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Effect of madhumeh an antibiotic polyherbal formulation on carbohydrate metabolism and antioxidant defence in Streptozotocin-Nicotinamide (STZ-NICO) induced diabetic rats

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Abstract

The present investigation was undertaken to validating the antidiabetic claim of the polyherbal formulation madhumeh and to investigate the antioxidant activity in streptozotocin– nicotinamide induced diabetic rat model. Evaluation of Antidiabetic activity of polyherbal formulation in Streptozotocin – Nicotinamide induced diabetic rats and Induction of diabetes in animal and estimation of biochemical parameters. Madhumeh possesses blood glucose lowering comparable to standard oral hypoglycemic agent glibenclamide. Madhumeh showed marked increase in total protein in diabetic treated and lower the level of triglyceride in serum. Body weight, food intake, water intake are improved in formulation treated rats compared to diabetic rats suggesting the returning of glucose uptake utilization back to normal levels. Ours study shows that the hepatic antioxidant enzyme (SOD, CAT and GSH) levels were increased significantly with a significant reduction of LPO on treatment. Madhumeh exhibited good free radical scavenging property.

Key-Words: Madhumeh, Polyherbal Formulation, Streptozotocin – Nicotinamide (STZ-NICO).

Introduction

Diabetes mellitus (DM) is a metabolic disorder affecting carbohydrate, fat and protein metabolism. The world wide survey reported that the diabetes is affecting nearly 10% of the population. Diabetes is likely to remain a significant threat to public health in the year to come, in the absence of effective and affordable interventions for both type of diabetes. The frequency of the disease will escalate world wide, with the major impact on the population of developing countries. Ayurvedic herbal drugs for diabetes are selected on the principle of taste, physiochemical properties, potency, post digestive effect and unique action. It has been argued that major antidiabetic activities from plants might originate from their antioxidant principle. These efforts may provide novel mechanism based application of traditional medicine used in this disorder [1]. DM is known to ancient Indian physicians as "Madhumeha". Many herbal products including several metals and minerals have been described of the care of DM in ancient literature. Since the allopathic medicine has large number of side effects, in recent years there has been an increase in the use of herbals by a majority of population throughout the world [2].

Polyherbal formulations with various active principles and properties have been used from ancient days to treat a whole range of human diseases. Generally, they are collection of therapeutic recipes that are formulated and prepared on the basis of the healing properties of individual ingredients with respect to the condition of sickness. Such herbal constituents with diverse pharmacological actions principally work together in a dynamic way to produce maximum therapeutic efficacy with minimum side effects. Nevertheless, these traditional medicinal preparations gradually lost their popularity and foothold among people due to the fast therapeutic action of allopathic system of medicine. In recent years however, renewed interest has grown on traditional herbal remedies because of the reportage of adverse side effects using synthetic drugs in allopathic medicine. At the same time, WHO also recommends further research on traditional method of treatment [3]. The present investigations was undertaken to study the effect of madhumeh, an antidiabetic polyherbal formulation, on carbohydrate metabolism and antioxidant defences in streptozotocin – nicotinamide induced diabetic rats.

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Material and Methods

5, 5ˋ – bithio bis-nitrobenzoic acid DTNB and Streptozotocin STZ were Sigma–Aldrich make. Ethylene diamine tetra acetic acid EDTA, Formaldehyde, Hydrogen Peroxide, n- Butanol, Pyridine and Tris-hydrochloride Tris-HCl were Qualigens make. Folin ciocalteaue Reagent, Alkaline copper reagent, Trichloro acetic acid TCA, Glacial acetic acid, Petroleum ether, Sodium dodecyl sulphate SDS and Potassium hydrogen phosphate were E-Merck make. Sodium pyrophosphate, Phenazonium methusalphate, Nicotinamide adenine dinucleotide NADH, Nitoblue tetrazolium NBT and Thiobarbituric acid TBA were Ottokemi make. Sodium citrate was Nice chemical Pvt. Ltd, Cochin make, Nicotinamide NICO was Himedia make. All the reagents were used as received without further purification.

