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Toxicological evaluation of *Diakure*, An antidiabetic polyherbal formulation

Shawn Tomy[1], Ujwala Tk[1], Sandra Celine[1], C Senthil Kumar[2], Sam Johnson Udaya Chander J[2]*

*Corresponding author:

Sam Johnson Udaya Chander J

[1]Pharm D Intern, RVS College of Pharmaceutical Sciences, Coimbatore, Tamil Nadu, India
[2]Asst. Professor, RVS College of Pharmaceutical Sciences, Coimbatore, Tamil Nadu, India

Abstract

Context and Purpose of the Study: DiaKure is a hypoglycemic polyherbal formulation prepared indigenously based on knowledge of traditional medical practitioners, which contains polyherbal mixture of *Vetiveria zizanioides* (root), *Hemidesmus indicus* (rhizome), *Strychnos potatorum* (seed), *Salacia reticulata* (bark), *Holarhena antidysenterica* (seed), *Cassia auriculata* (bark), *Trigonella graecum* (seed) and *Acacia catechu* (bark) and each individual herb has scientific background in treating diabetes by the folk medical practitioners in various communities of India. The main aim of present study is to conduct an acute and sub-acute toxicological evaluation on DiaKure (an anti-diabetic polyherbal formulation), which was indigenously developed. The powder formulation is made into a decoction for better effect and easy administration.

Materials and Methods: In acute toxicity tests, four groups of Wistar rats were orally treated with doses of 5, 50, 300 and 2000 mg/kg/day of DiaKure, and general behaviour, adverse effects, and mortality were recorded for up to 14 days. In sub-acute toxicity study, rats received DiaKure at the doses of 200, 500, and 1000 mg/kg/day for 28 days, and biochemical, hematological, and histopathological changes in tissues (liver, kidney, heart, and brain) were determined.

Main Findings: DiaKure did not produce any signs of toxicity or mortality in the acute toxicity test. Sub-acute toxicity study with DiaKure also did not show any change in food or water consumption, hematological, or biochemical profiles. Minimal rise in body weight was noted in group III rats. Further histological study shows no necrosis or infiltration. 1000 mg/kg-treated animal showed microvesicular steatosis in individual hepatocytes.

Implications: The above data showed that DiaKure could be safe for clinical use at a dose level less than or equal to 500 mg/kg. This toxicological evaluation gives this polyherbal mixture a scientific validation to the ancestral knowledge of various communities in India.

KEYWORDS: Acute toxicity, sub-acute toxicity, DiaKure, polyherbal, antidiabetic

Introduction

Diabetes mellitus (DM) is a metabolic disorder, which has multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism that results from defects in insulin secretion, insulin action, or both. There were 171 million people in the world with diabetes in year 2000 and this is likely to increase up to 366 million by 2030 [1]. In current practice, most of the diabetic patients were treated with standard anti-diabetic drugs such as sulfonylureas, biguanides, insulin, etc. These drugs have some kind of side effects like nausea, vomiting, abdominal pain, diarrhoea, headache, insulin resistance [2], anorexia, brain atrophy and fatty liver in chronic treatment [3], etc. In contrary, herbal formulations are high in efficacy with low incidence of side effects and low cost [4]. It is estimated that three quarters of the world population rely on herbal and traditional medicine for their primary healthcare needs [5]. Throughout history, herbs had been used by all cultures, but India has one of the oldest, richest, and most diverse cultural living traditions associated with the use of medicinal plants [6]. Indian plants, which are most effective and commonly studied in relation to diabetes and its associated complications are *Vetiveria zizanioides* [7], *Gymnema sylvestre, Hemidesmus indicus* [8], *Azadirachta indica, Strychnos potatorum* [9], *Salacia reticulata* [10], *Acacia catechu* [11], *Aegle marmelos, Holarhena antidysenterica* [12], *Cassia auriculata* [13], *Trigonella graecum* [14], *Coccinia indica, and Syzygium cumini* [15]. Keeping this scientific information in mind, a polyherbal anti-diabetic formulation (DiaKure) was formulated in the research laboratory of RVS College of Pharmaceutical Sciences in collaboration with Ayurveda Medical College Coimbatore in order to reduce the side effects of the traditional allopathic medications and also to reduce the economic burden of the diabetic patients. DiaKure consists of combination of eight herbs with each drug having scientific background of producing hypoglycemia. Medicinal plants are the source of

treatment for many diseases and ailments throughout the developing world [16] because they contain various bioactive principles, which have the potential to cause beneficial and/or detrimental effects [17]. Herbs can also cause detrimental effects due to the toxicity. The earliest report of toxicity of herbs originated from Galen, a Greek pharmacist and physician, who showed that herbs do not contain only medicinally beneficial constituents, but may also be constituted with harmful substances [18]. It is expected that individual herbs in DiaKure may cause some moderate-to-severe side effects due to complex nature of their chemical compositions. Hence, this study aims to establish safety of DiaKure through validated scientific toxicity studies and protocols. The acute oral toxicity test aims at establishing the therapeutic index i.e. defined as the ratio between LD_{50} and ED_{50} and, in sub-acute toxicity study, we find out the chances of toxicity by interpreting various hematological, biochemical, and histopathological results after long-term administration of drug. To the best of our knowledge, there are no references about the safe dosage of this herbal combination, so we thought it would be worthwhile to do the toxicity study in rodents.

