



Formulating anti-diabetic nutraceutical tablets based on edible plants from Tripura, India

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Abstract:

Nutraceuticals are food-based drugs that are used as dietary supplements to minimize chronic diseases. Diabetes is one of the most common chronic diseases all over the world. Recently, herbal nutraceuticals have taken a promising role in treating diabetes. We aimed to develop herbal nutraceutical tablets and evaluate its anti-diabetic activity using ob/ob mice. Five plant species were collected by field survey methods based on oral interviews with traditional healers of Tripura. The wet granulation method was applied to formulate the herbal nutraceutical tablet. Water- and fat-soluble vitamins were determined by reversed-phase high-performance liquid chromatography. Trace elements were analyzed by atomic absorption spectrophotometry. To evaluate the anti-diabetic activity of the herbal tablets, we determined serum hemoglobin, glycosylated serum protein, and oral glucose tolerance.

The newly formulated herbal nutraceutical tablets provided the optimal energy level. It contained sufficient amounts of essential minerals, such as iron (74.6 ± 2.7 mg/g), sodium (4.4 ± 0.4 mg/g), potassium (5.3 ± 0.7 mg/g), calcium (163.1 ± 2.2 mg/g), magnesium (39.2 ± 1.7 mg/g), and phosphorus (14.6 ± 2.1 mg/g). We also found optimal quantities of water-soluble vitamins, such as vitamin C (27.2 ± 4.3 mg/g), vitamin B1 (0.6 ± 0 mg/g), vitamin B3 (0.6 ± 0.2 mg/g), vitamin B6 (1.1 ± 0.2 mg/g), vitamin B12 (0.6 ± 0.2 mcg/g), and folic acid (82.6 ± 7.6 mcg/g), as well as fat-soluble vitamins, such as vitamin A (287.4 ± 6.3 mcg/g), vitamin D3 (2.6 ± 0.6 mcg/g), and vitamin E (0.7 ± 0 ng/g). Finally, the herbal nutraceutical tablet (200 mg/kg) significantly improved the anti-hyperglycemic effect on ob/ob mice (type 2 diabetes), compared to the standard drug, metformin (200 mg/kg).

The results suggest that the newly formulated herbal tablet may be recommended as an anti-diabetic nutraceutical drug.

Keywords: Edible medicinal plant, herbal nutraceutical, tablet dosage, anti-diabetic activity, dietary supplement

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INTRODUCTION

Modern lifestyle involves changes in essential food habits [1]. Due to improper diet, people suffer from various kinds of chronic diseases. Obesity, heart disease, and diabetes are among current global issues [2]. Nutraceuticals are food-based drugs which minimize diet-related illnesses. The word “nutraceutical” comes from “nutrition” and “pharmaceutical” [3]. Any pharmaceutical product manufactured from food sources has fundamental nutritional values and minimizes various chronic diseases [4].

Recently, nutraceuticals have gained extensive attention due to their better pharmacological functions

with fewer side effects. In 2017, 77% of Americans used nutraceuticals, and their number was rising daily [5]. Nutraceuticals are classified as dietary fiber, probiotics, prebiotics, polyunsaturated fatty acids, antioxidant, vitamins, polyphenols, and spices [6]. Herbal nutraceuticals are prepared from plants, fungi, algae, or their combinations. Various forms of herbal nutraceuticals (powder, tablets, capsules, liquid, etc.) are currently available in the market [7].

About 2500 years ago, Hippocrates wrote, “Let food be thy medicine and medicine be thy food.” This quotation is undoubtedly the principle of today [6]. The nutraceutical industry has great potential in some Asian

countries, such as China, Japan, India, South Korea, Thailand, Singapore, and Taiwan. With rich natural resources, skilled human resources, and excellent R&D facilities, these Asian countries are expected to lead the production of herbal nutraceuticals [8, 9].

