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Evaluation of the Impact of the Ayurvedic Formulation "Amruthotharam" on Obesity-Related Diabetic and Hepatic Disorders

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ABSTRACT

Diabetes mellitus (DM) is a non-communicable disease that affects people all over the world and is defined by ongoing hyperglycemia. Sulfonylureas, biguanides, -glucosidase inhibitors, and non-sulfonylurea secretagogues are a few oral hypoglycemic medications that are frequently recommended by doctors for managing diabetes. Oral hypoglycemic medication use causes noticeable negative effects, and there is currently no permanent viable treatment for DM recovery. Complementary and alternative therapies must be used to lower the incidence of disease until better medical methods are discovered. The search for an efficient medication, either by itself or in combination, to treat diabetes continues to be fruitless. This might have a viable replacement in the shape of herbal preparations, which are widely employed in conventional medical systems. In order to determine the impact of Amruthotharam kashaya prepared using a traditional method for a four-week treatment period on blood glucose levels as well as other biochemical parameters like total cholesterol, LDL, HDL, and VLDL in HFD-alloxan-induced diabetic rats, the present study was designed. Significant weight loss was also seen with diabetes management, and this was partially reversed after formulation administration. The formulation significantly decreased increased levels of a few particular biochemical markers and avoided other hyperglycemia-related complications. These findings offer scientific support for the anti-diabetic usage of a conventional formulation and imply that the administration of the formulation to rats can be used safely by humans because it lowers the levels of several biochemical factors that contribute to diabetes.

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INTRODUCTION

Diabetes is a metabolic condition that manifests as frequent urination, thirst, and hunger in addition to high or above-normal blood glucose levels (70–110 mg/dL). The main causes are either decreased insulin synthesis by -cells or diminished insulin sensitivity in cells. (Singab *et al.*, 2014) Diabetes raises the chance of obesity, heredity, ageing, hereditary insulin receptor and beta-cell function alterations, medication and infection abuse, and other cofactors. Type 1 diabetes, type 2 diabetes, and type 3 diabetes are the three forms of diabetes according to pathological reasons.

In type 1 diabetes, beta cells may be damaged or chemicals may be released into the bloodstream that acts as an antagonist to insulin. To put it another way, type 2 diabetes develops when the body is unable to properly utilise the insulin that the pancreas produces. This typically appears during middle age. Failure to manufacture insulin, which can be brought on by inconsistent secretion, is what leads to type 3 diabetes. Non-significant diabetes can have a number of different causes, including congenital diabetes (a genetic abnormality) or the destruction of beta cells, cystic fibrosis, steroid diabetes (caused by high doses of glucocorticoids), ageing, poor diet, obesity, sedentary lifestyle, sex infection, stress, and other factors.

Depending on the type of diabetes, different classes of anti-diabetic drugs may be employed; in type 1 diabetes, insulin may be administered externally; in other types of diabetes, the option depends on the type of diabetes. Despite the fact that these medications may have a number of negative side effects, including hypoglycemia, allergies, gastrointestinal problems, heart failure, lactic acidosis, and others (Patil *et al.*, 2011), they are typically given in combination. Alternative medicine and fresh methods for dealing with this health issue are therefore required, including plants that have antihyperglycemic benefits and fewer adverse effects.

Traditional medicine is preferred for a variety of reasons, including that it is more affordable, more in line with patient ideologies, allays worries about the side effects of chemical (synthetic) medications, satisfies the desire for more individualised healthcare, and facilitates greater public access to health information. Herbal remedies are typically used to treat chronic ailments rather than those that are life-threatening and to promote wellness.

Traditional treatments are also typically thought of as being natural and non-toxic. Using alloxan or streptozotocin to make experimental specimens diabetic, administering the extract and then analysing the results, or estimating various parameters in vitro, such as blood glucose level, triglycerides, insulin, free fatty acids, and cholesterol, is the most popular method for determining the potential of a drug to cure diabetes of specific herbs. (Pullaiah and Naidu, 2003)

One such remedy is Amruthotharam/Amrutottaram Kashayam, which treats metabolic disorders by reducing inflammation. It contains three herbal medicines—Guduchi (Tinospora cordifolia), Haritaki (Terminalia chebula), and Shunthi (Zingiber officinale)—in the proportions of 6:4:2 and has shown to be extremely effective in treating a variety of pathologies (Gupta, 2003).