Materials and Methods

Experimental animals

Wistar rats weighing between 150 g and 250 g were obtained from the animal house of the Department of Pharmacology, RVS College of Pharmaceutical Sciences. The animals were randomly selected, marked to permit individual identification, and kept in their cages (three per cage) for at least five days prior to dosing to allow for acclimatisation to the laboratory conditions with free access to food and water *ad libitum*. All animal experiments were conducted in compliance with (Organization for Economic Cooperation and Development) OECD Guidelines and approved by the Institutional Animal Ethics Committee (CPCSEA: 1012/c/06/CPCSEA) of the college.

Plant material and preparation of extract

Root of *Vetiveria zizanioides*, rhizome of *Hemidesmus indicus*, seed of *Strychnos potatorum*, bark of *Salacia reticulata*, bark of Acacia catechu, seed of Holarhena antidysenterica, seed of *Trigonella graecum* and seed of Cassia auriculata were collected from Tamil Nadu and Kerala. The above said herbs were identified and authenticated by Tamil Nadu Agricultural University, Coimbatore. The products were washed thoroughly and dried in shade. The dried herbs were grinded and formulated into a powder form with proper blending. The formulated powder was stored in a well-closed air-tight container.

Acute toxicity study

The acute toxicity study was carried out according to the OECD Guideline 423 [19, 20]. Animals were fasted prior to dosing (food, but not water, were withheld overnight). Following the period of fasting, the animals were weighed. In this study, twenty four Wistar rats divided into four groups of six (3 male and 3 female) rats each were used and were given 5, 50 and 300 and 2000 mg/kg of the decoction of DiaKure (p.o.). After drug administration, the food was withheld for three hours. The animals were observed continuously for the first two hours, then occasionally up to six hours and then daily up to 14 days post treatment to observe for any symptoms of toxicity or mortality. Daily observations on the changes in skin and fur, eyes and mucus membrane (nasal), autonomic effects (salivation, lacrimation, gauntness and piloerection) and gait, tremors and convulsion (central nervous system) were carried out and changes were noted. Lethal dose 50 (LD_{50}) and effective dose 50 (ED_{50}) of DiaKure were calculated by using the following formulae:

$$LD_{50} = \text{higher dose} - \Sigma (a \times b)/n$$

Where, a = dose difference
b = animal died
n = No. of animals in each group
$ED_{50} = LD_{50}/10$

Subacute toxicity study

Twenty four Wistar rats divided into four groups of six (3 male and 3 female) rats each were used. Three different doses viz., 200 mg/kg, 500 mg/kg and 1000 mg/kg were selected for the study. The doses were selected according to the OECD Guideline 407. Group 1 served as the control and received 4 ml/kg of distilled water while groups 2-4 received 200 mg/kg, 500 mg/kg and 1000 mg/kg of the DiaKure decoction for 28 days. All the rats were observed for any physiological and behavioral changes and mortality. The animals were weighed initially and every seven days. Food and water consumption was checked daily. On the first and last days of the study, all the rats were anaesthetized using chloroform and blood samples collected through retro-orbital plexus and used for the estimation of hematological parameters and biochemical parameters.

Hematological parameters

Blood samples were collected in collecting tubes containing EDTA and hematological tests including total hemoglobin (Hb), total white blood cell (WBC) count, differential leukocyte count, total red blood cell (RBC) count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count were performed with an automatic cell counter (ERMA PCE 210) in both control and DiaKure-treated groups [21].

Biochemical estimations

Blood was collected in collecting tubes and was centrifuged without additives at 3000 xg at 4 ℃ for 10 minutes. Serum was separated and stored at -20 ℃ until use. Biochemical parameters such as aspartate transaminase (AST), alanine transaminase (ALT), random blood glucose, urea, creatinine and lipid profile were determined by colorimetric assays with Reckon kit (Robonick) in both control and DiaKure-treated groups [21].

Organs weight and histology

After the blood collection, the rats were sacrificed and were quickly dissected. The liver, kidneys, heart, and brain were excised and observed for any signs of lesions and weighed after thorough washing with normal saline. The small pieces of each organ were kept in 10% neutral, buffered formalin solution for 48 hours and processed for histopathological studies. The pieces of organs harvested were dehydrated with alcohol of increasing grade (70, 80, 90, 95%, and absolute), cleared with xylene and embedded in paraffin. Micrometer sections were stained with hematoxylin and eosin and were examined by a pathologist under a light microscope, and photomicrographs of the samples were recorded.

Statistical analysis

Statistical comparison was performed using one way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons. All statistical analysis was performed using SPSS statistical version 17.0 software package (SPSS Inc., USA).

Results

Acute toxicity study

There was no mortality or signs of toxicity up to the limit dose of 2000 mg/kg in DiaKure-treated rats. All 24 rats were normal throughout the study and survived until the end of the 14-day experiment period. Experimental observations were recorded systematically for each group. Special attention was given for the observations of tremor, convulsion, salivation, diarrhoea, lethargy, sleep and coma. The observed data were charted in Table 1.

