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Investigation of Acute, Sub-Acute, Chronic Oral Toxicity and Mutagenicity of *Coleus forskohlii* Briq. Hydroethanolic Extract, Standardized for 10% Forskolin in Experimental Animals

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ABSTRACT

Objective: To investigate acute, sub-acute and chronic toxicity of *Coleus forskohlii* Briq. (Lamiaceae) (CF) hydroethanolic root extract standardized for 10% forskolin, in male and female Wistar rats by oral route. **Methods:** In the single dose oral toxicity study, groups of five healthy male and female rats were dosed orally with CF extract (10% forskolin) at 2000 mg/kg body weight and were observed once daily for 14 consecutive days for toxicity, general behavior and pharmacological effects. In sub-acute oral toxicity, the test substance was administered for 28 days with daily doses of 100, 300 and 1000 mg/kg body weight. In chronic oral toxicity study, groups of 20 male and 20 female rats were subjected to 10% forskolin for 180 days at daily dose levels of 500 mg/kg and 1000 mg/kg body weight. Ames test was also performed to evaluate the test substances' ability to induce reverse mutations. **Results:** No deaths were reported in all the toxicity studies performed. No significant changes observed in the hematology and serum biochemistry values from the control group animals. The body weight changes and necropsy results were normal. There was no apparent progression of organ damage in any of these toxicity tests. Furthermore, CF extract (10% forskolin) did not produce any significant toxic effects in Wistar rats at 1000 mg/kg body weight and had no potential to induce mutagenicity. **Conclusion:** In conclusion, the "No Observed Adverse Effect Level (NOAEL)" of CF extract was determined to be above 1000 mg/kg b.wt/day.

INTRODUCTION

Coleus forskohlii Briq. (Lamiaceae) is an aromatic herb indigenous to India. The herb (Fig.1) received a lot of attention over the past 40 years from medical researchers as the only significant plant source of forskolin, a bioactive diterpene compound with diverse pharmacological benefits. Plants of the *Coleus* species have been used as herbal medicine to treat various disorders of the cardiovascular, respiratory, gastrointestinal, and central nervous systems since ancient times [1]. The roots of this folk medicine, *Coleus forskohlii* (Makandi), have a long history of food use in India, in the form of pickle or condiment. In the northern parts of India, the paste of roots of the plant is used by local people as topical application on tumors and boils. In south India, the decoction of the roots is used as a tonic by the tribals of Trichigadi (Kotas). The active phytochemical forskolin in *C. forskohlii* (CF) was discovered in 1974 with vast array of effects on the body.

Fig. 1. *Coleus forskohlii* Briq.

Forskolin lowers intraocular pressure (IOP) in rabbits, monkeys, and man [2]. Extensive studies were done by Majeed et al and demonstrated successfully the IOP lowering effects of forskolin, in both animal [3] and human studies [4, 5] at different strengths. Ocular toxicity of forskolin has also been well established (Unpublished results, Muhammed et al.). Results from other studies suggest that CF may help mitigate weight gain in overweight females [6]. Forskolin could possibly be used as a therapeutic agent for weight management and treatment in obese men [7]. Additionally, forskolin is more effective than sodium cromoglycate in preventing asthma attacks in patients with mild persistent or moderate persistent asthma [8].

Despite its huge beneficial effects, its oral toxicity data has not been reported in any scientific literature till date. The present study was designed to investigate the acute, subacute, chronic toxicity of CF extract standardized for 10% forskolin in male and female Wistar rats. Also, with no available literature on its mutagenicity, bacterial reverse mutation assay of CF extract having 10% forskolin with an independent repeat assay was performed to evaluate its mutagenic potential, if any. Forskolin's (Fig. 2) IUPAC name is [(3*R*,4a*R*,5*S*,6*S*,6a*S*,10*S*,10a*R*,10b*S*)-6,10, 10b-trihydroxy-3,4a,7,7,10a-pentamethyl-1-oxo-3-vinyldodecahydro-1*H*-benzo[*f*]chromen-5-yl] acetate.

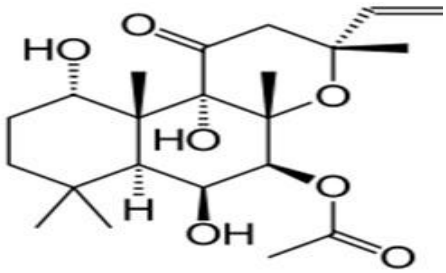


Fig. 2. Chemical structure of forskolin; (PubChem CID: 47936)

MATERIALS AND METHODS

Herb and Animals

Sufficient quantity of *Coleus forskohlii* (CF) hydroethanolic root extract standardized for 10% forskolin was supplied by Sami Labs Limited, Bangalore to carry out the toxicity studies. The test material supplied by Sami Labs was light brown to brown coloured powder and it was used for animal studies only after passing all the quality control procedures. While an assay by HPLC (G1377A, Agilent 1100, CA) revealed presence of 10.17% of forskolin in the supplied material, it passed other quality control procedures for microbes and heavy metals. Animals were housed in an environment controlled room at 22 ± 3 °C and relative humidity of 30% to 70% with the photoperiod of 12 h of light and dark cycles. Five rats of each sex per cage were housed in sterilized standard polycarbonate cages with steam sterilized clean paddy husk changed along with the cage thrice a week. Pelleted rat diet of fixed amount of 25 g was given to each animal daily and water given *ad libitum.*

Chemicals

For reverse mutation assay, methyl methane sulphonate (MMS), sodium azide, nitrofluorene and 2 amino - anthracene were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 9-Amino acridine was purchased from Fluka - Sigma Aldrich (St. Louis, MO, USA). The *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 strain WP2uvrA were purchased from Molecular Toxicology Inc. (Boone, NC, USA). Rat liver S9 fraction induced by aroclor in male Sprague - Dawley rats was purchased from Molecular Toxicology Inc. (Boone, NC, USA). The chemicals and solvents used throughout the experiments were of analytical grade.

