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Development and Evaluation of Antidiabetic Potential of Polyherbal Formulation in Streptozotocin Induced Animal Model

Mahendra Kumar Sahu*, Vijay Kumar Singh, S. Prakash Rao

Columbia Institute of Pharmacy, Tekari, Near Vidhansabha, Raipur, Chhattisgarh, India.

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***Corresponding author:** Mahendra Kumar Sahu, Department of Pharmacology, Columbia Institute of Pharmacy, Vill-Tekari, Near Vidhansabha, Raipur -493111, Dist-Raipur, (C.G.) India. Email:mahendrapharma0310@gmail.com, Mobile: 8109794919.

Abstract

Background

Diabetes mellitus (DM) is a group of disorders that results in too much sugar in the blood due to impairment of lipids, carbohydrates, proteins metabolism.

Aim and objectives

Development and Evaluation of Polyherbal formulation (PHF) and determination of antidiabetic potential of developed formulation in Streptozotocin induced animal model.

Methods

In the present study plant parts Azadirechta Indica (AI) leaves, Moringa Oleifera (MO) fruits and Andrographis Paniculata (AP) root and stem were collected and evaluated as per physico-chemical parameters and active chemical constituents were extracted using hydro alcoholic solvent. The active compounds present in all the three extracts were identified by preliminary phytochemical screening. PHF was prepared in a ratio of 1:1:1 quality of the finished product was evaluated on the parameter's angle of repose, loose bulk density, tapped bulk density, carr's index and hausner ratio as per the World Health Organization's (WHO) guidelines for the quality control of herbal materials. The acute toxicity study of PHF were performed as per OECD guideline 423, rats were orally administered 250, 500, 1000 and 2000 mg/kg over 14 days. The oral glucose tolerance test (OGTT) was performed at 200 and 400 mg/kg body weight. Antidiabetic activity of the PHF (200 and 400 mg/kg) was screened against streptozotocin (STZ) induced diabetes in rats and glibenclamide was used (5.0 mg/kg body weight) as standard drug. The investigational drug was administered for 14 days and the effect of the PHF on blood glucose levels was studied at 14th day after interventional period. At the end of the study, the blood samples were collected from all the animals for biochemical estimation.

Results

The plant parts AI leaves, MO fruits, AP stem and leaves were evaluated as per physicochemical parameters and they were found as per API. Preliminary phytochemical screening of hydroalcoholic extracts were revealed that presence of alkaloids, glycosides, saponins, flavonoids, carbohydrates, steroids, tannins and phenolic compounds in each extract. PHF were developed by mixing of each extract in the same ratio and evaluated. It was found to be angle of repose (θ) 29.1, loose bulk density 0.48 gm/ml, tapped density 0.54 gm/ml , carrs index 12.50%, hausner's ratio 1.13. Diabetes was induced by STZ and treated with PHF did not show any change in behavior and no mortality was observed during interventional period upto the dose level 2000 mg/kg. OGTT was performed by oral administration of PHF with dose 200 and 400 mg/kg body weight result was found to be gradually decreased in blood glucose level 75.75±1.92 mg/dl and 72± 2.73 mg/dl at 180 min from the study it was predicted that PHF possess Anti-hyperglycemic activity. Experimental study was shows that on repeated administration of PHF and glibenclamide

for 14 days, a sustained and significant decrease in the average blood glucose level of diabetic rats was observed. End of the interventional period biochemical parameters were studied and it was found to be level of SGOT and urea level remain constant at dose of 200 mg/kg, decrease in SGPT is near to standard and decrease in creatinine level is greater than Std at dose of 400 mg/kg.

Conclusion

PHF containing extracts of (Azadirecta indica, Moringa oeifera and Andrographis paniculata) showed significant antidiabetic and antihyperlipidemic activity which was close to standard drug. Along with remarkable reduction in Total Cholesterol (TC) level and increased in High Density Lipoprotein (HDL) STZ induced diabetes rats. The formulation has emerged as potential combination which can challenge the synthetic drug.

Key words: Diabetes mellitus; Azadirechta indica; Moringa Oleifera; Andrographis Paniculata; Polyherbal formulation; Glibenclamide.

