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Antidiabetic Effect of Polyherbal Formulation in Streptozotocin Induced Diabetic Rats

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ABSTRACT

The objective of the present study is planned to investigate the antidiabetic potential of polyherbal preparation in streptozotocin induced diabetic rats. Diabetes was induced in rats by single intraperitoneal injection of streptozotocin (50 mg/kg b.wt.). After 72 hrs rats with marked hyperglycaemia (fasting blood glucose ≥250 mg/dl) were selected and used for the study. Antidiabetic effect was evaluated by oral administration of polyherbal formulation consisting of (*Helicteres isora, Portulaca oleracea,* and *Caralluma attenuata*) at different doses of 100, 200 and 400 mg/kg b.wt. for 28 days. In streptozotocin diabetic rats, oral administration of polyherbal formulation in dose dependent manner show reduced blood glucose level, which was comparable to that of reference standard glibenclamide (5 mg/kg b.wt.). Significant decrease in body weight also was observed with diabetic control, which was partially restored upon administration of polyherbal formulation. Hence, these findings demonstrate that polyherbal formulation has potential to treat diabetes mellitus and its complications.

Keywords: Polyherbal formulation, streptozotocin, *Helicteres isora, Portulaca oleracea, Caralluma attenuata.*

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INTRODUCTION

Diabetes mellitus is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood sugar[1]. Diabetes is a disease condition distinguished by an inability to control blood glucose level due to the insufficient production of or heightened resistance to the hormone insulin[2]. Defective insulin secretion is the major cause for chronic hyperglycemia resulting in impaired function or serious damage to many of the body's systems, like eyes, kidneys, nerves, heart and blood vessels[3]. Since diabetes mellitus is a multi-factorial disease, the treatment is aimed to not only controlling blood sugar level to normal limit, but also at correcting the associated metabolic defects[4]. Early diabetes symptoms can be very mild and often even unnoticeable. Diabetes is characterized by chronic hyperglycemia, which causes glycation of body proteins that in turn leads to secondary complications affecting eyes, kidney, nerves and arteries. Along with hyperglycemia and abnormalities in serum lipid, diabetes is associated with micro and macro vascular complications the major causes of morbidity and death[5]. The cells of the liver, which are known as hepatocytes carry out many biochemical activities. Some of these biochemical activities carried include excretion of bile, carbohydrate metabolism, protein metabolism, synthesis of blood clotting factors, storage of iron and some vitamins, detoxification and lipid metabolism. It is therefore very obvious that any disease condition or adverse physiological conditions, which affect the hepatocytes, will cause concerted and tremendous metabolic derangement. In such conditions also, there will be an increase in the serum activities of the mitochondrial-bound liver enzymes since hepatocytic damage causes their release into the serum. It is therefore very pertinent to ascertain the effect of any ingestible food or drug on the serum activities of the liver enzymes so as to ensure the hepato-protectiveness of such food or drug. This can be achieved through liver function tests, which include estimation of plasma protein, aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT) and bilirubin[6].

Helicteres isora (Sterculiaceae) is a woody shrub or small tree 2-3 m tall, young shoots tellate hairy, bark thin and strong. Laves are simple, bifarious, oblong-obovate or roundish, cordate, scabrous, clothd with stellate hairs on both surfaces. Fruit of *H. isora* is short stalked with rough and twisted brown follicles. Each follicle contains 15-28 brown cubical seeds. The pericarp bears stellate lignified trichomes and has a number of large lysogenous mucilage cavities. The mesocarp and endocarp consist of fibres, the latter running tangentially. The testa has an outer