Table 1: Changes in wellness parameters observed for Diakure treated Wistar rats.

S.no	Response	Group 1 (5 mg/kg)		Group 2 (50 mg/kg)		Group 3 (300 mg/kg)		Group 4 (1000 mg/kg)	
		Before	After	Before	After	Before	After	Before	After
1	Alertness	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
2	Grooming	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
3	Anxiety	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
4	Roaming	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
5	Tremor	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
6	Convulsion	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
7	Depression	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
8	Gripping strength	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
9	Scratching	Present	Present	Present	Present	Present	Present	Present	Present
10	Defecation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
11	Writhing	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
12	Pupils	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
13	Urination	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
14	Salivation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
15	Skin and fur	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
16	Lacrimation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
17	Pilo erection	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
18	Nail status	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
19	Gauntness	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
20	Gait	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
21	Diarrhoea	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
22	Sleep	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
23	Coma	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
24	Lethargy	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
25	Mucous membrane	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

Sub-acute toxicity study

General observations

Oral administration of DiaKure at doses of 200, 500, and 1000 mg/kg body weight daily for 28 days did not produce any signs of toxicity or mortality. The animals did not show any changes in general behavior or other physiological activities and were found normal throughout the study.

Physical parameters

Little or no change was observed in food consumption and water intake in DiaKure (200, 500, and 1000 mg/kg)-treated groups compared with control group after 28 days of study period in rats (Tables 2 and 3). DiaKure caused a statistically significant ($p<0.01$) rise in body weight among animals in group III.

Table 2: Effect of 27 days treatment of DiaKure on body weight of Wistar rats.

Group	0th day	7th day	14th day	21st day	28th day
Group I (Control)	203.0±1.91	206.5±2.11	208.5±0.99	211.66±1.08	213.33±0.88
Group II (200 mg/kg)	208.0±2.90	208.0±2.0	210.66±1.05	212.33±0.99	213.67±1.58
Group III (500 mg/kg)	208.33±2.95	211.0±1.82	211.66±0.95	215.17±1.14	218.67±0.67**
Group IV (1000 mg/kg)	201.0±2.63	208.33±1.11	209.5±1.09	212.83±1.14	215.17±0.60

*Values are expressed as mean ± SEM of 6 animals (one-way ANOVA). The values are statistically different from control at p<0.01***

Table 3: Effect of 27 days treatment of DiaKure on food intake and water intake of Wistar rats.

Group	Item	0th day	7th day	14th day	21st day	28th day
Group I (Control)	Food	17.67±0.88	21.33±0.92	22.5±1.38	27.33±0.91	26.17±1.11
	Water	20.83±0.83	22.33±0.71	24.17±0.7	21.0±1.34	25.17±1.4
Group II (200 mg/kg)	Food	18.83±1.14	20.83±1.08	22.17±1.51	27.83±0.83	26.83±1.19
	Water	17.83±0.83	19.83±0.48*	24.83±1.01	22.0±0.82	24.67±0.88
Group III (500 mg/kg)	Food	21.33±1.26	21.0±1.18	23.83±1.01	24.5±0.99	28.67±0.88
	Water	20.83±0.95	20.67±0.67	27.5±0.99*	24.67±0.33*	26.0±0.73
Group IV (1000 mg/kg)	Food	22.0±0.97*	21.0±0.97	24.67±1.12	24.5±1.43	28.17±1.95
	Water	21.67±0.88	18.67±0.84**	25.66±0.49	25.33±0.76**	24.83±1.25

Values are expressed as mean ± SEM of 6 animals (one-way ANOVA). The values are statistically different from control at p<0.05, p<0.01***

Hematological studies

Statistically significant change in platelet count was observed among DiaKure-treated animals. DiaKure did not produce any statistically significant change in hemoglobin level, MCV, MCH, MCHC and WBC count when compared with the control group animals, which is shown in Table 4.

Table 4: Effect of 27 days treatment of DiaKure on hematological parameters of Wistar rats.

