Hypoglycemic and Antilipidperoxidative Effects of a Polyherbal Formulation, Diakyur, in Experimental Animal Models

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(Received April 5, 2007; Accepted August 11, 2007)

The present study investigates the effect of Diakyur, a polyherbal formulation composed of powder of *Cassia javanica* and dried, standardised aqueous extracts of *Cassia auriculata, Salacia reticulata, Gymnema sylvestre, Mucuna pruriens, Syzygium jambolanum, Terminalia arjuna* on blood glucose level of normal and diabetic animals as well as lipid peroxide level in normal and 28 day drug treated diabetic rats. The raw materials and the formulation were standardised by high performance thin layer chromatography (HPTLC) and high pressure liquid chromatography (HPLC) method of analysis. The hypoglycemic activity and glucose tolerance test were studied in normal and alloxan (150 mg/kg, i.p) induced diabetic rats and rabbits after administration of Diakyur at the dose of 1600 mg/kg, p.o. Blood glucose level was determined by *O*-toluidine method. Lipid peroxide levels of plasma, erythrocyte membrane, liver and kidney tissues were studied in alloxan induced diabetic rats after 28 days drug treatment. At the dose of 1600 mg/kg, p.o Diakyur showed a hypoglycemic effect at varying degree of significance ($p < 0.05$–0.001) in normal as well as alloxan induced diabetic rats and rabbits in comparison with respective control groups. Diakyur treatment in the glucose tolerance test showed the maximum effect at 180th min of glucose administration in both normal and alloxan diabetic animals. The drug treated alloxan diabetic rats showed significant ($p < 0.001$) reduction in plasma, erythrocyte membrane, liver and kidney lipid peroxide levels after 28 days treatment when compared to untreated alloxan diabetic rats. The results indicate the significant hypoglycemic activity of Diakyur in both rats and rabbits, whereas an antilipidperoxidative activity in diabetic rats.

Key words —— Diakyur, hypoglycemic activity, alloxan-induced diabete, antioxidant

INTRODUCTION

Type II diabetes results from defect(s) in insulin secretion, almost always with a major contribution from insulin resistance.[1)] It is a common disorder among the Indian population. Therapeutic options for diabetes are diet, exercise, oral hypoglycemic drugs and insulin therapy. In India, number of alternative medicines like Ayurvedic as well as siddha preparations have attracted great interest in Type II diabetes management. Diakyur is one such ayurvedic polyherbal formulation which contains crude powder of *Cassia javanica* and dried, standardised aqueous extracts of *Cassia auriculata*, *Salacia reticulata*, *Gymnema sylvestre*, *Mucuna pruriens*, *Syzygium jambolanum* and *Terminalia arjuna*, the herbal medicines commonly used by traditional medical practitioners for the treatment of Type II diabetes in community practice.

Salacia reticulata, *Gymnema sylvestre* and *Syzygium jambolanum* are proven antidiabetic drugs.[2–4)] *Terminalia arjuna* is a proven cardiotonic[5)] and antioxidant drug which protects the heart and blood vessels from the oxidative stress of free radicals.[6)] *Cassia auriculata*[7)] and *Cassia javanica* rich in bioflavonoids are hypocholesterolemic and hypolipidemic agents and preserve the favourable high-density lipoprotein (HDL): low-density lipoprotein (LDL) cholesterol ratio. *Mucuna pruriens* is reported to have antioxidant,[8)] antidiabetic[9)] and neuroprotective[10)] activities. In light of the above reports the hypoglycemic and antilipidperoxidative effects of Diakyur were studied in different experimental animal models.

MATERIALS AND METHODS

Drugs Used —— Each 600 mg capsule of Diakyur contained crude powder of *Cassia javanica* (17 mg) and dried standardised aqueous extracts of *Cas-*

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sia auriculata (165 mg), *Salacia reticulata* (22 mg), *Gymnema sylvestre* (50 mg), *Mucuna pruriens* (50 mg), *Syzygium jambolanum* (162.5 mg) and *Terminalia arjuna* (33.5 mg), which were standardised using the respective marker compounds.

Extraction and Standardization of Plant Materials —— The extraction and standardization of the plant extracts of Diakyur were carried out according to our previous study.[11)]

The dried powder was dissolved in water by constant stirring in a water bath to reflux, filtered and administered to the animals by oral intubation. Alloxan monohydrate used for the induction of diabetes was purchased from Sigma, Mumbai, India. Glibenclamide was used as the positive control.

