ORIGINAL ARTICLE

Antidiabetic activity of a polyherbal formulation (Karnim Plus)

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Karnim Plus, an herbal formulation marketed for diabetes, was investigated for its glucose tolerance, hypoglycaemic, and antidiabetic effects in rats. The glucose tolerance test was studied at 400 mg/kg. Hypoglycemic studies were carried out in normal rats at two dose levels, 200 mg/kg and 400 mg/kg. Antidiabetic effect was analyzed in alloxan-induced diabetic rats at two dose levels, 200 mg/kg and 400 mg/kg. Glibenclamide, 4 mg/kg, was used as the standard drug. The biochemical parameters such as glucose, urea, creatinine, serum cholesterol, and serum triglyceride were also assessed in experimental animals. The product showed its effectiveness in oral glucose tolerance test and antidiabetic activity, but it did not produce hypoglycemic effect. Treatment of diabetic rats with the product restored the elevated biochemical parameters significantly. The present study supports the use of this product as an antidiabetic.

Key words: Karnim Plus, antidiabetic activity, *Momordica charantia*, *Azadirachta indica*, *Picrorrhiza kurroa*, *Ocimum sanctum*, *Zingiber officinale*

INTRODUCTION

Diabetes mellitus (DM) is the name given to a group of disorders characterized by chronic hyperglycemia, polyurea, polydipsia, polyphagia, emaciation, and weakness due to disturbance in carbohydrate, fat, and protein metabolism associated with absolute or relative deficiency in insulin secretion and/or insulin action.[1]

Oral hypoglycemic agents are useful in the treatment of DM but their use is restricted by the pharmacokinetic properties, secondary failure rates, and accompanying side effects, and the World Health Organization expert committee on diabetes has listed as one of its recommendations that traditional methods of treatment for diabetes should be further investigated.[2] DM was known to ancient Indian physicians as 'madumeha'. Many herbal products including several metals and minerals have been described for the care of DM in the ancient literature. Ayurveda has been the first to give an elaborate description of this disease, its clinical features and the patterns, and its management by herbal or herbomineral drugs. Plant drugs are frequently considered to be less toxic and free from side effects than synthetic ones.[3] Keeping the above information in view, Karnim Plus (Syn. Herbajules Diamelon Plus in Malaysia), an indigenous polyherbal formulation was developed by the Unijules Life Sciences Ltd, Nagpur, India, containing the extracts of *Momordica charantia, Azadirachta indica, Picrorrhiza kurroa, Ocimum sanctum, and Zingiber officinale*. This formulation has been selected by us to evaluate its antidiabetic activity.

MATERIALS AND METHODS

Drugs, Chemicals, and Reagents

The polyherbal formulation Karnim Plus was procured from Unijules Life Sciences Ltd, Nagpur, India. Alloxan monohydrate (Spectrochem Pvt. Ltd., Bombay), glibenclamide (Ozone International, Mumbai), and accu chek active glucostrips (Roche Diagnostic India Pvt. Ltd, Mumbai) were provided by the central storehouse of B. R. Nahata College of Pharmacy, Mandsaur.

Procurement and Selection of Animals

Wistar albino rats of either sex weighing between 100 and 150 g were obtained from B.R.N.C.P. Mandsaur Animal House. These animals were used for the acute toxicity and antidiabetic activity studies. The animals were stabilized for 1 week, were maintained under standard condition at room temperature: 60 ± 5% relative humidity and 12-hour light–dark cycle. They had been given standard pellet diet and water *ad libitum* throughout the course of the study. The animals were handled gently to avoid giving them too much stress, which could result in an increased adrenal out put.

Acute Toxicity Studies

The acute toxicity study was carried out in adult female

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albino rats by the "fix dose" method of OECD (Organization for Economic Co-operation and Development) Guideline No.420. The fixed dose method as in Annex 2d, test procedure with a starting dose of 2000 mg/kg body weight, was adopted. The animals were fasted overnight and next day the product (suspended in 5% tween 80 solution) was administered orally at a dose level 2000 mg/kg. Then the animals were observed continuously for 3 hours for general behavioral, neurological, and autonomic profiles and then every 30 minutes for next 3 hours and finally for mortality after 24 hours till 14 days.[4]

Selection of Doses

For the assessment of hypoglycemic activity, two dose level were chosen in such a way that one dose was approximately one-tenth of the maximum dose during acute toxicity studies and the other high dose was twice that of one-tenth dose (200 mg/kg, 400 mg/kg).

Hypoglycemic Study in Normal Rats

Animals and experimental setup

Albino rats of either sex weighing 100-150 g were taken. The rats were kept fasting overnight with free access to water. During the experiment, the animals were divided into four groups of five animals in each group. The blood sample was taken by pricking the rat's tail. Polyherbal formulation was administered with glass syringe and microsuction canula no. 18.

Preparation of dosing

The doses of 200 and 400 mg/kg of Karnim Plus were administered as made by dissolving appropriate quantity in 5% tween 80 solution.