Parameters	Sex	Day	Control	200 mg/kg	500 mg/kg	1000 mg/kg
Hb	M	0	15.9±1.76	12.6±1.1	15.60±0.65	13.76±1.95
		28	14.3±1.29	13.73±1.97	12.76±1.07	15.80±1.77
	F	0	13.63±1.31	12.96±1.22	13.86±0.72	15.23±2.06
		28	16.53±2.06	15.3±0.65	16.23±1.9	14.33±2.35
RBC	M	0	7.33±0.32	9.11±0.34*	8.98±0.57*	7.89±0.23
		28	6.83±0.35	7.41±0.24	9.98±0.62***	5.9±0.18
	F	0	8.38±0.30	10.53±0.42***	6.98±0.20*	9.93±0.26***
		28	8.1±0.32	8.31±0.36	7.85±0.18	8.01±0.29
WBC	M	0	11.40±1.22	12.10±1.36	8.26±4.0	10.10±0.63
		28	10.30±0.79	10.40±0.80	13.0±0.61	13.53±1.12
	F	0	13.66±1.89	11.86±1.16	12.6±0.71	11.93±0.33
		28	12.46±1.5	12.60±1.05	12.9±1.16	12.16±1.42
Platelet Count	M	0	667.50±7.83	632.8±10.88*	627.0±3.17**	806.16±7.20***
		28	740.5±9.35	626.33±4.98***	705.33±7.17**	596.16±6.66***
	F	0	764.83±8.07	732.50±9.64*	751.33±7.28	856.50±5.42***
		28	831.33±10.56	734.33±7.92***	792.33±6.05**	700.16±5.08***
MCV	M	0	58.43±1.22	61.43±2.54	57.83±1.33	58.36±1.41
		28	55.0±1.73	54.40±0.80	59.2±0.96	54.0±0.90
	F	0	62.0±1.51	62.20±1.94	63.20±0.75	59.76±1.91
		28	57.13±0.84	53.13±1.56	63.10±1.30*	53.66±1.63
MCH	M	0	20.33±1.13	19.70±1.06	19.86±1.58	19.56±1.08
		28	18.96±1.30	20.53±1.09	19.66±1.84	19.90±0.96
	F	0	19.23±1.51	18.96±1.64	18.86±1.65	19.63±1.08
		28	20.03±1.52	18.86±1.47	19.90±1.51	18.76±1043
MCHC	M	0	31.20±1.51	30.50±1.45	32.40±2.37	29.86±2.10
		28	30.90±2.03	29.20±1.21	30.93±1.94	32.76±2.37
	F	0	30.96±1.72	31.50±2.49	31.30±2.65	30.03±2.94
		28	29.20±2.14	30.60±1.84	32.40±1.06	31.63±1.58
N	M	0	8.83±0.30	9.5±0.34	12.83±0.74***	9.33±0.42
		28	11.66±0.21	9.0±0.36***	12.33±0.33	10.0±0.36**
	F	0	9.5±0.42	8.66±0.33	11.66±0.21**	10.66±0.49
		28	12.83±0.47	11.83±0.40	12.0±0.51	13.16±0.40
L	M	0	67.16±1.62	87.83±1.30***	69.5±1.36	64.16±1.30
		28	71.33±1.68	79.50±1.52**	69.16±0.74	84.16±1.57***
	F	0	78.0±1.65	83.6±1.57	80.50±1.28	74.16±1.7
		28	81.50±1.38	81.0±1.73	84.83±1.13	83.5±0.99
E	M	0	2.0±0.44	1.16±0.47	1.66±0.88	1.66±0.33
		28	3.16±0.65	1.0±0.25	2.33±0.66	2.0±0.57
	F	0	1.66±0.61	1.33±0.55	2.33±0.33	1.66±0.66
		28	1.66±0.55	1.66±0.55	3.0±0.0	1.0±0.0
M	M	0	0	0	0	0
		28	0	0	0	0
	F	0	0.75±0.31	0.67±0.24	0.56±0.12	0.79±0.31
		28	0.69±0.22	0.65±0.28	0.88±0.22	1.02±0.11
B	M	0	0	0	0	0
		28	0	0	0	0
	F	0	0.6±0.69	0.4±0.9	0	0
		28	0.2±0.4	0.5±0.8	0.4±0.78	0.7±0.22

Values are expressed as mean ± SEM of 6 animals (one-way ANOVA); the values are statistically different from control at p<0.05, p<0.01** and p<0.001***.M-Male; F-Female; N-Neutrophils; L-Lymphocytes; E-Eosinophils; M-Monocytes; B-Basophils*

Biochemical analysis

All groups except group IV animals showed no significant change in biochemical parameters measured. Group IV animals treated with DiaKure 1000 mg/kg showed an elevated level of aspartate transaminase (AST) and alanine transaminase (ALT) after 28 days of study. Statistically significant variations were found in hepatic and renal biomarkers, shown in Table 5.

Table 5: Effect of 27 days treatment of DiaKure on biochemical parameters of Wistar rats.

Parameters	Sex	Day	Control	200mg/kg	500 mg/kg	1000 mg/kg
Random Glucose	M	0	87.13±2.42	88.26±3.79	87.63±3.47	87.40±3.53
		28	90.20±2.10	90.83±2.62	88.96±3.68	88.46±2.79
	F	0	83.36±1.70	90.93±3.11	87.76±1.73	90.30±2.16
		28	86.60±3.54	90.00±2.20	92.56±1.73	86.80±2.42
AST	M	0	30.63±2.08	33.16±2.7	32.83±2.5	32.9±2.0
		28	31.03±2.47	31.73±2.1	31.63±2.1	40.43±2.06*
	F	0	32.70±2.45	32.03±2.83	32.9±2.80	32.40±2.07
		28	31.50±2.45	31.0±1.72	31.80±2.52	42.16±2.07*
ALT	M	0	35.03±2.80	36.16±2.30	36.23±2.19	36.0±2.36
		28	35.36±2.33	34.60±2.25	35.66±1.44	44.60±2.34*
	F	0	33.90±2.40	35.76±1.90	35.26±1.93	34.06±2.27
		28	34.90±2.32	35.96±2.39	36.30±2.22	47.56±1.61**
Urea	M	0	18.53±3.23	27.70±2.95	31.0±3.23	27.93±3.75
		28	22.40±3.94	34.40±3.46	29.73±1.76	30.43±3.32
	F	0	19.23±2.88	28.03±1.77	34.93±4.26	41.56±2.79
		28	22.16±3.20	32.53±3.55	21.66±2.73	36.96±3.47*
Creatinine	M	0	0.39±0.26	0.52±0.19	0.44±0.06	0.52±0.05
		28	0.28±0.02	0.34±0.05	0.39±0.04	0.72±0.03***
	F	0	0.42±0.23	0.32±0.05	0.47±0.08	0.57±0.10
		28	0.47±0.16	0.36±0.06	0.59±0.10	0.94±0.09*