Ethics

All the animal toxicity studies were performed after having a favorable opinion from the institutional animal ethics committee and in accordance with the recommendation of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for laboratory animal facility published in the gazette of India, December 15th 1998. Though the test material was supplied in the form of powder by Sami Labs Limited, all the rules set forth in National Biodiversity Authority, an autonomous and statutory body under the Ministry of Environment and Forests, Government of India, 2004, were strictly adhered to in all aspects while utilization of the biological resources and traditional knowledge from the tribals prior to this study.

Toxicological evaluation

Acute oral toxicity [9]

Following a quarantine period of atleast five days, five healthy male and five healthy, non-pregnant and nulliparous female Wistar albino rats with a mean body weight of 251 g were randomly assigned to the treatment groups. Animals were housed 5/sex/cage in suspended wire cages. The test article was mixed with corn oil to make dosing by gavage possible. The dose was based on the dry weight of the test article. Single dose was administered orally by syringe at a dose level of 2000 mg/kg body weight (b.wt). Animals were observed 1, 2 and 4 h post dose and once daily for 14 days for toxicity and pharmacological effects. The animals were observed

twice daily for mortality. Body weights were recorded immediately pretest, weekly, and at study termination.

Repeated dose 28 day oral toxicity study [10]

Sixty Sprague Dawley, 30 male and 30 female, healthy rats were randomly divided into six groups of 5 animals per sex and the individual animal was fur marked with picric acid. These group animals were assigned to doses of 0 mg/kg b.wt (control), 0 mg/kg b.wt (recovery), 100 mg/kg b.wt, 300 mg/kg b.wt, 1000 mg/kg b.wt and 1000 mg/kg b.wt (recovery) respectively. Rats were assigned five per sex per cage wise and the females were nulliparous and nonpregnant. CF extract suspended in 0.1% carboxy methyl cellulose was administered to animals through oral gavage at respective dose levels of 100 mg/kg b.wt, 300 mg/kg b.wt, 1000 mg/kg b.wt (dose volume of 10 ml/ kg b.wt) to main and recovery group animals. The test substance suspensions were freshly prepared every day for 28 days. The control animals were administered vehicle only. Dose levels for the 28 day sub acute study were based on the recommended limit test dose of the OECD guidelines. There was no administration of test item formulation / vehicle during the 15 day period for the recovery group. All treated and control rats were observed twice daily throughout the study duration. The body weights, food consumption of all rats were recorded on test day 1 (immediately prior to oral application) and then on weekly basis (immediately prior to the sacrifice for necropsy). Laboratory investigations were carried out prior to sacrifice on completion of dosing period on day 29 and at the end of post-dosing recovery period on day 43, in animals' fasted over-night. Blood samples were collected from orbital sinus on the following morning using sodium heparin and analyzed using Rx Daytona (Randox) autoanalyser system for blood chemistry. Similarly, potassium EDTA tubes were used for hematology and analysis was performed using Beckman Coulter hematology analyzer. Prothrombin time analysis was conducted using citrate buffer. All animals were sacrificed on day 29, except for recovery group animals which were sacrificed on day 43. Necropsy of all animals was carried out and the weights of various organs were recorded. All findings such as clinical signs of intoxication, body weight changes, food consumption, hematology, blood chemistry, gross and histopathology observations were recorded.

Chronic (180 days) oral toxicity study [11]

Groups of twenty male and twenty female Wistar rats were subjected to daily administration of CF extract (10% forskolin) by oral gavage for 180 days at the dose levels of 0 mg/kg b.wt (control), 500 mg/kg b.wt and 1000 mg/kg b.wt, and were sacrificed after completion of 180 days to evaluate its toxicity. Additionally two groups of ten rats per sex at 0 mg/kg b.wt and 1000 mg/kg b.wt were further observed for a period of 28 days following the 180 days' treatment, for assessment of reversibility, persistence or delayed occurrence of toxicity. The rats were examined daily for signs of toxicity and mortality. They were subjected to detailed clinical examination before initiation of the study and weekly thereafter during the exposure recovery period, and at termination. Body weight and food consumption were recorded weekly. Laboratory investigations were performed on blood (hematology and serum chemistry) and urine at the end of three months and at termination of the study. Histopathological evaluation was performed in control and high dose group rats and on tissues showing gross pathological changes in low dose and recovery group rats.

Bacterial reverse mutation assay [12]

The test article, *Coleus forskohlii* extract, was tested in the bacterial reverse mutation assay with an independent repeat assay using *Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* tester strain WP2 *uvr*A in the presence and absence of Aroclor-induced rat liver S9. The assay was performed in two phases, using the plate incorporating method. The first phase, the preliminary toxicity assay, was used to establish the dose-range for the mutagenicity assay. The second phase, the mutagenicity assay, (initial and independent repeat assays), was used to determine the mutagenic potential of the test article. A solubility test was conducted to select the vehicle. The test was conducted using one or more of the following solvents in the order of preference as listed: water, dimethyl sulfoxide (DMSO), ethanol (EtOH), and acetone. The test article was dissolved to determine the vehicle, selected in order of preference, that permitted preparation of the highest soluble or workable stock concentration, upto 500 mg/mL. Standard protocol [13] was followed for conducting this test. Dimethyl sulfoxide (DMSO) was selected as the solvent of choice based on compatibility with the target cells and solubility of the test article.

Statistical analysis

The statistical analysis of the experimental data was carried out by the analysis (co-) variance followed by Student's 't' test and Cochran't' test. Statistical comparisons were evaluated at the 5 % ($P \leq 0.05$) significant level. In the case of recovery groups also, data was analyzed using the methods stated above. Comparison of means between treatment groups was performed. Student's 't' test was employed for bacterial reverse mutation assay results.

RESULTS

Acute oral toxicity

CF extract showed no mortality and did not exhibit any clinical signs of toxicity in the acute, sub-acute, and sub-chronic toxicity studies. Based on the acute toxicity findings, an LD_{50} value could not be determined, as the LD_{50} describes only one end point, i.e. death. Thus the results obtained in the study suggested that the LD_{50} of CF extract may be higher than 2,000 mg/kg b.wt, as no lethality was found in the rats exposed to that concentration throughout the experimental period of the study.