Animals Used —— Colony bred Wistar albino rats and naïve white rabbits were used for the study. The animals were properly fed with cereals, pulses, and green vegetables and water *ad libitum*. The rats were housed in polyvinyl chloride (PVC) cages and the rabbits were housed in pens with wooden partition. Rats of either sex weighing 125–150 g and rabbits of either sex weighing 1500–2000 g were used for the study, and were divided into six animals in each group. The study had approval from the Institutional Animal Ethical Committee (IAEC) of CPCSEA (Committee for the purpose of Control and Supervision of Experiments on Animals).

Toxicity Studies —— Acute and subacute toxicity studies were reported in our previous study.[11)]

Induction of Diabetes —— The animals were fasted for 18 hr and made diabetic by injecting alloxan monohydrate (150 mg/kg, i.p) dissolved in sterile normal saline.[12)] Diabetic state was confirmed when the blood sugar value was greater than 200 mg/dl.

Hypoglycemic Activity

Normal Fasted Animal Models —— Rats and rabbits were fasted for 18 hr before the experiment. The test drug was administered at the dose level of 1600 mg/kg, p.o. The control group received distilled water at the dose of 10 ml/kg, b.w. Blood samples were drawn at intervals of 0, 1, 3 and 4 hr after drug administration. Blood sugar estimation was done by *O*-toluidine method.[13)] Glucose tolerance test was done in fasted animals by administering 1.5 g/kg, b.w. to rats and 1 g/kg, b.w. to rabbits[14)] after 30 min of drug administration at the dose of 1600 mg/kg. Control animals received the respective loading of glucose by oral intubation. Blood samples were collected at intervals of 30, 60, and 180 min after glucose loading and blood sugar was estimated as above.

Diabetic Animal Models —— 18 hr fasted animals were made diabetic by alloxan (alloxan monohydrate, 150 mg/kg, i.p). Blood samples were collected before the administration of alloxan and after 5 days of alloxan administration. Diabetic state was confirmed when the blood sugar value was above 200 mg/dl. The blood samples were collected at the intervals of 1, 3 and 4 hr after drug administration. Glucose tolerance test (GTT) was performed as in normal fasted animals using alloxan diabetic rats and rabbits after 28 days of drug treatment.

Estimation of Lipid Peroxidation —— After drug treatment for 28 days, the alloxan-induced rats were sacrificed and the plasma lipid peroxide level was determined by the method of Yagi.[15)] Erythrocyte membrane was prepared according to the method of Dodge.[18)] Lipid peroxidation in liver, kidney tissues and erythrocyte membrane were estimated by thiobarbituric acid reactive substances (TBARS) by the method of Ohkawa[19)] and expressed in terms of n mol of Malondialdehyde (MDA) liberated per min/mg protein.

Statistical Analysis —— Data are expressed as mean ± S.E. of mean. Statistical analysis was done using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison. p values < 0.05 were considered as significant.

RESULTS AND DISCUSSION

In the present study, administration of Diakyur at the dose of 1600 mg/kg, p.o. was found to reduce blood sugar level in normal rats and rabbits (Table 1). The maximum reduction in blood sugar level was noted after 4 hr of drug administration. In case of rats, 37.23% reduction of the glucose level was observed and standard glibenclamide showed 44.64% reduction in blood glucose levels, whereas in case of rabbits 15.57% reduction was obtained for the drug treated group and 30.59% reduction in the standard treated group. The effect of Diakyur on glucose tolerance test in normal rats and rabbits is given in Table 2. In both glucose fed animals, administration of 1600 mg/kg, p.o. of Diakyur significantly ($p < 0.001$) increased the tolerance for glucose at the 180th min after glucose loading.

The effect of Diakyur in alloxan induced diabetic rats and rabbits is shown in Table 3. The fasting blood sugar level in these animals was found to be 246–253 mg/100 ml. Maximum reduction of

Table 1. Effect of Diakyur on Blood Glucose Levels of Fasted Normal Albino Rats and Rabbits

Groups	Treatment	Blood sugar level in mg/dl (%)			
		Fasting (0 hr)	1 hr	3 hr	4 hr
I	Control				
	i) Rats	107 ± 0.3	86.4 ± 1.3 (19.3)*	98.4 ± 8.7 (8.0)	101 ± 2.5 (5.6)
	ii) Rabbits	106.2 ± 3.2	108.4 ± 1.6 (2.1)	105.9 ± 6.7 (0.3)	103.7 ± 7.6 (2.4)
II	Diakyur (1600 mg/kg)				
	i) Rats	103.4 ± 3.6	80.3 ± 4.5 (22.3)*	75.7 ± 3.5 (26.8)**	64.9 ± 2.2 (37.2)**
	ii) Rabbits	103.4 ± 2.4	101.7 ± 3.2 (1.6)	99.6 ± 7.9 (3.7)	87.3 ± 3.5 (15.6)**
III	Glibenclamide				
	i) Rats (2 mg/kg)	102.8 ± 4.5	74.5 ± 4.3 (27.5)**	67.8 ± 3.6 (34.0)**	56.9 ± 2.3 (44.6)***
	ii) Rabbits (5 mg/kg)	98.7 ± 8.7	83.2 ± 4.5 (15.7)*	76.4 ± 5.4 (22.6)**	68.5 ± 6.2 (30.6)***

Values are mean ± SEM, $n = 6$ animals in each group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Comparison was made between Fasting value vs. 1st, 3rd and 4th hr. Values in the parenthesis indicates the percentage reduction compared to '0' hr.

