

## **Study of hypoglycemic effects and antioxidant potential of polyherbal formulation in rats**

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### **Abstract**

Present study was designed to evaluate the hypoglycemic effect and antioxidant potential of polyherbal formulation in streptozotocin (STZ) induced diabetic rats. Five different groups were made including 10 rats in each group. An aqueous suspension of herbal mixture containing 5 ingredients of equal amounts– leaves of *Azadirachta indica* (Margosa), leaves of *Gymnema sylvestre* (Gurmar), fruits of *Momordica charantia* (Bitter gourd), seeds of *Syzigium cumini* (Black plum) and seeds of *Trigonella foenum* (Fenugreek) was administered orally once a day at a dose of 500 mg/kg body weight for a period of 4 weeks. In these groups lipid peroxidation (LPO), reduced glutathione (GSH), glutathione *S*-transferase (GST), proteins, urea & cholesterol was estimated in liver and kidney as bioindicators of oxidative stress. The antidiabetic effect of herbal formulation was compared with antidiabetic drug 'Glibenclamide'. Our observations showed that herbal treatment restored the elevated blood glucose level to normal range in diabetic rats. The formulation also prevented the diabetes-induced decrease in the body weight of rats. Results showed an increased level of GSH and GST and significant decline in LPO with herbal treatment in the tissues of normal and diabetic animals, while no significant change was observed in GSH & GST level with glibenclamide treated diabetic group. Level of serum urea increased to almost two fold in diabetic rats as compared with the values observed in the control group. The urea level restored to normal range in diabetic rats after receiving the herbal mixture. Serum cholesterol was significantly lower in diabetic rats as compared to control. The combination of these five herbal drugs appeared more effective in low dose as an antidiabetic and antioxidant agent than the individual herbal drug, when used alone. This herbal formulation also appeared to be devoid of any significant toxic effect in animals.

**Keywords.** *Azadirachta indica*, *Gymnema sylvestre*, *Momordica charantia*, *Syzigium cumini*, *Trigonella foenum*, Diabetes mellitus, antioxidants

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### **Introduction**

Polyherbal drugs are considered to be more effective for the management of diabetes with Ayurvedic medicines. Diabetes mellitus represents a syndrome of metabolic disorder and complex pathophysiological interactions between hyperglycemia, insulin resistance and dysfunction of the  $\beta$  cells of pancreas. The available antidiabetic measures such as oral hypoglycemic agents and insulin, do not effectively control the delayed diabetic complications like nephropathy, neuropathy, retinopathy and cardiovascular diseases [1]. Streptozotocin (STZ), a  $\beta$ -cytotoxin, induces 'chemical diabetes' in a wide variety of animal species including rat by selectively damaging the insulin-secreting  $\beta$ -cells of the pancreas. Intraperito-

neal injection of STZ produces fragmentation of DNA of  $\beta$ -cells of pancreas which stimulates poly (ADP-ribose) and depletes NAD ultimately leading to destruction of  $\beta$ -cells and it is evidenced by clinical symptoms of hyperglycemia and hypoinsulinaemia [2,3]. Oxidative tissue injury has been suggested to play an important role in the pathogenesis of diabetes and associated complications [4]. However, much less information is available on the mechanism of action of plant products and their hypoglycemic potential when used in combination. Herbal products are generally considered to be least toxic and free from side effects when compared with their synthetic counterparts [5]. Studies have been undertaken in the past to assess herbal formulations containing several plant products for their cumulative hypoglycemic potential and their metabolic effects.

The serum glucose, lipid and cholesterol values for the rats are in agreement with those expected for streptozotocin diabetic rats [6]. In India there is no report of this herbal formulation when used in diabetic rats in low dose, therefore the present study was planned to assess the antioxidant potential and hypoglycemic effect of the herbal formulation in diabetic rats. Antioxidant potential of the herbal formulation was evaluated to understand its possible beneficial role in the pathogenesis of diabetes and associated tissue-damaging complications.

The results of the study can serve as a step toward the development of an antihyperlipidemic herbal therapy for diabetes.