Repeated 28 day oral toxicity

Body weight, food consumption, clinical and ophthalmological examination

In the 28 day toxicity study, both male and female animals from control and different dose groups exhibited normal body weight gain throughout the dosing period of 28 days. Animals from 1000 mg/kg recovery groups exhibited normal body weight gain at the end of recovery period on day 42, when compared with the body weight gain of control (Fig. 3a). During the dosing period and the post-dosing recovery period the quantity of food consumed by animals from different dose groups was found to be comparable with that of control animals (Fig. 3b). Animals were free of intoxicating clinical signs and survived throughout the dosing period of 28 days in the control and treated groups and also during the recovery period. The eyes of control and all dose group animals were examined prior to the initiation of the dosing, in week 4 of the study. It did not reveal any dose related changes in the ophthalmic examination (data not shown).

Hematological analysis

Hematological investigation's conducted at the end of dosing period on day 29 and at the end of recovery period on day 43, revealed following significant changes in the values of different parameters studied when compared with that of respective controls, however the values obtained were within normal biological and laboratory limits. Decreased MCHC values were obtained for animals from male 100 mg/kg and 300 mg/kg dose groups, sacrificed on day 29 ($p<0.05$) and increased MCH values were obtained for animals from 1000 mg/kg recovery dose group, sacrificed on day 43 ($p<0.05$). In females, decreased MCH values were obtained for animals from 100 mg/kg dose group, sacrificed on day 29 ($p<0.05$). Table 1 depicts hematological values of all the animal groups.

Table 1. Effect of 28 days oral exposure to CF extract on mean hematology data of male & female Wistar rats at necropsy

Parameter	Sex	Control	Control (Recovery)	Dose (mg/kg-d)			
				100	300	1000	1000 (Recovery)
Hb (g%)	Male	14.52 ± 1.06	16.24 ± 1.45	14.18 ± 1.05	14.02 ± 0.84	14.82 ± 1.01	15.86 ± 1.03
	Female	13.88 ± 1.52	14.56 ± 1.67	13.42 ± 1.12	13.46 ± 0.98	14.24 ± 0.72	15.00 ± 1.66
Total RBC (10^6/cmm)	Male	7.50 ± 0.40	4.45 ± 0.85	7.35 ± 0.41	7.37 ± 0.68	7.69 ± 0.46	7.84 ± 0.58
	Female	6.88 ± 0.77	7.40 ± 0.83	6.92 ± 0.65	7.03 ± 0.58	7.33 ± 0.59	7.08 ± 0.83
HCT (%)	Male	43.24 ± 3.21	45.20 ± 3.79	42.90 ± 3.02	43.06 ± 2.66	43.82 ± 2.73	43.98 ± 2.88
	Female	40.70 ± 4.23	40.44 ± 4.47	40.00 ± 2.75	41.92 ± 2.66	41.56 ± 2.03	42.38 ± 4.32
MCV (μm^3)	Male	57.66 ± 2.27	53.58 ± 2.43	58.36 ± 3.27	58.62 ± 3.37	56.96 ± 1.38	56.14 ± 1.24
	Female	59.22 ± 2.28	54.68 ± 2.46	57.90 ± 2.02	59.82 ± 3.64	56.78 ± 2.05	60.12 ± 5.96
MCH (pg)	Male	19.40 ± 0.76	19.24 ± 0.87	19.32 ± 1.14	19.06 ± 0.87	19.26 ± 0.58	20.26 ± 0.43
	Female	20.16 ± 0.43	19.66 ± 0.59	19.46 ± 0.49*	19.18 ± 1.21	19.44 ± 0.68	21.22 ± 1.66
MCHC (%)	Male	33.64 ± 0.19	35.90 ± 0.37	33.08 ± 0.31*	32.56 ± 0.92*	33.82 ± 0.42	36.08 ± 0.13
	Female	34.06 ± 0.64	35.92 ± 0.68	33.62 ± 0.59	32.16 ± 2.39	34.22 ± 0.24	35.38 ± 1.00
PLT (10^5/cmm)	Male	401.80 ± 42.68	400.80 ± 82.05	374.60 ± 28.96	429.60 ± 46.95	406.00 ± 109.12	339.20 ± 78.95
	Female	343.40 ± 87.08	359.60 ± 107.88	427.60 ± 33.03	444.00 ± 78.21	449.00 ± 59.21	296.40 ± 114.36
Total WBC (10^3/cmm)	Male	9.74 ± 1.52	13.48 ± 1.71	9.78 ± 2.16	9.44 ± 1.38	9.80 ± 1.60	11.36 ± 2.73
	Female	9.28 ± 1.72	8.80 ± 2.09	9.30 ± 1.25	9.62 ± 1.62	9.34 ± 1.82	9.62 ± 1.99
Neutrophil (%)	Male	21.40 ± 4.51	22.80 ± 1.79	21.80 ± 3.96	22.00 ± 3.24	21.40 ± 4.04	24.60 ± 2.88
	Female	21.20 ± 3.03	25.40 ± 2.41	21.40 ± 4.04	21.80 ± 4.32	21.80 ± 4.15	24.40 ± 2.07
Lymphocyte (%)	Male	74.80 ± 4.09	73.00 ± 2.24	74. 80 ± 3.70	75.00 ± 3.24	75.40 ± 4.51	71.60 ± 3.05
	Female	75.40 ± 2.97	70.80 ± 1.64	75.40 ± 3.36	75.20 ± 3.70	74.40 ± 3.65	71.80 ± 2.59
Eosinophil (%)	Male	1.00 ± 1.00	1.40 ± 0.55	1.20 ± 0.84	0.80 ± 0.84	1.00 ± 1.00	1.20 ± 0.45
	Female	1.20 ± 0.84	1.20 ± 0.45	1.20 ± 0.84	0.80 ± 0.84	1.20 ± 1.10	1.40 ± 0.55
Monocyte (%)	Male	2.80 ± 0.45	2.00 ± 0.71	2.20 ± 0.84	2.20 ± 0.84	1.80 ± 1.30	2.000 ± 0.71
	Female	2.20 ± 0.84	2.20 ± 0.84	2.00 ± 1.00	2.20 ± 0.84	2.60 ± 0.55	2.00 ± 0.71
Basophil (%)	Male	0.00 ± 0.00	0.80 ± 0.45	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.60 ± 0.55
	Female	0.00 ± 0.00	0.40 ± 0.55	0.00 ± 0.00	0.00 ± 00	0.00 ± 0.00	0.40 ± 0.55
Pt (Sec.)		15.20 ± 0.84	14.60 ± 1.14	14.80 ± 1.30	15.20 ± 0.84	14.80 ± 1.64	15.00 ± 1.58
		15.20 ± 1.30	15.00 ± 1.58	15.20 ± 0.84	15.40 ± 1.14	15.40 ± 1.14	15.00 ± 1.58

