Acute and Subacute Toxicity Studies on the Polyherbal Antidiabetic Formulation Diakyur in Experimental Animal Models

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The present study investigated the acute and subacute toxicity of Diakyur, a polyherbal antidiabetic formulation, in experimental animal models. Diakyur contains aqueous extract dry powders of *Cassia auriculata, Gymnema sylvestre, Mucuna pruriens, Syzygium jambolanum, Terminalia arjuna, Salacia reticulata,* and a crude powder of *Cassia javanica*. The raw materials were standardized by gravimetric, HPLC and High performance thin layer chromatography (HPTLC) methods for their respective bioactive marker compounds. In an acute toxicity study, Diakyur was administered orally at doses ranging from 100–12800 mg/kg p.o. and the animals were observed for any toxic symptoms up to 72 hr. The results indicated there were no toxic symptoms up to the dose level of 12800 mg/kg p.o. In a subacute toxicity study, Diakyur was tested at the dose of 1600 mg/kg p.o. once daily for 28 days. The animals were sacrificed on the 29th day and various blood biochemical parameters were measured. The liver, kidney, heart, adrenals, pancreas and uterus were processed for histopathological study. The results of the 28 day subacute toxicity study did not show evidence of any changes in body weight, food and water intake, hematological parameters, liver and kidney function tests when compared with the control animals. The vital organs of animals treated with Diakyur for 28 days did not show any histopathological evidence of pathological lesions. From the results it is concluded that Diakyur at the dose of 1600 mg/kg p.o. is safe for long-term treatment in diabetic conditions.

Key words —— Diakyur, polyherbal formulation, acute and subacute toxicity

INTRODUCTION

Herbal medicines are popular remedies for diseases used by a vast majority of the world's population. Herbal formulations, which have attained widespread acceptability as therapeutic agents in India, include nootropics, antidiabetics, hepatoprotective agents, and lipid-lowering agents. The pharmacological effects of many plants have been studied in various laboratories, whereas there are many limitations regarding the safety and efficacy of these preparations.[1)] Diakyur, a polyherbal antidiabetic formulation containing seven ingredients of herbal origin that is used in traditional medicine to treat type II diabetes, contains both antidiabetic and antioxidant principles. *Salacia reticulata*, *Gymnema sylvestre* and *Syzygium jambolanum* are proven antidiabetic drugs[2–4)] that alone or in combination act to control the hyperglycemia in diabetes. *Terminalia arjuna* is a proven cardiotonic[5)] and antioxidant drug that protects the heart and blood vessels from the oxidative stress of free radicals.[6)] *Cassia auriculata*[7)] and *Cassia javanica*, which are rich in bioflavonoids, are hypocholesterolemic and hypolipidemic agents that maintain a favorable High density lipo protein (HDL): Low density lipo protein (LDL) cholesterol ratio. *Mucuna pruriens* is a rejuvenator drug also found to have a neuroprotective action[8)] which may help to prevent the neuropathic complications of diabetes.

In the present study, the acute and subacute toxicity of Diakyur, a polyherbal formulation, were investigated to assess its safety and tolerability profile in long-term treatment.

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MATERIALS AND METHODS

Drugs —— Diakyur contains crude powder of *Cassia javanica* and dried extracts of *Cassia auriculata*, *Salacia reticulata*, *Gymnema sylvestre*, *Mucuna pruriens*, *Syzygium jambolanum* and *Terminalia arjuna*, which were standardized using their respective bioactive marker compounds.

Extraction and Standardization of Plant Materials:

Cassia auriculata seeds: 165 kg of plant material was refluxed 5 times with 825 l of water for 6 hr each time. It was then concentrated under vacuum at 70–75°C. The herb to extract ratio was 15:1. It was standardized for its total emodin content by High performance thin layer chromatography (HPTLC). The stationary phase used was Silicagel $60F_{254}$ (Merck), (Mumbai, India). The mobile phase used was ethyl acetate: methanol: water (10:1.35:1.0) and it was detected by spraying 10% ethanolic potassium hydroxide reagent.

Syzygium jambolanum seeds: 162.5 kg of plant material was refluxed 5 times with 815 l of water for 6 hr each time. It was then concentrated under vacuum at 70–75°C. The herb to extract ratio was 8:1. It was standardized for its total tannin content, which was determined by redox titration against 0.1 N potassium permanganate.

Terminalia arjuna bark: 33.5 kg of plant material was refluxed 5 times with 170 l of water for 6 hr each time. It was then concentrated under vacuum at 70–75°C. The herb to extract ratio was 15:1. It was standardized for its total tannin content, which was determined by redox titration against 0.1 N potassium permanganate.

Mucuna pruriens seeds: 50 kg of plant material was refluxed 5 times with 250 l of water for 6 hr each time. It was then concentrated under vacuum at 70–75°C. The herb to extract ratio was 10:1. It was standardized for its levodopa (L-DOPA) content, which was carried out by HPLC. The column used was Phenomenex C_{18} and the mobile phase was 0.1% v/v phosphoric acid in water: acetonitrile (95:05). Peak detection was performed at 281 nm.

