	Standard, Diabetic rats were treated with Glibenclamide 0.25 mg/kg once daily for 21 days
IV	Diabetic rats were treated with Polyherbal preparation at 250 mg/kg once daily for 21 days.
V	Diabetic rats were treated with Polyherbal preparation at 500 mg/kg once daily for 21 days.

Physiological Parameters:

Body weight of animals were measured using animal weighing balance.

Table 2: Evaluation of Organoleptic properties of Polyherbal Capsules (F1 to F8)

Evaluation Parameters	**Observations**							
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Color	Light green	Light green	Light green	Light green	Light green	Dark green	Dark green	Dark green
Odor Characteristics	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Taste	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter
Nature	Powder granules	Powder granules	Powder granules	Powder granules	Powder granules	Powder granules	Powder granules	Powder granules
Size of capsule	0	0	0	0	0	0	0	0

Table 3: Evaluation of Polyherbal Capsules (F1 to F8)

Evaluation Parameters	**Observations**							
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Moisture Content	1.13% w/w	1.06% w/w	1.05% w/w	0.96% w/w	1.16% w/w	1.04% w/w	0.98% w/w	1.09% w/w
pH	7.3	7.1	6.9	7.4	7.3	7.4	7.1	7.2
Average Weight	500 mg	500 mg	500 mg	500 mg	500 mg	500 mg	500 mg	500 mg

Weight Variations (%)	3.92	5.88	3.88	6.73	2.11	3.63	4.37	3.49
Disintegration Time	12min	10 min	13 min	12 min	10min	11 min	12 min	11 min

Table 4: Effect of Polyherbal preparation on body weight in Streptozotocin induced diabetes in rats.

Groups	**Treatment**	**Body weight (gm) (**mean± SEM**)**			
		Initial	**Day 7**	**Day 14**	**Day 21**
I	Normal Control	132.5±5.36	133.7±5.66	134.2±5.77	135.5±5.38
II	Diabetic control	132.9±6.22	130.2±6.59	130.0±6.58	129.3±6.38
III	Standard (Glibenclamide 0.25 mg/kg)	133.3±4.28	135.8±4.58	134.9±4.63	135.4±4.76
IV	Polyherbal preparation (250 mg/kg)	132.0±3.24	130.8±3.52	134.7±3.44	132.8±3.89
V	Polyherbal preparation (500 mg/kg)	132.9±7.56	129.4±7.22	133.5±7.74	134.7±7.84

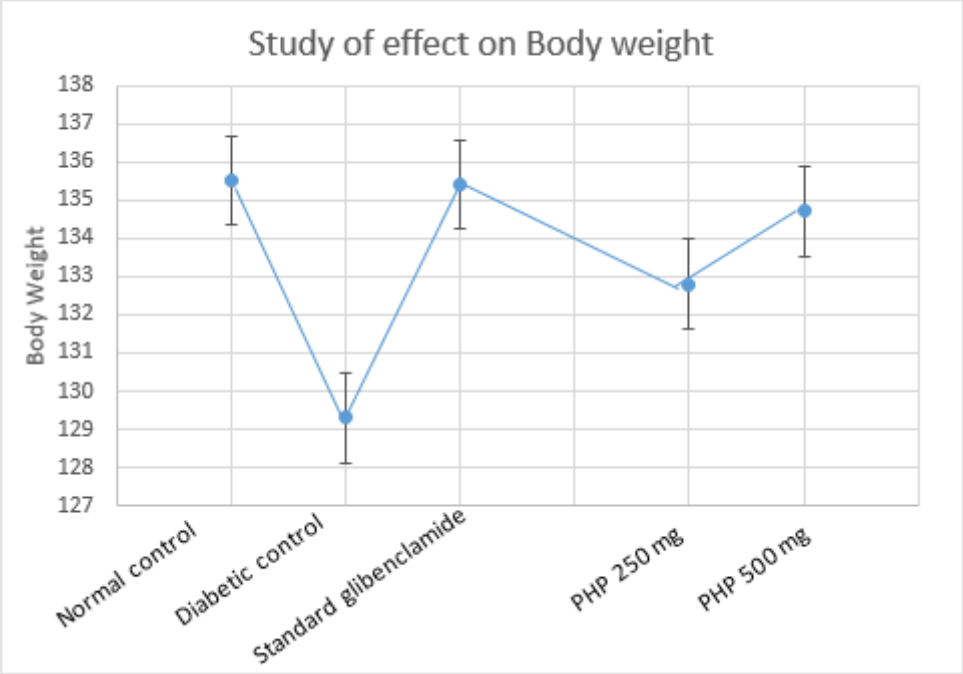

Fig 1: Graph showing study on body weight variation in different group of animals under different conditions

Table 5: Effect of Polyherbal preparation on the blood glucose level in Streptozotocin induced diabetic rats.

