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RESEARCH ARTICLE

ENHANCED ORAL ADMINISTRATION OF INSULIN BY THE HERBAL FORMULATION OF *DESMODIUM GANGETICUM*: AN *IN VIVO* STUDY

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ABSTRACT

Background: Oral administration of peptide hormones like insulin is challenging due to its structural instability, which is often broken by the digestive enzymes and other environmental factors during absorption in the intestine. The present study deals with the protective effect of the herbal drug Desmodium gangeticum on the stability of the peptide hormone, insulin, in oral dosage.
Hypothesis: Herbal extract of Desmodium gangeticum increase the oral stability of insulin for Diabetes treatment.
Method: Streptozotocin (STZ) induced diabetes was developed in male Wistar rats (170 - 190g) and treated with STZ (65mg/kg) in 0.02M citrate saline buffer. A blood glucose level exceeding 200 was considered diabetic. Rats were then treated orally with insulin in combination with Desmodium gangeticum for 4-5 days. After 12h of fasting, the rats were then decaptivated to obtain blood and tissue homogenates which were used to assess relevant parameters for biochemical assays.
Result: Combinatorial therapy of insulin and Desmodium gangeticum on oral dosage produced a significance decrease in blood glucose levels in STZ-induced diabetic rats, as evident by biochemical analysis.
Conclusion: The overall results suggest the aqueous extract of Desmodium gangeticum is a possible candidate for oral delivery of insulin for Diabetes treatment.

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INTRODUCTION

Insulin is a natural hormone which controls the level of blood glucose and is limited in tissues of diabetes mellitus (DM)-induced rats. Patients with Type 2 DM are insulin resistant, have relatively low insulin production, or both. Some patients with Type 2 diabetes may eventually require insulin when other medications fail to control blood glucose levels adequately. In such patients, injection of insulin through subcutaneous route had become unavoidable as invasive routes of rectal, nasal, pulmonary and ocular delivery still to prove to be a challenge. The oral route is suitable and the desired route of drug delivery, especially when repeated or routine administration is necessary. But the effect of digestive enzymes and micro environmental factors could possibly reduce the stability of the peptide hormone during absorption and when exposed to digestive enzymes. The entrapping of insulin in polymeric microspheres (Rosa *et al*., 2000, Jain *et al*., 2005) or by coating with polymer films gives hope to avoid the digestion of this polypeptide in the gastrointestinal tract (Nolan *et al*., 2004; Sukhishvili and Svetlana, 2005) but little is known about its side effects. However, no study has been done to transport insulin with the help of an herbal extract as a vehicle. *Desmodium gangeticum* belongs to the family leguminosae and ayurvedic medicines (indigenous) for diabetes relied on the extracts of this plant that was found to possess isoflavanoid glycoside (Avasthi and Tewari, 1995). It is also used as diuretics and found to improve digestion. More importantly, *Desmodium gangeticum* extract was found to play a role maintaining the prolonged haemostatic glycemic level in rats (Gandhi Siddhar Selvam, 2016). Initially, the efficacy of *Desmodium gangeticum* extract along with insulin was tested in Diabetes induced male Wistar rats and compared with controls, oral administration of insulin alone and those received insulin injections subcutaneously. The aim of this study is to evaluate the herbal formulation to core the insulin and reduced blood glucose in diabetic rats. Administration of insulin through oral individually does not produce a significant effect than the existing oral hypoglycemic agents. Thus it was therefore planned to study the effect of herbal coated insulin formulation on the blood glucose levels, lipid changes and oxidant stress in streptozotocin induced diabetic rats.

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MATERIALS AND METHODS

Preparation of aqueous extract of the roots of *Desmodium gangeticum* the plants after collection from the herbal garden were washed and cleaned. The plant parts of *Desmodium gangeticum* were dried in sunlight. The plant material was taxonomically identified and the voucher specimen A/C no. 8574 was retained in our laboratory for future reference. One kilogram of fresh secondary roots of *Desmodium gangeticum* was used. The sliced, air-dried roots of the plant were milled into fine powder. Soxhlet extraction with 2.5 l of distilled water at room temperature for 24 h with shaking was used to obtain the extracts. The aqueous extracts were filtered and concentrated to dryness under reduced pressure at 30±1°C. The resulting aqueous extract was freeze-dried, finally giving 18.66 g [i. e., 1.866% yield]. Aliquot portions of the crude root aqueous extract residue were weighed and dissolved in distilled water for the experiment.

