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RESEARCH ARTICLE

Formulation, Characterisation and assessment of Antidiabetic activity of Polyherbal tablet (PHF) in diabetic rat model

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Abstract

Diabetes mellitus is a complex and a diverse group of disorders that disturbs the metabolism of carbohydrate, fat and protein. The number of *diabetes mellitus* cases has been increasing worldwide in recent years. In 2000, the WHO estimated a total of 171 million of people with *diabetes mellitus* from the global population, and this report projected to increase to 366 million by 2030. Now a days polyherbal formulations made by different herbal pharmaceutical company is very much popular and acceptable for chronic use in case of diabetes, hypertension, bronchial asthma, hyperlipidemia, rheumatoid arthritis etc. As the incidence of diabetes is increasing day by day globally and the rate of occurrence of disease in India is the high, the popularity of polyherbal formulation is increasing day by day. Many physicians from different system of medicine refer to advice polyherbal formulations for long time use in patient of diabetes, as the manufacturer of herbal formulator claim the formulation is having negligible side effect. Present Study was done with Evaluation of antihyperglycemic Potential of prepared polyherbal Formulation. PHF i.e. with 100mg/kg and 150 mg/kg, the FBS level was reduced from 187.16 $\pm$ 3.25 to 133.16 $\pm$ 2.93 and 181.5 $\pm$ 4.42 to 136.33 $\pm$ 2.58, respectively. In the combination regimen of PHF (150 mg/kg) and Metformin (50mg/kg) the mean FBS level dropped from 180.3 $\pm$ 1.75 to 131.16 $\pm$ 2.14. In all these groups the mean FBS level has reached to the normal pre induction level. The daily single administration of PHF formulation (150mg/kg) and Metformin (50 mg/kg) significantly reduced blood sugar levels of Streptozotocin (STZ) induced diabetic rats. It is also important to study antihyperglycemic effect of the said Polyhedral Formulation in different animal Model to conclude exact pattern of Anti hyperglycaemic activity.

Keywords: Diabetes mellitus; polyherbal formulations; antihyperglycemic; Tamarindus indica; S.rebaudiana

Introduction

Diabetes mellitus is a metabolic disease characterized by hyperglycaemia. It occurs due to either defects in insulin secretion or insulin action, or both. The chronic hyperglycaemia is related with damage and malfunction of different organs, predominantly kidneys, eyes, nerves, blood vessels and heart. Symptoms of marked hyperglycaemia include polyuria, polydipsia, occasionally polyphagia, weight loss and blurred vision. [1]

Over the past 40 years, prevalence of diabetes mellitus has increased. The trend for future is a continuous increase of all ethnic groups, men or women, for all age groups worldwide [2]. This increase is observed above all in type 2 *diabetes mellitus* (T2DM) [3]**.** In 1995, nearly 135 million people were affected and an increase of 300 million cases is estimated for year 2025 [4]. Various complications of the disease are coronary artery disease (CAD) including obesity, dyslipidemia, hyperten-

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sion (HT) and physical inactivity [5] **.** Obesity increases the risk of CAD in adults and is associated with insulin resistance in normoglycemic as well as in individuals with type 2 DM [6] **.**

The fruit Tamarind is obtained from the plant *Tamarindus indica*. The plant is a long lived and large sized tree. It is a famous as well as common tree found in India. Its fruit is salted and stored in almost every house. The people of Deccan largely consume it. They say that 'life is very ticklish in absence of Imli'. It is also used largely as a flavour, stabilizer and binder in food preparations. Generally people use Imli to make pickles, curries, jam and sauce and to prepare majoon as well as jawarish. The seeds are made flour for making bread in famine seasons. The seeds are also fried and consumed by poorer. It is very useful for people of hot area and near to equator.

Tamarind seed powders were used for tannase production by *A. niger*. The tannase yield was 6.44 IU/g ds for tamarind seed powder.

According to Ethnobotanical and traditional literature, seeds act as anti-asthmatic, antiulcer as well as antioxidant agent. Vaginal Atony is treated by a pessary of seed kernel. The fried seed paste was used on anus after setting the tract in proper position for treatment of rectal prolapse. In Unani system, spermatorrhoea, nocturnal emissions as well as seminal debility are cured by use of roasted kernel and seeds. Homeopathic system uses seed for the treatment of stomachache. It is also used in cough and for the relaxation of uvula. The urethral discharge and polyuria are treated by administration of seed kernel pounded with milk. The red outer covering of seeds is very useful in diarrhea and dysentery. The seeds are used for treatment of colitis and other intestinal disorders. To feed cattle ground seeds are usually useful. Seeds are helpful in vaginal discharges and ulcers.