Hb – Hemoglobin, RBC – Red blood corpuscle, HCT – Hematocrit, MCV - Mean corpuscular volume , MCH – Mean corpuscular hemoglobin, MCHC – Mean corpuscular hemoglobin concentration, PLT – Platelet, WBC- White blood corpuscle, Pt – Prothrombin time. All the values are on Day 29, except for recovery groups wherein the values are on Day 43. Values are Mean ± SD. * P<0.05.

Biochemical analysis, gross and histopathological examination

Male and female biochemical investigations (Table 2) conducted at the end of dosing period on day 29 and at the end of recovery period on day 43, revealed no significant changes in the values of different parameters studied when compared with that of respective controls. In comparison with respective controls on day 29 and day 43, organ weight data of animals from different dose groups was found to be comparable (Table 3). Gross and histopathological examinations did not reveal any abnormality attributable to the treatment (data not shown).

Table 2. Effect of 28 days oral exposure to CF extract on mean serum biochemical data of male & female Wistar rats at necropsy

Parameter	Sex	Control	Control	Dose (mg/kg-d)			
			(Recovery)	100	300	1000	1000 (Recovery)
Total protein (g %)	Male	7.62 ± 0.10	7.42 ± 0.28	7.41 ± 0.29	7.45 ± 0.30	7.30 ± 0.30	7.26 ± 0.27
	Female	7.15 ± 0.18	7.18 ± 0.20	7.54 ± 0.33	7.33 ± 0.26	7.20 ± 0.16	7.35 ± 0.28
BUN (mg %)	Male	44.60 ± 1.52	44.00 ± 3.81	42.00 ± 4.24	45.00 ± 1.58	42.40 ± 3.65	45.20 ± 3.56
	Female	42.00 ± 2.00	41.80 ± 1.79	43.80 ± 2.59	43.00 ± 3.39	42.00 ± 2.74	41.80 ± 2.59
ALT (IU/L)	Male	62.60 ± 4.16	61.80 ± 2.17	63.40 ± 4.67	64.20 ± 2.17	62.40 ± 3.51	60.40 ± 3.91
	Female	64.20 ± 3.03	61.00 ± 2.55	62.00 ± 4.24	62.00 ± 3.74	61.40 ± 2.51	61.00 ± 3.00
AST (IU/L)	Male	115.60 ± 4.83	115.00 ± 7.81	115.20 ± 6.69	116.40 ± 2.41	115.20 ± 5.07	113.00 ± 5.43
	Female	109.00 ± 8.15	111.80 ± 7.89	111.20 ± 9.78	115.00 ± 6.40	116.80 ± 4.32	115.40 ± 5.27
AP (IU/L)	Male	345.82 ± 23.42	355.60 ± 28.32	342.80 ± 35.97	366.20 ± 11.08	340.80 ± 16.95	334.60 ± 16.62
	Female	343.80 ± 21.15	337.80 ± 25.97	336.40 ± 25.86	339.00 ± 31.50	345.80 ± 18.16	352.00 ± 22.62
Blood sugar (mg %)	Male	80.40 ± 2.88	81.00 ± 3.00	79.20 ± 1.92	82.40 ± 3.21	80.00 ± 2.55	81.00 ± 3.54
	Female	80.40 ± 3.05	81.80 ± 3.11	78.80 ± 2.39	79.60 ± 3.21	81.00 ± 3.54	80.20 ± 3.35
Calcium (mg %)	Male	9.38 ± 0.19	9.38 ± 0.29	9.24 ± 0.27	9.44 ± 0.26	9.22 ± 0.16	9.36 ± 0.34
	Female	9.20 ± 0.25	9.36 ± 0.34	9.34 ± 0.21	9.42 ± 0.29	9.28 ± 0.15	9.32 ± 0.34
Phosphorus (mg %)	Male	4.12 ± 0.18	4.00 ± 0.16	3.98 ± 0.15	4.10 ± 0.19	4.12 ± 0.08	3.98 ± 0.15
	Female	4.10 ± 0.07	4.08 ± 0.23	3.98 ± 0.15	4.08 ± 0.27	4.00 ± 0.23	4.14 ± 0.21
γ GT (U/L)	Male	16.20 ± 1.48	16.60 ± 2.19	16.80 ± 1.48	16.60 ± 1.52	16.20 ± 1.92	15.80 ± 1.64
	Female	15.60 ± 2.88	14.20 ± 2.68	13.60 ± 1.34	14.40 ± 2.51	15.60 ± 1.82	15.60 ± 2.30
Bilirubin (mg %)	Male	0.63 ± 0.04	0.63 ± 0.03	0.63 ± 0.03	0.62 ± 0.04	0.63 ± 0.04	0.60 ± 0.03
	Female	0.66 ± 0.03	0.62 ± 0.03	0.62 ± 0.01	0.62 ± 0.05	0.62 ± 0.04	0.61 ± 0.03