Animals

Male wistar rats (170-190 g) were procured from King Institute of Preventive Medicine, Chennai, India, and used for the investigation of oral delivery of insulin via *Desmodium gangeticum*. These studies were approved by the Institutional animal ethical committee (IAEC). The animals were housed under standard conditions of temperature (22±3° C) and relative humidity (30-70%) with a 12:12 light: dark cycle. The animals were fed with standard pellet diet (Amrit Feeds Ltd, Bangalore.) and water ad libitum. Animals were fasted for 18–24 h and streptozotocin (STZ; 65 mg/kg) in 0.02 M citrate saline buffer was administered intraperitoneally. A blood glucose level exceeding 200 mg/dl was considered diabetic.

Preparation of *Desmodium gangeticum*-mixed insulin

The combinatorial drug mixture was prepared by mixing 1mg/ml of aqueous extract of *Desmodium gangeticum* root and 40 IU/ml of human Insulin in the ratio of 1:1 and used for further experiments.

Group 1: Normal control

Normal rats (n=6) were injected with equivalent volume of physiological saline and fed a normal diet.

Group 2: Experimental diabetic control

STZ induced diabetic animals (n=6) were monitored for 4-5 days to stabilize the diabetic condition. Blood sugar level was measured in blood samples collected from orbital plexus. The diabetic state was confirmed 48h after injection of streptozotocin. Rats with a fasting blood glucose level above 200mg/dl were included in this group.

Group 3: Experimental diabetes and *Desmodium gangeticum* through intra peritoneal

Diabetes mellitus induced rats (n=6) by streptozotocin injected intra peritoneal, were monitored for 4-5 days. *Desmodium gangeticum* drug was administrated through intra peritoneal to the rats at the dose of 10 mg/kg body weight for a period of 30 days.

Group 4: Experimental diabetes and insulin through intra peritoneal

Diabetes mellitus induced rats (n=6) by streptozotocin injected intra peritoneal, were monitored for 4-5 days. Human Insulin was administrated through intra peritoneal to the rats at the dose of 40µl/kg body weight for a period of 30 days.

Group 5: Experimental diabetes and insulin mixed *Desmodium gangeticum* through intra peritoneal

Diabetes mellitus induced rats (n=6) by streptozotocin injected intra peritoneal, were monitored for 4-5 days. *Desmodium gangeticum* mixed Human Insulin ratio of 1:1 was administrated through intra peritoneal to the rats at the dose of 10mg/kg body weight for a period of 30 days.

Group 6: Experimental diabetes and *Desmodium gangeticum* through oral delivery

Diabetes mellitus induced rats (n=6) by streptozotocin injected intra peritoneal, were monitored for 4-5 days. *Desmodium gangeticum* was administrated through oral to the rats at the dose of 10mg/kg body weight for a period of 30 days.

Group 7: Experimental diabetes and insulin through oral delivery

Diabetes mellitus induced rats (n=6) by streptozotocin injected intra peritoneal, were monitored for 4-5 days. Human Insulin was administrated through oral to the rats at the dose of 40µl/kg body weight for a period of 30 days.

Group 8: Experimental diabetes and insulin mixed *Desmodium gangeticum* through oral delivery

Diabetes mellitus induced rats (n=6) by streptozotocin injected intra peritoneal, were monitored for 4-5 days. *Desmodium gangeticum* mixed Human Insulin (1:1) was administrated through oral to the rats at the dose of 10mg/kg body weight for a period of thirty days.

Group 9: Experimental diabetes mellitus and standard drug

The STZ induced diabetic animals (n=6) in the group 9 were fed with standard drug [Glibenclamide at the dose of 2 mg/kg body weight].
At the end of the experiment, the animals were made to fast for 12h and the rats were stunned by a blow at the back of the neck and killed by decapitation. Blood and tissues were removed aseptically at ice cold conditions.

Acute toxicity studies:

Wister albino rats (150-250g) maintained under standard laboratory condition was used. A total of 6 animals were used which received a single dose (2000mg/kg, body weight.) of *Desmodium gangeticum* drug. Animals were kept overnight fasting prior to drug administration. After the administration of *Desmodium gangeticum* drug, the food was withheld for 3-4 h. Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24h (with special attention during the first 4h) and daily thereafter for a period of 14 days.

