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Phytochemical screening and Safety evaluation of hydroalcoholic extract of *Dendrophthoe falcata* Ettingsh: Summary of acute and subacute toxicological data

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ABSTRACT

The hydroalcoholic extract (HEDF) from the aerial parts of the hemiparasitic plant D. falcata, was evaluated for acute and subacute toxicity with the hope that the results would provide information on the safety of this extract prior to the evaluation of its efficacy in humans. In rats, single intraperitoneal injections of HEDF (1.0 – 2.5 g/kg) induced a regular dose-dependent increase in the death rate and incidence of general behaviour adverse effects. In sub acute toxicity studies, three doses of 250, 475 or 950 mg/kg/day of HEDF were administered orally for a period of 28 days. Phytochemical analysis revealed that polyphenols, terpenes and steroids were major compounds. The LD_{50} value after acute intraperitoneal doses was 1.75 g/kg. In the open field arena, HEDF (475mg/kg and 950mg/kg p.o.) reduced the number of rearing episodes and locomotion, while number of urine spots increased. In subacute tests, haematological analysis showed a significant ($p < 0.01$) increase in WBC count. In the blood chemistry analysis, a transient decrease in AST activity was observed whereas cholesterol level was decreases in animals that received high dose of extract. Pathologically, neither gross abnormalities nor histopathological changes were observed. Collectively these data demonstrate that HEDF are relatively safe in rats; however, assessment of hepato-biliary function should be done during chronic use in humans.

Key words: *Dendrophthoe falcata*, phytochemical screening, Acute and subacute toxicity study, LD_{50} values, haematology, blood chemistry analysis.

INTRODUCTION

A World Health Organization survey indicated that about 70 – 80% of the world's populations rely on non-conventional medicine, mainly of herbal sources, in their primary healthcare. This is especially the case in developing countries where the cost of consulting a western style doctor and the price of medication are beyond the means of most people [1]. Although medicinal plants may produce several biological activities in humans, generally very little is known about their

toxicity and the same applies for the mistletoe species *Dendrophthoe falcata* (L.f) Ettingsh, as safety should be the overriding criterion in the selection of medicinal plants for use in healthcare systems [2].

D. falcata, commonly known as 'Banda' (Hindi) is an evergreen hemiparasitic shrub with bark smooth grey, leaves opposite unequal, thick 1.6 - 25.4 cm long, flowers single, orange-red or scarlet softly pubescent, berries soft ovoid-oblong,1.3cm diameter and indigenous to India, Srilanka, Thailand, Indo-china, Australia [3]. The aerial parts are used in wounds, menstrual troubles, asthma, psychic disorders, pulmonary tuberculosis, consumption and mania by the tribal of India[4, 5]. Leaf paste is used in skin diseases [6]. Its paste is applied on boils, setting dislocated bones and extracting pus [7]. Mistletoe is known as one of medicinal plant used in traditional therapy such as cough medicine, cancer treatment and after birth treatment [8, 9]. Traditional physicians of Korku, a tribe inhabiting the forest areas of Melghat region of Amravati district, Maharastra state of India, use *Dendrophthoe falcata* as antifertility agent in women [10]. Leaf paste is also used in abortion [6]. In addition to its medicinal value, the fruit of *D. falcata* tastes sweet and is consumed as a food [11]. The plant has been scientifically proved to have antilithiatic, diuretic, cytotoxic, immunomodulatory, antioxidant, wound healing, chemopreventive and antitumor activities [12-17]. The merit of the traditional use of *D. falcata* has also been reported by the isolation and identification of several possible active chemical constituents such as oleanolic acid derivatives, β-sitostirol, stigmasterol [18], kaemferol, quercetin–3-O-ramnoside, rutin, quercetin, myrecitin and their glycosides [19], (+)-catechin, leucocyanidine, gallic acid, chebulinic acid [20] and some pentacyclic triterpenes, kaempferol-3-O-α-l-rhamnopyranoside and quercetin-3-O-α-l-rhamnopyranoside, etc. [11]. In the present study, we have described a range of toxicological tests carried out to investigate the biosafety of a hydroalcoholic extract from aerial parts of *Dendrophthoe falcata* after acute and subacute exposure of the extracts to experimental rats.