Groups	Treatment	Blood Glucose Level (mg/dl)			
		Day 3	Day 7	Day 14	Day 21
I	Normal Control (NC)	103.0±8.22	101.2±8.66	103.4±6.25	105.7±6.36
II	Diabetic control	218.0±8.59[a***]	283.0±11.26 [a***]	323.7±8.55 [a***]	307.7±5.46 [a***]
III	Standard (Glibenclamide 0.25 mg/kg)	208.6±8.75 [a***]	155.0±5.52 [a***, b***]	123.0±6.59 [a***, b***]	95.8±4.32 [a***, b***]
IV	Polyherbal preparation (250 mg/kg)	201.0±8.84 [a***]	198.0±6.67 [a***, b***, c***]	172.2±9.24 [a***, b***, c***]	143.3±8.36 [a***, b***, c***]
V	Polyherbal preparation (500 mg/kg)	206.0±8.54 [a***]	186.0±10.76 [a***, b***, c***]	143.5±6.83 [a***, b***, c***]	118.2±7.48 [a***, b***, c***]

Values are mean ± SEM from a group of six animals. *p<0.05, **p<0.01 and***p<0.001

a- Significance difference as compare to normal control group

b- Significance difference as compare to Diabetic control group

c- Significance difference as compare to standard treated group

DISCUSSION AND CONCLUSION

Extraction of Plant Material

The shade dried coarsely powder of selected medicinal plant *Swertia chirayita* leaves and fruits of *Ficus glomerata* are extracted with ethanol in a soxhlet apparatus. The solvents are removed by distillation under reduced pressure and the resulting semisolid mass is vacuum dried using rotary flash evaporator.

The percentage yields of alcoholic extract of selected medicinal plant along with their color, nature and pH are presented in Table 2 shows comparative percentage extractive value. The Percent yield of *Swerita chirayita* is found to be maximum as compare to *Ficus glomerata*.

Evaluation of PHF (Capsule)

The polyherbal capsules were evaluated for their description, uniformity of dosage units, weight variation, disintegration time, and moisture content and compared with Indian pharmacopoeia standards. The results for various formulation (F1 to F8) were presented in Table 3. From the detailed result it was found that the formulation code, F5 is showing better results as compared to other formulation codes.

The acute toxicity studies revealed the non-toxic nature of the herbal formulation. Experiment was carried out on normal healthy rats at 2000mg/kg, there was no mortality observed at this dose in rats.

Diabetes mellitus (DM) is a chronic disease characterized by hyperglycemia resulting in insulin resistance and/or insulin secondary deficiency caused by the failure of beta- (β-) pancreatic cells.

Streptozotocin-induced hyperglycemia in rodents is considered to be a widely used model for the preliminary screening of agents. Streptozotocin(N-{methylnitrocarbamoyl}-D-glucosamine), is a potent DNA methylating agent and acts as a nitric oxide donor in pancreatic cells. ß-cells are particularly sensitive to be damaged by nitric oxide and free radicals because of their low levels of free radical scavenging enzymes.

In the present study, diabetes was induced in animals by a single dose of freshly prepared Streptozotocin administered through intraperitoneal route. The treatment started after diabetes induction that is on day 3rd to 21 days.

The change in body weight is one of the evaluation criteria in disease progression. The body weight of animals in different groups during the study period is shown in Table 4. The body weight was monitored on initial, day 7, day 14 and day 21. The body weight continuously declines throughout the treatment majorly in diabetic control group animals while in the remaining groups overall no gain no loss was recorded.

The findings of diabetes by blood glucose level provides an excellent and simple tool to measure the anti-diabetic activity of the study drug. The evaluation of blood glucose level was significantly increased in diabetic control group animal. Animal treatment with polyherbal preparation at dose 250 mg/kg & 500 mg/kg showed a significant anti-hyperglycemic activity. The maximum reduction in blood glucose levels was observed in animals receiving glibenclamide (Table 5).

On the basis of results of this preliminary study it is concluded that polyherbal preparation has dose-dependent anti-diabetic activity. The present study shows that the polyherbal preparation at dose 500 mg/kg has significant anti-diabetic activity. Further studies may be carried out to confirm its anti-diabetic activity.

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