The current study's objective was to assess the formulation made using a conventional manner for its antidiabetic capability.

MATERIALS AND METHODS

Material

The crude forms of herbal drugs *Tinospora cordifolia*, *Terminalia chebula*, *and Zingiber officinale* were procured from the local market of Ratnagiri.

Method

Preparation of Amruthotharam Kashaya (F1)

Amruthotharam Kashaya was prepared by mixing 3 parts of *Tinospora cordifolia* (Guduchi), 2 parts of *Terminalia chebula*(Hareda) and 1 part of *Zingiber officinalis* (Ginger). The prepared formulation was then studied for their phytochemical studies.

Qualitative and Quantitative Evaluation of Phytochemicals in Amruthotharam kashaya (F1)

Qualitative Evaluation of Phytochemicals in F1

F1 formulation was tested for the occurrence of phytochemicals, including glycosides, alkaloids, cardiac glycosides, flavonoids, tannins, cyanogenetic glycosides, and saponin.

Quantitative Analysis of Phytochemicals in F1

Quantitative analysis of bitters in Tinospora cordifolia extract

50 ml of methanol was used to extract 3 g sample. The process was performed for 30 min. The extracts were filtered, and the residue was extracted twice with 50 ml of methanol for 15 minutes each time. In a water bath, the whole filtrate mixture was evaporated to dryness in a steel dish. About 10 ml of distilled water was added to the residue in the steel dish, which was then extracted four times with 25 ml of ethyl acetate in a separating flask.

In a previously-weighed beaker on a water bath, the top organic layer was combined, continue the evaporation till a dehydrated product was obtained. The beaker was weighed after being cooked in an oven at 105°C for one hour. (Kaur *et al.*, 2016; Khasnabis *et al.*, 2015; Yang *et al.*, 2018)

Quantitative analysis of tannins in Terminalia chebula extract

The sample of about 1 gm was weighed and added to a conical flask. Add 100 ml of distilled water to a conical flask containing the sample drug. Shake the prepared solution for 2 hrs and then be allowed to stand for overnight. Filtered the solution and pipette out 10 ml of filtrate in a conical flask.

Table 1: Quantitative analysis of bitters, tannins and volatile oil

Sr. No.	Parameters	F1 (%)
1.	Quantitative analysis of bitters	2.48
2.	Quantitative analysis of tannins	38.02
3.	Quantitative analysis of volatile oil	1.45

Table 2: Change in body weight

Groups	Change in Body weight	
	Before	After 4 weeks of treatment
Normal group	187.52 ± 2.06	200.13 ± 1.52
HDF+alloxan-induced diabetes group	258.09 ± 1.28	293.70 ± 1.93
Formulation F1	252.72 ± 1.85	223.72 ± 3.10
Standard	254.10 ± 1.80	221.55 ± 1.66

Table 3: Change in blood glucose level

Groups	Change in fasting glucose level in the blood (mg/dl)		Change in postprandial glucose level in the blood (mg/dl)	
	After induction of diabetes	after 4th week of treatment with F1	After induction of diabetes	after 4th week of treatment with F1
Normal group	90.67 ± 0.82	94.17 ± 1.33	117.67 ± 0.82	120.33 ± 0.52
HDF+ alloxan-induced diabetes group	188.00 ± 4.82	214.83 ± 2.86	184.00 ± 2.53	222.17 ± 2.14
Formulation F1	185.67 ± 3.83	161.33 ± 2.07	182.17 ± 1.94	137.33 ± 1.97
Standard	188.50 ± 2.51	155.33 ± 3.33	184.33 ± 2.42	130.17 ± 1.17