Values are expressed as mean ± SEM of 6 animals (one-way ANOVA); the values are statistically different from control at p<0.05, p<0.01** and p<0.001****.*
M-Male; F-Female

Lipid profile

Lipid profile parameters like total cholesterol, LDL, VLDL, HDL and triglycerides were found to be normal for DiaKure-treated rats when compared with control groups. This can be seen in Table 6.

Table 6: Effect of 27 days treatment of DiaKure on Lipid profile of Wistar rats.

Parameters	Sex	Day	Control	200 mg/kg	500 mg/kg	1000 mg/kg
Total Cholesterol	M	0	93.70±3.79	93.96±3.01	91.36±2.80	87.66±3.19
		28	98.76±2.76	92.30±2.84	89.90±1.96	88.53±3.25
	F	0	109.66±3.83	91.33±3.16**	92.90±2.46**	89.26±2.05**
		28	113.80±2.55	93.63±2.62**	98.56±3.03***	89.10±2.40***
Triglycerides	M	0	87.03±6.32	104.90±1.96*	98.53±2.80	59.70±3.66**
		28	112.33±7.03	104.93±2.80	98.90±1.82	61.60±1.53***
	F	0	113.80±5.48	104.86±2.46	97.16±2.88*	60.73±1.23***
		28	99.33±7.56	106.10±2.54	98.90±1.82	58.86±1.76***
HDL	M	0	41.16±5.52	42.93±2.07	39.90±2.75	38.46±3.65
		28	42.83±4.16	44.40±2.42	40.76±2.88	39.10±3.52
	F	0	45.43±4.27	43.93±3.30	41.03±3.03	40.53±3.17
		28	44.53±2.86	46.03±2.44	39.80±3.43	38.36±2.89
LDL	M	0	38.23±4.12	34.43±2.34	34.50±3.67	28.30±2.33
		28	36.46±4.68	33.60±2.71	32.70±3.10	29.50±2.03
	F	0	32.76±4.10	34.86±3.07	33.08±2.14	30.03±2.83
		28	48.33±4.07	36.30±2.48	33.76±3.27*	29.13±3.09**
VLDL	M	0	17.96±2.23	22.46±3.40	17.90±1.74	14.50±2.23
		28	25.70±4.93	23.46±3.00	18.76±3.41	13.50±2.44
	F	0	22.30±3.09	23.16±3.15	20.56±3.18	14.63±2.17
		28	24.56±3.88	24.20±2.70	18.46±2.46	14.13±1.24

Values are expressed as mean ± SEM of 6 animals (one-way ANOVA); the values are statistically different from control at p<0.05, p<0.01** and p<0.001***. M-Male; F-Female; HDL-high density lipoprotein; LDL-low density lipoprotein; VLDL-very low density lipoprotein*

Organs weight and histology

The organs like kidney, heart, and brain isolated in various groups did not show any abnormalities in their gross examinations and difference in their mean weights both in treated and control groups (Table 7).

Table 7: Effect of 27 days treatment of DiaKure on isolated organs of Wistar rats.

Group	Brain	Heart	Kidney	Liver
Group I (Control)	1.68±0.02	0.98±0.04	1.45±0.08	5.12±0.11
Group II (200 mg/kg)	1.77±1.02	0.95±0.09	1.53±0.07	5.38±0.17
Group III (500 mg/kg)	1.72±0.06	1.03±0.08	1.48±0.08	5.33±0.14
Group IV (1000 mg/kg)	1.62±0.11	1.03±0.11	1.45±0.08	5.17±0.14

Values are expressed as mean ± SEM of 6 animals (one-way ANOVA). Values are not statistically different.

The following figures show the photomicrographs of brain, liver, kidney, and heart of DiaKure-treated animals.