Experimental Work

Plants

Gymnema sylvestre, Melia azadirachta, Eugenia jambholana and Aegle marmelos (Linn.) were received as gift sample from Ultra College of pharmacy, Madurai (India).

Animals

Healthy adult male Wistar albino rats weighing about 250-300 g were used for the study. The animals were housed in polypropylene cages, maintained under standard conditions (12 h light/12-h dark cycle 25 ± 3^{0}C: 35- 60% humidity) were fed standard rat pellet diet and water adlibitium. The study was approved by institutional animal ethics (18/UCP/IAEC/2007/011) committee of Ultra College of pharmacy, Madurai (India).

Instruments

Research Centrifuge (REMI-24) and Homogenizer were received from Remi. U.V Spectrophotometer, PH meter and Colorimeter received from Systronics. Deep Freezer (Kadavil electro Mech. Ind.), BOD incubator, Auto analyzer (RAD)

Induction of Diabetes in Animal [4]

The animal model of type 2 diabetes mellitus (NIDDM) was induced in overnight fasted animals by a single I. P. administration of 60 mg/kg STZ, dissolved in ice told citrate buffer pH (4-3), 15 min after the I.P administration of 120 mg/kg NICO. Hyperglycemia was confirmed by the elevated blood glucose levels determined at 72 h and then on day 7 of the injection. Only rats confirmed with permanent NIDDM were used in the diabetic study. In the present study glibenclamide (2.5 mg/kg b.w) was used as standard. Fasting blood glucose level were estimated on 0, 5 and 12th days of the study. Body weight, serum, cholesterol and triglyceride of the animal were noted during study. Any changes in skin, ear, eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous system, somatomotor activity and behavior pattern were noted. Also sign of tremors, convulsion, salivation, diarrhoea, lethargy and coma were noted. The onset of toxicity and sign of toxicity were also noted with water and food intake. One group of the normal animal was also maintained throughout the study.

Collection of Blood Sample and Blood Glucose Determination

Blood samples were collected from tail vein of the animal and blood glucose level was determined by collecting blood in eppendorff tubes containing 50 microlitre of anticoagulant (10% trisodium citrate solution) from normal and STZ – NICO. An induced diabetic rat, plasma was separated by centrifugation at 5000 rpm for 10 min for total cholesterol and triglyceride using kits from Span diagnostic Ltd, India. At the end of the study, all the serving animals of the respective groups were sacrificed by an over dosage of ether anaesthesia. The liver was excised, rinsed in ice-cold normal saline followed by cold 0.15 M Tri HCl (pH-7.4), blotted dried and weighed A 10% w/v homogenate was prepared in 0.15 M Tris HCl buffer and was used for the estimation of lipid peroxidation (LPO) and GSH (glutathione). The rest of the homogenate was centrifuged at 1500 rpm for 15 min at 4^{0}C. The supernatant, thus obtained was used for the estimation of SOD and catalase (CAT) and total protein.

After exsanguinations, the animal pancrease were removed immediately and wash with ice-cold saline. The pancreases were fixed in 10% neutral buffered formalin. Sections of 3-5mm thickness were stained with hematoxylin and eosin (H and E) for histopathological studies. Dose calculation done according to Meeh's formula and dose of animal (Rats) calculated on the basis of body surface area [5].

Estimation of Biochemical Parameters

Lipid peroxidation

The level of Thiobarbituric acid reactive substances (TBARS) in the liver as a marker for Lipid peroxidation was measured by the method of Ohkawa [6]. Mixture of 0.4 ml of 10% liver homogenate, 1.5 ml of SDS (8.1 %.), 1.5 ml acetate buffer (pH 3.45) and 1.5 ml of TBA (08%) solution was heated at 95^{0}C for 1 hrs. After cooling, 5.0 ml of n-butanol/pyridine (15:1) was added. The absorbance of the n-butanol-pyridine layer was measured at 532 nm.