Table 4: Evaluation of biochemical parameters

Parameters	Normal diet	HFD+ Alloxan induced diabetes	Treated with formulation F1	HFD+ Alloxan-induced diabetic rats treated with Metformin
Total cholesterol levels (mg/dl)	115.13 ± 1.97	289.86 ± 1.35	175.58 ± 1.70	173.5 ± 1.60
Triglycerides levels (mg/dl)	24.59 ± 1.01	106.63 ± 1.97	43.72 ± 1.48	44.93 ± 1.31
HDL levels (mg/dl)	47.53 ± 0.85	19.62 ± 0.52	33.83 ± 1.00	32.67 ± 1.04
LDL levels (mg/dl)	51.78 ± 0.80	111.6 ± 1.53	66.26 ± 1.62	65.25 ± 2.10
VLDL levels (mg/dl)	14.95 ± 1.05	57.08 ± 1.19	32.65 ± 1.76	33.02 ± 0.97

The distilled water of about 750 ml and 25 ml of indigo sulphonic acid was added to the above filtrate and titrated against 0.1N potassium permanganate solution.

By excluding the amount of sample, blank reading was performed.

Quantitative analysis of Volatile oil in Zingiber officinale

The sample of 10 gm was weighed and added to a round bottom flask. Glycerin and distilled water of about 75 ml and 175 ml, respectively, were added to the above round bottom flask.

A few glass beads and 6 stripes of filter paper (7cm x1cm) were also added to it. Attached the flask to the Dean stark apparatus. The oil was collected from the mixture after heating.

***In- vivo* Studies**

The experiment was done out on both sexes of Wister albino rats weighing between 200- 250 g. The animals were kept in a well-ventilated animal unit with a 12-hour light/dark cycle.

For 28 days, the 3 experimental groups of six animals each were given a customized high cholesterol diet and 6 rats were provided a regular diet; Group I- Received a normal diet, Group II- Received HFD with alloxan-induced diabetes, Group III- Received formulation F1, Group IV- HFD- alloxan-induced diabetic rats treated with Metformin.

All administrations were done orally. The animals fasted for 12 hours after their previous meal. On day 28, blood samples were taken through retroorbital puncture under light anaesthesia.

Blood was centrifuged to obtain serum, which was then analyzed for confirmation of the beginnings of diabetes. In an alloxan-induced diabetes animal, the blood glucose activity of the test product (amruthotharam) was measured. (Ighodaro *et al.*, 2017)

Diabetes mellitus was induced in the 3- 6 rats with a single intraperitoneal dosage of around 125 mg/kg alloxan monohydrate 5 percent (dissolved in normal saline).

After the confirmation of diabetes mellitus induction on/after the fifth alloxan treatment, fasting blood glucose level was enhanced in rats with a blood glucose level of 150mg/dl who were included in the investigation (day0).

The maximum dose for a human is 250 mg/kg twice a day. Therefore, the animal equivalent dose (AED) is calculated based on body weight using the following equation,

$$AED(mg/kg) = Human\ dose(mg/kg) \times Km\ ratio$$

Where, Km is correction factor.

Treatment with F1 was begun on the 6th day of alloxan treatment (day 1) & continue for 4 weeks. All administrations were done by oral route. The blood sample was collected from retro-orbital puncture of each animal under mild anaesthesia (isofluorane) and then were subjected to analyze for blood sugar level and HbA1c. Then compared with control & standard group.

Collection and Analysis of Blood Sample

After the induction of diabetes on 5^{th}, 7^{th}, and 9^{th} week blood samples were collected and evaluated for blood glucose fasting, postprandial glucose level and blood lipid profile.

Histopathological Study

The purpose of histopathology on the harvested pancreas and liver part was to see the effect of the formulation F1. After completion of treatment, i.e. after 4 weeks, the animals were sacrificed. The pancreas were collected from the group of animals I, II, and IV to be evaluated for a diabetic condition. The liver of animals (group I, III) were collected and evaluated for the liver condition due to a high-fat diet. The staining of the liver segment was done according to normal histological techniques and observed using microscopic techniques.

RESULTS AND DISCUSSION

All the aqueous extracts of plants such as *Terminalia chebula* (Hareda), *Tinospora cordifolia*(Guduchi) and *Zingiber officinalis* (Ginger) were gifted from Amsar Pvt. Ltd. Colvale Goa.

Methods for Preparation of Formulations

The formulation F1 was prepared by mixing raw drugs such as guduchi, hareda and ginger in 3:2:1 by traditional techniques.