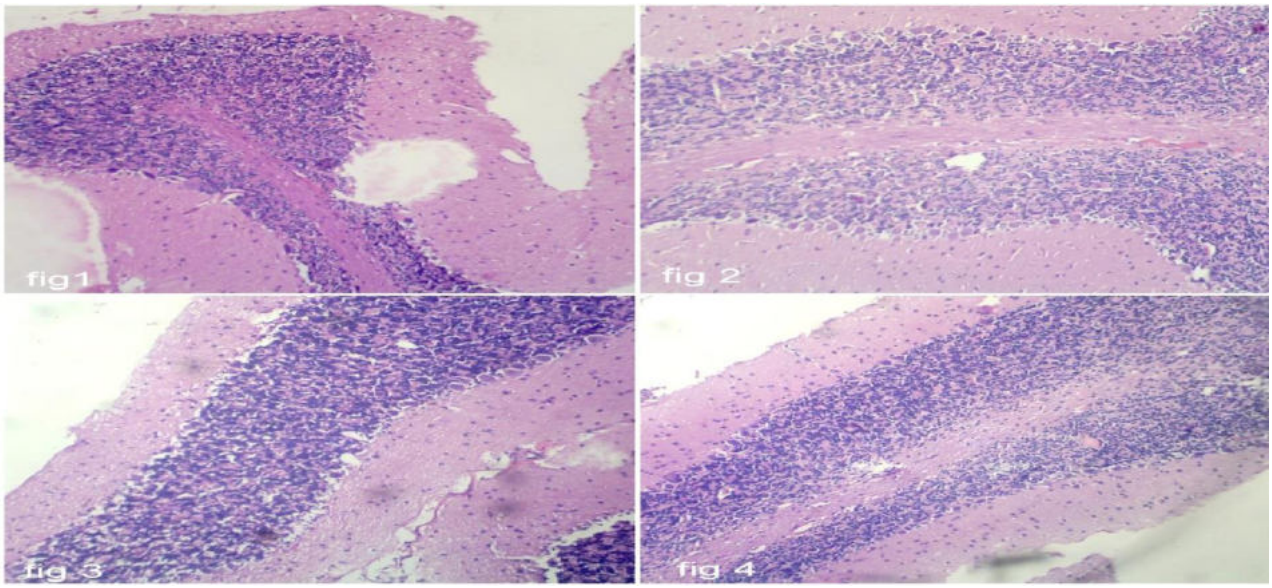

Figure 1: Histology of Brain of control group and DiaKure-treated groups. Brain section from control group (fig 1) shows normal cerebellum with brain parenchyma showing normal morphology, no evidence of neuronal degeneration. Fig 2, 3 and 4 shows brain cells from DiaKure 200, 500 and 1000 mg/kg-treated groups respectively, which also exhibits normal cerebellum with brain parenchyma showing normal morphology and no evidence of neuronal degeneration.

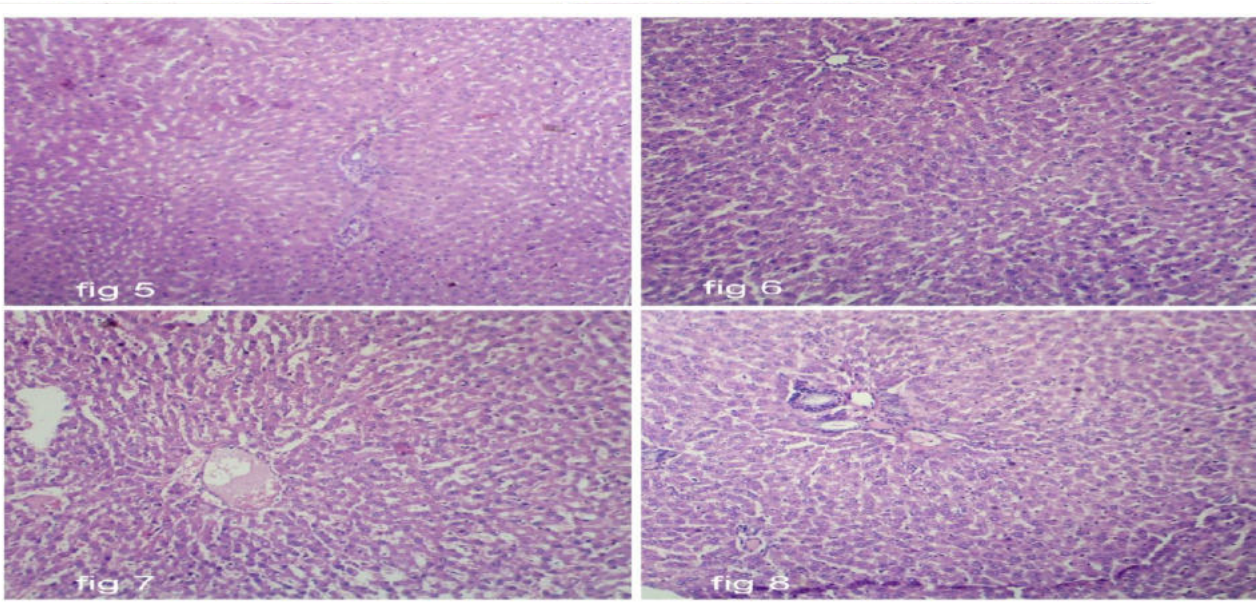

Figure 2: Histology of liver of control and DiaKure-treated animals. Section of liver from control animals (fig 5) revealed normal lobular architecture and there is no evidence of binucleation cytoplasmic vacuolation or inflammation; the hepatocytes of 200 and 500 mg/kg treated groups (fig 6 and 7) also show normal lobular architecture and there is no evidence of binucleation cytoplasmic vacuolation or inflammation, but the 1000 mg/kg treated group (fig 8) showed normal lobular architecture, but individual hepatocytes show microvesicular steatosis. The central vein showed congestion and the portal triad showed bile duct hyperplasia. The sinusoids were dilated.