Seeds are rich in phenolic compounds, polymeric tannins, glycosides, fatty acids, flavonoids, saponins, alkaloids. There is also presence of fatty acids, essential amino acids in seeds. Auxins are available in seeds. Tamarind seeds have 2-hydroxy-3′, 4′ -dihydroxyacetophenone (TAO), methy l-3, 4-dihydroxybenzoate (TA1), 3, 4-dihydroxyphenylacetate (TA2) and (-)-epicatechin. Seed also consist of acetic acid, arabinose, dihydroxylphenyl acetate. Oil obtained from seeds consists of lauric, palmitic, myristic, stearic, arachidic, behenic, lignoceric acids, loeic and linoleic acids. Dry form of kernel has 17.1-20.1% protein, 6.0-7.4% fat, 65.1-72.2% carbohydrates and some crude fibre and ash. Whereas roasted kernel includes calcium 121 mg and phosphorous 237 mg per 100 gm. It also contains proteins like prolamines and albumins [4].

For hundreds of years, indigenous peoples in Brazil and Paraguay have used the leaves of stevia as a sweetener. The Guarani Indians of Paraguay call it kaa jheé and have used it to sweeten their yerba mate tea for centuries. They have also used stevia to sweeten other teas and foods and have used it medicinally as a cardiotonic, for obesity, hypertension, and heartburn, and to help lower uric acid levels.

In addition to being a sweetener, stevia is considered (in Brazilian herbal medicine) to be hypotensive, diuretic, cardiotonic, and tonic. The leaf is used for obesity, cavities, hypertension, fatigue, depression, sweet cravings, and infections. The leaf is employed in traditional medical systems in Paraguay for the same purposes as in Brazil [5–7].

Lufa cylindrical belongs to cucurbitaceae family having kingdom Plantae according to botanical classification. The different plant parts such as leaves, flower and seeds have been used traditionally for medicinal purpose. The plant part traditionally used for intestinal worm, Chronic bronchitis, protozoal disease and for certain kind of fungal infection [8].

Materials and Method

Plant material and Extraction

Stevia (S.*rebaudiana*) leaves were collected from palisree mela (local festival) of Western Odisha from a vendor who cultivate stevia in small scale for commercial purpose.

Lufa cylindrical belongs to cucurbitaceae family having kingdom Plantae according to botanical classification. The leaves of the plant were collected from jamadarpli forest area of sambalpur district, Odisha.

Stevia leaves were washed to remove dust and subjected to aqueous extraction after air drying. The dried ground leaves were mixed with hot water (65 C) at the ratio of 1:45(w/V). The mixture was shaken and kept at room temperature for 24 hour. It was sired at least 3-4 times per day. After 24 h,the mixture was filtered through what man filter paper and the filter was evaporated by using vacume evapoarator.

The leaves of *lufa cylindrica* were dried in shade and powdered to get a coarse powder. About 800gm of dry coarse powder was extracted with ethanol (40-60^{0}C) by continuous hot percolation using Soxhlet apparatus. The extraction was continued for 72hours. The ethanolic extract was filtered and concentrated to a dry mass by using vacuum distillation.

Ether and ethanol extracts of fruits were prepared sequentially by standard procedures in soxhalation apparatus. Matured unripe fruits were shade dried until properly dried, crushed in a mechanical grinder into fine powder. The powder (500 g) was extracted sequentially with 1 litres of ether, 1 litres of ethanol in a Soxhlet apparatus at 65°C until the powder became exhausted totally. The resulting extracts were filtered, concentrated and

dried in vacuo (yield 7.60, 8.25 and 8.75% w/w, respectively). The extracts were stored in desiccators for use in subsequent experiments [9].

Preparation of Polyherbal Tablet

The dried Plant extract of all proposed plants were mixed with different excipients using wet granulation method for preparing later solid pharmaceutical form. These prepared granules of each form were compressed into tablet using compressing machine at Gayatri College of Pharmacy [10].

Characterisation of Polyhedral Tablet

The Prepared Polyherbal Tablet had been undergone characterisation under following parameter such as particle size measurement, colour and appearance, angle of Repose, Wet variation, Hardness and Friability test were conducted successfully by taking standard parameters.

Screening of Antidiabetic Potential of Polyherbal formulation (PHF)

Animals *Sprague Dawley* (SD) rats of either sex weighing between 150-200 gm were procured from Scientific Trader, Baleswar. They were housed in polypropylene cages (six rats / cage) and maintained under controlled room temperature (20-24°C) and with relative humidity of 45-55% under 12:12hr light and dark cycle. They were provided with standard lab diet and water ad libitum and kept for 1 week to acclimatize with the laboratory condition before starting the experiment.

The rats were grouped into 06 groups and each group has 6 rats. Group 1: considered as control group; Group 2: considered as treatment group, treated with standard antidiabetic compound, metformin. Group 3, 4 & 5 each were considered as test group, treated with polyherbal formulation "PHF" in different doses. Group 6 consist of 06 rats and treated with combination of both "PHF" and standard drug "metformin" [11–13].

Acute toxicity study

The acute toxicity study was performed as per OECD (guidelines 425) and LD_{50} was calculated accordingly. The animals were examined at every 30 min up to a period of 3 h and then occasionally for additional 4 h period, finally 24 h mortality was recorded. All the animals found to be safe [14].