Table 1. Effect of Herbal drug on blood glucose, insulin, levels in the serum of diabetic rats

Groups	Blood Glucose (mg/dl)	Insulin (µU/ml)
G1:Normal	82.95 ± 4.05	15.52 ± 1.20
G2:Diabetic control	284.09 ± 4.14	07.85 ± 0.69
G3:*Desmodium gangeticum* intra peritoneal	139.41 ± 4.24	09.11 ± 1.66
G4:Insulin intra peritoneal	117.27 ± 3.36	11.96 ± 1.67
G5:Insulin mixed *Desmodium gangeticum* intra peritoneal	120.65 ±2.28	11.98± 0.56
G6:Insulin Oral	246.18 ±2.26	14.89± 1.58
G7:*Desmodium gangeticum* Oral	158.23 ±2.52	11.75± 1.86
G8:Insulin mixed *Desmodium gangeticum* Oral	93.18± 1.42	12.01 ± 0.97
G9:Glibenclamide	133 ± 4.02	08.02 ± 0.82

Values are mean ± standard error (n=6 rats), Group 2 is compared to groups 1, 3, 4 to 9, Group 8 is compared to group 4 and 9,* Not significant in all cases p<0.004

Table 2. Effect of herbal drug on blood cholesterol, HDL cholesterol, Triglycerides and LDL+VLDL cholesterol levels in the serum of diabetic rats

Groups	Total cholesterol (mg/dl)	HDL cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL+VLDL cholesterol (mg/dl)
G1:Normal	86.81 ± 1.07	50.22 ± 0.93	28.85± 0.98	45.00 ± 0.58
G2:Diabetic control	207.64 ± 3.07	90.29 ± 2.11	85.97 ± 1.88	130.41 ± 1.13
G3:*Desmodium gangeticum* intra peritoneal	163.89 ± 1.23	63.54 ± 1.76	54.63 ± 0.65	76.62 ± 1.88
G4:Insulin intra peritoneal	180.55 ± 0.67	77. 46 ± 1.34	74.88 ± 1.11	95.57 ± 0.98
G5:Insulin mixed *Desmodium gangeticum* intra peritoneal	170.86 ± 1.87	55.25 ± 3.68	65.43± 2.54	63.54 ± 3.23
G6:Insulin Oral	199.23 ± 2.89	85.36 ± 4.68	80.32 ± 1.35*	119.63±1.26
G7:*Desmodium gangeticum* Oral	135.32 ± 3.52	54.87 ± 1.56	52.34 ± 2.56	59.86 ± 2.35
G8:Insulin mixed *Desmodium gangeticum* intra Oral	102.08 ± 1.48	52.81 ± 1.64	43.96 ± 1.30	48.37 ± 2.70
G9:Glibenclamide	159.57 ± 0.86	79.53 ± 0.78	55.26 ± 1.25	80.50 ± 2.87

Values are mean ± standard error (n=6 rats), Group 2 is compared to groups 1, 3, 4 to 9, Group 8 is compared to group 4 and 9,
* Not significant In all cases p<0.004

Table 3. Effect of ORAL INSULIN drug on the activities of antioxidant in Kidney on normal and STZ induced diabetic rats

ANDIOXIDENT	TBARS (mM/100g wet tissue)	Catalase (µM of H_2O_2 consumed /min/mg protein)	SOD (U*/mg protein)		GPx (µg of GSH consumed/min/mg protein)	GSH (nmol/mg)
			Mn SOD	CuZn SOD		
G1:Normal	0.60 ± 0.02	7.60 ± 0.50	9.8 ±1.1	52.5 ±4.1	26.98 ± 1.60	14.55 ± 0.52
G2:Diabetic control	0.86 ± 0.03	4.20 ± 0.48	5.9 ± 0.8	35.2±3.2	16.55 ± 1.55	7.43 ± 0.71
G3:Desmodium gangeticum intra peritoneal	0.67 ± 0.01	5.87 ± 0.53	6.8 ± 1.7	47.3 ±4.2	20.02 ± 1.80	10.70 ± 0.46
G4:Insulin intra peritoneal	0.84 ± 0.02	4.86 ± 0.42	7.1 ± 0.9	39.5 ±3.9	19.76 ± 1.97	9.19 ± 0.91
G5:Insulin mixed Desmodium gangeticum intra peritoneal	0.56 ± 0.02	5.98 ± 0.56	6.3 ± 0.4	44.00 ±2.9	23.68 ±1.56	8.96 ±0.82
G6:Insulin Oral	0.59 ± 0.03	6.07 ± 0.35	6.0 ± 0.2	43.6 ±4.2	22.65 ±1.87	9.14 ±0.98
G7:Desmodium gangeticum Oral	0.74 ± 0.03	4.84 ± 0.27	6.1 ± 0.2	36.4 ±5.1	19.65 ±1.65	8.56 ±0.56
G8:Insulin mixed Desmodium gangeticum intra Oral	0.55 ± 0.01	6.83 ± 0.10	8.9 ± 0.3	49.6±3.5	27.28 ± 1.48	13.03 ± 0.87
G9:Glibenclamide	0.69±0.03	5.87±0.28	6.1 ± 1.0	40.25±2.7	21.35±1.78	9.25±0.25