layer with rectangular thin walled cells, followed by another layer with lignified palisade cells and rows of brown pigment cells[7]. *Portulaca oleracea* L., a member of family Portulacaceae, is a warm climate, annual, green herb, with branched and succulent stems which are decumbent near the base and ascending near the top to a height of 15-30 cm. The plant is fleshy, stout and succulent (water content of over 90%), with obovate to spathulate, obtuse opposite leaves tapering towards the base. The flowers are small, yellow, and sessile in clusters of 3-5 on the forks and tips of the branches, opening in the morning only. The fruit is oblong and transversely dehiscent. The seeds are orbicular and 0.5 mm in diameter[8,9]. *Caralluma attenuata* Weight, also known as *Caralluma fimbriata*,, belongs to the family Asclepiadaceae. *C. attenuata* is known as 'Dugdha' in hindi which is a thick, succulent perennial herb growing wild in dry hill slope regions of India. It is eaten raw as a cure for diabetes (personal information from users) and the juice of the plant along with black pepper is suggested in the treatment of migraine[10].

MATERIALS AND METHODS:

Collection of plant materials

Helicteres isora, seeds of *Portulaca oleracea*, and whole plant of *Caralluma attenuata* were collected from the Jadaav Nursery, Udaipur and authenticated by Botanist of Rajasthan College of Agriculture, Udaipur, Rajasthan. Plants were preserved in herbarium of the institution

Chemicals

Streptozotocin was obtained from Sisco Pharmaceuticals Limited, Mumbai. Glibenclamide was obtained as a gratis sample from Zydus Cadila, Ahmedabad. All the other chemicals used for the present study were of analytical grade. All the diagnostic kits were procured from Lab-care diagnostics Ltd., India.

Preparation of polyherbal formulation

The plants materials were air dried under shade at 25±2°C and then pulverized by a mechanical grinder and sieved through 120 meshes separately. Each plants material was defatted with petroleum ether to remove all the fatty substances. The defatted material was further extracted with 50% aqueous alcohol. Individual extracts were concentrated to dry mass using rotary evaporator under controlled temperature (25-40°C). Dried powder of extract of *Helicteres isora, Portulaca oleracea and Caralluma attenuata were* mixed in the same ratio to form the polyherbal formulation.

Experimental animals

Healthy adult wistar albino rats weighing 150-200gm of either sex were obtained from the Geetanjali Medical College and Hospital, Udaipur. They were kept in the departmental animal house at temperature 26±2°C and relative humidity 44–56 %, light and dark cycles of 10 and 14 hrs respectively for one week before and during the experiments. Animals were provided with standard rodent pellet diet and the food was withdrawn 18-24 hrs before the experiment though water was allowed ad libitum. All studies were performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by the animal care committee, CPCSEA, India.

Preparation of doses

Suspension of finally powdered polyherbal formulation were prepared in 1% (w/v) sodium carboxymethyl cellulose (CMC) and administered orally through orogastric tubes at the following doses of 100, 200 and 400 mg/kg. The control animals received vehicle only.

Acute toxicity studies

The present study of the polyherbal formulation was conducted according to the Organization for Economic Cooperation and Development (OECD) revised fixed dose procedure for acute toxicity testing (OECD guideline 420, 2001). Healthy wistar albino rats of either sex (150-200gm) were administered a limit dose of 2000 and 5000 mg/kg of the polyherbal formulation and animals were observed for mortality and clinical signs for the first hour, then hourly for 3 hrs and finally periodically until 48 hrs. All of the experimental animals were maintained under close observation for 14 days, and the number of rats that died within the study period was noted. The LD50 was predicted to be above 2000 or 5000 mg/kg, if three or more rats survived[11]. No abnormal behavioral, neurological, autonomic changes and death was observed till the end of the 14 day. Hence, the median lethal dose (LD50) of the polyherbal formulation was then greater than 2000 mg/kg. Polyherbal formulation was found to be safe up to the dose of 2000 mg/kg. Therefore dose of 100, 200 and 400 mg/kg b.wt. were selected for the experiment.

Induction of diabetes

Rats were fasted overnight before being injected with streptozotocin (STZ) at a dose of 50 mg/kg. Diabetes was induced in rats by single intraperitoneal injection of STZ dissolved in freshly prepared 0.01M citrate buffer, pH 4.5[12]. After 72 hrs rats with marked hyperglycemia (fasting blood glucose ≥250 mg/dl) were selected and used for the study.