Experimental induction of Diabetes mellitus

Diabetes mellitus (Type 2 diabetic) was induced in the rats with normal blood glucose levels by a single injection of streptozotocin (STZ) 35mg/ kg body weight I.P in 0.1M sodium citrate buffer, PH 4.5. In contrast rats in control group were injected with 0.1M citrate buffer solution only. After injection of STZ the animals were kept for observation for 48 hrs.

Table 1 The grouping of rats into different groups for the treatment of plant extract and the standard drug.

group	drug	Dose (mg/kg)	treatment
I.	Normal saline	1ml/kg	Control
II.	metformin	50.00	Standard
III.	PHF	50	Test
IV.	PHF	100	Test
V.	PHF	150	Test
VI.	Metformin +PHF	50+150	Combination

Blood Sample Collection The blood samples were collected through the tail vein puncturing with a needle. A drop of blood of size 0.3 to 1.0 μl required. The drop of blood obtained by pricking with the lancet provided with glucometer for estimation of FBS and PPBS (Dubois 2014). FBS and PPBS at 1hr and 2hr were estimated with the help of glucometer weekly for 4 weeks. The glucometer include a clock that was set for date and time and memory for past test results. It enables to keep a record of blood glucose levels over days and wee

Results and Discussion

The mean FBS level of all the rats before induction of diabetics was found to be 126.5 $\pm$ 1.378 mg/dl (Table 1). In contrast the mean $\pm$ S.D of FBS level for different treatment groups over a time period from week 0 to week 4 was included in Table 2. In the control group the mean FBS has dropped from 181.16 $\pm$ 8.50 in Week 0 to 181.16 $\pm$ 2.32. This is far above the mean level before induction, even after 4 weeks. In Metformin (50mg/kg) treated group the FBS level was declined from 190.5 $\pm$ 2.66 to 138.83 $\pm$ 2.23. Similarly in PHF(50mg/kg) treatment group the FBS was declined from 182.3 $\pm$ 6.02 to 135.33 $\pm$ 3.01 which is significant and nearer to the pre induction level. In slightly higher concentration of treatment of PHF i.e. with 100 mg/kg and 150 mg/kg, the FBS level was reduced from 187.16 $\pm$ 3.25 to 133.16 $\pm$ 2.93 and 181.5 $\pm$ 4.42 to 136.33 $\pm$ 2.58, respectively. In the combination regimen of PHF (150mg/kg) and Metformin (50mg/kg) the mean FBS level dropped from 180.3 $\pm$ 1.75 to 131.16 $\pm$ 2.14. In all these groups the mean FBS level has reached to the normal pre induction level.

One way ANOVA has been applied for comparison among different treatments groups and followed by Dunnetts multiple comparison tests for comparison between pair of treatment effects.

Table 2 Descriptive statistics of fasting blood sugar at different time period with different doses of drug treatment

Time Period	Mean FBS ± SD (mg/dl)					
	Control	Metformin 50mg/kg	DiaM 50 mg/kg	DiaM 100 mg/kg	DiaM 150 mg/kg	Metformin 50mg/kg +DiaM 150 mg/kg
Week-0	181.16 ± 8.50	190.5 ± 2.66	182.3 ± 6.02	187.16 ± 3.25	181.5 ± 4.42	180.3 ± 1.75
Week-1	179.13 ± 3.54	144.66 ± 4.50	140.16 ± 1.94	144.5 ± 4.59	141.16 ± 2.86	142.16 ± 3.54
Week-2	179.66 ± 1.75	141.33 ± 2.58	139.33 ± 1.63	139.83 ± 1.94	139.16 ± 1.94	136.16 ± 2.04
Week-3	180.5 ± 1.87	140.5 ± 2.26	133.16 ± 1.83	134.16 ± 1.47	135.16 ± 1.94	133.33 ± 2.66
Week-4	181.16 ± 2.32	138.83 ± 2.23	135.33 ± 3.01	133.16 ± 2.93	136.33 ± 2.58	131.16 ± 2.14

Conclusion

The daily single administration of PHF formulation (150mg/kg) and Metformin (50 mg/kg) significantly reduced blood sugar levels of Streptozotocin (STZ) induced diabetic mice. This Anti hyperglycaemic effect is believed to be due to the ability of PHF extract to stimulate glucose uptake into cells. Corosolic acid, lagerstroemin, tannic acid and penta-O-galloyl-D-glucopyranose (PGG) are the known active components in the leaf extract. Metformin (50 mg/kg) did not cause significant glucose reduction. Further study in the field of Formulation needed to overcome manufacturing difficulty as well as making a suitable herbal solid doses form. It also important to study antihyperglycemic effect of the said Polyhedral Formulation in different animal Model to conclude exact pattern of Anti hyperglycaemic activity.

Conflict of Interest

There is no conflict of interest

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