Values are mean ± standard error (n=6 rats), Group 2 is compared to groups 1, 3, 4 to 9, Group 8 is compared to group 4 and 9 In all cases p<0.004

Table 4. Effect of ORAL INSULIN drug on the activities of antioxidant in liver on normal and STZ induced diabetic rats

ANDIOXIDENT	TBARS (mM/100g wet tissue)	Catalase (µM of H_2O_2 consumed /min/mg protein)	SOD (U*/mg protein)		GPx (µg of GSH consumed/min/mg protein)	GSH (nmol/mg)
			Mn SOD	CuZn SOD		
G1:Normal	2.86 ± 0.18	89.41 ± 7.67	12.15 ± 0.97	89.3 ±3.1	45.80± 1.55	31.36± 2.11
G2:Diabetic control	8.65 ± 0.61	55.90 ± 4.46	06.35 ± 0.61	55.8±3.8	35.61 ± 0.92	20.22 ± 2.08
G3:Desmodium gangeticum intra peritoneal	4.97 ± 0.65	74.57 ± 3.53	07.80 ±0.73	61.7 ±3.2	39.02 ± 1.80	26.70 ± 2.26
G4:Insulin intra peritoneal	5.84 ± 0.54	61.86 ± 5.42	07.11 ±1.9	62.5 ±2.9	39.76 ± 1.97	25.19 ± 1.91
G5:Insulin mixed Desmodium gangeticum intra peritoneal	3.96 ± 0.25	77.98 ± 4.56	08.34 ±1.4	64.3 ±2.9	40.68 ±1.56	27.96 ±2.12
G6:Insulin Oral	4.09 ± 0.26	71.07 ± 4.35	9.87±1.4	65.6 ±3.6	41.65 ±1.87	26.14 ±2.08
G7:Desmodium gangeticum Oral	6.14 ± 0.18	59.84 ± 2.27	06.1 ±1.2	59.7 ±4.1	39.65 ±1.65	21.56 ±1.56
G8:Insulin mixed Desmodium gangeticum intra Oral	2.89 ± 0.21	81.85 ± 1.62	11.40 ± 1.14	77.8±3.5	44.12 ± 1.62	28.76 ± 1.90
G9:Glibenclamide	6.25±0.61	53.87 ± 4.85	10.37 ± 0.88	64.75 ±3.4	38.34 ± 1.18	22.20 ± 1.70

Values are mean ± standard error (n=6 rats), Group 2 is compared to groups 1, 3, 4 to 9, Group 8 is compared to group 4 and 9 In all cases p<0.004

Daily cage side observation included changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart and blood pressure), autonomic (salvation, lacrimation, perspiration, pilorection urinary incontinence and defecation changes (OECD guidelines).

Biochemical analysis

Fasting Glucose level in the plasma was estimated by the method of Wooton (1964). Cholesterol in the serum and tissues was extracted by the method of Folch *et al*.,(1957) and estimated by Abell *et al*., (1957) method. HDL cholesterol was estimated by Libermann-Burchard reaction (Richterich and Colombo, 1981). LDL+ VLDL cholesterol was calculated by Friedwald formula (Friedwald *et al*., 1972). Triglycerides were estimated by the method of Van (1961) with a modification that flurisil was used to remove phospholipid. Free fatty acid in the serum was extracted and estimated by the method of Falholt *et al*., (1973). Thiobarbituric acid reactive substances (TBARS) (Ohkawa *et al*., 1979) was measured as a marker of lipid per-oxidation and endogenous antioxidants, e.g., superoxide dismutase (SOD): Cu Zn SOD and Mn SOD (Marklund, 1982: Geller and Winge, 1983), catalase (Aebi, 1984), & glutathione peroxidase (GPx) (Wendel, 1981) were carried out in a UV- 1601 Shimadzu spectrophotometer. Protein concentration was measured with Folin phenol reagent, following the procedure described by Lowry (Lowry *et al*., 1951). Lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) were determined by the method of King (1965). Blood glucose was measured by the glucose-oxidase method. Insulin was assayed by radio-immunoassay kit method.

