International Journal of Multidisciplinary Innovative Research.
ISSN: 2583-0228 Volume 1, Number 1 (Jul' 2021) pp. 79-87

https://www.ciir.in

Anti-Diabetic Herbal Formulation Used for Diabetes

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ABSTRACT: Diabetes mellitus refers to a group of metabolic disorders that leads to high blood sugar level. The mixture of herbs is needed instead of mono-drug therapy for the control of the condition, for example, multifactorial metabolic disorders, diabetics, which have multiple complications, including hyperlipidemia, hepatic toxicity, immunodeficiency etc. The present paper deals with the development of polyherbal formulation for the treatment of diabetes, wherein different herbs i.e. Glycine max extract, Zingiber officinalis (rhizome) and curcumin (a phenolic antioxidant) are mixed with sodium Lauryl sulfate and were subjected under a method for the preparation of the herbal solution. The formulation was then analyzed for their in-vitro cytotoxicity wherein it was found that the herbal formulation does not showed any cytotoxicity after 24 hrs. Similarly, glucose utilization HepG2 cells was also determined wherein it was observed that the cells were able to utilize higher concentration of glucose. Protein glycation assay was also performed for the formulation wherein it was observed that the extract exhibited protein glycation of 24.5%.

KEYWORDS: Curcumin, Diabetes, Glycine max extract, Zingiber officinalis.

INTRODUCTION

According to American Diabetic association, 2002, Diabetic mellitus is a progressive illness confirmed by via blood sample that needs long-term treatment [1]. The more patients know about the condition, the more they can make effective management choices. Dietary care and exercise are important both in treatment and management of diabetic and findings of the study group on diabetes prevention show that improvements in lifestyle have cut diabetic occurrence by 58% [2]. A significant component of therapy is in type 1 diabetes complications, in which insulin substitute is completely deficient [3]. Insulin releasing from the pancreas is modified in type 2 diabetic mellitus and also can be of a complete deficit in quantity, which means that its substitution is also involved in management particularly when the diabetic mellitus is long in life (Figure 1).

Diabetic in 2017 was 426 million in the country, and by 2048 this number is estimated to hit 729 million. A new study has revealed that about 28 million (27.3 percent) men aged 25 years living in Pakistan with diabetes. Under this group, the percentage of type 2 diabetes is 90–95% [4]. A lot of synthetic medications were used to treat diabetes including meglitinides, biguanides, sulfonylurea and thiazolidiniones. However, there were also some side effects, such as elevated weight, toxicity, hypoglycemia and susceptibility to medications. This would lead to natural drugs being focused for fewer or nil adverse impacts and affordable forthcoming plans for drug progression [5].

Plants are potential source of numerous bioactive plant agents such as terpenes, peptides carotenoids, oil, phenolic acids and carbohydrates which make important contributions to the management of several diseases, including high blood pressure and cardio-vascular diseases. Glycine Max belongs to the family of legumnosae. It is a perennial with upright or ascending stem reaches a height of 1/2 to 6 feet, clad in important reform, ovate-lanceolate, unobtrusive, with allied racemes, white or lilac, violet to rot, natural to personality; 3 cm long, loosely hairy, 3-4 grains treadmill, compact, purple, chocolate or black. Soyabese is a resident of South East Asia and is believed to have come from a slim, prosthodic plan on the foundation of genetics. In Far East, Soyabean is a major vegetable crop [6].

Among the globe's leguminous plants is Soyabean. It is primarily cultivated as a global food crop. In Beijing, Japan and other Eastern Asian countries it is cultivated specifically as food grain. The beans are eaten orange, dry or brown, entirely or separately. Green beans are utilized as vegetables; in cookies and desserts, fried and salted seeds are utilized. The beans are grounded in flour and utilized for goods made from bakery. For industrial uses, the fatty oil derived from seeds is utilized. The soy was utilized as a full milk, tofu, tempesh and soy-sauce or refined. Scientists around world continue to have highest priority for growing popularity of soy foods in primarily for chronic diseases prevention [7][8]. Even FDA has decided to reduce risk of coronary heart attacks in a diet comprising soya proteins. The value in soy as antioxidant effect has grown.