Table 2. Effect of Diakyur in Fasted Rats and Rabbits after Glucose Loading

Groups	Treatment	Blood sugar level in mg/dl (%)			
		0	30 min	60 min	180 min
I	Control				
	i) Rats	90.3 ± 2.3	105.1 ± 7.8***	93.2 ± 3.7*	92.3 ± 2.8**
	ii) Rabbits	118.1 ± 0.8	166.7 ± 3.1***	150.5 ± 3.2**	120.2 ± 1.1***
II	Diakyur (1600 mg/kg)				
	i) Rats	88.2 ± 4.2	99.0 ± 4.7**	88.5 ± 2.1*	86.23 ± 3.1**
	ii) Rabbits	112.3 ± 2.3	136.2 ± 2.1***	122.2 ± 1.5**	110.6 ± 1.8***

Values are mean ± SEM, $n = 6$ animals in each group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Comparison were made between 0 vs. 30 min (hyperglycemia) and 30 vs. 60 min and 180 min (control of glycemia).

Table 3. Effect of Diakyur on Blood Sugar Level of Alloxan Induced Diabetic Rats and Rabbits

Groups	Treatment	Blood sugar level in mg/dl (%)			
		Fasting (0 hr)	1 hr	3 hr	4 hr
I	Control				
	i) Rats	252.4 ± 3.5^{ns}	262.3 ± 2.7^{ns}	256.5 ± 4.3^{ns}	250.2 ± 4.1^{ns}
	ii) Rabbits	248.3 ± 2.5^{ns}	257.4 ± 3.8^{ns}	250.3 ± 4.2^{ns}	244.1 ± 6.2^{ns}
II	Diakyur (1600 mg/kg)				
	i) Rats	247.3 ± 4.1	199.3 ± 2.1 (19.4)*	187.4 ± 3.6 (24.2)**	182.3 ± 1.2 (26.3)***
	ii) Rabbits	253.2 ± 1.5	203.4 ± 6.2 (19.7)*	189.8 ± 4.7 (25.0)**	178.4 ± 3.5 (29.5)***
III	Glibenclamide (2 mg/kg)				
	i) Rats	248.5 ± 2.3	229.3 ± 2.8 (7.7)*	203.4 ± 5.4 (18.2)**	173.0 ± 5.2 (30.4)***
	ii) Rabbits	246.4 ± 2.5	224.4 ± 4.6 (8.9)*	195.3 ± 5.2 (20.7)**	165.3 ± 3.5 (32.9)***

Values are mean ± SEM, $n = 6$ animals in each group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, ^{ns}—not significant. Comparison was made between Fasting value vs. 1st, 3rd and 4th hr. Values in the parenthesis indicates the percentage reduction compared to '0' hr.

blood glucose level was observed at the 4th hr after administration of Diakyur. Effect of the drug was comparatively less than the standard drug (Glibenclamide). In the untreated animals, blood glucose levels did not change significantly. After 28 days of treatment with the drug there was significant reduction (0 min) in blood sugar level in rats but not in rabbits. The effect of Diakyur on GTT in alloxan induced diabetic rats and rabbits after 28 days treatment is shown in Table 4, which indicates that significant reduction was observed at the 180th min of glucose loading. The biochemical study on lipid peroxidation of plasma, erythrocyte membrane liver and kidney of alloxan diabetic rats after treatment for 28 days (Table 5) showed significant reduction ($p < 0.001$) in the drug treated group when compared with the alloxan diabetic group.