Introduction

Importance of Herbal in Mankind

Herbal drugs play an important role in the development of potent therapeutic agents. Furthermore, it has proven their potential for the prevention of several ailments. Earlier human beings started their studies on diseases and its treatments, but there was no evidence found that people have prehistoric use of synthetic medicines for their sickness [1]. However they struggled to make use of the things, which could easily procure. The most common thing was found in their surrounding was plants and animals. Several plants were found suitable as a food supplement; some were poisonous and have medicinal importance [2]. Keeping this information in consideration, herbs were transferred from their origin to generation as folk medicine. So the herbal medicine was known from ancient times. This is only because of the belief that many herbal medicines are known to be free from side effects. Furthermore, it is fact that the discovery of the new synthetic drug is time consuming & an expensive. In the present scenario, the demand for herbal products is growing exponentially. All over the world pharmaceutical companies are currently conducting extensive research on plant materials for their probable medicinal value [3]. Research needs in the field of herbal medicines are enormous; the identification of active compounds from the plants source is still remaining a challenge. So, there should be research based confirmation on either whole herbs or extracted compounds are superior. The issue of herb–herb and herb–drug interactions is also an important issue, which requires increased awareness and study, as polypharmacy and polyherbacy are common. The new technologies, such as nanotechnology and novel emulsification methods are used in the formulation of herbal products, which mainly affect bioavailability and the efficacy of herbal components and this also needs study. This can lead to reinvestigation of some agents that failed earlier trials and can be restudied and redesigned using new technologies to determine whether they can be modified for better efficacy and fewer side effects [4]. Today, there is an urgent need to develop safer drugs for the treatment of various disorders. As a result, there is a growing interest in the pharmacological evaluation of various plants used in traditional systems of medicine [5].

Diabetes Mellitus

Diabetes Mellitus (DM) is a metabolic disorder associated by impairment in the metabolism of carbohydrate, fat and proteins which was recognized by insufficient insulin secretion or mounting resistance to its action [6]. DM develops due to obesity which is also an increasing problem worldwide, Induces atherosclerosis and other metabolic syndromes [7-10]. According to the requirements of insulin DM was classified into two main categories; insulin dependent diabetes mellitus (Type 1), and non-insulin dependent diabetes mellitus (Type 2) [11]. Which were proposed by WHO in 1980 and 1985 changed new classification system were identified four types of diabetes mellitus, Type 1 insulin dependent diabetes mellitus, Type 2 non-insulin dependent diabetes mellitus and Type 3 is Maturity Onset Diabetes of the Young (MODY) as well as Gestational Diabetes Mellitus (GDM) was classified as Type 4 [12].

Materials and Methods

Drug and Chemicals used

Glibenclamide (USV Pharma Ltd. India), Straptozotocin (Lab chemicals, India), one touch glucometer (Johnson & Johnson, India), Ethanol (Qualigens, India) and other chemicals were used of analytical grade.

Collection, identification and authentification of plant materials

In the present study, the fresh leaves of Azadirechta Indica, fruits of Moringa oleifera and fresh leaves and roots of Andrographis Paniculata were collected in febuary, 2018, from Raipur, Chhattisgarh, India. The plants were identified and authenticated by Dr. S. Prakash Rao, Department of Phytochemistry and Pharmacognosy, Columbia Institute of Pharmacy, Raipur, Chhattisgarh, India.

Quality assessment/Physiochemical evaluation of plant materials

Each plant parts were crushed and converted into fine powders than quality assessment of plant materials was done as per the standard procedure of Ayurvedic Pharmacopeia of India. Different parameters were tested with the methods describe in API.

Foreign organic matter

According to Ayurvedic Pharmacopeia of India, Foreign matter is described as any material that consist of part of organ or organ part from which the drug is derived. The plant should be free from any foreign particle like dust, insects, faecal matter etc. The percentage of foreign matter should not be more than the limit prescribed in monograph. There should not be any contamination in drug material used for developing the polyherbal formulation (PHF).

Procedure

100-500 gm of plant materials were weighed and spread as a thin layer and was inspected first with naked eyes and then with the use of lens (6x). All the foreign matter were Separated, weighed and percentage was calculated.

Determination of Total ash value

3 gm of dried powered sample was weighed in silica dish and it was incinerated at a temperature not exceeding 450°C until it get free from carbon. The incinerated material was cooled, weighed and percentage of ash was calculated with reference to air dried drug.

Determination of Acid insoluble ash value Ash obtained was boiled with 25 ml of dil. HCL for 5 minutes filtered and insoluble matter was collected in crucible and washed with hot water and ignited till constant weight. The percentage of acid insoluble ash was calculated with respect to air dried drug.