GSH

GSH was determined by the method Beutter [7]. 0.2 ml of tissue homogenate mixed was with 1.8 ml of EDTA

solution. To this 3.0 ml of precipitating reagent (1.67g of metaphosphonic acid, 0.2g of EDTA, disodium salt, 30 g sodium choride in 1000 ml of distilled water was added mixed thoroughly and kept for 5 min before centrifugation. To 2 ml of filtrate, 4.0 ml of 0.03M disodium hydrogen phosphate solution and 1ml of DNTB 5.5 elithio bis – nitrobenzoic acid. Reagents were added and absorbances were recorded for a reaction time for 5 min at 412 nm.

SOD

The activity of SOD in tissue was assayed by the method of Kakkar [8]. The assay mixture contained 1.2 ml sodium pyrophosphate buffer (pH- 8.3), (0.1 ml, 0.025 M) phenazine methosulphate (186 mm), 0.3 ml NBT (300 mM), 0.2 ml NADH (780 mM) and approximately diluted enzyme preparation and water in total volume of 3 ml. After incubation at 30^0C for 90 sec, the reaction was terminated by the addition of 1.0 ml of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4.0 ml n-butanol. The color intensity of the chromogen in the butanol layer was measured at 360 nm against n-butanol for 8 min.

CAT

Catalase was assayed according to the method of Maehly and Chance [9]. The estimation was spectrophotometrically following the decrease in absorbance at 230 nm. The tissue was homogenized in M/150 phosphate buffer (pH-7) at $1-4^0C$ and centrifuge at 500 RPM. The reaction mixture contained 0.01M phosphate buffer (pH-7), 2 mM of H_2O_2 and the enzyme extract. The specific activity of catalase is exporessed in term of U / mg protein. A unit is defined as the velocity constant per second.

Total Protein

The protein estimation of tissue homogenate was measured by the method of Lowry [10]. 0.5 ml of tissue homogenate was mixed with 0.5 ml of 10% TCA and centrifuge for 10 min. The precipitate obtained was dissolved in 1.0 ml of 0.1 N Hacl. From this an aliquate was taken for protein estimation. 0.1 ml g aliquate was mixed with 5.0 ml of alkline copper reagent and allowed to stand at room temperature for 10 min. 0.5 ml of folin's phenol reagent was added and the blue color developed was read offer 20 min at 640 nm.

Results and Discussion

Diabetes was induced with single i.p. injection of STZ 60 mg/kg and Nicotinamide 120 mg/kg body weight. The STZ was freshly dissolved in citrate buffer (0.01 M, pH- 4.5). STZ is widely used as chemical inducer for type I diabetes. In some clinical practice, a high dose of NA is occasionally administered to diabetics to prevent the progress of IDDM [11]. So nicotinamide administered prevent the excessive destruction of β-cells thereby NIDDM will result instead of IDDM. In the study significant hyperglycemia was achieved within 72 hrs after STZ - nicotinamide injection. Animals with more than 200 mg/dl of blood sugar were considered to be diabetic and used for study. STZ-NICO treatment increased the glucose level about three folds. Treatment with Madhumeh significantly (p < 0.001) decreased the blood glucose level from 5th day onward, while there was no improvement in diabetic rats. The reduction in blood glucose levels was comparable to that of standard drug glibenclamide.

Glibenclamide is an oral sulphonylurea with a hypoglycemic effect. Glibenclamide is indicated as an adjunct to diet to lower the blood glucose level in patients with non-insulin dependent (Type 2) diabetes mellitus were hyperglycemia cannot be controlled by diet alone. Although glibenclamide has been showed various side effects, it was generally used as reference drugs in anti-diabetic activity test. [12]There is a sudden loss of body weight in diabetes. Polyphagia and polyurea are also the significant indices of diabetes [13] which are presented in Table 1. These changes in glucose, food, water intake are due to the lack of insulin shown in Table 2. The herbal formulation treatment could normalize these changes showing that the insulin secretion should have been corrected either by stimulation of β-cells or by repair of β-cells. The antihyperglycemic activity of the constituents of madhumeh has already been reported [13].