Tripura is India's state located in the northeastern part of the subcontinent between 22°7' and 24°2' north latitudes and 91°0' and 92°0' east longitudes. Its highest temperature is 35.60°C, and yearly rainfall is 2000 mm. These excellent climatic conditions make Tripura a favorable place for various edible medicinal plants, or plants with nutritional benefits and bioactive compounds for the human body [10, 11].

According to literature, the forest of Tripura is a good source of edible medicinal plants with anti-diabetic properties [12]. Diabetes is a big issue in the Indian health care system. Type 2 diabetes is the most common in adults with uncontrolled diet. Every year, nearly 1 million Indians die due to diabetes. This chronic disease afflicts 2.8% of the global population [13]. Treatment with herbal medicine improves insulin secretion and reduces intestinal glucose absorption in diabetics [14].

This study aimed to formulate herbal nutraceutical tablets from selected edible medicinal plants of Tripura, India, and evaluate its anti-diabetic activity using ob/ob mice (type II diabetes model).

STUDY OBJECTS AND METHODS

Ethnomedicinal data collection. Ethnomedicinal data about five edible plant parts (*Musa paradisiaca* fruit, *Musa paradisiaca* stem, *Cmorphophallus paeoniifolius* corn, *Artocarpus heterophyllus* seed, and *Colocasia esculenta* leaf) were collected from oral interviews with traditional healers from three ethnic communities (Tripuri, Reang, and Jamatia) of Tripura. All the interviewees were adults aged over forty [15]. The scientific names of the collected plant specimens were identified with the help of a plant taxonomist and the book, "The Flora of Tripura State" [16].

Processing of plant materials. After identification, we selected plant parts with medicinal properties, washed them thoroughly with distilled water, and then properly air-dried them. The dried samples were ground to fine powder. The powder material was packed in a sealed container and preserved at room temperature for further experiments [17].

Experimental animals. In our experiments, we used laboratory mice as model organisms. Adult Swiss albino mice (18–25 g) of both sexes were used for acute toxicity tests. Male ob/ob mice (type II diabetes mice weighing 50–55 g, aged 12 weeks) and male C56BL6 mice (normal mice weighing 28–32 g, aged 12 weeks) were used for an anti-diabetic activity test. The temperature (20°C) and humidity (53%) of the animal house were controlled and maintained throughout the 12 h/12 h light/dark cycle. Food and water were available except during the fasting period. The care and handling of the animals were in line with

the regulations of the National Institutes of Health. The Institutional Ethics Committee (No. 1667/GO/a/12/CPCSEA) approved the study protocol [18].

Acute toxicity test. Acute toxicity tests of five different plant powder samples were measured by the method described by Ali *et al.* with some modification [19]. The Swiss albino mice (18–25 g) of both sexes were divided into two groups, the control group and the experimental group, ten animals in each. The control group received only distilled water, whereas the experimental group received different doses (5, 50, 300, 800, 1200, and 2000 mg/kg body weight) of the powder sample orally. The mice were then kept under observation up to 72 h for mortality or symptoms of toxicity [19].

Formulation of herbal tablet. We used the wet granulation method to prepare novel herbal anti-diabetic nutraceutical tablets. Equal amounts of previously prepared plant materials (*M. paradisiaca* fruit, *M. paradisiaca* stem, *C. paeoniifolius* corn, *A. heterophyllus* seed, and *C. esculenta* leaf) were placed in a rapid mixture granulator, with a 10% starch solution added dropwise into the binder. The damp masses were screened through a sieve #10 and then dried. The dried granules were screened through a sieve #20 and stored in a desiccator until they were ready for tablet compression. The prepared granules were compressed in a single punch tablet press machine (Manesty Type F3, Liver Poole, England) with a punch diameter of 0.75 cm and a compression pressure of 933 Pa (N/m²). The die volume matched the tablets' weight to confirm that 600 mg was obtained [20].

Evaluation of granules. Tablet granules were evaluated by Wadher *et al.* methods with some modification. Particularly, we determined their bulk density, tapped density, Hausner quotient, Carr's compressibility index, flow rate, and angle of repose [21].