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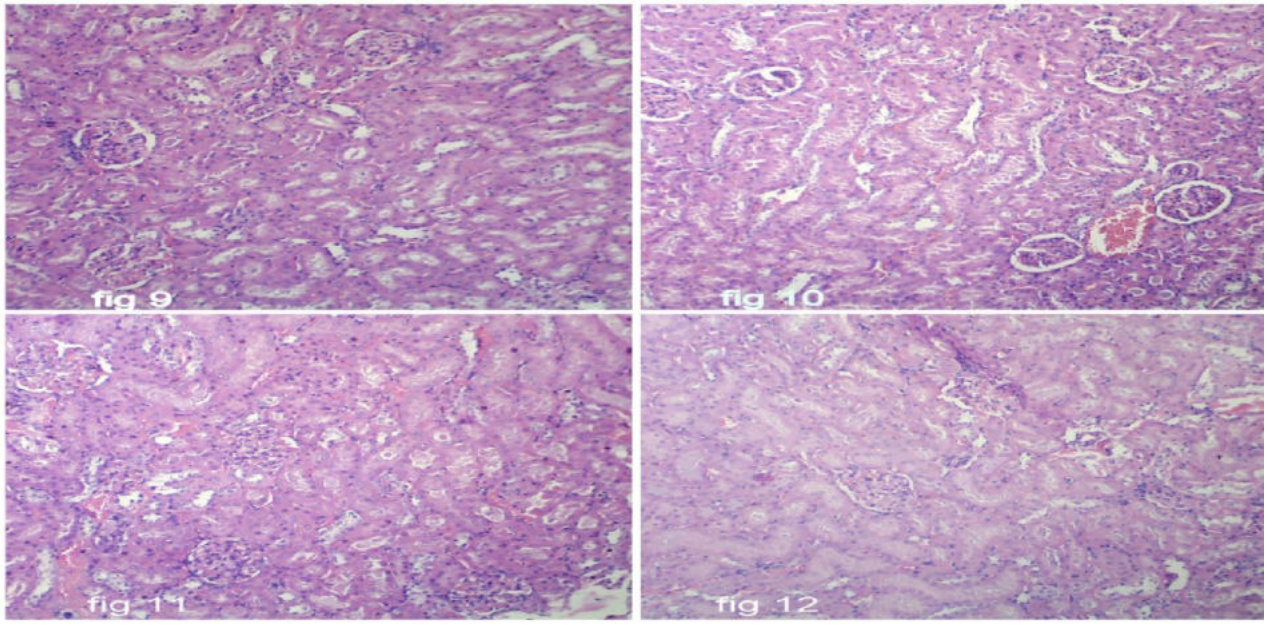

Figure 3: Histology of kidney of control and DiaKure-treated animals. Section of kidney from control animal (fig 9) shows normal medulla, cortex and glomeruli. The tubulointerstitial compartments show unremarkable findings. The collecting duct of the medulla also shows normal morphology. There is no evidence of inflammation or necrosis. Renal histology of DiaKure-treated groups (fig 10, 11 and 12) were also found to be same as control group.