Histopathological analysis of pancreas

Histopathology results also confirm the biochemical result related to pancreatic function. Figure 1 shows the degeneration of islets observed with diabetic were not present in the treated pancreas showing the recovery of normal carbohydrate metabolism.

Table 3 shows the effect of Madhumeh on level of, triglyceride, cholesterol total protein level and TBARS. Cholesterol and triglyceride level (p<0.001) were decreased in treated diabetic rats. The treatment with Madhumeh and glibenclamide produced significant decresed in cholesterol and triglyceride when compared to diabetic animals. Results are shown in Table 3. Circulating triglyceride levels are increased in diabetics [14]. This altered Triglyceride level is corrected in treated group showing the regulation of carbohydrate and lipid metabolism by the herbal formulation.

A significant (p < 0.001) increase in liver TBARS was observed in STZ – NICO induced diabetic animals when compared to diabetic control animals. TBARS level of treated animal showed (p < 0.001) decrease and glibenclamide treatment showed significant (p <

0.001) decrease when compared to STZ – Nicotinamide induced diabetic animals.

Nakakimura et al [15] have reported that concentration of lipid peroxidation increases in the kidney of diabetic rats and an increased level of TBARS in an index of lipid peroxidation. In Madhumeh and glibenclamide treated diabetic rats, the TBARS levels were low which may be due to the free radical scavenging action of active ingredients.

Table 4 shows the effect of Madhumeh on SOD, CAT and GSH. A significant ($p < 0.001$) decrease in GSH in STZ – NICO induced diabetic animals when compare to normal. The liver GSH level in diabetic rats treated with madhumeh significantly ($p < 0.001$) increased and glibenclamide showed significantly ($p < 0.001$) increased when compare to STZ – NICO induced diabetic animals. GSH is a major endogenous antioxidant which counterbalances free radical mediated damage. It is well known that GSH is involved in the protection of normal cell structure and function by maintaining the redox homeostasis, quenching of free radicals and by participating in detoxification reactions. Significant decrease in the levels of GSH has been observed in Madhumeh treated rats when compared to STZ induced diabetic rats. The decrease in liver GSH levels represents increased utilization due to oxidative stress [16].

A significant ($p < 0.001$) increased in liver SOD was observed in induced diabetic animals when compare to control. The liver level of diabetic treated with madhumeh and glibenclamide showed significant ($p < 0.001$) decreased when to STZ – Nicotinamide induced diabetic animals. A significant ($p < 0.001$) decreased in the liver CAT level was observed in induced diabetic animals when compare to normal animals. The liver level of diabetic treated with madhumeh and glibenclamide shows significant ($p < 0.001$) increased when to STZ – NICO induced diabetic animals as presented in Table 4. SOD protects tissues against oxygen free radicals by catalyzing the removal of superoxide radical ($O_2^{\cdot-}$), which damages the membrane and biological structures [17]. Catalase has been shown to be responsible for the detoxification of significant amounts of H_2O_2 [18]. SOD and catalases are the two major scavenging enzymes that remove the toxic free radicals in *vivo*. Madhumeh and glibenclamide treated rats showed decreased lipid peroxidation that is associated with increased activity of SOD and CAT as shown in table 4.

Thus, it may be concluded from the results of the present investigation that Madhumeh possesses antioxidant activity in diabetic conditions and its antidiabetic potency is also validated.