## **Study design**

### **Preparation of polyherbal formulation**

Fresh fruits of *M. cherantia* (Karela), leaves of *A. indica* (Neem) and seeds of *S. cumini* (Jamun) were collected, cleaned and dried under shade. Dried leaves of *G. sylvestre* (Gurmar) and seeds of *T. foenum* (Methi) were purchased. The plants were botanically authenticated. Herbal ingredients were powdered separately and stored. Herbal preparation was made fresh before use by mixing the five ingredients in equal ratio (w/w) and homogenizing in water with the help of a pestle and mortar.

### **Experimental design**

Diabetic rats having fasting blood glucose in the range of >200 mg/dl were selected for the study. This work has been approved by ethical committee of C.S.M.M.U. Rats of either sex were divided into the following five equally sized groups (10 rats) for 4 weeks of study

Group I. Control: Without any treatment

Group II. Diabetic: Diabetic rats without any treatment

Group III. Herbal treated-control: Non-diabetic rats given herbal treatment

Group IV. Herbal treated-diabetic: Diabetic rats given herbal treatment

Group V. Diabetic treated Glibenclamide: Diabetic rats given Glibenclamide treatment

### **Induction of diabetes**

Albino rats (100-150 g) maintained under standard laboratory conditions, were fasted overnight prior to STZ administration (Sigma, USA). Rats were injected with STZ at a dose of 70 mg/kg of body weight (BW) (in cold 0.9% NaCl) via intraperitoneal (IP) route. The induction of diabetes was confirmed by determination of high fasting blood glucose level with polydipsia and polyuria on the fifth day of STZ administration. Control animals were treated with normal saline. Rats with fasting blood glucose level >200 mg/dl were selected for experimentation. All the animals were sacrificed after recording the final

body weight. Blood glucose was again estimated at the end of herbal treatment before sacrificing the rats.

### **Sample collection**

At the end of fourth week blood was collected by heart puncture in an EDTA vial (2 mg EDTA/ml blood) for glucose estimation and plain vial for blood urea and cholesterol estimation. Immediately afterwards, the rats were sacrificed by decapitation and liver and kidney were taken out by dissection. Tissues were washed with ice-cold KCl-Tris buffer (1.15% KCl buffered with Tris-HCl, 0.01M, pH 7.4) and homogenized with the help of Potter-Elvehjem homogenizer in ice-cold KCl-Tris buffer containing 0.001M EDTA for GSH estimation and without EDTA for estimation of lipid peroxide and GST.

### **Determination of blood glucose [7]**

Fasting blood glucose level of animals was estimated by glucometer (Accuchek Instant, Germany) using a drop of blood taken from the tail vein. At the end of herbal treatment blood collected in EDTA vial and blood glucose was determined spectrophotometrically by glucose oxidase method using commercially available kit (Accurex Biochemical Pvt. Ltd. India).

### **Determination of reduced glutathione and lipid peroxidation [8]**

The level of GSH in the homogenates of liver and kidney was estimated as protein free sulfhydryl content using Ellman's reagent Malonyldialdehyde (MDA), a product of unsaturated fatty acid peroxidation, was estimated in tissue homogenate as a measure of thiobarbituric acid reactive substances (TBARS) formed from LPO.

### **Estimation of glutathione S-transferase [9]**

The activity of glutathione S-transferase (GST) in 2000g x 10 min supernatant of tissues was determined using 1-chloro 2, 4- dinitrobenzine (CDNB) as the substrate

### **Determination of urea and cholesterol [10]**

Blood urea and serum cholesterol were estimated spectrophotometrically using commercially available kits (Accurex Biochemical Pvt. Ltd. India) by enzymatic methods

### **Protein content [11]**

Protein contents in liver and kidney were estimated by using bovine serum albumin as the standard.

### **Statistical analysis**

The data was statistically evaluated and the significance of treatment in different group of rats was calculated using student's t-test. All the results were expressed as mean +SE.