Determination of Alcohol soluble extractive value

5gm of powdered drug was macerated with 100 ml of alcohol in cork fitted conical flask. Solution was shaken frequently for 6 hrs and was allowed to stand for 18 hrs. After 18 hr. content was filtered and 25 ml of filtrate was evaporated to dryness in a shallow dish at 105°C to constant weight and percentage of alcohol soluble extractives was calculated with reference to air dried drug.

Determination of water-soluble extractives

5gm of powdered drug was macerated with 100 ml of water in cork fitted conical flask. Solution was shaken frequently for 6 hrs and allowed to stand for 18 hrs. After 18 hr. content was filtered and 25 ml of filtrate was evaporated to dryness in a shallow dish at 105°C to constant weight and percentage of water soluble extractives was calculated with reference to air dried drug. The data generated in respect of above findings will be used as in-house standards.

Preparation of hydro-alcoholic (HA) extracts

The plant parts were washed, shade dried and powdered. In order to prepare the PHF, about 500gm of Azadirecta Indica (leaves), 500gm of Moringa Oleifera (fruits) and 500gm of Andrographis Paniculata (roots and leaves) powders were soaked overnight separately in 1000-1200 ml of Petroleum Ether(PE). After 3 days the suspension were filtered and PE was to be evaporated overnight. Again the dried powders were separately resuspended in a Stoppered container with the HA solvent. Allowed to stand at room temperature for a period of 7days. Additionally, extract was concentrated to dryness in a rotary evaporator (Buchi type) under reduced pressure and controlled temperature (37–40°C) to get percentage yield.

Preliminary phytochemical screening of HA extracts

Crude extract of plants were subjected to different chemical tests to detect the presence of various phytochemical constituents as per procedure adopted in literature.The details are incorporated below in the following table. Results of the entire chemical test are discussed in Results.

Constituent	Chemical Test	Procedure	Azadirechta Indica	Moringa Oleifera	Andrographis Paniculata
Alkaloids	Mayer's reagent test	Extract+ Dil. HCL + 3ml Mayer's reagent	Yellow precipitate obtained	Yellow precipitate obtained	Yellow precipitate obtained
	Dragondroff's test	Extract + Dil. HCL+ 3ml Dragendroff's reagent	Reddish brown precipitate	Reddish brown precipitate	Reddish brown precipitate
Glycosides	Legal's test	Extract + 10%	NaoH + Sodium	Nitroprusside	Blue colour
Saponins	Flavonoids	Foam test Extract + water	shaken vigorously	Persistance	Foam
	Lead acetate	test	Extract solution	lead acetate	Yellow

			of		
Carbohydrate's	Fehling's test	1ml Fehling A+ 1ml Fehling mixed and boiled for a minute	Brick red precipitate formed	Brick red precipitate formed	Brick red precipitate formed
Steroid's	Salkowski test	Extract(2ml)+2ml+ chloroform + 2ml conc. H2SO4	Choloroform layer turned red and acid layer green	Choloroform layer turned red and acid layer green	No Chloroform layer Formed
Tannin's and Phenolic Coumpounds	FeCl3 test	Extract+ FeCl3	Deep blue Coloured	Deep blue coloured	Deep blue Coloured

Table 1: Preliminary phytochemical screening of HA extracts

Design and Development of PHF

From the extracts of three plants Azadirechta Indica (leaves), Moringa Oleifera (fruits) and Andrographis Paniculata (roots and leaves), formulation have been made by blending the extracts in ration 1:1:1.

Evaluation of Polyherbal formulations

Prepared PHF was evaluated on following parameters:

Angle of repose

Angle of repose was determined by using funnel method. The accurately weighed blend was taken in a funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touches the apex of the heap or head of blend. The drug excipient blend was allowed to flow through the funnel freely on to the surface. The diameter of the powder cone was measured and angle of repose was calculated using the following equation:

$\tan \theta = h/r$ Where, h = height of powder cone formed, r = radius of the powder cone formed

Loose bulk density

Apparent bulk density was determined by pouring a weighed quantity of blend into graduated cylinder and measuring the volume and weight. LBD = Weight of the powder / volume of the packing.

Tapped bulk density

It was determined by placing a graduated cylinder, containing a known mass of drug excipient blend. The cylinder was allowed to fall under its own weight on to a hard surface from the height of 10 cm at two second intervals. The tapping was continued until no further change in volume was noted.

TBD = Weight of the powder/vol of the tapped packing

Compressibility index

The Compressibility index of the blends was determined by Carr's compressibility index.