Grouping of animals

Group I Kept as normal control, i.e., neither treated with Karnim Plus nor standard.
Group II Treated with standard oral hypoglycemic drug, i.e., Glibenclamide (4 mg/kg).
Group III Treated orally with Karnim Plus 200 mg/kg.
Group IV Treated orally with Karnim Plus 400 mg/kg.

Determination of hypoglycemic activity

Test samples were given orally using oral gastric gavages to the fasted animals. The blood glucose concentrations of the animals were measured at the beginning of the study and the measurements were repeated 1, 2, and 4 hours after the initiation of the experiment.[2,5,6,]

Oral Glucose Tolerance Test in Normal Rats

Animals and experimental setup

Albino rats of either sex weighing 100-150 g were taken. The rats were kept fasting overnight with free access to water. During the experiment the animals were divided into three groups of five animals in each group. The blood sample was taken by pricking the rat's tail. Polyherbal formulation was administered with glass syringe and microsuction canula no. 18.

Preparation of dosing

The dose 400 mg/kg of Karnim Plus were administered as made by dissolving appropriate quantity in 5% tween 80 solution.

Grouping of animals

Group I Kept as negative control, i.e., neither treated with Karnim Plus nor standard.
Group II Treated with standard oral hypoglycemic drug, i.e., Glibenclamide (4 mg/kg).
Group III Treated orally with Karnim Plus 400 mg/kg.

Determination of OGTT activity

The blood glucose concentrations of the animals were measured at the beginning of the study. Then the rats were orally treated with 3 g/kg body weight glucose solution after 30 minutes of the product and drug treatment. The measurements were repeated after 30, 90 and 150 minutes after the glucose load.[2,6]

Antidiabetic Activity

Induction of diabetes

Animals were fasted for 24 hours then a single intraperitoneal injection of freshly prepared alloxan (120 mg/kg dissolved in 0.9% saline) was injected. After that the animals were left aside for 4 hours and then 10% glucose solution was placed in the cages for 24 hours. The diabetes was confirmed by estimation of blood glucose level (BGL) on the third day. Rats having BGL >250 mg/dl were used for study and during the experiment the animals were divided into five groups of six animals in each group.

Grouping of animals

Group I Kept as normal control, i.e., neither treated with Karnim Plus nor standard.
Group II Kept as negative control i.e. treated with alloxan (120 mg/kg i.p.).
Group III Treated with standard oral hypoglycemic drug, i.e., Glibenclamide (4 mg/kg) after the third day of treatment with alloxan (120 mg/kg i.p.).
Group IV Treated orally with Karnim Plus 200 mg/kg after the third day of treatment with alloxan (120 mg/kg i.p.).
Group V Treated orally with Karnim Plus 400 mg/kg after the third day of treatment with alloxan (120 mg/kg i.p.).

Determination of antidiabetic activity

The blood glucose concentrations of the animals were

measured at the beginning of the study and the measurements were repeated on 3rd, 7th, and 11th day of the experiment.[2,6,7]

Biochemical determinations

After the 11th day of treatment, blood was collected from the orbital plexus of overnight fasted rats. The serum was separated and urea, creatinine, triglycerides, and cholesterol level were determined by using urea Berthelot test kit, creatinine mono reagent test kit, triglycerides test kit, and cholesterol test kit (Span diagnostic Ltd, Surat), respectively.

Statistical analysis

The data were expressed as mean ± SEM. The data of hypoglycemic activity, oral glucose tolerance test (OGTT), and antidiabetic activity were analyzed by one-way analysis of variance (ANOVA) followed by "Dunnett's test." A *P* value <0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Acute toxicity studies on female rats showed no mortality at a dose of 2000 mg/kg, during a time period of 14 days. During the study, no noticeable responses were seen in the rats. This helps to predict that it does not contain any type of toxicity and is safe.

In the hypoglycemic activity studied, Karnim Plus did not show any significant activity at dose levels of 200 and 400 mg/kg, whereas standard drug glibenclamide produced significant activity [Table 1]. In the OGTT, Karnim Plus at a dose of 400 mg/kg significantly reduced the blood glucose level at 30 minutes after glucose administration. Standard drug glibenclamide produced activity at all the time interval tested [Table 2].

Karnim Plus showed significant antidiabetic activity at 7th and 11th days at both 200 and 400 mg/kg dose levels [Table 3]. In diabetic rats urea, creatinine, cholesterol, and triglycerides level increased. After treatment with formulation, the formulation significantly reduced the biochemical parameters [Table 4].