Experimental design

Rats were divided into six groups, with six animals in each group.

Group I : Normal Control; rats were received only 1% CMC (1ml/kg/day, p.o.)

Group II : Diabetic Control; rats were received only 1% CMC (1ml/kg/day, p.o.)

Group III : Diabetic rats treated with glibenclamide at a dose of 5 mg/kg/day, p.o.

Group IV : Diabetic rats treated with polyherbal formulation at a dose of 100 mg/kg/day, p.o.

Group V : Diabetic rats treated with polyherbal formulation at a dose of 200 mg/kg/day, p.o.

Group VI : Diabetic rats treated with polyherbal formulation at a dose of 400 mg/kg/day, p.o.

Antidiabetic activity of polyherbal formulation was evaluated by estimation of blood glucose levels and body weight measurement on the day 0, day 7, day 14, day 21 and day 28 of the study by using commercially available kit (Accu-Chek Active Test Meter).

Statistical analysis:

The results were expressed as mean±SEM and statistical difference was evaluated by using one-way analysis of varience (ANOVA) followed by Dunnett's test and compare with respective control group. A difference in the mean P value $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION:

On the basis of acute toxicity study there is no abnormal behavioural, neurological, autonomic changes and death was observed till the end of the 14 day. Hence, the median lethal dose (LD50) of the polyherbal formulation was then greater than 2000 mg/kg. Polyherbal formulation was found to be safe up to the dose of 2000 mg/kg. Therefore dose of 100, 200 and 400 mg/kg b.wt. of polyherbal formulation were selected for all the experiments. On repeated administration of polyherbal formulation daily up to 28 days exhibited significant antidiabetic activity in streptozotocin induced diabetic rats. The blood glucose level was significantly elevated in diabetic rats as compared to normal rats. As shown in Table 1, oral administration of polyherbal formulation in dose dependent manner show decrease in the blood glucose as compared to diabetic control rats. At the end of 28 days of the treatment, there was a decrease of blood glucose levels with the glibenclamide and polyherbal formulation (100, 200 and 400 mg/kg b.wt.) respectively when compared with diabetic control group. As shown in Table 2, STZ diabetic rats showed significant reduction in body weight as compared to normal group. There were the significant changes in body weight at the end of 28 days treatment. The body weight of normal rats, treated with polyherbal formulation and standard drug treated group, increased significantly, whereas body weight of diabetic control group rats decreased.

Table 1: Antidiabetic effect of polyherbal formulation on blood glucose levels in streptozotocin induced diabetic rats

Group	Treatment	Blood Glucose Levels (mg/dl)				
		Day 0	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Group I	Normal Control	86.18 ± 2.26	81.31 ± 0.33	83.00 ± 0.81	80.21 ±2.10	82.26 ± 1.19
Group II	Diabetic Control	261.27 ± 1.73	282.51 ± 3.21	298.64 ± 3.10	319.25 ± 1.71	343.12 ± 0.38
Group III	Diabetic rats treated with glibenclamide in a dose of 5 mg/kg b.wt.	264.50 ± 0.70	221.33 ± 1.28	162.76± 2.23*	117.30 ± 1.32**	103.44 ± 1.20**
Group IV	Diabetic rats treated with polyherbal formulation at a dose of 100 mg/kg b.wt.	265.15 ± 0.89	246.62 ± 1.4	181.77± 1.32*	132.58 ± 0.66*	118.47 ± 1.33**
Group V	Diabetic rats treated with polyherbal formulation at a dose of 200 mg/kg b.wt.	266.06 ± 0.17	243.54 ± 1.30	178.37 ± 1.06	126.2 ± 1.44*	113.21 ± 1.77**
Group VI	Diabetic rats treated with polyherbal formulation at a dose of 400 mg/kg b.wt.	264.13 ± 0.7	232.39 ± 0.60*	171.67 ± 2.52*	120.17 ± 1.70*	108.44 ± 1.29**