Compressibility index (%) = (TBD-LBD) x 100/TBD

Hausner ratio

It is the measurement of frictional resistance of drug and ideal range should be 1.2-1.5. It is determined by using the following formula:

Hausner ratio= TBD / LBD

Acute toxicity study of PHF as per OECD guidelines

Preparation of Formulations

For dosing 100 ml of each formulation was prepared by dissolving 5 gm of formulation in

100 ml of distilled water (so, 1ml contain 50 mg of drug).

Experimental Animals

Adult Wistar rats (180 ± 10 g) of either sex were obtained from Columbia institute of pharmacy, Raipur, Chhattisgarh, india. The animals were housed in large, spacious polyacrylic cages at an ambient room temperature with 12h light/12h dark cycle. Rats had free access to water and rodent pellets diet (Hindustan Lever Ltd, Bangalore, India). The study was approved by the Institute Animal Ethics Committee and all the animal experiments were carried out according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, Regd. No. 1321/PO/ReBi/S/10/10/CPCSEA.

Acute toxicity study of PHF

Acute toxicity studies were carried out in adult female albino rats weighing between 130-180 gm by Acute Oral Toxicity method of OECD Guideline No 423. They were administered (orally) with varying doses (250, 500, 1000 and 2000 mg/kg body weight) for each of six formulations. Animals were divided into 5 groups of three animals each and were acclimatized for 5 days. Prior to dosing animals were kept fasted overnight and next day each formulation were administered orally at a dose level of 250, 500, 1000 and 2000 mg/kg body weight. Rats were observed for clinical signs of toxicity continuously for 2 hours and occasionally for further 4hours for general behavioral and finally for any mortality after 24 hours till 14 days. No mortality was observed during a time period of 14 days.

Oral glucose tolerance test of formulation

Selection of dose

Two dose level were chosen in such a way that one dose was approximately one-tenth of the maximum dose used during the acute toxicity studies, second dose was the twice that of one tenth dose (200mg/kg, 400mg/kg b. wt)

Initial Screening of all the PHF for Anti-hyperglycemic activity (Oral Glucose Tolerance Test)

Formulation was screened for anti-hyperglycemic activity to get the information on their efficacy so that the formulation which is not effective could be modified. Formulation was analyzed for anti-hyperglycemic and antihyperlipidemic activity in normal healthy rats by conducting Oral Glucose Tolerance Test (OGTT). Initial testing was carried out at different dose levels of formulation (200 and 400 mg/kg b. wt). Overnight fasted rats were weighed and divided in to five groups with 5 rats in each group for each formulation as given below. After 30 minutes, rats of all groups were loaded orally with glucose 2g/kg b. wt. Blood glucose level was determined by glucometer before and at 30 min, 60 min, 120 min, 150 min and 180 min after loading with glucose.

Group Design for OGTT study

Group I – Normal Control treated with vehicle i.e (2ml/kg) distilled water

Group II- Standard given Glibenclamide (5mg/b.wt)

Group III- treated orally with F-A 200 mg/kg b.wt.

Group IV- treated orally with F-A 400 mg/kg b.wt.

Antidiabetic activity

Study protocol

Induction of diabetes and experimental study Diabetes was induced in rats by intra-peritoneal injection of Streptozotocin (45 mg/kg b.wt) which was dissolved in normal saline. After 72 h of STZ administration blood glucose level was measured by one touch glucometer (Johnson & Johnson, India) to confirm diabetes. Blood samples were drawn by picking the rat tail. The diabetic rats with blood glucose levels ≥250 mg/dl were selected for the studies. After 72 hr. of STZ injection animal with BGL ≥250 mg/dl were divided into different groups (with 5 animals each) for anti-diabetic study of Formulations. Following groups were prepared:

Group I –Normal control (given distilled water)

Group II-Negative control (treated with STZ 45 mg/kg b.wt i.p)

Group III-Standard (Treated with Glibenclamide 5mg/kg b.wt after 3rd day of STZ injection)

Group IV-Treated orally with Formulation A with dose of 200 mg/kg b.wt after 3rd day of STZ injection

Group V- Treated orally with Formulation A dose of 400 mg/kg b.wt after 3rd day of STZ injection

Study was conducted for 14 days. Treatment was started from 3rd day. Standard drug and Formulations given daily for 14 days and blood glucose levels were measured with the help of one touch glucometer (Johnson & Johnson, India) on 3rd day (assume as 0 hrs.), after 3 hrs, 5th day, 10th day and 14th day of experiment. Blood sample was taken by picking the rat tail vein and for the measurement of other biochemical parameters blood sample was withdrawn from retro orbital plexus of rats.