Evaluation of herbal tablets. Weight variation, thickness, hardness, friability, and *in vitro* disintegration time were determined by Wadher *et al.* methods with some modification [21].

Total moisture, total carbohydrate, complete protein, total fat, total ash, and total caloric value of the herbal tablets were determined by Debnath *et al.* method with some modification [17]. Total dietary fiber was measured by Ozoliņa *et al.* method with some modification [22].

Concentrations of minerals were determined by an atomic absorption spectrophotometer. Debnath *et al.* method with some modification was applied to measure the content of minerals in the tablets [17].

Vitamins C, B₁, B₃, B₆, and folic acid were determined by Antakli *et al.* method with some modification, using the RP-HPLC system [23].

Instrumental conditions:

Column: C18 BDS (10 cm×4.6 mm; 3 μm);

Mobile phase: A = Hexane-1-sulfonic acid sodium (5.84 mM): acetonitrile (95:5) with 0.1% triethylamine as solvent (A) at pH 2.5; B = 5.84 mM of hexane-1-

Table 1 Ethnomedicinal use report on edible medicinal plant parts selected

Botanical name and family	Local name	Plants parts	Ethnomedicinal use
<i>Musa paradisiaca</i> (Musaceae)	Kola	Unripe fruit	Diabetes, hypertension, ulcers, diarrhea
<i>Musa paradisiaca</i> (Musaceae)	Kola	Stem	Diabetes, high blood pressure, high acidity
<i>Cmorphophallus paeoniifolius</i> (Araceae)	Batama	Corn	Helminths, liver disease, digestive and gastric disorders, diabetes
<i>Artocarpus heterophyllus</i> (Moraceae)	Kathal	Seed	Ulcers, constipation, diarrhea; excessive accumulation of fluid in tissues
<i>Colocasia esculenta</i> (Araceae)	Kocho	Leaf	Diabetes, microbial infection, liver disease

sulfonic acid sodium: acetonitrile (50:50) with 0.1% triethylamine as solvent (B) at pH 2.5, pH = 3.54;

Flow rate: 1.6 mL/min;

Injected volume: 20 μ L;

Absorbance recorded: Vitamins C and B₁ = 246 nm, vitamin B₃ = 260 nm, vitamin B₆ = 290 nm, vitamin B₉ = 282 nm.

Determination of fat-soluble vitamins. Vitamins A, D₃, and E were determined by using the reversed-phase high-performance liquid chromatography as reported by Xue *et al.* with some modification [24].

Instrumental conditions:

Column: dC18 (particle diameter 5 μ m, 150 \times 4.6 mm i.d.);

Mobile phase: methanol:water = 98:2;

Flow rate: 1.00 mL/min;

Injected volume: 10 μ L;

Absorbance recorded: vitamin E = 230 nm, vitamin A and D₃ = 265nm.

Anti-diabetic activity. Experimental design. The animals were randomly divided into four groups of six animals for test purposes, namely: a) a normal group (completely healthy mice) treated with 0.5% sodium carboxymethyl cellulose; b) a vehicle control group (ob/ob mice) treated with 0.5% sodium carboxymethyl cellulose; c) a positive control group treated with 200 mg/kg of metformin via gavage; and d) an experimental group treated with 200 mg/kg herbal nutraceutical tablet via gavage. The experiment lasted four weeks. At the end of the experiment, all the animal groups fasted overnight, and blood samples were collected from the tail vein. Before blood collection, the animals were given pentobarbital as an anesthetic agent [18].

Determination of serum hemoglobin and glycosylated serum protein. Hemoglobin (HbA1c) and glycosylated serum protein were measured by respective kits (Merck Millipore, Germany) according to the manufacturer's instruction [18].

Oral glucose tolerance test. After four weeks of treatment with herbal nutraceutical tablets, the animals were made to fast overnight, and glucose solution (2 g/kg of body weight) was administered orally. After that, their blood samples were collected every 30 min (0, 30, 60, 90, and 120 min). The blood glucose was measured by a glucose meter (i-QARE DS-W®) [18].