The soy proteins is equivalent to other pulse proteins in energy physiology and the level of digestibility of the biochemical values. Soya is regarded as an aid for the regeneration of acidosis in a specific diet. Scientists around world continue to have highest priority for growing popularity of soy foods in primarily for chronic disease protection [9], [10]. Even FDA has decided to lower the risk of cardiovascular heart attacks in a diet comprising soya proteins. Their involvement in soy has grown as antioxidant and flavonols in particular. Curcuma longa belonges to a family of Zingiberaceae. The Curcuma genus, consisting of approximately 50, is a community of the Hedychieae and comprises of a relatively small population of rhizomateous, permanent species distributed across tropical and subtropical Asia. Critical research on C turmeric is written by Govindarajan. Ethnobotanical data were checked on some Curcuma species and it was perceived that Curcuma has a powerful form of wide spectrum hepatoprotective product that is utilized in Indonesia and is useful for treating liver disease. In the ethnic group of Tanzania and Tobago long was primarily utilized for endoparasites, internally and externally accidents and associated maternity. In Nepal, turmeric is utilized as a diet [11], [12].

The Curcuma genus has different drugs for cancer and tumorgensis. The anti-cancer and antitumor properties of Curcuma longa have been reported by Anto et al, Mutation Res, 1992, at 370:127-131. Curcumin's inhibiting effect on the production of DNA and RNA in cultivated HeLa cells has been shown. Azoxymethanol (40 M) can be blocked in colonic neoplasia in the mice by dietary curcumin. Many studies have published on antibiotic actions of Curcuma, and antimicrobial features are well known. Banerjee and Nigam documented the activity of different Curcuma species in antibacteria and antichemistry. Property of C molluscicidal. It has been recorded longa [13], [14]. The insecticide properties of multiple Curcuma species. In severe, subacute, and inflammatory process models of mice and rats, turmeric has demonstrated an anti-inflammatory activity.

To evaluate the functional effects of pancreatic damages and to assess the recovery effectiveness of possible therapeutic medicines, creation of clinically validated models of alloxane-induced

diabetic is necessary. There is also much under-estimation of role of medicinal herbs in enhanced insulin secretion and functions as a non-diabetic tablet formulation. The recovery theory indicates that alloxane-disturbing agents impair their pancreatic activity as the impact of alloxane-determining agents declines with time through the application of our current herbal formulation research [15][16]. Medications such as glibenclamide, which include penformin, are relaxing for diabetic behaviour. Studies have also demonstrated that the medicinal wording has the benefit of boosting the pancreas activity by the alloxane-caused diabetes and is utilized as a strong anti-diabetic composition in the care of diabetic patients as pills [17]. The purpose of present study is to prepare herbal formulation for treating diabetes by combining herbal drugs and prepare a dosage form that effectively treats type 2 diabetes. In vitro tactics have been utilized to visualize impact of anti-diabetic drug of the intended formulation.