## **Results**

We evaluate the antidiabetic and antioxidative potential of

polyherbal formulation containing leaves of *Azadirachta indica* & *Gymnema sylvestre*, fruits of *Momordica charantia* and seeds of *Syzygium cumini* & *Trigonella foenum* (Table I). Each of these plants has been reported to have antidiabetic and antioxidative activities. Our findings showed 15% increase in the body weight of control (normal) rats in 28 days. The weight of diabetic rats reduced significantly (12%) but treatment with herbal mixture restored the weight of diabetic animals. Remarkably, the body weight gain (25%) in the control (normal) group of rats receiving herbal mixture was more than that observed in the control animals, while no gain in body weight was observed in glibenclamide treated group (Table II).

An increase of 2-3 folds in the fasting blood glucose level was observed 5 days after STZ administration, which persisted for another 4 weeks of the study period. Herbal and glibenclamide treated groups showed returned to normal value of blood glucose. Herbal treatment did not elicit any significant change in the glucose level in the control group of rats (Table III). Results of different biomarkers were shown in Table IV. The levels of TBARS measured as MDA, resulting from lipid peroxidation in the liver and kidney of control (normal) rats were 229, 230. There was a significant increase of 56 and 80 % in LPO in the liver and kidney respectively of diabetic animals as compared to control rats. Herbal treatment significantly reduced the

level of TBARS to below control values in the liver and kidney of normal animals. Enhanced LPO observed in the tissues of diabetic rats was restored to control or below control values following treatment with the herbal formulation and glibenclamide.

The basal level of GSH in the liver and kidney of control rats were 9.62, and 4.10  $\mu\text{mole/g}$  tissue respectively. The level of GSH in liver and kidney of diabetic animals decreased by 20, 48% respectively, when compared with their respective control values. Herbal treatment of diabetic animals restored the GSH level to control or below the control values in the tissues investigated while no increase in GSH level was observed in glibenclamide treated group. Herbal treatment also raised the GSH content in the kidney of control rats without affecting the hepatic level. STZ induced diabetes caused a mild decrease in the hepatic and renal GST level. Chronic treatment with the herbal formulation resulted in significant rise of GST levels in all the tissues of diabetic rats. Herbal treatment also increased the GST levels in liver (40%), and kidney (38%) as compared to respective values in control rats. Total protein contents of liver and kidney were significantly decreased by 21% and 17 % respectively in diabetic rats as compared to control animals. Herbal and glibenclamide treatment increased the proteins of liver and kidney to control values.

**Table 1.** Composition of ingredient(s) present in polyherbal formulation

Botanical name	Common name	Family	Part used	Ingredients Used (mg)
<i>Azadirachta indica</i>	Margosa(Neem)	Meliaceae,	leaves	100
<i>Gymnema sylvestre</i>	Gudmar	Asclepiadaceae.	leaves	100
<i>Momordica charantia</i>	Bitter gourd	Cucurbitaceae	fruits	100
<i>Syzygium cumini</i>	Black plum	Myrtaceae	seeds	100
<i>Trigonella foenum</i>	Fenugreek	Fabaceae)	seeds	100

**Table 2.** Effects of herbal formulation on body weight in different groups

S. No.	Body Weight	Groups				
		Control	Diabetic	herbal treated control	herbal treated diabetic	Glibenclamide treated diabetic
1	Before herbal treatment	129 + 5.9	135 + 4.5	117 + 5.2	131 + 5.1	128 + 5.6
2	After herbal treatment	147 + 7.1	118 + 3.9*	146 + 8.3*	145 + 7.3	135 + 4.6

\*  $p < 0.01$  as compared with initial weights of the same group of the rats

**Table 3.** Effect of herbal formulation on blood glucose in normal (control) and diabetic rats.

S. No.	Fasting blood glucose (mg/100ml)	Groups				
		Control	Diabetic	herbal treated control	herbal treated diabetic	Glibenclamide treated diabetic
1	Before herbal treatment	73 + 3.4	211 + 17.7	78 + 2.3	227 + 23.6	237 + 16.3
2	After herbal treatment	80 + 4.4	182 + 25.1	81 + 3.5	95 + 7.1*	98 + 8.7*