Statistical analysis. For the analysis of granules, herbal tablets, proximate compositions, minerals, and

vitamins, the data were expressed as mean \pm SDs. For the evaluation of anti-diabetic activity in ob/ob mice, the data were expressed as mean \pm S.E.M. One-way ANOVA was used to determine significant differences among groups, after which the modified Student's t-test with the Bonferroni correction was applied to compare individual groups. All statistical analyses were performed with SPSS 17.0 software. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Ethnomedicinal study. The field survey showed that the five edible medicinal plants were applied by the healers of Tripura to cure different diseases (Table 1). Ethnomedicinal studies play a vital role in finding medicinal plants that can be used to produce novel crude drugs. They also verify the protection of cultural heritage [11]. The data that we collected from the informants of healers clearly proved that the plants we had selected were of medicinal importance.

Acute toxicity test. Our results indicated no changes of behaviour or mortality caused by the plant samples at the highest dose of 2000 mg/kg b.wt. This meant that this dose had no lethal or toxic effect. Toxicity assessment is one of the crucial steps prior to human uses of any pharmaceuticals or food ingredients. Acute toxicity studies determine adverse effects of any active compound after oral ingestion of a single or multiple doses [19]. We found that the five plant samples under study had no toxic effect.

Evaluation of granules. All the physical parameters of the herbal granules under study were found to be satisfactory (Table 2). The Carr's compressibility index indicates the strength of the powder/granules, while the Hausner ratio determines the powder/granules' inter-particulate friction. Both parameters are used to analyze the powder/granules' flow rate. The Carr's compressibility index of less than 10 or the Hausner ratio of less than 1.11 indicate an "excellent" flow rate, while the Carr's compressibility index higher than 38 or the Hausner ratio higher than 1.60 indicate a "very poor" flow rate. Both parameters depend on the bulk density and the tap density of the powder/granules [25]. The angle of repose is another parameter used to evaluate the powder/granules' flow rate. Its value of less than 30° indicates an "excellent" flow rate, while the value greater than 56° indicates a "very poor" flow rate [26].

Table 2 Physical parameters of herbal granules

Physical parameters	Herbal granules prepared with 10% starch solution
Bulk density, g/mL	0.46 ± 0.01
Tapped density, g/mL	0.48 ± 0.01
Hausner quotient	1.07 ± 0.01
Carr's compressibility, %	6.72 ± 0.53
Angle of repose, °	32.29 ± 0.99
Flow rate, g/sec	6.67 ± 0.57

*The values represent mean ± SD for three samples

Table 4 Proximate composition of herbal nutraceutical tables

Proximate composition	Total amount, %
Moisture	62.44 ± 2.11
Carbohydrates	1.88 ± 0.52
Protein	0.95 ± 0.65
Fat	0.43 ± 0.53
Ash	1.78 ± 0.53
Dietary fiber	1.66 ± 0.67
Calories, kcal/g	15.19

*The values represent mean ± SD for three samples

Table 6 Profiles of water soluble and fat-soluble vitamins in herbal nutraceutical tablets

Vitamin	Total amount
C (ascorbic acid), mg/g	27.2 ± 4.3
Vitamin B ₁ (thiamin), mg/g	0.6 ± 0.0
Vitamin B ₃ (niacin), mg/g	0.6 ± 0.2
Vitamin B ₆ (pyridoxine), mg/g	1.1 ± 0.2
Vitamin B ₁₂ (cobalamin), mcg/g	0.6 ± 0.2
Vitamin B ₉ (folic acid), mcg/g	82.6 ± 7.6
Vitamin A (retinol), mcg/g	287.4 ± 6.3
Vitamin D ₃ (cholecalciferol), mcg/g	2.6 ± 0.6
Vitamin E (tocopherol), ng/g	0.7 ± 0.0

*The values represent mean ± SD for three samples

Our results showed an excellent flow rate in the granules prepared with a 10% starch solution (Table 2).