Qualitative and Quantitative Evaluation of Phytochemicals in Amruthotharam kashaya (F1)

Qualitative Evaluation of Phytochemicals in F1

The formulation F1 was further evaluated for the presence or absence of various phytochemicals such as alkaloids, glycosides, flavonoids, cardiac glycosides, cyanogenetic glycosides, tannins, saponin qualitatively.

The phytochemicals such as cardiac glycosides, cyanogenetic glycosides and saponins were found to be absent in F1.

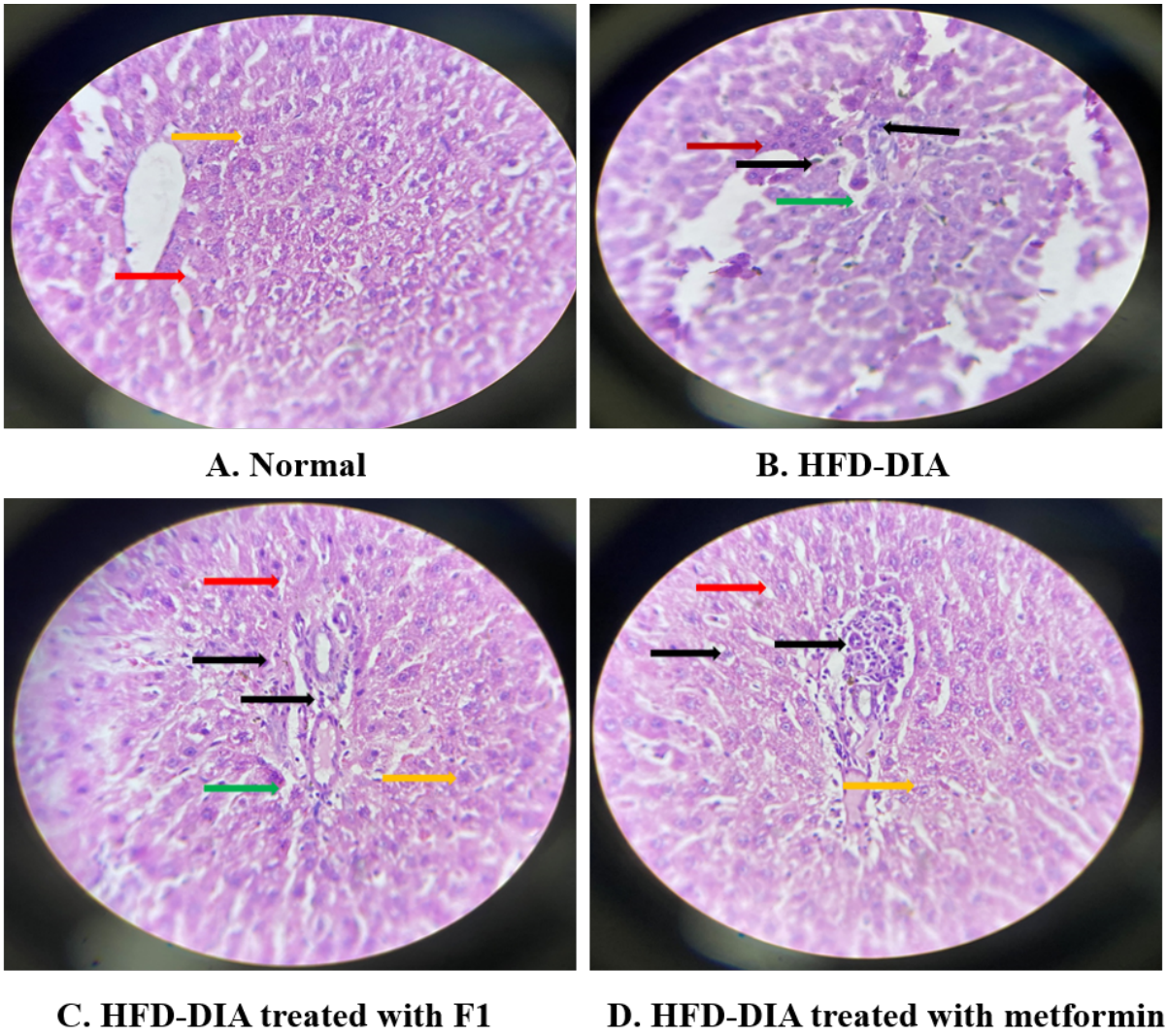

Figure 1: Histopathology of Liver

From Figure 1,

⇒ Yellow arrow : Normal hepatocytes

⇒ Red arrow : Normal sinusoids

⇒ Brown arrow : Degenerated liver

⇒ Green arrow : Dilated sinusoids

⇒ Black arrow : Infiltration cells (sign of inflammation)