Statistical analysis

All the data are reported as mean±SD. The results were statistically analyzed by one way analysis of variance (ANOVA) by SPSS software 12.00, followed by Duncans multiple range test (DMRT). $P<0.05$ was considered to be significant.

RESULTS

Insulin and glucose level

Table 1 shows plasma insulin and glucose levels in normal and diabetic rats. In normal rats, plasma insulin concentrations were elevated when the insulin was given intraperitoneal and as insulin mixed with *Desmodium gangeticum*. A subsequent decline in blood glucose level confirms the activity of insulin delivered in both routes (intraperitoneal and oral). In fact, oral administration of insulin did not show a significant elevation of plasma insulin levels. A similar pattern of results were observed in diabetic rats also. But, to our surprise the elevation of plasma insulin levels were much higher than that of normal animals when insulin-mixed *Desmodium gangeticum* was used as the vehicle to transport insulin. We predict that the above results may be due to insulin secretagouge action of *Desmodium gangeticum* -mixed insulin.

Biochemical parameters

Table 2 shows the level of blood cholesterol, triglycerides, HDL cholesterol and LDL+VLDL cholesterol in normal and experimental rats.

Cholesterol, triglycerides, HDL cholesterol and LDL+VLDL cholesterol were significantly higher in the diabetic control rats as compared to the normal rats. The significant reduction in blood cholesterol, triglycerides, HDL cholesterol and LDL+VLDL cholesterol was shown in the rats fed with aqueous extract of *Desmodium gangeticum* when compared to the glibenclamide treated rats. However both drug showed a significant improvement the level of blood cholesterol, triglycerides, HDL cholesterol and LDL+VLDL cholesterol when compared to the diabetic control rats. Table 3 and 4, indicates the significant increase in the concentration of TBARS and decrease in other antioxidant enzymes in the liver, kidney of diabetic rats compared to the normal control rats. Oral administration insulin mixed *Desmodium gangeticum* (10mg/kg) decreased the level of TBARS and significantly increased the antioxidant enzymes namely SOD, catalase, GPx and GSH in the tissue of diabetic rats in 30 days. For all the biochemical parameters studied, oral treatment with insulin mixed *Desmodium gangeticum* drug at the dosage of 10mg/kg exhibited significant effect in all the biochemical parameters studied.

DISCUSSION

The study undertaken to evaluate the pharmaceutical activity especially hypoglycemic activity of the insulin mixed *Desmodium gangeticum* drug produced desirable results. The oral insulin produced a marked decrease in blood glucose levels in streptozotocin-induced diabetic rats. The drugs (insulin mixed *Desmodium gangeticum)* (1:1) showed significant results at a dosage of 10mg/kg. It has to be further studied if the drug induced pancreatic ß-cells to secrete more insulin or acted by inhibiting glucose absorption through gastrointestinal tract like other herbs (*P. marsupium* and *M. charantia*) (Mukhtar *et al*., 1985). Further, the drug is found to be safe up to a dosage of 2g/kg as it showed no deleterious effects in this dose. Diabetes mellitus is associated with dyslipidemia that is characterized by increased cholesterol levels along with elevated levels of HDL cholesterol, LDL and VLDL cholesterol and triglycerides (Barrett-Connor, 1992). A marked decrease in the levels of these biochemical parameters was observed in diabetic rats treated with insulin mixed *Desmodium gangeticum* combination. Another major change in diabetes is the oxidative stress.

As mentioned earlier, this oxidative stress damages the pancreatic tissue further reducing insulin secretion. Non-protein thiols like glutathione are one of the primary defenses to counteract the oxidative stress. A decreased activity of glutathione, SOD, catalase and GPx was observed in kidney and liver tissues of diabetes induced rats (Ihm *et al*. 1999; McCord *et al*., 1971). The observed decrease may be due to the utilization of non-protein thiols by increased oxygen free radicals produced in hyperglycemia conditions. The treatment with insulin mixed herbal drug under study increased the levels of antioxidant enzymes- SOD, catalase, GPx, GSH to almost their normal blood levels. This drug is found to be more effective than the standard drug glibenclamide. Thus, we conclude from this study that the insulin mixed formulation has a significant antihyperglycemic, antioxidant and cardioprotective activities. This drug proves to have a uncanny role in treating diabetes mellitus. A further study at molecular level on the mechanism of this drug can give us a clear insight on its mode of action that makes it more effective than the standard drug (glibenclamide).

Conflict of Interest

There are no known conflicts of interest associated with this publication and there has been no significant financial support for this work.

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