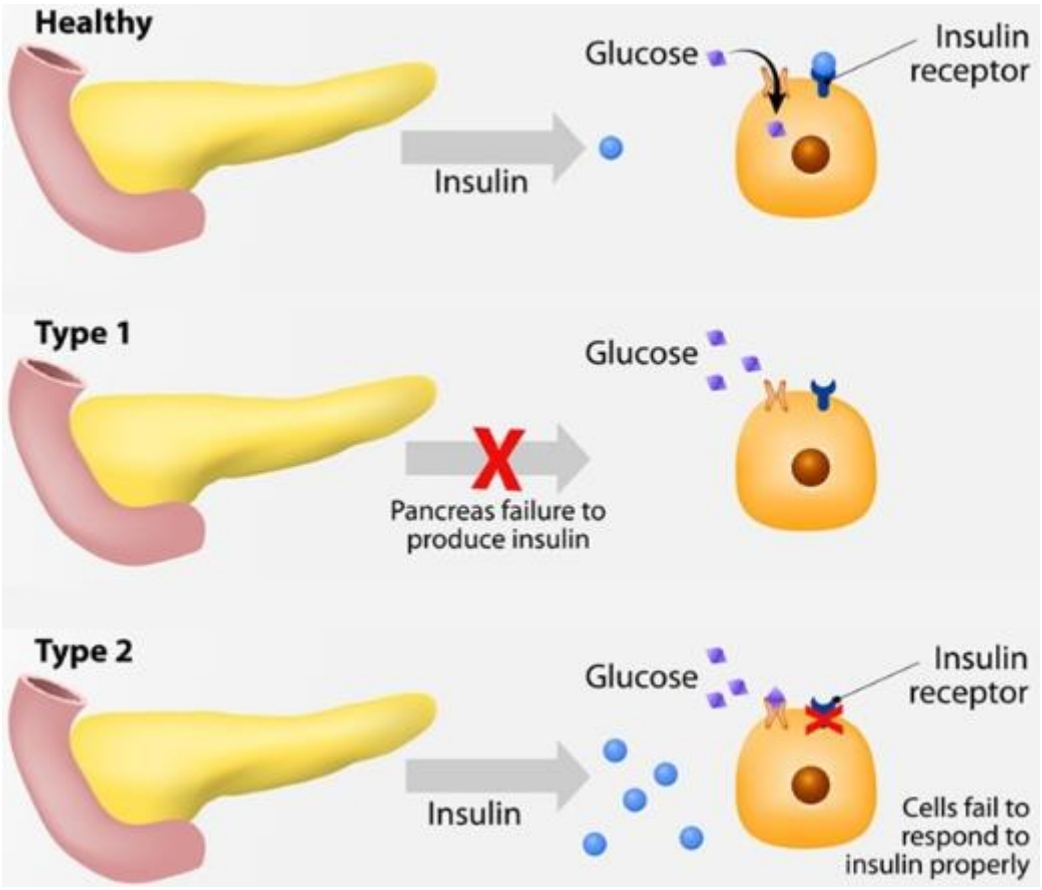


Figure 1: Diabetes, i.e. type 1 and 2 are represented herein. Type 1 caused due to lack of production of appropriate amount of insulin from the pancreas. Type 2 diabetes is caused due to impropriate response of the body cells against insulin formed [18]**.**

In-vitro anti-diabetic activity of *Brachylaena elliptica* was observed by a group of researchers which includes cytotoxicity test, glucose uptake test and alike wherein ti was observed that the herb was potent towards treating diabetes but still was not much significant when compare with the marketed formulations [19].

Research Question

- Why there is a need to treat diabetes mellitus by using herbal formulation?

METHODOLOGY

1. *Design*

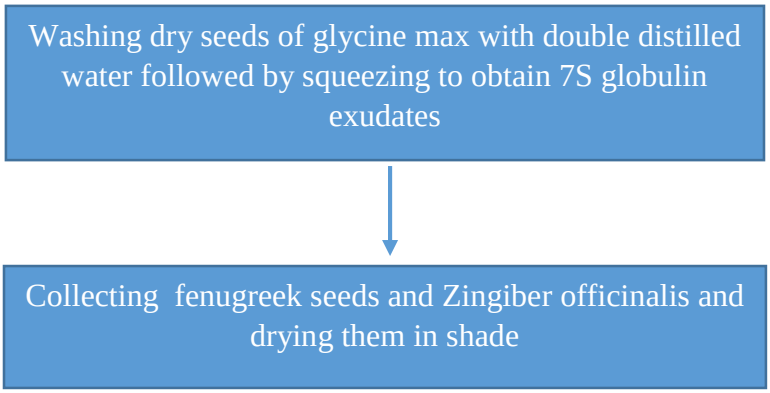
Selection of the herbs was done after extensive search of the review of literature of the herbal sample. The herbal composition was prepared by a well-known method and then the prepared formulation was then subjected to in vitro analysis in order to determine the potential of the intended composition. Phytochemical analysis was also done.

2. *Samples*

The samples include herbs that were utilized for the preparation of the anti-diabetic herbal formulation. The herbs were procured from the reliable resource. Fenugreek seeds were procured from the seeds market, Delhi. Further, Glycine max extract, Zingiber officinalis (rhizome) and curcumin (a phenolic antioxidant) were also procured from a reliable source.