Evaluation of herbal tablets. All the physical parameters of the herbal tablets under study were found to be satisfactory (Table 3). The weight variation test is employed to ensure that each tablet/capsule in the batch contains the same amount of drug ingredients. Checking a tablet's thickness is necessary for packaging since very thick tablets are not suitable for packaging. A tablet's friability and hardness tests are closely related to each other. They determine the physical strength of a tablet. Disintegration refers to the mechanical breakup of a compressed tablet into small granules at a specific time point. The disintegration test provides critical safety data on the drug's bioavailability in the body [27, 28].

In our experiments, 20 tablets were used to check the average uniformity of weight and 10 tablets to check the average uniformity of thickness and hardness. For

Table 3 Physical parameters of herbal nutraceutical tablets prepared with 10% starch solution

Physical parameters	Value
Uniformity of weight, mg	610.5 ± 1.70
Uniformity of thickness, mm	3.78 ± 0.041
Hardness, kg/cm ²	3.88 ± 0.078
Friability, %	0.65 ± 0.020
Disintegration time, s	295.33 ± 1.52

*The values represent mean ± SD for three samples

Table 5 Minerals profile of herbal nutraceutical tablets

Minerals	Total amount, mg/g
Iron	74.6 ± 2.7
Sodium	4.4 ± 0.4
Potassium	5.3 ± 0.7
Calcium	163.1 ± 2.2
Magnesium	39.2 ± 1.7
Phosphorus	14.6 ± 2.1

*The values represent mean ± SD for three samples

the friability and disintegration tests, the samples were prepared in triplicate. According to the results (Table 3), the physical parameters of our newly formulated tablets were within the limits established by the United States Pharmacopoeia.

Determination of proximate composition. The proximate composition of the herbal nutraceutical tablets is represented in Table 4. The moisture content is an essential parameter because high moisture affects the physical stability of food products. Their shelf life also depends on the total moisture content [29]. As we can see in Table 4, our newly formulated herbal tablets had a low moisture content (62.44 ± 2.11 %), indicating high physical stability.

Carbohydrates, proteins, and fats hold a special place in human nutrition. The human body requires them in relatively large amounts for normal functioning. These three macronutrients provide energy (measured in calories) in the human body [30]. Our newly formulated herbal nutraceutical tablet contained optimal amounts of carbohydrates (1.88 ± 0.52 %), proteins (0.95 ± 0.65 %), and fats (0.43 ± 0.53 %), as well as provided a good amount of energy (15.19 kcal).

So, the herbal nutraceutical tablets developed may be recommended to people with unbalanced energy levels. Dietary fiber is a complex mixture of polysaccharides. Diets with a high content of fiber alleviate constipation [31]. Our herbal nutraceutical tablet contained a good amount of dietary fiber (1.66 ± 0.67%), so it may be used to reduce constipation.

Minerals determination. Iron is an essential microelement for producing blood. Anemia is the most common disease caused by iron deficiency. Every year, approximately 30% of patients suffer from