The raw materials were extracted using the suit-

Table 4. Effect of Diakyur on GTT in Alloxan Induced Diabetic Rats after 28 Days Treatment

Groups	Treatment	Blood sugar level in mg/dl (%)			
		0	30 min	60 min	180 min
I	Control				
	i) Rats	270.3 ± 5.3	302.5 ± 2.7**	340.8 ± 2.9^{ns}	270.3 ± 3.6*
	ii) Rabbits	287.2 ± 1.5	299.5 ± 5.6*	339.0 ± 2.7^{ns}	298.8 ± 5.6^{ns}
II	Diakyur (1600 mg/kg)				
	i) Rats	217.4 ± 4.5	230.8 ± 8.6*	203.6 ± 3.2*	182.8 ± 9.8**
	ii) Rabbits	298.8 ± 5.6	278.6 ± 3.7*	216.7 ± 5.4*	179.2 ± 4.7**

Values are mean ± SEM, $n = 6$ animals in each group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, ^{ns}—not significant. Comparison were made between 0 vs. 30 min (hyperglycemia) and 30 vs. 60 min and 180 min (control of glycemia).

Table 5. Effect of Diakyur on Lipid Peroxidation Level of Plasma, Erythrocyte Membrane, Liver and Kidney of Alloxan Diabetic Rats after Treatment for 28 Days

	Parameters		
MDA (nmoles of MDA liberated /min/mg protein)	Control (Group I)	Alloxan diabetic rats (Group II)	Diakyur treated alloxan diabetic rats (Group III)
Plasma LPO	0.07 ± 0.005	0.22 ± 0.023***	0.094 ± 0.005***
Erythrocyte LPO	1.22 ± 0.086	2.09 ± 0.012***	1.31 ± 0.054***
Liver LPO	0.97 ± 0.09	1.37 ± 0.06***	0.92 ± 0.14***
Kidney LPO	0.87 ± 0.07	1.39 ± 0.21***	0.84 ± 0.12***

Values are mean ± SEM, $n = 6$ animals in each group. $^{***}p < 0.001$. Comparison was made between Groups I vs. II and II vs. III.

able solvents and were standardised for their active constituents. The formulation Diakyur was also compared by its high performance thin layer chromatography (HPTLC) fingerprinting with the in-house reference standard. The results of the present study indicate that the drug Diakyur at the dose of 1600 mg/kg showed hypoglycemic effect in all experimental models with varying degrees of significance ($p < 0.05$–0.001) when compared to control animals. At the end of the 28 day treatment in alloxan induced diabetic rats, there was a significant decrease in the blood sugar level of rats but not in rabbits, whereas in GTT both rats and rabbits showed significant reduction in the blood sugar level. Since the GTT profile is more relevant to establish the hypoglycemic activity of the test compound rather than estimating the random blood glucose level, the results obtained in GTT were considered.

Alloxan, a well-known diabetogenic agent is widely used to induce Type II diabetes in animals.[16,17] Alloxan and its reduction product dialuric acid establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter, highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β cells.[20] Thus alloxan induced diabetes mellitus served as a pathological biomodel for testing a substance with supposed antioxidant activity *in vivo*.[21] One of the targets of the reactive oxygen species is DNA of pancreatic islets. Its fragmentation takes place in β cells exposed to alloxan.[22,23] The increase in oxygen free radicals in diabetic conditions is mainly because of the effect of the diabetogenic agent alloxan. The low insulin level increases the activity of the enzymes fatty acyl coenzyme and coenzyme A oxidase, which inhibits β-oxidation of fatty acids resulting in lipid peroxidation[22,23] of various tissues like liver, kidney, pancreas and brain. Increased lipid peroxidation (LPO) levels in various tissues like liver, kidney and brain of diabetic rats were reported earlier.[24,25] In the present study, the drug treated group showed reduction in lipid peroxide levels in erythrocyte membrane, liver and kidney tissues of diabetic rats, which indicates that Diakyur inhibits the oxidative damage due to the presence of antioxidant herbal molecules[26–29] in the formulation, thereby reducing the toxicity of alloxan.

This antioxidant capacity has significant therapeutic importance in reducing the late complica-

tions of diabetes like atherosclerosis and related complications. Since the composition of the formula is polyherbal in nature, it is difficult to study the pharmacokinetics of the formula.

Whether the drug can be used as a primary oral antidiabetic drug should be supported by randomized double blind clinical trials in Type II diabetic patients. On the basis of the above results it could be concluded that Diakyur, a combination of seven herbal ingredients, exerts significant hypoglycemic and antilipidperoxidative activities. This could be due to the different types of bioactive principles of plant origin, which serve as hypoglycemic agents to reduce the blood sugar level in diabetic animals.

In conclusion, diabetes mellitus is a well-known clinical entity with various late complications like retinopathy, neuropathy, nephropathy etc., Diakyur is a polyherbal formulation which has significant hypoglycemic activity as well as antilipidperoxidative activity so that it can be used as an adjuvant along with allopathic treatment of medicine to treat diabetes as well as to delay the late complications of diabetes. Further detailed studies will be carried to prove the antioxidant capacity of Diakyur by of estimating the enzymic and nonenzymic antioxidants.

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