MATERIALS AND METHODS

Plant material and extract preparation

Fresh aerial parts of *D. falcata*, grown on the host plant *Azadirachta indica* were collected in the month of March from the thick forest areas of Similipal biosphere reserve, Mayurbhanj district of Orissa, India. *Dendrophthoe falcata*(L.f)Ettingsh (Loranthaceae) was preliminarily authenticated by Dr. N.K. Dhal, Department of Natural products, Regional Research Laboratory (RRL), Bhubaneswar, India which was latter on confirmed from Botanical Survey of India, Hawrah, West Bengal, India (CNH/I-I/32/2010/Tech.II/237). One set of the herbarium has been preserved in our laboratory for future reference. The aerial parts were air-dried, pulverized to a coarse powder in a mechanical grinder, passed through a 40 mesh sieve and extracted in a soxhlet extractor with ethanol-water (8:2). The extract was decanted, filtered with Whatman No. 1 filter paper and concentrated at reduced pressure below 40 ^{0}C through rota vapor to obtain dry extract (20.6% w/w). Hydroalcoholic extract from the aerial parts of *Dendrophthoe falcata* (HEDF) was kept at 4^{0}C.

Phytochemical screening

An attempt was also made to observe the presence and absence of different phytochemical constituents in the hydroalcoholic extract. The test for flavonoids, sterols and tannins were carried out by using the methods previously described by Tona et al. (1998) [21]. Two milligrams of each extract were separately dissolved in 2 ml of the adequate solvent. The detection of major chemical groups was carried out by thin-layer chromatography (TLC) on silica gel 60 F_{254} from Merck (Dramstadt, Germany) (layer thickness, 0.25mm) as follows: for

flavonoids, TLC was developed in n-Butanol/acetic acid/water 4:1:5, then spots were visualized with 1% $AlCl_3$ solution in methanol under ultraviolet (UV) 366 nm. Terpenes and steroids were detected with Libermann-Burchard as a reagent using n-hexane/CH_2Cl_2 1:9 as a mobile phase. A range of colors are produced after heating sprayed plates for 10 min at 100^0C. Tannins were detected with $FeCl_3$. Each class of tannins gave a specific coloration. And alkaloids (Dragendorff's test), glycosides (Molisch's reagent), fixed oil (spot test), proteins (Ninhydrine test) were detected according to standard methods [22].

Animals used

Wistar albino female rats (130 - 160 g) were selected for the experiment. Six rats were taken for each group. The rats were used after acclimatization to the laboratory environment for a 7-day period. They were kept in the departmental animal house at 26±2°C at relative humidity 44–55% and light dark cycles of 10 and 14 h, respectively. Animals were provided with rodent diet and water *ad-libitum*. The animal experiment was performed according to the institute's animal ethical committee approval and guidelines Reg. no. 621/02/ac/CPCSEA of Birla Institute of Technology, Mesra, India.

Acute toxicity studies

For acute toxicity study evaluation, the hydroalcoholic extract of *Dendrophthoe falcata* was aseptically suspended in Tween – 80 (1% v/v) and administered in single intraperitonial (i.p.) doses of 0, 1, 1.25, 1.5, 1.75, 2, 2.25 and 2.5 g/kg. The general behaviours of the rats were continuously monitored for 1h after dosing, periodically during the first 24h (with special attention given during first 4h) [23], and then daily thereafter, for a total of 14 days. Changes in the normal activity of rats and their weights were monitored and the time at which signs of toxicity or death appeared. All surviving animals were euthanized with diethyl ether at day 14 and the external appearance of viscera, lungs, stomach, intestine, liver, kidney, spleen and brain were carefully noted and any apparent and significant features or differences from the norm were recorded.

Determination of LD_{50} values by Graphical method

The LD_{50} determination for HEDF was performed as described by Graphical method [24] from the acute toxicity data, in intra peritoneal route of administration. The toxicological effect was assessed on the basis of mortality, which was expressed as a LD_{50} value. The percentage of mortality was converted to probits and the values were plotted against log dose. The LD_{50} was the dose intersected by probit 5. In the groups with no dead animals and in the groups with only dead animals, the obtained percentages were corrected using the following formulae:

Correction formula for 0% dead group = 100(0.25/n)
Correction formula for 100% dead group = 100[(n - 0.25)/n]

Where n represents the number of animals in the group. After correction, the percentages were converted into probits. The values thus obtained were plotted against log dose. The LD_{50} value was determined by finding the dose that was intersected by probit 5.