Gymnema sylvestre leaves: 50 kg of plant material was refluxed 5 times with 250 l of an 8:2 mixture of water and alcohol for 6 hr each time. It was then concentrated under vacuum at 70–75°C. The herb to extract ratio was 10:1. Total gymnemic acid content was standardized by a gravimetric method of determination.

Salacia reticulata bark: 22 kg of plant material was refluxed 5 times with 110 l of an 8:2 mixture of water and alcohol for 6 hr each time. It was then concentrated under vacuum at 60°C. The herb to extract ratio was 23:1. Total saponin content was determined by a gravimetric method.

Cassia javanica bark: 15 kg crude powder was used for the formulation and it was standardized by HPTLC method of analysis. The raw material was compared with an in-house reference standard for its total anthraquinone content.

Standardization of the Diakyur Formulation:

The extracts obtained from the authenticated plant specimens were mixed in the right proportion and maintained as reference standard. The batches were compared with the reference standard by HPTLC analysis. One g of the material was refluxed with 20 ml of alcohol in a water bath for 1 hr. It was then filtered and the filtrate was concentrated to 5 ml. The concentrate was spotted and developed in a mobile phase consisting of toluene, ethyl acetate, and formic acid (6:4:0.4). The dried plate was then scanned at 254 nm. The fingerprinting of the batches was compared with the reference standard.

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.

Animals —— Colony bred Wistar albino rats and Swiss albino mice were used. The animals were fed cereals, pulses, and green vegetables and had free access to water. They were housed in Polyvinyl Chloride (PVC) cages. Rats of either sex weighing 125–150 g and mice of either sex weighing 18–22 g were used and approved by the Institutional Animal Ethical Committee (IAEC) No. 07/017/03, Institute of Basic Medical Sciences (IBMS), University of Madras, India.

Acute Toxicity Study —— Swiss albino mice of either sex weighing 18–22 g were randomly distributed to 8 different groups with 6 animals in each group. The animals were fasted overnight and the drug was administered orally at dose levels of 100, 200, 400, 800, 1600, 3200, 6400 and 12800 mg/kg of body weight. The animals were closely observed for the first 12 hr for any toxic symptoms and for 72 hr for mortality rate.[9)]

Subacute Toxicity Study —— Wistar albino rats of either sex weighing 125–150 g were assigned to each group (6 per group). Group I received distilled water for 28 days and Group II received the test drug Diakyur at the dose of 1600 mg/kg p.o., once daily for 28 days. Body weight, food intake and water intake were monitored. The animals were sacrificed

on day 29, blood samples were collected for hematological parameters like hemoglobin (Hb), red blood cell (RBC) count,[10] white blood cell (WBC) count,[11] erythrocyte sedimentation rate (ESR),[12] differential count (DC) [neutrophils (N), lymphocytes (L), eosinophils (E), basophils (B)] and biochemical parameters like serum glutamate oxaloactete transaminase (SGOT),[13] serum glutamate pyruvate transaminase (SGPT),[13] alkaline phosphatase (ALP),[14] blood urea nitrogen (BUN),[15] and serum creatinine.[16]

Statistical Analysis —— The data are presented as the mean ± S.E. Results were analyzed statistically using one-way Analysis of one way variance (ANOVA) followed by Tukey's multiple comparison test. The minimum level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

The extract of *Cassia auriculata* was standardized for its total emodin content (0.6% w/w), *Salacia reticulata* (saponins: 20% w/w), *Gymnema sylvestre* (Gymnemic acid: 20% w/w), *Mucuna pruriens* (L-DOPA: 5% w/w), *Syzygium jambolanum* (total tannins: 12% w/w), *Terminalia arjuna* (Total tannins: 20% w/w), and *Cassia javanica* (total anthraquinones: 0.1% w/w). The formulation Diakyur was compared by its HPTLC fingerprinting with the in-house reference standard.

Acute Toxicity Study

In the acute toxicity study, Diakyur up to the dose level of 12800 mg/kg of body weight did not exhibit any lethality or toxic symptoms. Further dosing to estimate the LD_{50} of the drug was not performed. According to Organisation for Economic Cooperation and Development (OECD) guidelines for acute oral toxicity, an LD_{50} dose of 2000 mg/kg and above is categorized as unclassified and hence the drug is found to be safe. As 1600 mg/kg of body weight was well tolerated by the animals without any behavioral changes during long-term treatment, further studies were carried out with 1600 mg/kg of body weight.