The levels of serum lipids are usually elevated in DM and such an elevation represents a risk factor for coronary heart disease. This abnormal high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots mainly due to the action of insulin. Under normal circumstances, insulin activates the enzyme lipoprotein

Table 1: Effect of Karnim Plus on the blood glucose level (mg/dl) of normal rats

Group	Dose	Blood glucose level			
		0 hour	1 hours	2 hours	4 hours
Normal control	5% tween 80 (1 ml/kg)	88.40 ± 1.28	89.80 ± 1.24	87.80 ± 5.91	83.40 ± 3.57
Glibenclamide	4 mg/kg	94.00 ± 13.12	44.20 ± 10.09**	47.60 ± 5.55**	47.20 ± 6.32**
Karnim Plus	200 mg/kg	74.80 ± 4.19	80.20 ± 6.26	94.60 ± 2.04	65.80 ± 6.54
Karnim Plus	400 mg/kg	87.60 ± 8.48	95.40 ± 4.80	93.40 ± 3.09	87.20 ± 5.00

N = 5, ** *P* <0.01 vs negative control (ANOVA followed by Dunnet's test). Values are expressed in mean ± SEM

Table 2: Oral glucose tolerance test of Karnim Plus on blood glucose level (mg/dl) of normal rats

Group	Dose	Blood glucose level			
		0 minute	30 minutes	90 minutes	150 minutes
Normal control	5% tween 80 (1 ml/kg)	81.00 ± 2.70	115.80 ± 7.04	106.40 ± 4.77	89.20 ± 1.83
Glibenclamide	4 mg/kg	82.80 ± 2.42	75.80 ± 3.79**	64.00 ± 5.21**	58.80 ± 2.08*
Karnim Plus	400 mg/kg	83.00 ± 5.29	97.80 ± 1.50*	97.80 ± 3.06	97.60 ± 5.94

N = 5, * *P* <0.05, ** *P* <0.01 vs negative control (ANOVA followed by Dunnet's test). Values are expressed in mean ± SEM

Table 3: Antidiabetic activity of Karnim Plus in alloxan induced diabetic rats

Group	Dose	Blood glucose level (mg/dl)			
		0 day	3 day	7 day	11day
Normal control	5% tween 80 (1ml/kg)	83.33 ± 4.12	81.33 ± 2.77	81.00 ± 2.22	79.17 ± 1.80
Negative control	5% tween 80 (1ml/kg)	82.16 ± 2.96	335.17 ± 29.72	345.83 ± 27.52	370.50 ± 25.52
Glibenclamide	4 mg/kg	82.83 ± 2.32	329.67 ± 23.77	158.33 ± 6.81**	115.00 ± 5.74**
Karnim Plus	200 mg/kg	86.00 ± 3.51	312.67 ± 11.07	261.50 ± 7.48**	218.83 ± 5.63**
Karnim Plus	400 mg/kg	88.83 ± 2.59	310.67 ± 11.75	251.50 ± 6.60**	206.17 ± 5.73**

N=6, * *P*<0.05, ** *P* < 0.01 vs Negative control (ANOVA followed by Dunnet's Test), Value expressed in mean ± SEM

Table 4: Effect of Karnim Plus in biochemical parameters (mg/dl)

Group	Urea	Creatinine	Cholesterol	Triglycerides
Normal control	41.41 ± 0.59	0.85 ± 0.03	81.80 ± 1.82	99.20 ± 1.71
Negative control	73.88 ± 2.37	1.65 ± 0.02	173.77 ± 2.55	165.33 ± 2.81
Glibenclamide	28.68 ± 1.60**	1.11 ± 0.02**	87.25 ± 1.42**	67.33 ± 0.70 **
Karnim Plus (200 mg/kg)	67.68 ± 0.86*	1.33 ± 0.02**	121.70 ±1.84**	101.58 ± 1.75**
Karnim Plus (400 mg/kg)	65.95 ± 0.72 **	1.30 ± 0.01**	109.88± 2.71**	91.30 ± 1.20 **

N=6, * p<0.05, ** p < 0.01 vs Negative control (ANOVA followed by Dunnet's Test), Value expressed in mean ± SEM

lipase, which hydrolyses triglycerides. However, in a diabetic state, lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridemia.[7] Also insulin deficiency is associated with hypercholesterolemia. Insulin deficiency may be responsible for dyslipidemia, because insulin has an inhibitory action on HMG-CoA reductase, a key rate-limiting enzyme responsible for the metabolism of cholesterol-rich LDL particles. The mechanisms responsible for the development of hypertriglyceridemia and hypercholesterolemia in uncontrolled diabetes in humans are due to a number of metabolic abnormalities that occur sequentially.[8] In our study also, the diabetic rats showed hypercholesterolemia and hypertriglyceridemia and the treatment with product significantly ($P < 0.01$) decreased both cholesterol and triglyceride levels. This implies that the product can prevent or be helpful in reducing the complications of lipid profile seen in some diabetics in whom hyperglycemia and hypercholesterolemia coexist quite often.

The diabetic hyperglycemia induced by alloxan produces elevation of plasma levels of urea and creatinine, which are considered as significant markers of renal dysfunction.[9] The results in Table 4 showed a significant increase in the level of plasma urea and creatinine in the diabetic groups compared to control level. These results indicated that diabetes might lead to renal dysfunction. While, after treatment of alloxan-diabetic rats with product, the level of urea and creatinine were significantly ($P < 0.05$) ($P < 0.01$) decreased compared to the mean value of the diabetic group. This further confirms the utility of this product in diabetes-associated complications.

To conclude, the product Karnim Plus, manufactured by Unijules Life Sciences Ltd., Nagpur, was proven to have antidiabetic effect and this formulation could be used as an alternative remedy for the treatment of diabetes.

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