**Table 4.** Effect of biochemical parameters in different groups

S. No.	Biochemical parameters	Tissues	Groups				
			Control	Diabetic	herbal treated control	herbal treated diabetic	Glibenclamide treated diabetic
1	Lipid peroxidation	Liver	229±9.0	359±15.6*	147±3.5*	192±7.4**	184 ±6.5**
		Kidney	230±9.7	415±12.6*	147±11.3*	225±4.7**	211 ±7.1**
2	GSH	Liver	9.62 + 0.26	7.62 + 0.19***	9.14 + 0.15	8.66 + 0.24**	9.12 ±.22
		Kidney	4.10 + 0.20	2.10 + 0.13***	6.37 + 0.22***	4.80 + 0.88**	5.2 ±.20
3	GST	Liver	367 + 16	342 + 19	514 + 14***	505 + 30****	390 ± 16
		Kidney	50.3 + 0.9	41.7 + 0.9***	69.9 + 4.9***	82.7 + 5.5****	55.2 ± 1.6
4	Protein	Liver	208 + 2.9	163 + 7.2*	273 + 6.4*	242 + 8.0**	260 + 7.2**
		Kidney	210 + 5.9	173 + 5.3*	213 + 6.3	200 + 4.6**	240 + 5.6**

\*  $P < 0.01$  as compared with control rats\*\*  $P < 0.01$ , as compared with diabetic rats.\*\*\*  $P < 0.001$  as compared with control rats\*\*\*\*  $P < 0.001$ , as compared with diabetic rats.**Table 5.** Effect of herbal formulation on serum urea and cholesterol levels in different groups

S. No.	Parameters	Groups				
		Control	Diabetic	herbal treated control	herbal treated diabetic	Diabetic treated Glibenclamide
1.	Serum urea (mg %)	31.4 + 2.4	67.2 + 6.5	35.0 + 3.3	32.0 + 3.0****	33.2 + 2.8****
2.	Serum Cholesterol (mg %)	66.4 + 5.4	69.9 + 9.7	68.3 + 7.9	41.0 + 4.7**	56 + 4.6

\*\*  $P < 0.001$ , as compared with diabetic rats.\*\*\*\*  $P < 0.01$ , as compared with diabetic rats.

## Discussion

Diabetes mellitus (DM) is characterized by hyperglycemia associated with impairment in insulin secretion and/or insulin action as well as alteration in intermediary metabolism of carbohydrates, proteins and lipids. In future annual incidence rate of DM will increase worldwide, especially in India. It has been proposed that approximately 57 million Indians will be affected by DM by the year 2025 [12].

The present study was conducted to determine the antidiabetic as well as antioxidative effects of a polyherbal formulation, in streptozotocin induced diabetic rats. This study selected the streptozotocin induced diabetic rat as an experimental model because it is commonly used model to study the effects of antidiabetogenic agents [13]. In present study the doses of five herbal drugs used in the herbal formulation were significantly less than the individual doses used by other workers.

Previous studies [14, 15] have reported antidiabetic effect of the extracts of these plants portions at remarkably higher doses in experimental animals. Our results showed that oral treatment of diabetic rats with a crude herbal formulation (500mg powder /kg) containing equal amounts of *A. indica*, *G. sylvestre*, *M. charantia*, *S. cumini*, and *T. foenum* restored the elevated blood glucose levels to normal range indicating marked antidiabetic activity of the formulation. In another study [16] no signifi-

cant changes were noticed in blood glucose, serum lipid levels and kidney parameters in normal rats treated with 'Glyoherb' suspension alone. Daily oral administration of 'Glyoherb' suspension in 200, 400 and 600 mg/kg doses for 28 days produced a dose-dependant decrease in blood glucose levels. It also produced a significant decrease in elevated serum triglyceride, cholesterol, VLDL, LDL, serum urea, creatinine and in antioxidant parameters in a dose dependant manner.

Tushar [17] observed that treatment with 'Diashis' in STZ-induced diabetic rats resulted in a significant ( $P < 0.01$ ) recovery in the activities of hepatic hexokinase, glucose-6-phosphate dehydrogenase, and glucose-6-phosphatase along with correction in the levels of fasting blood glucose, glycated hemoglobin, and liver and skeletal muscle glycogen. Decrease in the activities of hexokinase and glucose-6-phosphate dehydrogenase in diabetes may be due to diabetes induced-oxidative injury as free radicals are scavengers of structural and functional protein including enzyme in cells [18]. Significant decrease in antioxidant enzymes like catalase (CAT), peroxidase (Px), and glutathione-S-transferase (GST) [19]. in diabetes may be due to low levels of insulin or due to high levels of advanced glycosylated end products [20,21].