3. *Methodology*

Dry mature seeds of glycine max i.e. Soya bean was washed in purified double distilled water of pyrogen free, and were then immersed in hot water at 55°C for 4 hrs. The seeds were then squeezed in silicon cloth to obtain white exudates of 7S globulin. Then the plants constituents of fenugreek seeds, Zingiber officinalis (rhizome) were collected and dried in shade. The dried material was then grinded to obtain a powder and then extracted with water for 5 days to obtain a solvent. Further the solvent is decanted and filtered to remove the plants debris followed by concentrating under vacuum at 45°C. Then the 500 ml of fenugreek seeds extract and 500 ml of Zingiber officinalis extract was lyophilized to obtain a powdered extract. Further, 500 gm of exudates of 7S globulin and extracts of fenugreek seeds and Zingiber officinalis were mixed followed by addition of 20 gm of curcumin to obtain a herbal solution. 20 gm of Starch was added to the herbal solution and heated until starch was dissolved in the herbal solution. The solution was then cooled and volume was made with water upto 150 ml to obtain an anti-diabetic herbal solution [19]. The prepared formulation was then subjected to phytochemical screening and in vitro analysis to determine the effect of the anti-diabetic formulation. Figure 2 shows the diagrammatic representation of methodology adopted to develop an anti-herbal diabetic formulation.



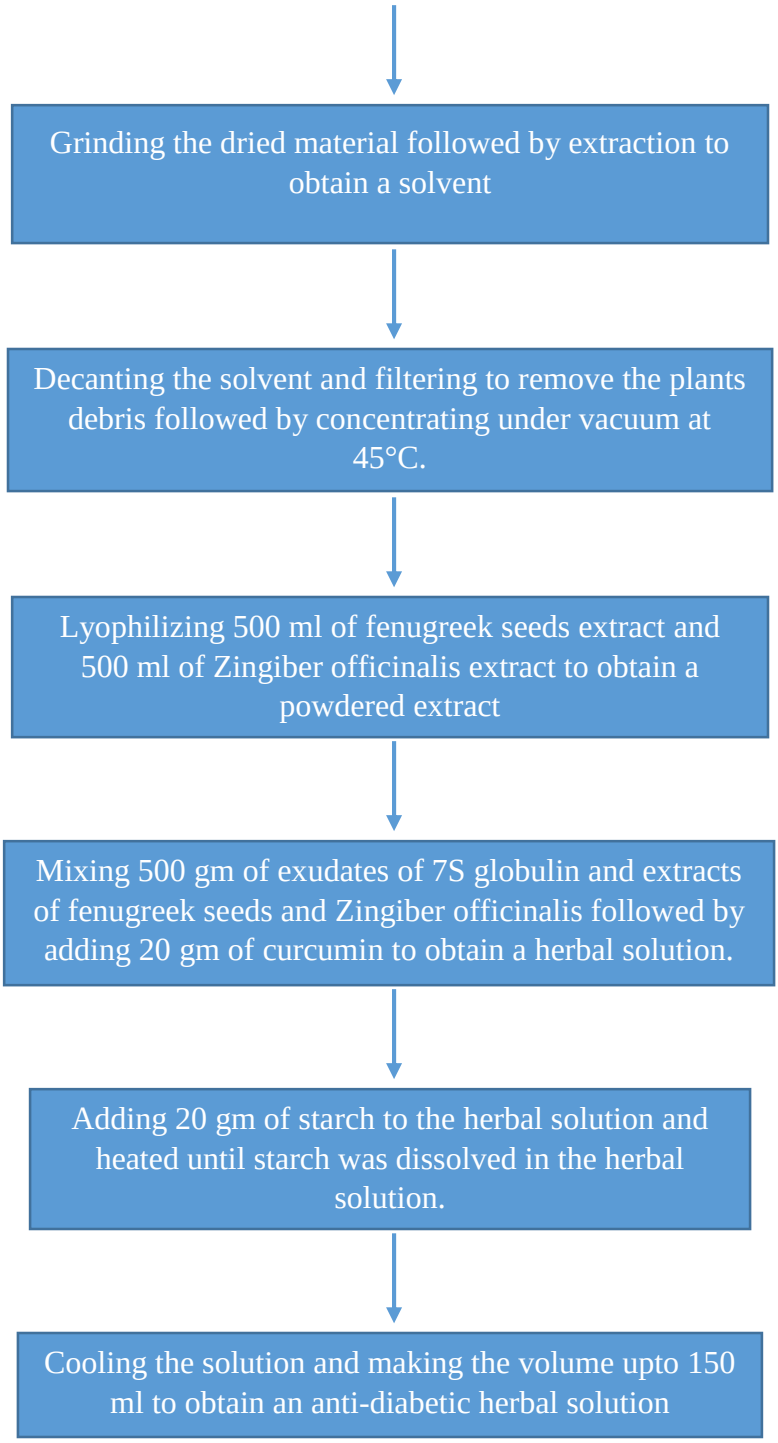


Figure 2: Flow chart of the methodology adopted to formulate herbal drug formulation to treat diabetes mellitus.