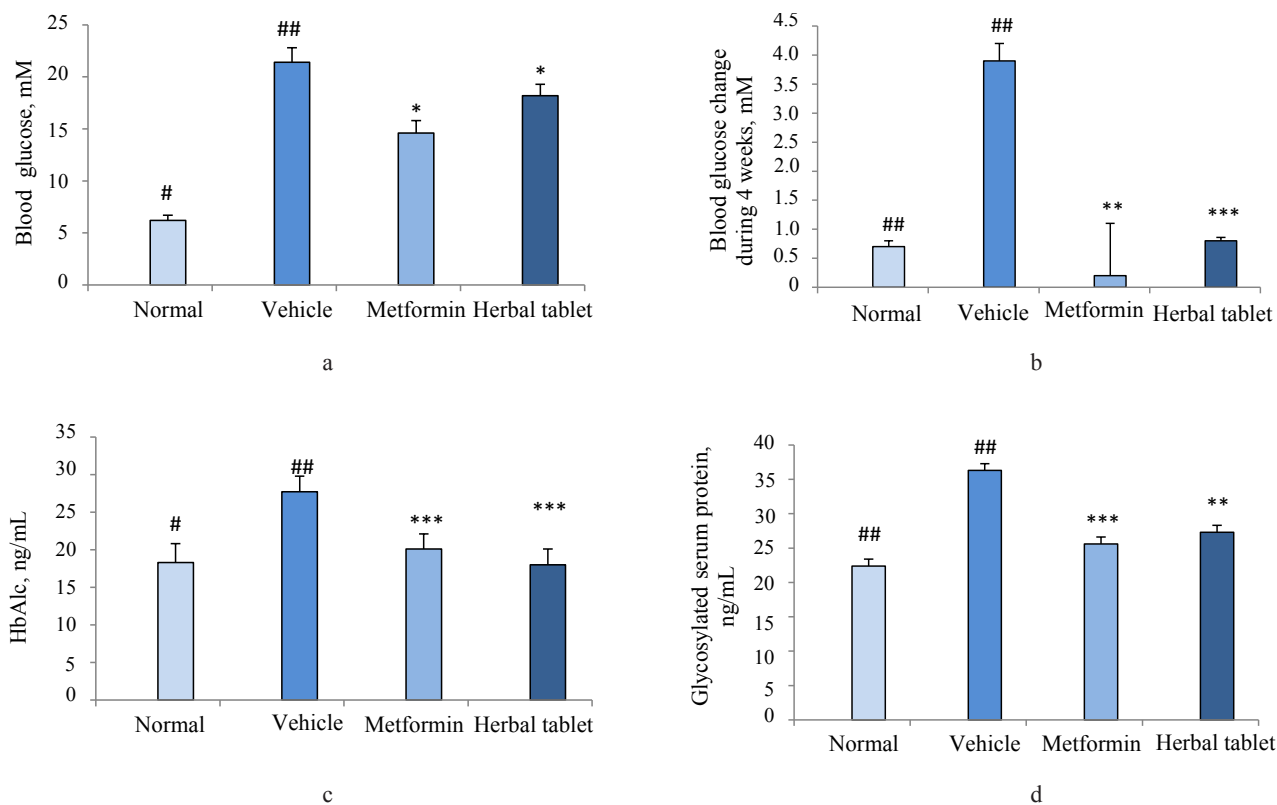


Figure 1 Carbohydrate metabolism parameters of ob/ob mice treated with sodium carboxymethyl cellulose (normal group), sodium carboxymethyl cellulose (vehicle control group), metformin (positive control group), and herbal nutraceutical tablet (experimental group). Each group contained six animals

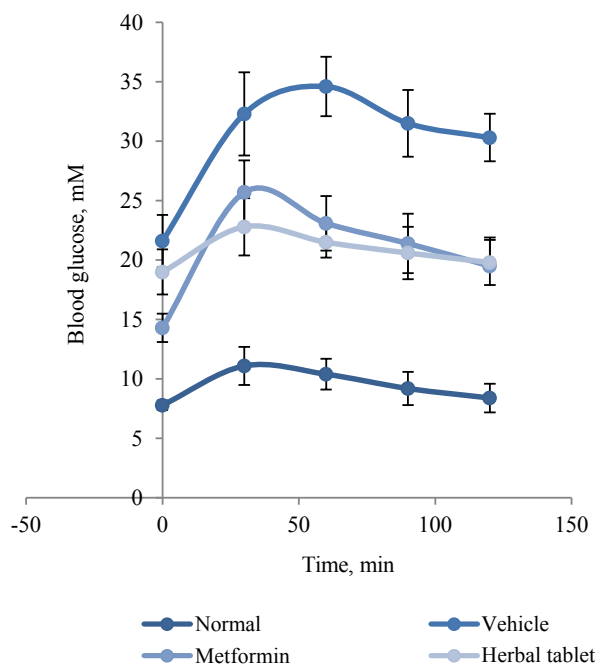


Figure 2 Change of blood glucose with time in ob/ob mice treated with sodium carboxymethyl cellulose (normal group), sodium carboxymethyl cellulose (vehicle control group), metformin (positive control group), and herbal nutraceutical tablet (experimental group). Each group contained six animals