Open field test

The open field apparatus was a white rectangular (77 cm × 55 cm × 7 cm [l × w × h]) enclosure with a flat floor divided into subdivisions of 121 cm^2 each. It was kept in a quiet room and illuminated by centrally positioned 40W tungsten lamp pending 150 cm from the floor level. Rats were individually exposed to the open field for 10 min, 15 min after being treated with HEDF (250, 475 and 950 mg/kg body wt) or its vehicle and an observer sat adjacent to the

apparatus recorded the following behavioral parameters: ambulation (number of subdivisions traversed), rearing (number of rearing episodes), grooming (number of grooming episodes), number of urine spots and defecation (number of fecal bolus left in the arena).

Subacute oral toxicity

Dosing of animals in repeated dose toxicity

The repeated dose toxicity was carried out following OECD guideline 407 [25]. This toxicity study was conducted on four groups of rats (0 mg/kg control, 250 mg/kg low dose (~ 0.05 × LD_{50} oral), 475 mg/kg medium dose (~ 0.1 × LD_{50} oral) and 950 mg/kg high dose (~ 0.2 × LD_{50} oral) [26]) for 28 days. From another group of six animals, blood was collected by sacrificing the individuals and the baseline measurements of haematology and blood chemistry analysis were done. HEDF was orally administered using gavage to test groups, Tween – 80 (1%v/v) was administered to control group. The maximum volume administered was not greater than 2 ml/100g body weight.

Body weight changes

All rats were weighed and observed for any physiological and behavioral changes on each alternate day during the treatment period. Any rat that died during the test period was tested pathologically, and all animals were examined at the end of the test period.

Vital organ weights

Qualitative data on weights of vital organs such as the brain, heart, lungs, liver, spleen, pancreas, kidney and ovaries of the rats were assessed immediately on an AB265-S Mettler Toledo, electronic balance for subsequent analysis by carefully dissecting each organ from sacrificed animal into 10% solution of buffered formalin (pH 7.4).

Biochemical and haematological analyses

At the end of the experimentation (the 28^{th} day), all surviving animals were fasted overnight, and anesthetized afterwards for blood collection from the right ventricle. Blood samples were collected into three tubes: (1) 3.2% buffered sodium citrate tubes; (2) heparinized centrifuge tubes; (3) dry non-heparinized centrifuze tubes. A blood analysis (haematology, coagulation and chemistry) was carried out. The blood in the sodium citrate tubes were used for prothrombin time (PT) and partial thromboplastin time (PTT) estimation [27]. The heparinized blood was used for the haematological study which included red blood cell count (RBC), haemoglobin concentration (Hb), hematocrit (Ht), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet (PLT) [28], total white blood cell count (WBC) and white blood cell differential count [29]. The non-heparinized blood was allowed to coagulate before being centrifuged and the serum separated. The serum was assayed for albumin [30], aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) [31], total protein [32], total bilirubin [33], creatinine [34], triglyceride, cholesterol [35], glucose and blood urea nitrogen (BUN) [36].

Histopathological analysis

After blood collection for biological analysis, all the animals were euthanized and the principal vital organs were removed and macroscopically analyzed. After macroscopic analysis, representative fragments of liver, kidney, lung, pancreas, heart, spleen, brain and ovary were subsequently fixed in 10% solution of buffered formalin (pH 7.4) and enclosed in paraffin. Five-micrometer sections were obtained and cooled with haematoxylin-eosin for evaluation under an optical microscope [37].

Statistical analysis

Pharmacological data were analysed by one-way analysis of variance (ANOVA) followed by Dunnett's *t* test with equal sample size. The difference was considered significant when *p* value < 0.05. All the values were expressed as mean ± standard error mean (SEM).

RESULTS

Phytochemical screening

The results of preliminary phytochemical screening of the crude ethanolic extract revealed the presence of steroids, terpenes, glycosides, tannins, proteins, flavonoids and fixed oil (Table 1).