Madhumeh possesses blood glucose lowering comparable to standard oral hypoglycemic agent glibenclamide. Madhumeh showed marked increase in total protein in diabetic treated and lower the level of triglyceride in serum. Body weight, food intake, water intake are improved in formulation treated rats compared to diabetic rats suggesting the returning of glucose uptake utilization back to normal levels. The hepatic antioxidant enzyme (SOD, CAT and GSH) levels were increased significantly with a significant reduction of LPO on treatment. Madhumeh exhibited good free radical scavenging property.

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Table 1: Effect of Madhumeh on glucose level in diabetic rats

Group	Treatment	Day 0	Day 5	Day 12
I	Normal	84.83 ± 0.87	84.83 ± 0.87	85.00 ± 0.93
II	Diabetic control	221 ± 0.60	231.83 ± 0.70	238.5 ± 0.42
III	Madhumeh treated	219 ± 0.67 (0.9%) NS	184.5 ± 0.84* (20.41%)	164.5 ± 0.61* (31.61%)
IV	Glibenclamide treated	222.5 ± 0.84 (0.6%) NS	183.33 ± 0.55* (20.92%)	159.8 ± 0.60* (32.99%)

Value are mean $\pm$ SEM (n=6) * values are statistically significant compared to diabetic group at $p < 0.05$

Table 2: Effect of Madhumeh on body weight, food and water intake in rats

Group	Treatment	Body weight (g)		food intake g/rat/day	water intake ml/rat/day
		Initial	final		
I	Normal	224.50 ± 0.22	233.5 ± 0.23	11.50 ± 0.76	16.83 ± 0.14
II	Diabetic	207.50 ± 0.20	191.00 ± 0.19	19.33 ± 1.67	22.00 ± 1.27
III	Madhumeh	211.00 ± 0.21*	223.5 ± 0.22*	14.00 ± 0.85**	14.50 ± 0.076**
IV	Glibenclamide	216.00 ± 0.21*	226.0 ± 0.22*	13.167 ± 0.19**	12.667 ± 1.22**

Value are mean $\pm$ SEM (n=6) *, ** values are statistically significant compared to diabetic group at $p < 0.05$, $p < 0.001$ respectively.

Table 3: Effect of Madhumeh on triglyceride, cholesterol, total protein and TBARS

Treatment	Triglyceride mg/dl	Cholesterol mg/dl	Total protein mg/dl	TBARS nmol/mg protein
Normal	60.83 ± 0.40	65 ± 0.57	6.20 ± 0.30	0.150 ± 0.03
Diabetic	122.00 ± 0.73	75.66 ± 0.84	3.450 ± 0.31	0.334 ± 0.07
Madhumeh	45.16 ± 0.94*	40.00 ± 1.06*	4.737 ± 0.08*	0.177 ± 0.04*
Glibenclamide	39.5 ± 0.76*	36.82 ± 0.87*	5.110 ± 0.31**	0.167 ± 0.002*

Value are mean $\pm$ SEM (n=6) *, ** values are statistically significant compared to diabetic group at $p < 0.05$, $p < 0.01$ respectively.

Table 4: Effect of Madhumeh on SOD, CAT and GSH

Treatment	SOD U/mg Protein	CAT U/mg Protein	GSH U/ mg Protein
Normal	2.353 ± 0.06	3.24 ± 0.01	5.20 ± 0.12
Diabetic control	0.175 ± 0.03	1.56 ± 0.49	8.38 ± 0.740
Madhumeh treated	$0.548 \pm 0.004^*$	$2.20 \pm 0.07^{**}$	$3.66 \pm 0.076^*$
Glibenclamide treated	$1.428 \pm 0.089^{**}$	$1.69 \pm 0.020^{***}$	$3.40 \pm 0.12^{**}$