Assessment of Biochemical parameters

At the end of 14th day of experiment, 2-4 ml blood sample was withdrawn from retro-orbital plexus of rats and centrifuged at the 5000 rpm for 15-20 min; serum was separated and taken out with the help of syringe. Serum of rats was used for the analysis of other biochemical parameters through Auto analyzer.

Results and Observation

Physiochemical evaluation of plant materials

It was observed that all physicochemical evaluation parameters contain i.e. foreign organic matter, Total ash, Acid insoluble ash, Alcohol extractive and water-soluble extractives of plant drug was found to be within Ayurvedic pharmacopeia limits.

Percentage yield of all the HA plant extracts

The percentage yields of all HA plant extract are given in table 3.

Preliminary Phytochemical screening of HA plant extracts

Results of phytochemical screening are shown in table 4. It was found that Azadirechta Indica, Moringa Oleifera and Andrographis Paniculata contain all tested phytochemical compounds.

Design and Development of PHF

PHF was made in such a way so that it covers most of targeted sites in body to decrease the blood glucose level for their anti-diabetic action. For formulations quantity of doses used in developing the formulation was calculated on the basis of therapeutic doses reported in literatures.

Evaluation of Polyherbal formulations

The various combinations of dried powdered extracts of Azadirechta Indica, Moringa Oleifera, Andrographis Paniculata were prepared and evaluated on the parameters like angle of repose, loose bulk density, tapped bulk density, carr's index and hausner ratio. Preformulation study of the granules showed that all the evaluated parameters were within the acceptable limit.

Acute toxicity study of PHF formulation

STZ induced diabetic rats treated with PHF did not show any discernible change in behaviour up to the dose level of 2000 mg/kg body weight. No sign of mortality was observed during the observation of 14 days (table 6).

Oral glucose tolerance test (OGTT) of PHF

At 30 min after the administration of 2gm/kg glucose orally, the plasma glucose level issignificantly increased and the blood glucose level decreases gradually with the dministration of formulations. Results are given in table: 6 and results expressed in Mean ± SD in table 7.

Parameter	Azadirecta Indica		Moringa Oleifera		Andrographis Paniculata	
	Obtained value	API limit	Obtained value	API limit	Obtained value	API limit
Foreign organic matter	0.002%	NMT 0.3%	1.87±.05%	NMT 2%	1.78±01%	NMT 2%
Total ash value	3.48±23%	NMT 5%	4.36±.22%	NMT 5%	1.55±.25%	NMT 12%
Acid insoluble ash value	0.43±.01%	NMT 0.6%	1.33±.25%	NMT 2%	0.30±.03%	NMT 0.5%
Alcohol extractive value	8.30±0.72%	NLT 6%	11.69.±54%	NMT 12%	9.59±.36%	NLT 7%
Water soluble extractive value	30.78±0.51%	NLT 28%	22.42±.76%	NMT 23%	7.34±.74%	NLT 5%

(NMT-Not more than, NLT –Not less than)

Table 2: Results of Physico-chemical evaluation of the plant material

Name of Plant Drug	Powdered Plant Drug (gm)	Solvent used Ethanol:Water (10:90)	Percentage yield
Azadirechta Indica	250 gm	1000 ml	11.00%
Moringa Oleifera	250 gm	1000 ml	24.09%
Andrographis Panichulata	250 gm	1000 ml	15.23%

Table 3: Percentage yield of HA plant extracts

Constituent	Azadirechta Indica	Moringa Oleifera	Andrographis paniculata
Alkaloids	+	+	-
Glycosides	+	+	+
Saponins	+	+	+
Flavonoids	+	+	+
Carbohydrate's	+	+	+
Steroid's	+	+	+
Tannin's and Phenolic Compounds	-	+	+

Table 4: Preliminary Phytochemical screening of HA plant extract

Batch	Angle of repose	Loose bulk density	Tapped bulk density	Carr's index	Hausner's ratio
PHF	29.1	0.48	0.54	12.50	1.13