Table 1: Phytochemical screening of extract from *D. falcata* aerial parts.

Phytoconstituents	Observations
Alkaloids	-
Flavonoids	++++
Glycosides	++
Steroids	++++
Terpenes	++++
Tannins	++++
Fixed oil	+
Proteins	+

Note: (-): not detectable. (+): Low quantities. (++): average quantities. (++++): high quantities. Based on the intensity of colour.

Table 2: Toxicity of a single dose of a hydroalcoholic extract of *Dendrophthoe falcata* administered by intraperitonial route to experimental rats

Dose (mg/kg body wt)	Log dose	D/T	Percent mortality	Mortality latency (h)	Corrected mortality (%)	Probit	Symptoms
0	-	0/6	0	-	-	-	No
1000	3.0	0/6	0	-	4.1	3.25	Piloerection, hypoactivity
1250	3.097	1/6	17	>24, <96	17	4.05	Piloerection, ataxia
1500	3.176	3/6	50	>24,<48	50	5.00	Hypoactivity
1750	3.243	3/6	50	>12,<48	50	5.00	Lethargy
2000	3.301	4/6	67	>12,<24	67	5.44	Hypoactivity, lethargy Ataxia
2250	3.352	5/6	83	>1, <12	83	5.95	Hypoactivity, lethargy, ataxia
2500	3.398	6/6	100	>1, <12	95.83	6.75	Hypoactivity, lethargy, ataxia

All treated animals were carefully observed for any sign of toxicity. D/T, dead/treated rats; No, no symptoms; latency, time elapsed between dosing and death.

Acute toxicity studies

Except for a reduced locomotion (hypoactivity) and piloerection, no signs of toxicity were noted in rats treated with single intraperitonial dose of 1 g/kg body wt HEDF (Table II). There was a regular dose-dependent increase in mortality and decrease in mortality latency of rats after the i.p. administration of *Dendrophthoe falcata* extract. The first rat died between 24 to 96 h after injection of the 1.25 g/kg dose of HEDF, and the maximum frequency of death occurred at 2.5 g/kg within 1 to 12 h after the injection. In addition to death, the plant extract also induced minor to accentuated hypoactivity, ataxia and lethargy.

Determination of LD_{50} value

From the acute toxicity study data, the LD_{50} was also determined and was found to be 1.75 g/kg for intraperitonial administrations (Table 2, Fig. 1).

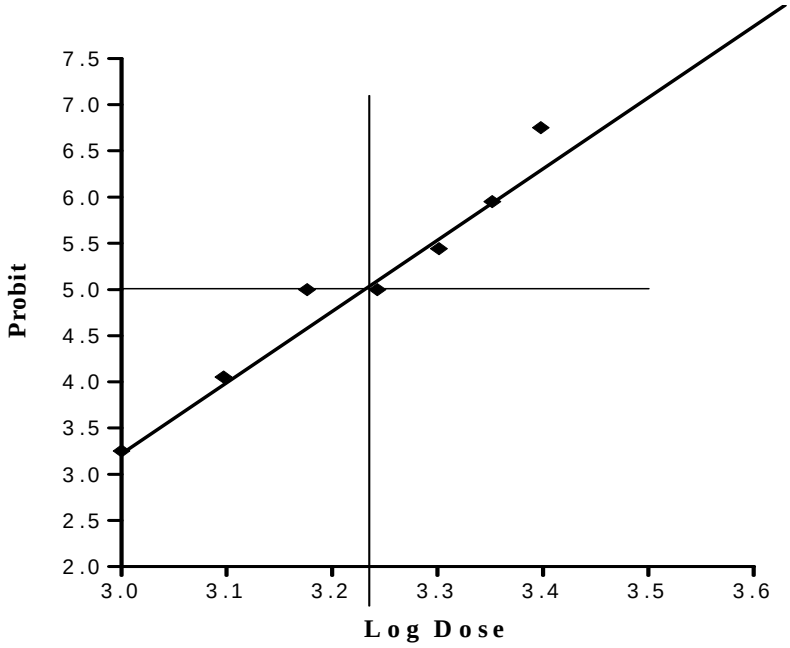

Fig.1. Determination of LD_{50} value for the hydroalcoholic extract of aerial parts of *D. falcata* after, intraperitoneal administration; LD_{50} = log 3.24 = 1738.00mg/kg ~ 1.75 g/kg.