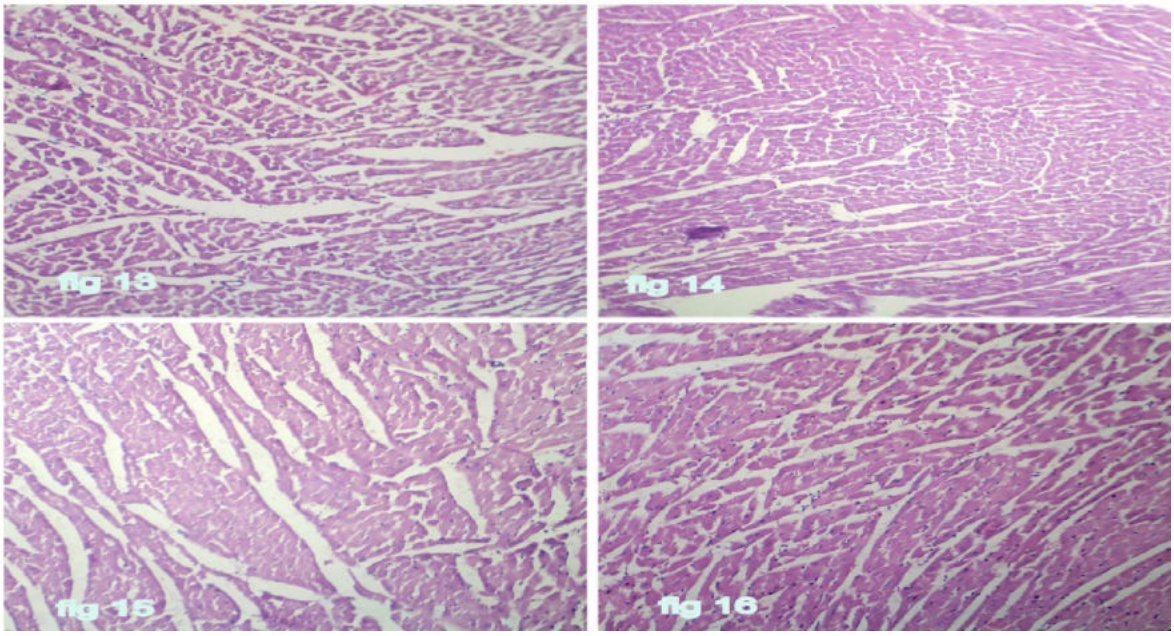

Figure 4: Histology of heart of control and DiaKure-treated animals. Section of heart from control animal (fig 13) showed normal myocardium with myocytes. The blood vessels were unremarkable. There was no evidence of myocytic degeneration or edema or inflammation; the DiaKure-treated rats (fig 14, 15 and 16) also exhibit the same microscopy.

Discussion

In the present study, acute and subacute toxicology of antidiabetic polyherbal formulation DiaKure, prepared from different types of herbal antidiabetic drugs with proven activity, was evaluated.

DiaKure did not show any signs or symptoms of toxicity in rats at doses up to 2000 mg/kg p.o., indicating that it has no toxicity at the maximal doses tested in this work. The acute toxicity study indicated that DiaKure at a dose 2000 mg/kg caused neither visible signs of toxicity nor mortality. The LD50 and ED50 of the drug were estimated as 2000 mg/kg and 200 mg/kg respectively. If LD50 is 2000 mg/kg, it could be Generally Regarded As Safe (GRAS). This finding is in agreement with Clarke and Clarke [22], who reported that any compound or drug with oral LD50 estimates greater than 1000 mg/kg body weight could be considered to be of low toxicity and safe. However, it is suggested that variables such as animal species, strain, age, gender, diet, bedding, ambient temperature, caging conditions, and time of the day can all affect the LD50 values obtained and as such were considerable uncertainties in extrapolating the LD50 obtained for species to other species. This clearly suggests that LD50 may not be considered as a biological constant [23]. Oral administration of DiaKure at doses of 200, 500, or 1000 mg/kg body weight daily for 28 days did not produce any signs of toxicity or mortality. The animals did not show any changes in general behavior or other physiological activities and were found normal throughout the study. A 28-day study provides information on the effects of repeated oral exposure and can indicate the need for further longer-term studies. It can also provide information on the selection of concentrations for longer-term studies. All animals were observed for morbidity and mortality twice daily. No signs and symptoms of toxicity, changes in behavior or other physical and physiological abnormalities were observed during the experimental period except for a positive rise in body weight of animals in Group III.

Hematological examinations showed a statistically significant change in platelet count among DiaKure-treated animals. DiaKure did not produce any statistically significant change in hemoglobin level, MCV, MCH, MCHC and WBC count. Biochemical parameters like random blood glucose, urea, creatinine, and lipid profile did not show any difference with the above doses of DiaKure compared to control group. The group IV, DiaKure 1000 mg/kg-treated animals, showed an elevated level of aspartate transaminase (AST) and alanine transaminase (ALT) after 28 days of study, indicative of hepatocellular effects. Measurements of additional enzymes (of hepatic or other origin) and bilirubin may provide useful information under certain circumstances. Lipid profile parameters like total cholesterol, LDL, VLDL, HDL and triglycerides were found to be normal for DiaKure-treated rats when compared with control groups. The organs like kidney, heart, and brain isolated in various groups did not show any abnormalities in their gross examinations and difference in their mean weights both in treated and control groups. Several studies have shown that medicinal plant products are not completely safe, particularly in the liver [24, 25]. The histological study of the liver of group IV showed that the lobular architecture was maintained, but individual hepatocytes showed microvesicular steatosis, central vein showed congestion, portal triad showed bile duct hyperplasia, and the sinusoids were dilated. A full histopathological examination should be carried out on the preserved organs and tissues of all animals in the control and high-dose groups to ascertain the extent of effect.

Conclusion

Complementary and alternative medicines (CAMs) such as herbal remedies require thorough safety and efficacy evaluation due to their growing use all over the world [26]. Although many traditional herbal remedies were available and some have been verified by clinical trials, their safety is often still questioned by consumers. Herbal remedies are considered safer and less damaging to the human body than synthetic drugs. However, the lack of standardization has been a major concern regarding use of herbal medicines [27]. By these acute and sub-acute toxicity studies of anti-diabetic polyherbal formulation DiaKure, it is concluded that it could be very useful in its future clinical study. The formulation did not show any signs of toxicity up to 2000 mg/kg of dose in acute toxicity study. Dose up to 500mg/kg was found to be safe in sub-acute toxicity study. 1000 mg/kg of DiaKure showed minimal signs of hepatotoxicity after 28 days of oral administration. Further studies are needed to characterize the pharmacological and safety profile of the formulation.

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Conflict of Interest

None.

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