Albumin (mg %)	Male	3.46 ± 0.20	3.58 ± 0.42	3.56 ± 0.14	3.50 ± 0.37	3.51 ± 0.16	3.53 ± 0.28
	Female	3.51 ± 0.39	3.53 ± 0.35	3.29 ± 0.33	3.59 ± 0.25	3.32 ± 0.23	3.40 ± 0.39
Creatine (mg %)	Male	0.94 ± 0.04	0.92 ± 0.03	0.95 ± 0.04	0.96 ± 0.04	0.94 ± 0.04	0.95 ± 0.04
	Female	0.95 ± 0.04	0.93 ± 0.04	0.95 ± 0.04	0.96 ± 0.05	0.93 ± 0.03	0.96 ± 0.04
CPK (IU/L)	Male	61.40 ± 3.85	62.80 ± 3.83	61.80 ± 3.90	64.20 ± 5.63	62.20 ± 2.59	61.00 ± 3.46
	Female	65.20 ± 3.49	60.40 ± 3.51	61.00 ± 3.54	63.80 ± 4.15	63.40 ± 3.71	62.00 ± 3.39
Sodium (mmol/L)	Male	135.80 ± 3.63	137.00 ± 3.67	137.20 ± 4.92	137.60 ± 3.58	138.40 ± 3.44	136.80 ± 3.77
	Female	138.80 ± 3.96	138.40 ± 4.04	136.60 ± 4.83	135.60 ± 4.83	138.80 ± 4.76	138.40 ± 4.39
Potassium (mmol/L)	Male	3.52 ± 0.17	3.53 ± 0.31	3.53 ± 0.14	3.59 ± 0.22	3.57 ± 0.17	3.40 ± 0.24
	Female	3.49 ± 0.13	3.44 ± 0.21	3.37 ± 0.09	3.72 ± 0.27	3.46 ± 0.18	3.50 ± 0.32
Chloride (mmol/L)	Male	102.40 ± 1.34	103.00 ± 2.55	102.80 ± 2.39	103.40 ± 0.55	103.60 ± 2.19	102.20 ± 2.17
	Female	103.40 ± 1.14	103.20 ± 2.17	102.20 ± 1.10	101.80 ± 2.59	104.00 ± 1.22	103.40 ± 1.82
Cholesterol (mg %)	Male	62.20 ± 3.83	59.20 ± 1.64	62.00 ± 3.54	64.20 ± 2.95	62.00 ± 3.39	62.80 ± 3.11
	Female	63.40 ± 3.78	62.60 ± 3.65	61.00 ± 3.46	63.20 ± 4.76	64.60 ± 2.07	60.60 ± 3.71
Triglycerides (mg %)	Male	105.40 ± 4.28	104.60 ± 2.07	104.60 ± 3.21	106.00 ± 3.81	106.40 ± 2.88	104.40 ± 2.97
	Female	104.20 ± 3.63	105.00 ± 3.74	107.00 ± 3.67	107.40 ± 2.88	105.80 ± 2.59	105.20 ± 2.77
LDH (IU/L)	Male	346.20 ± 14.45	363.60 ± 20.76	356.60 ± 13.89	338.60 ± 18.13	347.00 ± 20.02	354.00 ± 21.18
	Female	363.20 ± 33.73	344.60 ± 28.57	347.40 ± 18.84	351.00 ± 27.08	350.60 ± 26.31	347.40 ± 23.20

BUN – Blood urea nitrogen, ALT – Alanine transaminase, γ GT - Gamma-glutamyl transferase, CPK - Creatine phosphokinase, LDH - Lactate dehydrogenase

All the values are on Day 29, except for recovery groups wherein the values are on Day 43. Values are Mean ± SD.

Table 3. Effect of 28 days oral exposure to CF extract on mean absolute organ weight (g) of male & female Wistar rats at necropsy