Table 5: Evaluation parameters of dried PHF

Group	No of rats	Wt. of rats (gm)	Dose of formulation	Calculated dose (mg)	No. of dead animals
I	3	150.23	250 mg/kg b. wt	37.55	Nil
		148.79		37.19	
		150.12		37.53	
II	3	151.40	500 mg/kg b. wt	75.70	Nil
		145.62		72.81	
		156.01		78.00	
III	3	150.92	1000 mg/kg b. wt	150.92	Nil
		150.12		150.12	
		152.34		150.34	
IV	3	155.03	2000 mg/kg b. wt	310.06	Nil
		142.34		284.68	
		145.73		291.46	

Table 6: Results of Toxicity study of Formulation

Group	Treatment	No. of rats	Weight of rats (gm)	Dose	Calculated dose	Fasting BGL mg/dl	After loading with glucose 2g/kg b. wt. (Oral Glucose Tolerance Test)				
							30 min	60 min	120 min	150 min	180 min
I	Control	5	152.23	2ml/kg b. wt	0.30ml	68	102	116	128	130	127
			149.62		0.29	69	99	110	118	127	123
			155.24		0.31	63	98	113	120	135	129
			161.08		0.32	60	95	120	127	142	135
			153.12		0.30	65	92	115	122	135	130
II	Glibenclamide	5	149.58	5mg/kg b. wt	0.067mg	64	98	86	75	71	62
			151.32		0.067	66	103	90	76	68	58
			166.71		0.075	60	104	89	79	69	58
			168.54		0.075	65	99	80	73	66	60
			160.03		0.072	67	107	91	79	65	61
III	Fomulation	5	168.48	200mg/kg b. wt	33.69	67	103	89	90	78	76
			162.72		32.54	69	110	108	95	79	75

			154.13		30.82	71	111	111	99	82	74
			150.34		30.06	69	107	86	93	76	79
			152.16		30.43	70	103	91	89	81	75
IV	Formulation	5	170.02	400mg/kg	68.00	60	100	95	85	80	78
			148.53	b. wt	59.41	61	105	97	79	76	60
			156.61		62.64	63	99	87	79	72	68
			157.09		62.83	66	95	89	84	80	76
			162.08		64.83	60	108	96	86	79	80

Table 7: Results of OGTT (Blood glucose levels are expressed as Mean± SD)

GROUP	TREATMENT	FASTING BGL	30 min	60 min	120 min	150 min	180 min
I	Control	65.0±3.67	81.63±3.83	96.28±3.70	103.22±4.35	112.45±5.71	128.8±4.38
II	Glibenclamide	64.40±2.70	102.2±3.70	87.2±4.43	76.4±2.60	67.8±2.38	59.8±1.78
III	Formulation	69.2±1.48	106.8±3.76	94.4±4.97	93.2±4.02	79.2±2.38	75.75±1.92
IV	Formulation	62.0±2.54	99.4±3.64	92.8±4.49	82.6±3.36	77.4±3.43	72.0±2.73

Table 8: Average OGTT (Blood glucose level expressed in Mean± S.E.)

Findings of OGTT study

It was found that PHF with dose of 200 mg/kg body weight showed effective decrease in blood glucose i.e. 75.75±1.92 mg/dl and dose 400 mg showed 72± 2.73 mg/dl at 180 min. From the study it was predicted that PHF possess Anti-hyperglycemic activity.

Antidiabetic activity

Experimental study

Albino wistar rats of either sex (150-180gm body weight) were used for this study; they were acclimatized and given proper diet. The study was approved by the Institute Animal Ethics Committee and all the animal experiments were carried out according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. Results showed the significantly increase in blood glucose level in STZ treated diabetic rats. Glucose levels measured in blood of normal and experimental rats are given in table 8. On repeated administration of vehicle, PHF and glibenclamide for 14 days, a sustained and significant decrease in the average blood glucose level of diabetic rats was observed.

Parameters	Group I (Control)		Group II (Negative control)		Group III (Standard)		Group IV 200 mg		Group V 400 mg	
	0 day	After 15th day	0 day	After 15th day	0 day	After 15th day	0 day	After 15th day	0 day	After 15th day
Cholesterol	47.5 48.7	50.7 49.0	85.6 91.6	95.9 112.6	113.4 96.7	58.4 48.3	96.4 120.4	78.2 91.3	84.5 93.9	49.3 58.6