3.1 MTT Assay

MTT was conducted in accordance to protocol given by Mossmann with minor modifications. NHEK-293 cells (1×10^4) were subjected to various amount of anti-diabetic herbal formulation (0, 10, 20, 30, 40 and 50 µg/ml) for 24 hrs following which, cell viability was obtained

by aforementioned assay. To evaluate Growth inhibitory effect of the antidiabetic herbal formulation on NHEK-293 cells after 24 h treatment, it was found that the complex did not show any growth inhibitory activity on the Normal human embryonic kidney 293 (HEK-293) cell-lines. This implies, the formulation has no growth inhibitory activity on normal cells (Figure 3) [19].

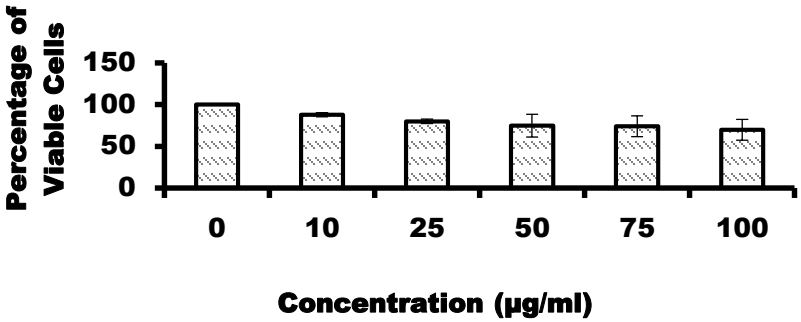


Figure 3: Cytotoxicity assay on NHEK 293 cells. NHEK 293 cells of fibroblast origin were treated with an increasing amounts of anti-diabetic formulation after 24 h. Anti-diabetic formulation showed negligible cytotoxicity even after 24 h.

3.2 Glucose utilization on HepG2 cells

HepG2 cells were freed into phosphatebuffered saline by transitory acquaintance to 0.25 percent Trypsin, suspended within naive growth mode, and then subjected into a 96well culture plate (Nunc, Denmark),6,000 cells per well, allowed to abide by and protrude for three days in incubator (humidified) with five percent CO2 at 37°C. Also included were pair of cellfree rows for serving as blanks. Plant extracts (2×5 µl) at concentration (25µl) of on third day after seeding, without altering the medium, at a dose of 25 µl. Aspiration was done to remove Spent culture medium after 48 h of incubation and swapped by a 25µl of incubated buffer (RPMI medium thinned with PBS 0.1 percent BSA and 8mm glucose) that again incubated at 37 °C for more 3 hrs. The positive controls were metformin (0.2 µg/ml) and berberine (19 µg/ml), while negative control (untreated) included only an extractfree incubation buffer. And 10 µl of incubated medium extracted from every well after incubation and conveyed to different 96well plate where 200 µl of glucose oxidase reagent (SERAPAK Plus, Bayer) was applied for determining glucose levelwithin medium. Absorbance was assessed at 492 nm expending a Multiscan MS microtitre plate scanner after incubating for 15 minutes at 37 °C (LabSystems) [19][20]. Amount of glucose used was measured as the dissimilarity amongst the wells that are cell-exempted and cell-comprising wells (Figure 4).