Behavioral effects in the open field arena

As shown in table 3, numbers of rearing episodes and locomotion were markedly reduced, while frequency of grooming behaviour and defecation remained unaltered, in rats exposed to the open field arena 15 min after the oral dose of HEDF at single oral doses of 250, 475 and 950 mg/kg body wt. The preceding results indicated that locomotor exploration was decreased by oral treatment with HEDF, a judgment that is consistent with the signs of hypoactivity recorded at acute toxicity study. Also, the number of urine spots were increased significantly in the open field arena, when compared with the vehicle treated group.

Table 3: Effect of a single oral dose of *Dendrophthoe falcata* hydroalcoholic extract on the open field behaviour of rats

Treatment Group	Dose (mg/kg)	Locomotion (N)	Rearing (N)	Defecation (N)	No. of urine spots (N)	Grooming (N)
Control	0	162±13.08	46.6±5.2	3±0.6	1.67±0.17	4.0±0.58
I	250	102±4.16**	37.6±1.7	2±0.5	2.33±0.3*	3.3±0.61
II	475	72.3±6.38**	28.3±4.3*	1.60±0.3	2.5±0.22*	3.0±0.51
III	950	54±8.72**	28±2.08*	2±0.57	2.83±0.4**	3.3±0.6

Group I was treated with control (tween 80, 1%v/v); II, III & IV treated with the plant extract. N, Number; Locomotion (number of squares traversed), rearing and grooming episodes, number of urine spots and defecation (fecal boluses) were measured during 10 min, starting 15 min after treatment by gavage with the extract or its vehicle (distilled water) alone. Values are shown as mean ± standard error mean (S.E.M.). Comparisons were made between control group (I) with II, III & IV; *statistically significantly different from control ($p < 0.05$); ** statistically significantly different from control ($p < 0.01$).

Subacute toxicity

General signs

Except for hypoactivity that appeared within 5-10 min and persisted for a few hours (1-12 h) after dosing, no deaths or significant changes in general behaviour or other physiological activities were observed at any point in the present study after the subacute exposure of 28 days to the experimental animals.

Body weight and vital organ weight changes

There were no significant changes either in corporal weight (Fig. 2) or in the weight of the principal organs (Table 4) of the extract treated and control group of animals. All animals exhibited a gain in body weight.