	55.0	60.2	98.4	126.4	126.5	49.4	98.9	71.7	117.8	80.8
	50.3	48.1	88.2	102.4	109.4	46.7	104.4	79.4	111.9	72.9
	62.0	55.3	112.4	126.6	89.4	38.7	85.6	59.2	107.8	66.6
Triglycerides	59.8	61.7	166.0	212.9	155.2	71.2	125.3	79.5	115.7	64.5
	76.2	80.4	97.0	140.2	93.4	49.6	134.2	99.16	96.9	46.8
	60.4	60.9	113.8	143.8	133.4	73.5	128.7	93.1	129.5	77.8
	68.6	69.9	99.2	124.5	148.9	70.5	106.8	75.7	133.7	84.4
	68.4	67.6	84.3	111.7	138.6	67.3	112.4	78.8	118.9	64.6
SGOT	10.8	11.2	68.2	72.3	49.1	15.2	050.3	30.2	56.7	25.4
	25.2	26.7	59.5	74.6	63.2	20.2	47.6	18.3	45.2	20.0
	20.5	11.4	52.6	62.0	58.4	26.3	49.2	21.4	43.8	16.3
	31.6	30.2	49.0	60.1	41.8	13.1	60.4	39.2	58.4	18.4
	13.7	18.2	70.1	82.4	60.2	21.5	54.2	22.3	53.5	34.3
SGPT	24.6	26.3	62.4	71.2	68.3	17.2	71.9	20.1	75.2	26.2
	29.2	31.4	58.0	78.8	73.2	25.3	69.8	28.3	60.0	19.3
	30.1	32.6	59.5	69.2	69.6	21.3	59.2	31.2	63.4	20.1
	18.0	22.3	70.1	81.9	72.4	19.6	81.3	45.2	71.3	18.3
	15.6	18.7	68.0	85.6	68.6	20.4	90.0	48.2	78.6	21.3
Creatinine	0.48	0.52	1.54	1.72	1.59	0.92	1.61	1.33	1.77	1.53
	0.53	0.58	1.63	1.69	1.63	1.11	1.67	1.30	1.64	1.49
	0.61	0.63	1.62	1.82	1.57	0.72	1.73	1.52	1.58	1.24
	0.42	0.69	1.58	1.92	1.88	0.69	1.59	1.10	1.91	1.31
	1.12	1.15	1.42	1.78	1.92	1.23	1.79	1.58	1.62	0.62
Urea	30.2	31.2	86.7	91.2	75.2	23.2	69.3	38.4	78.3	41.2
	29.2	30.4	40.4	94.2	77.6	26.2	72.5	40.1	77.9	38.3
	24.6	25.6	73.8	80.1	68.3	19.4	77.9	44.6	66.1	29.4
	28.2	26.2	78.4	86.6	88.7	31.4	82.3	50.3	81.5	50.2
	22.3	24.0	93.4	98.3	90.2	35.3	73.4	39.2	69.8	30.4
HDL	40.1	35.0	21.2	15.2	30.0	40.0	17.6	21.0	18.4	26.4
	35.8	40.3	20.2	12.0	20.1	41.3	21.3	29.4	23.7	29.0
	34.4	41.7	18.9	10.0	18.6	35.3	24.6	30.4	21.0	29.4
	35.3	38.5	19.2	12.2	15.2	36.2	20.0	28.6	39.4	45.0
	41.0	36.2	30.2	18.1	20.2	38.1	19.2	24.6	28.3	36.4
LDL	22.0	24.7	58.8	62.3	58.4	25.0	66.8	42.0	59.7	31.2
	30.2	32.3	60.2	71.4	56.4	24.8	62.8	45.1	55.8	36.0
	24.4	20.2	65.4	69.2	68.3	29.4	72.1	56.3	60.3	34.6
	28.6	29.0	71.2	76.7	67.7	20.4	75.6	60.1	71.7	50.0
	23.4	30.4	80.4	80.4	60.0	25.4	68.3	52.4	67.8	38.4

Table 9: Effect of PHF on change in biochemical parameters of blood plasma in albino wistar rats from 0 day to 14th day.

Biochemical parameters

Serum TG, Total cholesterol, LDL cholesterol were found to be increased significantly (P<0.0001) in STZ induced diabetic rats (shown in table: 9) as compared to non-diabetic control. HDL cholesterol was found to be significantly decreased in diabetic rats. Treatment with PHF produces a significant reduction in elevated serum TG, TC, LDL-cholesterol level in diabetic rats. In Biochemical Parameters PHF (400 mg and 200 mg) showed maximum decrease in SGPT, Urea and LDL Cholesterol level i.e. 69.8% near to glibenclamide, 43.36% and 39.6%.