Quantitative Analysis of Bitters, Tannins and Volatile oil in F1

The formulation F1 was evaluated for the number of bitters, tannins and volatile oil given in Table 1.

Pharmacological Activity

The animals were grouped into 4 groups containing 6 rats in each group. The animals were fed a high-fat diet for four weeks of period. Furthermore, the animals were treated with alloxan to induce diabetes. The process was carried out for four weeks of a time period for the determination of fasting glucose levels and postprandial glucose levels in the blood. The treatment was carried out to evaluate the change in body weight and glucose level in blood after 4 weeks of treatment with formulation F1.

Body weight changes

The body weights (gm) of all the animals were measured before and after 4 weeks of the study given in Table 2. There was a significant reduction in body weight observed when treated with F1.

Change in blood glucose level

The fasting glucose level and postprandial glucose level in the blood were measured and given in Table 3. There was a significant decrease in fasting glucose level and postprandial glucose level was observed when treated with F1.

Effect on serum biochemical parameters

The effect of formulation F1 was studied for differ-

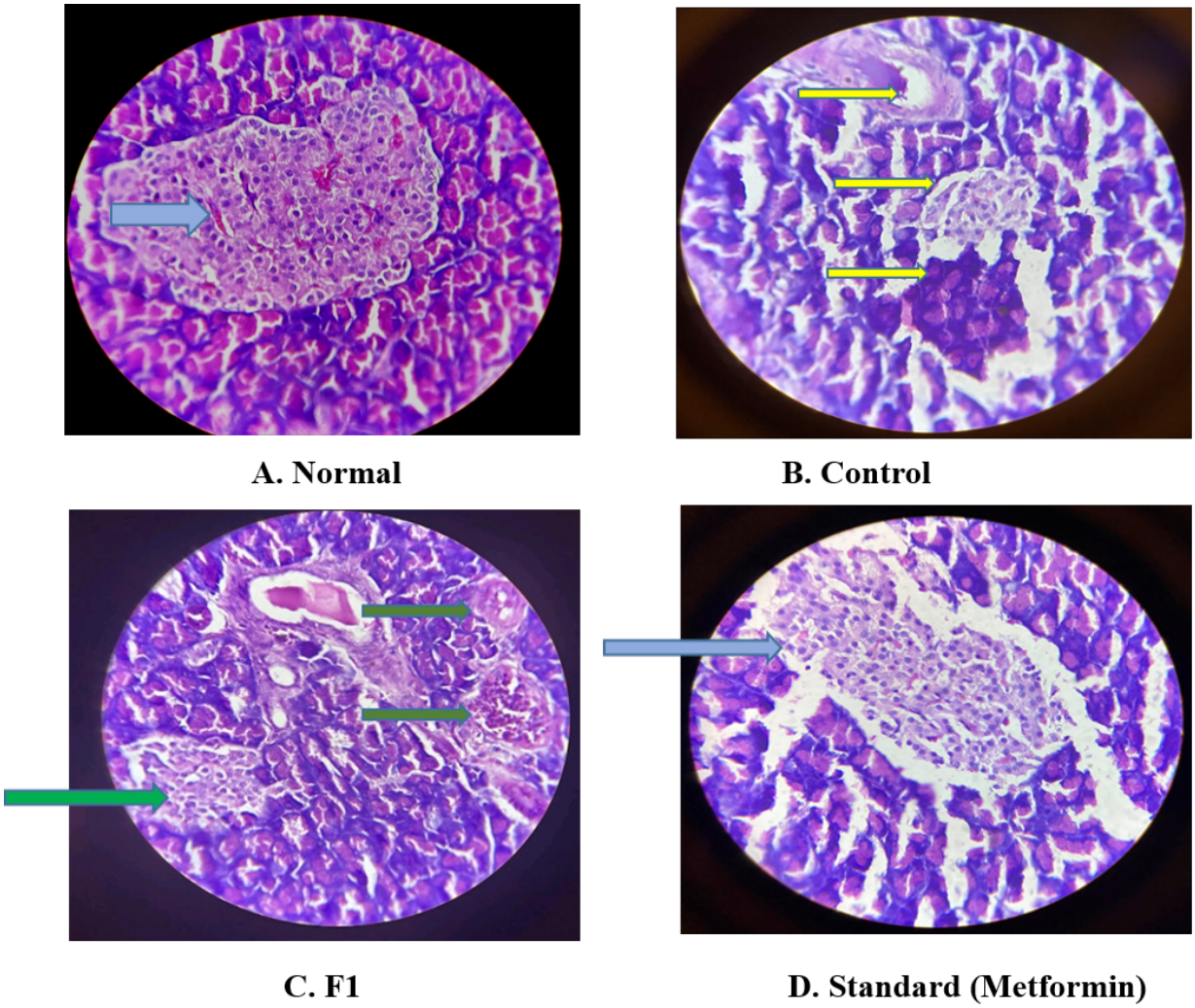

Figure 2: Results of Histopathology of Pancreas

ent parameters given in Table 4. The formulation was found to be effective in lowering total cholesterol level, triglycerides levels, LDL and VLDL levels, as well as improving HDL levels in serum.

Histopathological Evaluation

Histopathology of liver

Histopathology of liver tissue of rats treated with HFD- alloxan-induced diabetes showed infiltered cells (Figure 1B) when compared to normal healthy control rats liver tissue, which showed normal hepatocyte and normal sinusoids (Figure 1A). The rats with high-fat diet alloxan-induced diabetes, when treated with formulation F1 showed a decreased number of infiltered cells (Figure 1C) when compared with the metformin (Figure 1D).

Histopathology of pancreas