***<0.05, **P<0.01 Values are mean±SEM, n=6, when compared with diabetic control by using one way ANOVA followed by Dunnett's multiple comparison test.**

Table 2: Effect of polyherbal formulation on body weight in streptozotocin induced diabetic rats

Group	**Treatment**	**Change In Body Weight (gm)**	
		Initial	**Final**
Group I	Normal Control	164.40±1.87	189.67±1.39
Group II	Diabetic Control	162.00±2.16	110.33±0.70
Group III	Diabetic rats treated with glibenclamide in a dose of 5 mg/kg b.wt.	160.13±1.14	180.22±1.52*
Group IV	Diabetic rats treated with polyherbal formulation at a dose of 100 mg/kg b.wt.	160.27±0.22	176.17±0.71*
Group V	Diabetic rats treated with polyherbal formulation at a dose of 200 mg/kg b.wt.	158.57±1.20	178.17±2.10*
Group VI	Diabetic rats treated with polyherbal formulation at a dose of 400 mg/kg b.wt.	159.33±1.67	179.50±1.15*

***<0.05 Values are mean±SEM, n=6, when compared with diabetic control by using one way ANOVA followed by Dunnett's multiple comparison test**

Diabetes mellitus is probably the fastest growing metabolic disease in the world and as knowledge of the heterogeneous nature of the disease increases so does the need for more challenging and appropriate therapies. Traditional plant remedies have been used for centuries in the treatment of diabetes[13-15], but only a few have been scientifically evaluated. Therefore, we have investigated the antidiabetic effect of polyherbal formulation in STZ-induced diabetic rats. Polyherbal formulation showed a dose dependent effect on fasting blood glucose at 100, 200 and 400 mg/kg in diabetic rats. So, detailed studies were carried out with the graded doses of polyherbal formulation 100, 200 and 400 mg/kg b.wt. Induction of diabetes with STZ is associated with the characteristic loss of body weight, which is due to increased muscle wasting[16], and due to loss of tissue proteins[17]. Diabetic rats treated with the polyherbal formulation showed an increase in body weight when compared to the untreated diabetic rats which may be due to its protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis and may also be due to the improvement in glycemic control. STZ is a β-cytotoxin, induces 'chemical diabetes' in a wide variety of animal species including rat by selectively damaging the insulin-secreting β-cells of the pancreas. Intraperitoneal injection of STZ produces fragmentation of DNA of β-cells of pancreas which stimulates poly (ADP-ribose) and depletes NAD ultimately leading to destruction of β-cells and it is evidenced by clinical symptoms of hyperglycemia and hypoinsulinaemia[18,19]. STZ-diabetes in type 1 diabetics produce a significant increase in glucose levels associated with decrease in insulin levels[20]. Treatment with polyherbal formulation showed significant decrease in fasting blood glucose levels which was near to healthy control. The antidiabetic plant extracts may involve one or more compounds which decrease blood glucose levels suggesting that the natural constituents could act synergistically to induce a hypoglycemic effect as described by Marles and Fransworth[21–23].

In the present study, antidiabetic effect of polyherbal formulation was evaluated in streptozotocin induced diabetic rats. 28 days treatment with polyherbal formulation (100, 200 and 400 mg/kg b.wt.) and glibenclamide lowered elevated blood glucose level, which was reported high in diabetic control animals. Maximum reduction in the blood glucose level noted with polyherbal formulation 400 mg/kg. Thus polyherbal formulation proved antidiabetic activity in diabetic rats, which was comparable to standard drug used i.e. glibenclamide.

CONCLUSION:

On the basis of our study, we conclude that the polyherbal formulation have beneficial effects on blood glucose levels. Since streptozotocin effectively destroys pancreatic β-cells and causes

persistent hyperglycemia, the mechanism of action of polyherbal formulation might involve actions other than pancreatic β-cells insulin release or secretion. The antidiabetic effect of the polyherbal formulation could be due to increased utilization of glucose by peripheral tissues, improved sensitivity of target tissues for insulin or it may be due to improved metabolic regulation of glucose.

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