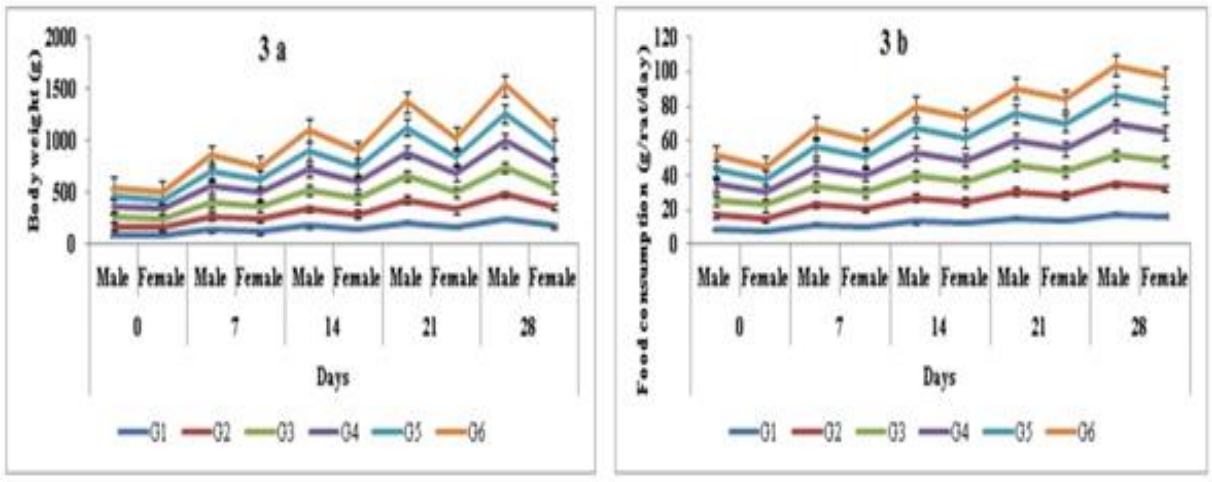
Organ	Sex	Control	Control Recovery	Treated			
				100	300	1000	1000 (Recovery)
Brain	Male	2.075 ± 0.027	2.055 ± 0.086	2.073 ± 0.197	2.146 ± 0.027	13.55 ± 0.83	1.911 ± 0.113
	Female	2.037 ± 0.161	1.904 ± 0.158	2.075 ± 0.179	1.937 ± 0.115	2.020 ± 0.068	2.133 ± 0.228
Liver	Male	11.283 ± 1.071	10.321 ± 1.811	12.295 ± 1.34	11.090 ± 0.565	12.507 ± 2.081	11.661 ± 0.904
	Female	8.438 ± 1.763	7.347 ± 0.772	9.971 ± 1.431	9.331 ± 1.354	9.441 ± 0.677	8.567 ± 0.111
Kidney	Male	2.547 ± 0.198	2.520 ± 0.121	2.616 ± 0.315	2.702 ± 0.208	2.519 ± 0.173	2.833 ± 0.202
	Female	1.903 ± 0.076	1.858 ± 0.307	1.951 ± 0.066	1.764 ± 0.200	1.699 ± 0.133	1.990 ± 0.216
Adrenal	Male	0.047 ± 0.004	0.052 ± 0.007	0.052 ± 0.005	0.050 ± 0.004	0.050 ± 0.005	0.055 ± 0.007
	Female	0.061 ± 0.008	0.064 ± 0.011	0.066 ± 0.007	0.067 ± 0.005	0.059 ± 0.005	0.065 ± 0.009
Heart	Male	1.039 ± 0.048	1.188 ± 0.193	1.171 ± 0.141	1.103 ± 0.107	1.038 ± 0.079	1.288 ± 0.135
	Female	0.789 ± 0.040	0.710 ± 0.102	0.836 ± 0.118	0.818 ± 0.083	0.818 ± 0.070	0.850 ± 0.089
Spleen	Male	1.403 ± 0.271	1.404 ± 0.336	1.802 ± 0.568	1.787 ± 0.500	1.374 ± 0.569	1.238 ± 0.146
	Female	1.556 ± 0.467	1.233 ± 0.414	1.755 ± 0.617	1.337 ± 0.324	1.067 ± 0.147	1.471 ± 0.375
Thymus	Male	0.428 ± 0.063	0.501 ± 0.049	0.511 ± 0.126	0.499 ± 0.144	0.403 ± 0.093	0.469 ± 0.120
	Female	0.424 ± 0.073	0.355 ± 0.048	0.468 ± 0.076	0.420 ± 0.099	0.387 ± 0.093	0.456 ± 0.084
Testes	Male	2.673 ± 0.239	2.629 ± 0.240	2.775 ± 0.197	2.616 ± 0.379	2.713 ± 0.187	3.012 ± 0.106
Epididymis	Male	0.649 ± 0.033	0.953 ± 0.094	0.687 ± 0.056	0.659 ± 0.064	0.644 ± 0.049	1.005 ± 0.98
Ovary	Female	0.081 ± 0.006	0.091 ± 0.004	0.078 ± 0.074	0.090 ± 0.011	0.083 ± 0.013	0.101 ± 0.022

Values are Mean ± SD.

Chronic (180 days) oral toxicity

Body weights and food consumption

In male rats, the body weights were found to be similar in all groups upto week 16. During later weeks, body weights of treatment group rats tend to be lower than control group, however, this was not statistically significant. The reduction in body weight gain was found to be reversed in recovery animals after stopping the treatment. Such difference was not seen in female animals. Body weight gain by female rats treated with CF extract (10% forskolin) at 500 mg/kg and 1000 mg/kg b.wts was found to be comparable to that by the control rats throughout the treatment period. Also, during recovery period, the weight gain by male and female rats from high dose group was found to be comparable to that by the control group rats (Fig. 3c). The values of average food consumption by male and female rats treated with CF extract (10% forskolin) at and upto 1000 mg/kg b.wt, remained comparable to those of the control group rats. The average daily good consumption per rat per day, computed over the period of 26 weeks, by male rats receiving CF extract (10% forskolin) at 500 mg/kg b.wt and 1000 mg/kg b.wt was 100% and 97 % respectively of that by control rats. Similarly, the average daily food consumption by female rats receiving the test article at 500 mg/kg b.wt and 1000 mg/kg b.wt was 99% and 97% respectively of that by control rats. After cessation of treatment, the values of food intake during the recovery period were found to be comparable among the control and the high dose group (Fig. 3d).