Value are mean $\pm$ SEM (n=6) *,**, *** values are statistically significant compared to diabetic group at $p<0.001$, $p<0.01$, $p<0.05$ respectively.
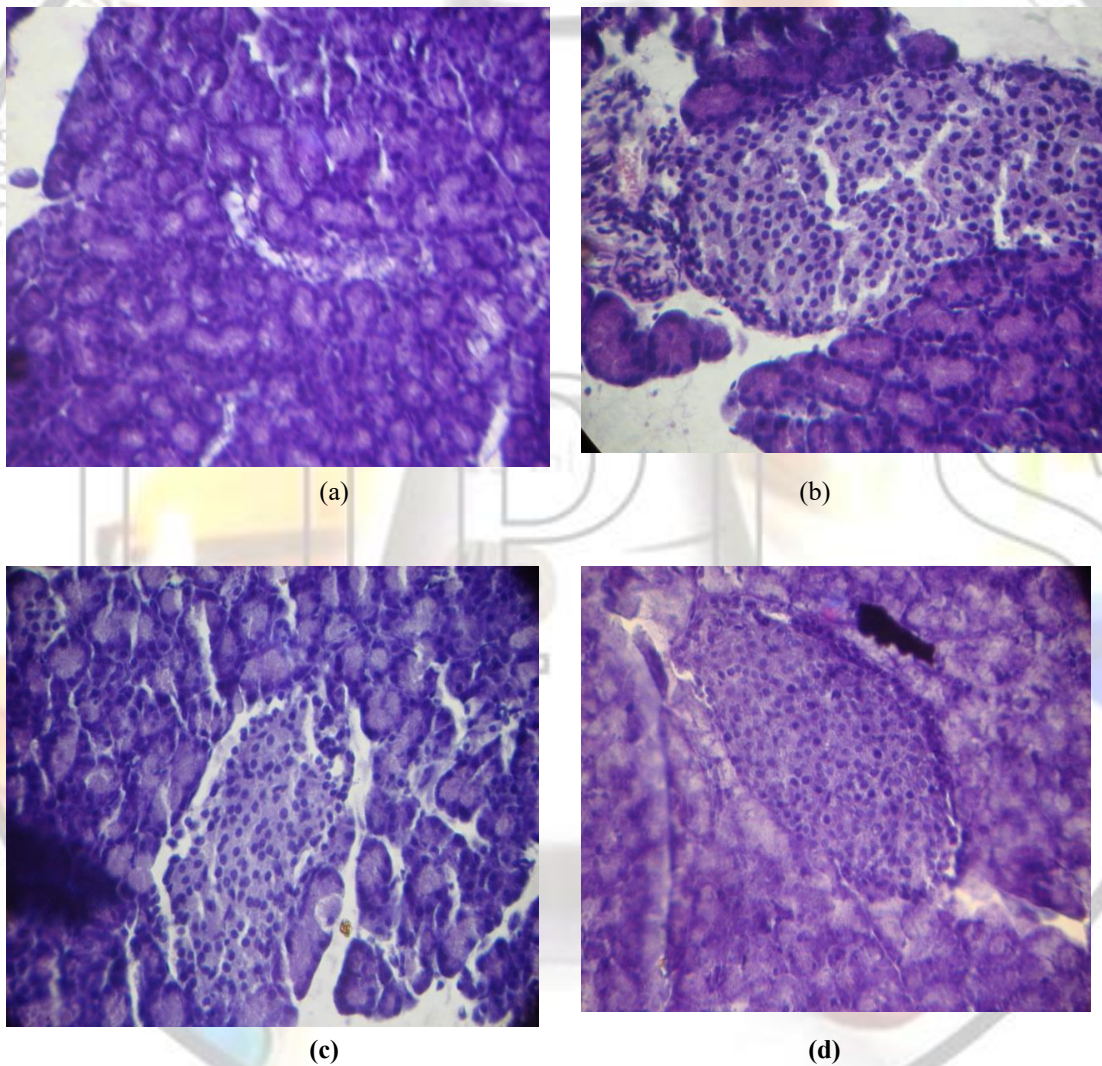

Fig. 1: Histopathological analysis of pancreas: (a) Normal, (b) Diabetic Control, (c) Madhumeh, (d) Glibenclamide treated at 45x