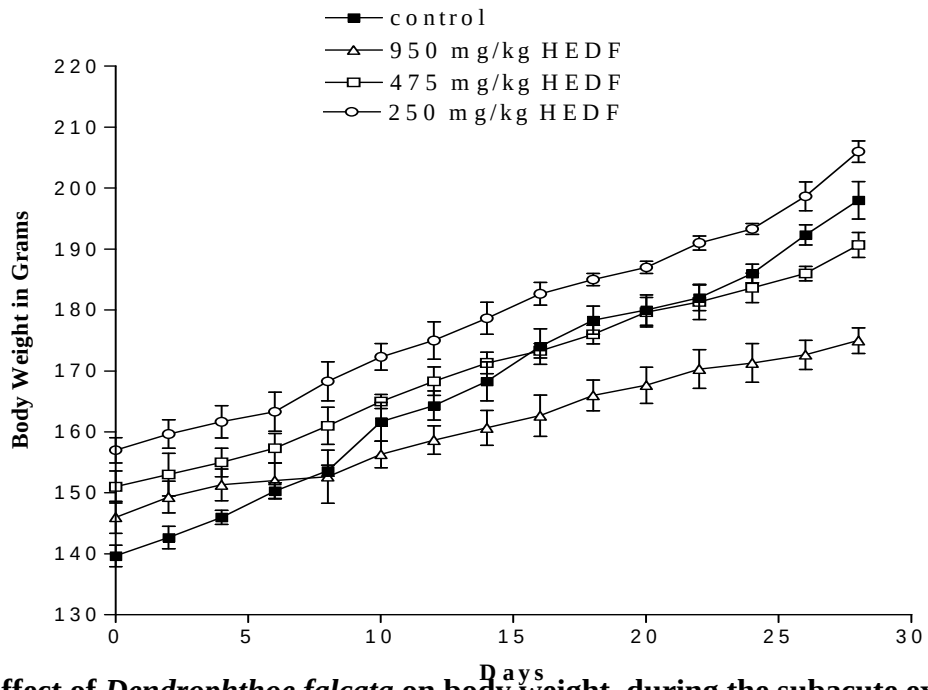

Fig. 2: Effect of *Dendrophthoe falcata* on body weight, during the subacute exposure to wistar rats (28 days).

Table 4: Effect of *Dendrophthoe falcata* hydroalcoholic extract on relative weight of vital organs with respect to body weight

Vital organs	Control group (g/100g)	Group I (250 mg/kg body wt)	Group II (475 mg/kg body wt)	Group III (950 mg/kg body wt)
Brain	0.79±0.25	0.78±0.02	0.81±0.01	0.79±0.03
Heart	0.44±0.01	0.45±0.015	0.45±0.03	0.45±0.01
Lungs	0.54±0.02	0.44±0.018	0.49±0.008	0.48±0.01
Liver	4.82±0.2	4.36±0.21	4.66±0.26	4.46±0.29
Spleen	0.34±0.01	0.33±0.031	0.33±0.01	0.32±0.02
Kidney	0.79±0.03	0.78±0.035	0.75±0.03	0.74±0.04
Stomach	0.83±0.01	0.82±0.006	0.85±0.03	0.82±0.02
Pancreas	0.53±0.02	0.5±0.01	0.54±0.02	0.51±0.01
Ovaries	0.064±0.02	0.073±0.012	0.07±0.005	0.06±0.005
Mortality (%)	Nil	Nil	Nil	Nil

Value are expressed as mean ± standard error mean (S.E.M.) for six rats. Comparisons were made between control group with Group I, II and group III; statistically no significant ($p > 0.05$) difference was observed as compared to the control.

Table 5: Hematological values of rats in subacute toxicity of the hydroalcoholic extracts from *Dendrophthoe falcate*

Parameters	Dose group (data expressed in mean ± S.E.M, n = 6)				
	Control		HEDF treated (mg/kg body wt) at day 28		
	Group C_0 (Day 0)	Group C_{28} (Day 28)	Group I (250)	Group II (475)	Group III (950)
Hb	15.3±0.3	14.5±0.37	13.3±0.4	13.067±1.09	14.46±0.78
Ht	40.3±0.88	39.067±1.9	38.3±1.45	40.9±0.73	37.67±0.46
RBC	8.4±0.8	8.7±0.26	8±0.265	8.3±0.39	8.14±0.33
MCH	19.23±1.14	18.5±0.56	20.067±0.88	20.66±0.42	19.67±0.21
MCHC	28.5±0.5	32.76±1.3	30.36±0.49	29.5±0.54	32.9±2.7
WBC	6.2±0.49	6.8±0.29	9.86±0.52**	11.1±0.41**	13.93±0.31**
PLT	943.3±41.5	936.6±38.94	942±28.9	923.3±82.2	961.3±81.87
Lym	81.8±1.36	84.3±0.88	76.9±0.66**	75±0.57**	70.6±0.8**
Neu	12.8±1.3	11±1.0	20.6±0.49**	21.4±0.52**	26.1±0.56**
Mon	2.43±0.26	2.73±0.32	2.03±0.29	1.9±0.12	1.93±0.35
Eos	1.6±0.13	1.4±0.09	1.62±0.13	1.4±0.1	0.99±0.06

*HEDF, Hydroalcoholic extract of D. falcata ; Hb, Hemoglobin concentration(g/dl); Ht, Hematocrit (%) ; RBC, Red blood cell (× 10^6 mm^{-3}); MCH, Mean corpuscular haemoglobin (pg); MCHC, Mean corpuscular haemoglobin concentration (g/dl) ; WBC, White blood cell (×10^3 mm^{-3}) ; Lym, Lymphocytes (%) ; Neu, Neutrophiles (%) ; Mon, Monocytes (%); Eos, Eosinophiles (%). Comparisons were made between group I (baseline measurement) with group II (vehicle treated), III, IV & V (Extract treated); ** statistically significantly significant (p <0.01) form control at day 0 and day 28.*