Subacute Toxicity Study

In the subacute toxicity study, the Diakyur treated groups did not show any significant changes in body weight increment at weekly intervals compared to the control group (Fig. 1). The weights of

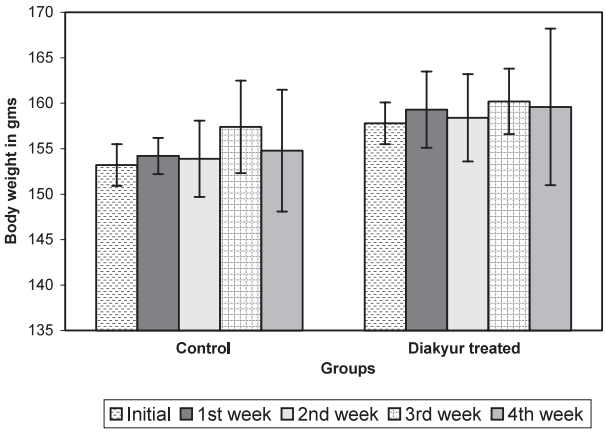

Fig. 1. Effects of Diakyur on Body Weight Increment in 28 Day Treatment
Data are the mean ± S.E. of 6 animals (One-way ANOVA).

Table 1. Effects of 28 Day Oral Administration of Diakyur on Organ Weights in Rats

Group	Treatment	Organ weight (g/100 g body weight)		
		Liver	Kidney	Heart
I	Control	2.98 ± 2.1	0.71 ± 1.2	0.35 ± 0.5
II	Diakyur	3.1 ± 0.7	0.68 ± 0.8	0.37 ± 1.1

Data are the mean ± S.E. of 6 animals (One-way ANOVA).

the liver, kidney, and heart (Table 1) were unaltered in the experimental groups compared with the control group. The hematological and biochemical parameters (hepatic and renal function tests) (Tables 2, 3 and 4) did not show any significant changes in the Diakyur treated groups when compared to the control group. The histopathological section of various organs such as the liver, kidney, heart, pancreas and uterus revealed normal architecture on comparison with the control group.

In the subacute toxicity study, the Diakyur treated groups did not show any significant changes in body weight increment, indicating that it did not have any adverse effects on body weight, which is used to assess the response to therapy of drugs[17] and to indicate the adverse effects of a drug.[18] The organ (liver, kidney and heart) weights in the test drug treated groups remained normal, indicating that Diakyur was not toxic in these vital organs. Furthermore, the histopathology results indicated it was not toxic in the liver, kidney, heart, adrenals, pancreas and uterus since they all exhibited normal architecture. There were no significant changes in any liver function parameters, such as SGPT, SGOT, ALP, and liver glycogen, compared to the control group. Increase in these parameters would have

Table 2. Effects of 28 Day Administration of Diakyur on Hematological Parameters in Rats

Group	Treatment	Hb (%)	RBC (mm^3)	WBC (mm^3)	Differential count percentage			
					N	L	E	B
I	Control	11.5 ± 0.2	7.58 ± 2.3	9.7 ± 12.4	70.2 ± 2.1	29.2 ± 2.1	1.0 ± 0.3	—
II	Diakyur	12.4 ± 0.9	7.43 ± 3.1	10.0 ± 9.2	69.3 ± 5.6	30.1 ± 0.1	0.98 ± 0.7	—

Data are the mean ± S.E. of 6 animals (One-way ANOVA). Hb: Hemoglobin, RBC: Red blood cells, WBC: White blood cells, N: Neutrophils, L: Lymphocytes, E: Eosinophils, B: Basophils.

Table 3. Effects of 28 Day Administration of Diakyur on Hepatic Function in Rats

Group	Treatment	Liver glycogen (mg%)	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)
I	Control	135.2 ± 0.6	23.4 ± 4.5	57.4 ± 7.8	63.2 ± 2.3
II	Diakyur	143.2 ± 5.3	25.9 ± 6.4	61.1 ± 2.1	62.4 ± 3.6

Data are the mean ± S.E. of 6 animals (One-way ANOVA). SGPT: Serum glutamate pyruvate transaminase, SGOT: Serum glutamate oxaloacetate transaminase, ALP: Alkaline phosphatase.

Table 4. Effects of 28 Day Administration of Diakyur on Kidney Function in Rats

Group	Treatment	Blood urea (mg%)	Serum creatinine (mg%)
I	Control	17.1 ± 0.5	1.04 ± 0.8
II	Diakyur	16.9 ± 0.4	0.98 ± 0.7

Data are the mean ± S.E. of 6 animals (One-way ANOVA).

indicated hepatocyte damage.[19] The normal levels of blood urea and serum creatinine (Table 3) indicate that the test drug did not interfere with renal function and that renal integrity was preserved.[20] Also, there were no significant changes in various hematological parameters such as Hb, RBC, WBC, ESR and differential count compared to the control group, which indicates that Diakyur may not be toxic and does not affect circulating red cells, hematopoiesis, or leukopoiesis.

The present findings suggest that Diakyur is nontoxic since no marked changes in hematological, biochemical, and histopathological parameters were observed. Thus, at normal therapeutic doses, Diakyur is considered to be safe for long-term treatment in diabetic conditions.

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