Effect of formulations F1 on the histological profile of the pancreas in untreated normal, HFD-alloxan-induced diabetic, and HFD-alloxan-induced diabetic wistar rats (original magnification 40). (See Figure 2A.) NPAN: hematoxylin and eosin (H/E) stained slices of pancreas from a normal control rat showing normal islet of langerhans, indicated by blue arrows. (See Figure 2B.) PHFD-Al: Pancreatic slice of an HFD-alloxan-induced diabetic rat demonstrating no/destroyed islet of langerhans and beta cells, as shown by yellow arrows. (See Figure 2C.) PF1: Pancreatic slice of HFD-alloxan-induced diabetic rats treated with Formulation -1 (Kasaya) at 1.5gm/kg body weight demonstrating a modest number of islets of langerhans (orange arrows) and a normal islet of langerhans with many beta cells (green arrows). (See Figure 2D.) PMET: pancreatic slice of diabetic rats treated with Metformin demonstrating normal pancreatic islet of langerhans with an increased beta-cell population (blue arrows).

CONCLUSION

The present study was conceived with a view to provide scientific and pharmacological evidences for

the hypoglycemic potential of the Amruthotharam kashaya prepared by traditional method. The pharmacological study was carried out to determine the formulation's effectiveness against diabetes. Body weight change, fasting glucose level change, and postprandial sugar level in blood were all included in the evaluation. The study also investigates how formulation F1 prepared by the traditional method influence blood biochemical indicators such as total cholesterol, triglyceride level, HDL level, LDL level, and VLDL level. F1 has been proven to aid in the reduction of total cholesterol, triglyceride levels, LDL levels, VLDL levels, and the increase of HDL levels in the blood when compared with standard. Histopathology of liver tissue treated with HFD-alloxan-induced diabetes demonstrated infiltered cells as compared to normal healthy control rats liver tissue, which displayed normal hepatocytes and sinusoids. The number of infiltered cells was decreased in rats with HFD-alloxan-induced diabetes who were treated with formulations F1. The overall results concluded that the formulation was found to be effective against diabetes.

Conflict of Interest

The authors declare that they have no conflict of interest.

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