Hematological and Plasma biochemical data

The status of bone marrow activity and intravascular effects were monitored by haematological examination as summarized in table 5. The haematological parameters, hematocrit, haemoglobin concentration, platelets, red blood cells, MCH and MCHC in the treated rats did not differ significantly ($p > 0.05$) from that of the control group and all the values remained within normal limits at the end of the experimental period. While the leukocyte count showed a significant ($p < 0.01$) increase in all three extract treated groups compared to the control. This variation is well correlated with a significant increase in neutrophils ($p < 0.01$) and lymphocytes ($p < 0.01$). Moreover neutrophils and lymphocytes were found to be significantly elevated in all groups.

As shown in Table 6, no significant treatment-related changes in the levels of plasma analytes such as albumin, ALT, ALP, total protein, total billirubin, creatinine, triglyceride, glucose and BUN activities were observed at the termination of the study. However, there appeared to be a significant increase in the serum AST activities for the control, low dose (250 mg/kg) group, when compared with that for the control group at day 0 ($p < 0.01$). These differences may be biologically relevant but were not considered treatment-related because they did not occur with any consistent relationship to the dose. The difference in AST levels was even observed in the control group at the termination of the study (control 28th day) versus control group at the start (control day 0), suggesting that the AST activity levels increased with age (time) [38]. Also, the cholesterol level was decreased significantly for the high dose (950 mg/kg/day) treated group.

Table 6: Blood chemistry values of rats in subacute toxicity of the hydroalcoholic extracts from *Dendrophthoe falcate*

Parameters	Dose group (data expressed in mean ± S.E.M, n = 6)				
	Control		HEDF treated (mg/kg body wt) at day 28		
	Group C_0 (Day 0)	Group C_{28} (Day 28)	Group I (250)	Group II (475)	Group III (950)
ALB	3±0.11	3.2±0.37	3.33±0.22	3.6±0.058	3.83±0.12
AST	183.7± 5.93	288.5± 5.54*	262.5± 6.02*	194.7± 6.28**	175.2± 2.8**
ALT	51± 1.08	55.7± 1.79	52.25± 2.71	45.75± 2.28	44.75± 1.54
ALP	107±2.85	113.7±2.72	99.2±3.09	99.7±1.79	97.7±1.25
TOP	6.73±0.12	6.46±0.37	6.2±0.351	7.06±0.145	6.26±0.27
TOB	0.23±0.02	0.26±0.01	0.21±0.005	0.231±0.008	0.237±0.029
CRE	0.65±0.03	0.58±0.02	0.6±0.01	0.61±0.015	0.55±0.02
TRG	40.65±0.33	43.76±1.86	40.03±0.48	40±0.61	40.33±0.3
COL	70.3±0.88	68±1.53	64.67±1.20	63.6±0.89	62.3±1.2**
PT	11.34±0.73	12.11±0.8	11.96±0.68	11.58±0.68	13.46±0.49
PTT	29.4±0.94	30.16±0.66	27.63±3.57	25.3±3.06	26.63±2.76
GLU	136±4.04	150.7±5.18	134.2±5.6	146.75±6.9	142.2±4.75
BUN	17.6±1.2	18.5±0.52	19.4±1.3	18.6±1.4	20±1.34

HEDF, Hydroalcoholic extract of D. falcata; ALB, Albumin (g/dl); AST, Aspartate transaminase (UI/L); ALT, Alanine transaminase (UI/L); ALP, Alkaline phosphatase (UI/L); TOP, Total protein (g/dl); TOB, Total bilirubin (mg/dl); CRE, Creatinine (mg/dl); TRG, Triglycerides (mg/dl); COL, Cholesterol (mg/dl); PT, Prothrombin time; PTT, Partial thromboplastin time; GLU, Glucose (mg/dl); BUN, Blood urea nitrogen (mg/dl) ; * Statistically significantly different from control at day 0 ($p < 0.01$), ** Statistically significantly different from control at day 28 ($p < 0.01$).