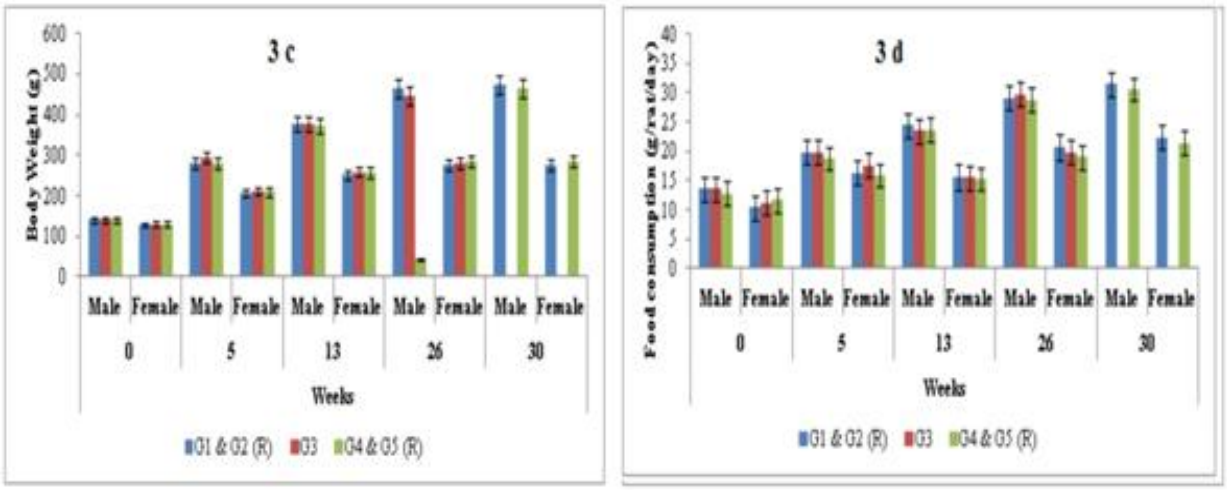


Fig. 3. Effects of CF extract on body weight and food consumption in male and female Wistar rats in 28 day and 180 day toxicity studies. Increase in body weight and food consumption of the treated group animals throughout the study periods was not statistically significant from the control group animals. **Fig. 3a. and 3b.** G 1 – Control, G 2 – Control (recovery), G 3 – 100 mg /kg-d, G 4 – 300 mg /kg-d, G 5 – 1000 mg /kg-d, G 6 1000 mg /kg-d (recovery); **Fig. 3c. and 3d.** G 1 – Control, G 2 – Control (recovery), G 3 – 500 mg /kg-d, G 4 – 1000 mg /kg-d, G 5 –1000 mg /kg-d (recovery).

Hematological analysis

At the end of three months and at the end of treatment period, the group mean values of hematological parameter such as hemoglobin, packed cell volume, total and differential WBC counts, total RBC count, RBC indices, platelet count and clotting time of male and female rats treated with CF extract (10% forskolin) at and upto the level of 1000 mg/kg b.wt were found to be comparable with those of the control animals. Values of hemoglobin, packed cell volume, total and differential WBC counts, total RBC count, RBC indices – platelet count and clotting time of male and female rats treated with CF extract (10% forskolin) at 1000 mg/kg b.wt were found to be comparable with those of the control rats at the end of recovery period (Table 4).

Table 4. Effect of 180 days oral exposure to CF extract on mean hematology data of male & female Wistar rats at necropsy

Parameter	Sex	G1 Control	G2 Recovery (R) Control	Treatment Dose (mg/kg-d)			G1 Control	G2 Recovery (R) Control
				G3 500	G4 1000	G5 1000 (R)		
				Day 93				
Hb (g/dL)	Male	13.70±0.55	14.20±0.61	13.57±1.36	13.48±0.95	13.80±0.41	12.36±0.59	12.73±0.52
	Female	14.22±0.61	14.18±0.84	13.88±0.89	13.59±1.69	13.98±0.90	13.23±0.54	12.91±1.04
PCV (%)	Male	39.01±0.55	39.97±1.75	38.99±3.54	39.15±2.65	39.92±1.12	41.13±1.69	37.59±1.63
	Female	39.59±1.75	39.62±1.89	39.29±2.48	38.14±4.79	39.48±2.46	38.36±1.51	37.74±2.71
Total RBC (10^6/cmm)	Male	8.02±0.42	8.22±0.36	8.00±0.87	8.10±0.61	8.19±0.32	6.91±0.37	7.54±0.35
	Female	7.90±0.41	7.89±0.38	7.78±0.58	7.54±0.93	7.96±0.54	7.45±0.34	7.37±0.46
MCH (pg)	Male	17.10±0.74	17.30±0.80	17.01±0.77	16.66±0.96	16.87±0.54	17.90±0.68	16.91±0.72
	Female	18.02±0.71	17.98±0.51	17.86±0.67	18.05 ±0.73	17.58±0.44	17.77±0.71	17.52±0.74
MCV (fL)	Male	48.66±1.70	48.68±2.04	48.94±2.34	48.41±2.63	48.80±1.74	59.59±2.15	49.92±1.95
	Female	50.15±1.63	50.26±1.41	50.57±1.52	50.63±1.78	49.65±1.16	51.52±1.72	51.23±1.92
MCHC (g/dL)	Male	35.13±0.44	35.53±0.38	34.76±0.50	34.42±0.46	34.57±0.45	30.04±0.39	33.87±0.39
	Female	35.92±0.44	35.78±0.57	35.32±0.47	35.64±0.41	35.41±0.29	34.48±0.48	34.19±0.49
Total WBC (10^3/cmm)	Male	9.51±1.32	9.64±1.96	12.40±6.00	10.09±2.22	10.42±2.62	14.32±1.85	9.89±2.60
	Female	9.00±2.10	8.64±2.26	10.03±2.28	9.16±3.05	8.65±2.51	7.33±1.21	7.06±2.85
Neutrophil (%)	Male	2.025±2.51	21.40±2.32	19.84±2.36	21.35±3.56	21.00±4.27	20.89±2.90	21.20±2.86
	Female	20.55±2.84	19.70±3.47	20.40±3.75	19.80±3.21	20.80±3.52	20.95±4.03	19.50±3.31
Lymphocyte (%)	Male	77.10±2.55	75.10±2.18	77.37±2.56	75.60±3.20	75.80±4.08	74.42±3.22	75.00±3.20
	Female	75.30±3.33	75.90±3.31	75.60±3.56	76.70±3.37	75.30±4.03	74.20±3.96	76.30±3.80
Eosinophil (%)	Male	1.00±0.00	1.20±0.42	1.05±0.23	1.20±0.41	1.20±0.42	1.58±0.51	1.50±0.53
	Female	1.35±0.59	1.50±0.53	1.50±0.51	1.40±0.50	1.30±0.48	1.50±0.51	1.30±0.48
Monocyte (%)	Male	1.65±0.67	2.30±1.06	1.74±0.81	1.85±0.67	2.00±0.67	3.11±1.20	2.30±1.16
	Female	2.80±1.20	2.90±1.45	2.50±0.83	2.10±0.97	2.60±1.26	3.35±1.04	2.90±1.52
PLT (10^6/cmm)	Male	61.77±12.57	63.54±13.02	68.91±13.86	71.73±10.96	76.76±10.52	57.14±14.62	65.85±16.01
	Female	60.85±12.53	69.47±10.62	65.50±10.11	69.90±14.18	77.68±13.66	67.64±7.79	63.18±13.73
Clotting time (Sec.)	Male	107.45±5.92	109.10±5.28	107.68±4.22	107.50±6.05	109.10±4.75	103.37±2.87	103.60±4.14
	Female	104.00±2.49	104.60±1.78	104.15±1.04	105.55±1.88	105.40±1.07	101.80±3.30	102.40±3.78