A decrease in GST levels in liver and kidney has been reported in diabetic rats[22]. In our study, mild decreased activity of GST in liver and significant decreased activity in kidney tissue was found. In the present study the herbal

formulation significantly increased GST level in all tissues suggesting insulin like role of these herbal drugs. Reduced GST activity in diabetic rats could be due to decreased availability of GSH and /or loss of catalytic efficiency of the enzyme resulting from oxidation of sulfhydryl and other group by ROS [23].

Our findings are in agreement with Patel SS [24] that polyherbal formulation (Dihar) produced significant decrease in serum creatinine and urea levels in diabetic rats. Administration of Dihar to diabetic rats significantly reduced the levels of lipid peroxidation and increased the activities of antioxidant enzymes. They also observed significant decrease in reduced glutathione, superoxide dismutase, catalase levels and increase in thiobarbituric acid reactive species levels in the liver of STZ-induced diabetic rats.

Treatment with Garlip and tolbutamide resulted in a significant reduction of blood glucose and increase in plasma insulin. Garlip also resulted in a significant decrease in tissue lipids and lipid peroxide formation. The decreased lipid peroxides and tissue lipids clearly showed the anti-hyperlipidemic and antiperoxidative effect of Garlip apart from its antidiabetic effect [25]. Treatment with Diasulin and Glibenclamide resulted in a significant reduction of blood glucose and increase in plasma insulin. Diasulin also resulted in a significant decrease in tissue lipids and lipid peroxide formation[26].

Chattopadhyay [27] showed complete reversal of elevated blood glucose level and relatively greater efficacy of *A. indica*, after 2 h an oral dose of ethanolic extracts of *A. indica* and *G. sylvestre* leaves to STZ- diabetic rats.

The hypoglycemic activity of the herbal formulation used in the present study may be due to presence of several compounds having hypoglycemic activity and their multiple sites of action. This polyherbal formulation has no general toxic effect as body weights remain similar to those in the control. In oxidative injury lipid per oxidation increased as an indicator of tissue injury [28]. Decreased GSH levels in liver and kidney tissue of diabetic rats recorded in the current study is in agreement with previous reports[29]. Based on the findings of the present study, it may be concluded that an increase in oxidative stress occurs in diabetes as an increased level of LPO and decreased level of antioxidants (GSH & GST).

The herbal formulation demonstrated pharmacological activity in reversing the altered parameters due to diabetes mellitus. Combination of five herbal drugs in low doses appeared to be more effective as antidiabetic and antioxidant agents than the individual herbal drug when used alone. The herbal formulation reinforces the constitutive cellular defense system by mimicking the endogenous

LPO and enhancing the levels of antioxidant tissue defenses, GSH and GST.

Halim [30] reported a significant reduction in serum lipids when water extract of dried powder of root and leaves (200 mg/kg body wt) of *A. augusta* and *A. indica* respectively was administered orally to alloxan diabetic rats once a day for 8 weeks.. Aqueous extract also decreased the formation of lipid peroxides estimated as thiobarbituric acid reactive substance, (TBARS), and increased antioxidants (superoxide dismutase, catalase, and glutathione peroxidase and glutathione transferase) in erythrocytes. There was reduction in LPO as TBARS in liver and, kidney. It also prevented decrease in body weight. They concluded that *Abroma Augusta* roots and *A. indica* leaves when given together as water extract had hypoglycaemic action and had better effect than given alone.

This formulation did not exert any toxic effects in STZ-induced impaired kidney and liver functions. It was rather found to be improving kidney and liver functions. It possesses potential antioxidant activity as it decreases lipid peroxidation and enhances antioxidant status in diabetic rats. The antidiabetic activity of formulation may be attributed to its antioxidant properties also. Thus, data from the present study indicate antidiabetic and antioxidant properties of a poly herbal formulation against STZ-induced diabetes in albino rats.

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