anemia globally, particularly 51% in India [32]. Our herbal nutraceutical tablet had a significant amount of iron (74.6 ± 2.7 mg/g), so it may alleviate the effects of iron deficiency (Table 5). Dietary potassium and sodium are two electrolytes that play a vital role in regulating fluid and blood volume [33]. The tablets developed contained a fair amount of sodium (4.4 ± 0.4 mg/g) and potassium (5.3 ± 0.7 mg/g), so it may be used to regulate the body’s fluid and blood volume. Calcium, phosphorus, and magnesium are three crucial micronutrients for healthy bone and teeth formation, as well as metabolic functions [34]. Our herbal nutraceutical tablets contained a considerable amount of these minerals, namely 163.1 ± 2.2 , 14.6 ± 2.1 , and 39.2 ± 1.7 mg/g, respectively. Therefore, the newly developed herbal tablets can help bone formation and improve metabolic functions in the human body.

Determination of vitamins. We determined the values of water-soluble (C, B₁, B₃, B₆, B₁₂, and folic acid) and fat-soluble vitamins (A, D₃, and E) in our herbal nutraceutical tablets (Table 6). We found rich amounts of vitamin C (27.2 ± 4.3 mg/g) and vitamin A (287.4 ± 6.3 mcg/g). Vitamin C is a water-soluble vitamin that works as an antioxidant and improves the immune function of the human body [35]. Vitamins B₁, B₃, B₆, and B₉ are essential for maintaining the nervous system, digestion, protein metabolism, red blood cells,

and skin health. Vitamins A, D₃, and E are common fat-soluble vitamins that support such body functions as vision, hair growth, bone maintenance, immune system regulation, oxidative stress prevention, etc. [36]. Our herbal nutraceutical tablets contained fair amounts of vitamins D₃, E, and B group (Table 6). Therefore, they may be used to treat diseases caused by their deficiency.

Anti-diabetic activity. The treatment of ob/ob mice with our herbal nutraceutical tablets (200 mg/kg body weight) significantly decreased their fasting blood glucose, serum hemoglobin, and glycosylated serum protein (Fig. 1). The effectiveness of the herbal tablet was comparable to that of metformin, a standard drug (200 mg/kg body weight).

A fasting blood glucose test, which is generally called a fasting plasma glucose test, measures the amount of glucose in the blood and determines the patient's risk of prediabetes or diabetes [37]. Our newly formulated herbal tablet (200 mg/kg) reduced the fasting blood glucose level (Fig. 1a and 1b). Serum hemoglobin and glycosylated serum protein are important indicators for the long-term glycemic control [38]. Our experiment showed that the herbal tablet (200 mg/kg) reduced their levels, compared to metformin (200 mg/kg) (Fig. 1c and 1d).

An oral glucose tolerance test determines the body's response to glucose. This test can be used to detect

type 2 diabetes [39]. The oral administration of the herbal tablet to ob/ob mice significantly reduced their blood glucose level (Fig. 2). Therefore, this tablet may be used as an anti-diabetic drug.

We also found that the herbal nutraceutical tablet (200 mg/kg) significantly enhanced oral glucose tolerance (Fig. 2). This means that this tablet can be used to alleviate type 2 diabetes mellitus through progressing insulin sensitivity.

CONCLUSION

Our study showed that the newly formulated herbal tablet contained optimal amounts of macro- and micronutrients, water, and fat-soluble vitamins. The tablet also provided significantly higher hypoglycemic activity compared to the standard drug, metformin. The results suggested that the herbal tablets developed may be recommended as an anti-diabetic herbal remedy.

CONTRIBUTION

The authors were equally involved in writing the manuscript and are equally responsible for plagiarism.

CONFLICT OF INTEREST

The authors have declared no conflict of interest in relation to this manuscript.

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