Treatment	Dose	Biochemical parameter maximum decreased from day 0th to day 15th	% decreased	Remarks
PHF	200 mg/kg b. wt.	SGOT Urea	57.2% 55.25%	Level of SGOT and urea level was found to be remain constant.
	400 mg/kg b. wt.	SGPT LDL TC,TG, Creatinine	69.8% 39.6%	Decrease in SGPT is near to standard. Decrease in creatinine level is greater than Std.

Table 10: Analysis of other Biochemical Parameters

Discussion

PHF have been developed with combinations of (3 Plants) antidiabetic activity was investigated in albino wistar rats with glibenclamide as standard, STZ was used to induce diabetes in rats. Formulation showed significant decrease in Blood glucose level with improvement in slight loss of body weight, Albino wistar rats were divided into V groups with n=5 and the diabetic rats received the formulation, vehicle and standard drug. Although formulation showed good antidiabetic activity. It showed 65.8% decrease in average blood glucose level which was very closer to standard drug glibenclamide. i.e. 66.2%. Reason for this superior activity of Formulation may be its potential active constituents which could possess better antidiabetic activity and the second main reason may its synergism (herb-herb interactions) which may be more compatible when formulated together and thus produce more effective results. As mentioned in results all the formulations gives dose dependent antidiabetic effect in this combination of medicinal plants. It was proved to be fruitful and comparable to standard against glibenclamide. PHF showed good antidiabetic activity with dose of 400 mg (i.e. 62.4 %) decrease in blood glucose level. On the basis of best synergistic effect, the lipid content except HDL was found to be increased in STZ diabetic rats. HDL Cholesterol was found to be more increased in combination as compared to individual. All combinations improve the conditions of hypercholesterolemia. PHF showed a greater increase in HDL % level to 57.12 % than those of standard. It has been observed through literatures that plants constituents like glycosides, alkaloids, flavonoids all these constituents have proved to be strong antidiabetic agent through different mechanism.

Conclusion and direction for future use

Since Ancient times medicinal plants as single drug and in combination with other herbal drugs are using in the treatment of various chronic and non-chronic disorders. Ayurveda is one of the most traditional systems of medicine which describes the methodology to use the medicinal plants as healing power in treating the disease. Polyherbalism is also the best concept of Ayurveda, which consists of magical power of healing the disease. Ayurveda is one of the reliable and trustworthy medicine systems. In developing countries mostly 75-95% of populations rely on herbal drugs. Deep research and investigation still needed on this magical system of medicines. Research Studies pertaining to safety, toxicological studies, Standardization, clinical trial studies are still required to grow Ayurveda and increasing its wide acceptability. Numbers of commercialized standardized herbal drugs are quiet less in market since we are lacking in developing the regulatory standards implemented protocols. Diabetes mellitus has appearing as dreadful disorder for society. It directly impacts our metabolic system by making it sluggish in catabolic activities. It is mainly characterized by hyperglycemia resulted from decrease insulin secretion. This dreadful disease can lead to many more complications like blindness,

kidney failure and organ dysfunction. Several synthetic drugs are available in market but with long use of these drugs could lead to serious side effect including the kidney failure there is greater risk of using these synthetic drugs for long term. Study of ancient Ayurvedic books like Charak Samhita and Sushastra Samhita revealed that drugs used in Ayurvedic formulations worked synergistically on root cause of disease. Therefore a quality control drug will be effective in management of diabetes. In view of above 3 plants, based on their reported mode of action PHF was made. PHF was subjected to acute toxicity study and found to be safe up to dose of 2000 mg/kg b.wt. After this oral glucose tolerance test (OGTT) was performed in animal model for preliminary assessment of antidiabetic activity. The antidiabetic activity was studied in albino wistar rats as per standard protocol. The diabetes was induced by use of Streptozotocin (STZ). For the study of antidiabetic activity PHF was given in 2 doses of 200 mg/kg b.wt and 400 mg/kg b.wt. for 14 days. The blood samples of each rat were analyzed for various biochemical parameters. The results showed that PHF containing extracts of (Azadirecta indica, Moringa oeifera and Andrographis paniculata) showed significant antidiabetic and antihyperlipidemic activity which was close to standard drug. Along with remarkable reduction in Total Cholesterol (TC) level and increased in High Density Lipoprotein (HDL) STZ induced diabetes rats. The formulation has emerged as potential combination which can challenge the synthetic drug.

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