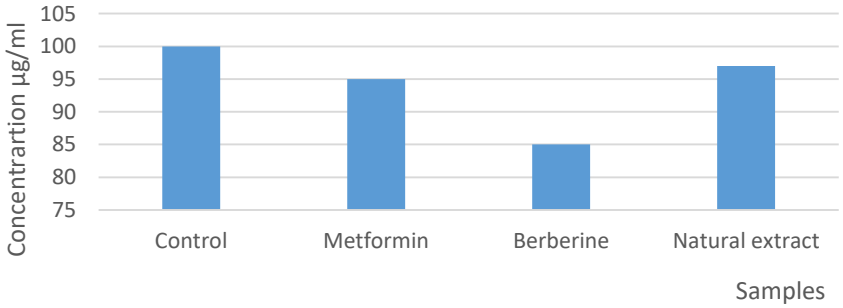


Figure 4: Consequence of the herbal extract on the glucose exploitation on HepG2 cells.

3.3 Alpha-Glycosidase Inhibition Assay

Alphaglucosidase inhibition assay was demonstrated with slight modification utilizing a methodology defined by Sancheti andfriends. In short 5 µl of plant extracts (50,100 and 200) µg/ml was applied to 20 µl of prepared 50 µg/ml alphaglucosidase solution in a 96well plate. 60 µl of 67 mM potassium phosphate buffer (having pH 6.8) subsequently was supplemented. 10 µl of10 mM ρ-nitrophenyl-α-D-glucoside solution (PNPGLUC) then was administered after 5 minutes of incubation. 25 µl of 100 mM sodium carbonate was supplemented after incubation and absorbance determined at 406 nm [19].

3.4 Protein Glycation Assay

In short, in black microplates, 50 µl of protein solution (100 mg of gelatin in 5ml of distilled water) applied to 10µl of glyceraldehyde solution. At 37 ° C for 24 h, plate was wrapped and incubated. 40 µl of plant extracts (prepared at 50 µg/ml and 100 µg/ml) was applied after incubation.While aminoguanidine (100 µM) was utilized as a positive control, a blank comprisingclean water as substitute of extract serving as a negative control (untreated) [19]. Fluorescence determined at 370 nm (excitation), 440 nm (excitation), respectively (emission).

RESULT AND DISCUSSION

1. *MTT ASSAY*

In MTT assay it was observed that 24 hrs no toxicity was observed on the cells.

2. *Glucose utilization on HepG2 cells*

Glucose utilization on HepG2 cells observed to be that the polyherbal anti-diabetic formulation was able to utilize glucose uptake efficiently (Figure 4).

3. *Alpha-Glycosidase Inhibition Assay*

It was observed that the polyherbal anti-diabetic formulation did not showed any effect on alpha-glycosidase but exhibited weak effect at tested concentration.

4. *Protein Glycation Assay*

It was observed that the herbal formulation exhibited enhanced effect on protein glycation which was about 24.5%. (Figure 5)

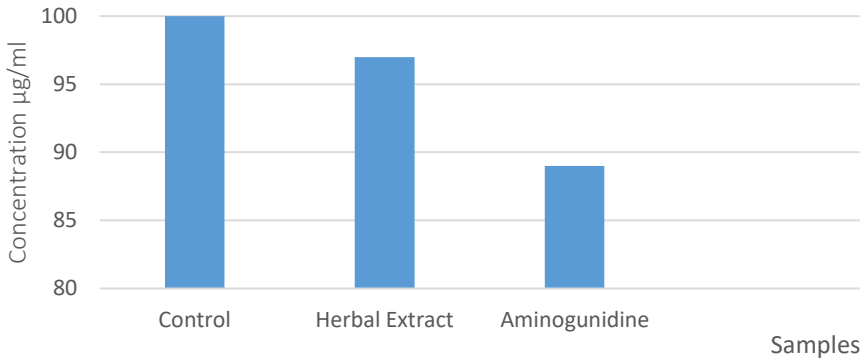


Figure 5: Protein glycation assay of the polyherbal formulation

CONCLUSION

In the present study the polyherbal formulation was prepared by employing various herbs which includes Glycine max extract, Zingiber officinalis (rhizome) and curcumin (a phenolic antioxidant). Further, the formulation was then analyzed for their in-vitro cytotoxicity wherein it was found that the herbal formulation does not showed any cytotoxicity after 24 hrs. Similarly, glucose utilization HepG2 cells was also determined wherein it was observed that the cells were able to utilize higher concentration of glucose. Protein glycation assay was also performed for the formulation wherein it was observed that the extract exhibited protein glycation of 24.5%.

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