Histopathological analysis

Pathological examinations of the tissues on a gross basis indicated that there were no detectable abnormalities. No alterations were seen in the microscopic examination of the internal vital organs; the cellular appearances were unremarkable in all extract treated and control groups.

DISCUSSION

Although poisonous plants are ubiquitous [39], herbal medicine is used by up to 80% of the population in developing countries. Despite widespread use, few scientific studies have been undertaken to ascertain the safety and efficacy of traditional remedies. In the present study, the hydroalcoholic extract of *Dendrophthoe falcata* was found to be non-toxic via intraperitonial route in rats, at least up to maximum doses of 1000 mg/kg body wt acutely and 950 mg/kg subacutely. Mortality and other adverse effects occurred only in rats receiving relatively higher doses of HEDF (LD_{50} = 1.75 g/kg via i.p. route). Based on the classification of Loomis, and Hayes [40] (1996), viz. that substances with LD_{50} between 500 and 5000 and between 5000 and 15,000 mg/kg body weight are regarded as being slightly toxic and practically non-toxic, respectively. The present results suggested that the biosafety of *D. falcata* falls between these categories. In the subacute toxicity study, HEDF, despite the dose used, did not appear to affect the body weight and caused no significant changes in their food intake and utilization of food indicating normal metabolism in the animals and suggesting that, at the oral doses administered, the extract did not hold back the growth of rats. The number of urine spots increased in open field arena for all the doses of HEDF. These findings are related to the results previously described by Alleykutty et al. (1993) [12], which has been shown that the extract of the plant possess diuretic activity. Also, the open field test showed that HEDF, at single oral doses reduced exploratory behaviour of the animals, a finding that is consistent with the hypoactivity noted in the acute and sub acute toxicity assays.

Hepatic and renal function has been monitored by the evaluation of the serum level of transaminases (ALT, AST) and alkaline phosphatase, respectively [41, 42]. Among these AST and ALT are well known enzymes used as biomarkers predicting possible toxicity [43]. Generally, damage to the parenchymal liver cells will result in elevations of both these transaminases [44]. In this study, the AST activity levels were increased only in the control rats (on day 28) and those treated with low dose of the extract (250 mg/kg body wt) when compared with the control rats on day 0 (baseline measurement), while the AST activity level was maintained at the other relatively high dose treatment. This suggests that the AST level increased with age, but that ongoing treatment with high dose of HEDF removed this elevation, sturdily signifying that *D. falcata* may have a liver protecting effect. This finding is well collaborative of the previous report that, the extracts from *D. falcata* have been shown to lower the transaminase levels, induced by the hepatotoxicant [45]. Certainly, Huang et al. proved that AST level increased with age [38]. Apart from liver, AST is also present in a wide variety of tissues including heart, skeletal muscle, kidney and brain. Moreover, AST can exist as a microenzyme by forming a complex with an immunoglobulin and the immunoglobulin-complexed macromolecule can cause an elevation in serum AST activity, which may be detected in blood chemistry analysis and erroneously be considered to indicate the presence of liver disease [46]. It is thus possible that *D. falcata* might influence these mechanisms to lower the AST levels along with acting on the liver.

The oxidative effect of various hepatotoxicants is the main cause of the intoxication of liver [47]. The HEDF extract react as a hepatoprotector since it was shown to be a polyphenolic rich extract particularly in flavonoids that are antioxidant components [18]. For instance, flavonoids reduced

glutathione *tert*-butylhydroperoxide-induced and the lipid peroxidation that may strongly contribute to cellular damage [48, 47]. No alteration in liver weight was observed, as well as histopathological analyses revealed no significant change, as shown by a normal lobular architecture and portal space containing arterioles, venule and bile ducts. The change in WBC count induced by the hydroalcoholic extract of *D. falcata* is also well concerted with the earlier study [13].

In conclusion, this study provides precious preliminary data on the toxicity profile of *D. falcata* that should be constructive for the planning of future preclinical and clinical studies of this plant medicine. The *D. falcata* hydroalcoholic extract appears to be relatively non-toxic, causes no apparent organ damage and can be used as therapeutic agent in treating the reported diseases effectively. This article establishes the safety of *D. falcata* for trial from the systematic scientific stand-point.

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