Hb – Hemoglobin, PCV– Packed cell volume, RBC – Red blood corpuscle, MCV - Mean corpuscular volume, MCH – Mean corpuscular hemoglobin, MCHC – Mean corpuscular hemoglobin concentration, WBC - White blood corpuscle. PLT – Platelet. Values are Mean ± SD

Biochemical analysis

The test article, CF extract (10% forskolin) upto the dose level of 1000 mg/kg, did not induce any changes in the plasma levels of total protein, albumin, alanine aminotransferase, asparate aminotransferase, cholesterol, alkaline phosphatase, glucose, creatinine, urea, urea nitrogen, total bilirubin, calcium, phosphorus, sodium and potassium in male and female rats at the end of three months and at termination of the treatment. Values of total protein, albumin, alanine aminotransferase, asparate aminotransferase, cholesterol, alkaline phosphatase, glucose, creatine, urea, urea nitrogen, total bilirubin, calcium, phosphorus, sodium and potassium of male and female rats treated with CF extract (10% forskolin) at 1000 mg/kg b.wt were found to be comparable with those of the control rats at the end of recovery period (Table 5).

Table 5. Effect of 180 days oral exposure to CF extract on mean serum biochemical data of male & female Wistar rats at necropsy

Parameter	Sex	Control G1	G2 Recovery (R) Control	Treatment Dose (mg/kg-d)			Control G1	G2 Recovery (R) Control
				G3 500	G4 1000	G5 1000 (R)		
				Day 93 to 96				
Total Protein (g/dL)	Male	6.55±0.28	6.86±0.31	6.75±0.25	6.13±0.66	6.49±0.50	6.75±0.44	6.92±0.55
	Female	6.33±0.73	5.44±0.76	5.77±1.05	6.69±1.18	7.23±0.39	7.34±0.49	7.27±0.36
Albumin (g/dL)	Male	2.50±0.15	2.48 ±0.20	2.48±0.17	2.32±0.13	2.51±0.16	3.12±0.47	3.01±0.54
	Female	2.57±0.19	2.72±0.30	2.86±0.24	2.78±0.30	2.77±0.29	3.34±0.45	3.34±0.57
ALT (IU/L)	Male	69.73±11.96	76.03±25.44	76.36±26.79	81.24±27.50	71.61±14.52	93.53±12.01	92.64±20.47
	Female	71.31±19.37	76.11±10.66	67.48±16.97	72.58±17.11	80.26±22.56	119.62±32.07	76.04±16.34
AST (IU/L)	Male	142.85±23.64	149.70±24.04	144.68±31.61	165.75±38.49	158.40±25.08	180.89±32.23	168.42±31.31
	Female	150.05±46.51	152.30±16.83	164.25±52.26	153.45±23.51	190.50±40.74	183.20±40.06	160.55±28.53
ALP (IU/L)	Male	137.76±44.56	115.46±23.98	126.15±56.72	187.68±56.40	125.81±34.39	140.81±48.66	180.06±146.39
	Female	108.08±28.81	146.73±26.23	110.04±37.16	146.28±101.31	123.40±49.37	101.72±42.97	93.13±33.12
Glucose (mg/dL)	Male	75.70±18.90	77.83±6.75	63.74±11.09	63.59±19.55	71.19±7.56	69.84±4.80	73.94±9.99
	Female	102.27±24.74	77.54±7.04	71.03±13.27	103.79±17.21	69.38±8.85	84.05±8.49	74.84±7.43
Total Cholesterol (mg/dL)	Male	68.31±6.92	67.57±8.18	66.10±10.07	63.23±8.89	64.92±6.20	75.04±7.10	81.04±12.37
	Female	63.90±10.00	75.88±9.29	78.89±9.78	71.75±9.82	92.33±27.37	81.12±9.39	81.82±9.90
BUN (mg/dL)	Male	17.10±3.59	15.88±2.54	16.38±2.94	20.50±3.21	13.54±1.42	16.69±2.43	14.19±1.86
	Female	21.90±3.14	19.59±1.79	17.88±4.25	23.01±2.52	21.40±4.03	16.84±2.32	16.70±2.60
Creat. (mg/dl)	Male	0.98±0.06	0.89±0.14	0.91±0.18	0.98±0.08	1.02±0.05	0.88±0.06	0.80±0.06
	Female	0.94±0.22	0.94±0.04	0.97±0.12	0.97±0.08	1.03±0.16	0.97±0.07	0.83±0.07
Sodium (mmol/L)	Male	151.91±1.18	150.70±1.40	150.16±1.34	150.09±2.00	150.59±1.52	154.29±1.75	153.41±1.86
	Female	151.51±1.91	151.38±1.97	151.51±2.14	150.28±2.03	151.36±1.54	153.04±1.66	152.86±2.29
Potassium (mmol/L)	Male	3.35±0.21	3.54±0.18	3.61±0.34	3.42±0.30	3.75±0.30	3.57±0.28	3.81±0.49
	Female	3.58±0.36	3.48±0.27	3.50±0.42	3.55±0.35	3.76±0.54	3.81±0.61	3.66±0.54
Urea (mg/dL)	Male	36.59 ±7.68	33.98±5.44	35.06±6.30	43.87±6.88	28.98±3.04	35.72±5.21	30.37±3.97
	Female	46.86±6.71	41.92±3.82	38.26±9.08	49.23±5.38	45.80±8.62	36.04±4.96	35.73±5.56
Total Bilirubin (mg/dL)	Male	0.42±0.07	0.30±0.06	0.33±0.07	0.39±0.06	0.40±0.10	0.78±0.63	0.41±0.09
	Female	0.37±0.07	0.40±0.11	0.42±0.12	0.40±0.12	0.34±0.07	0.50±0.09	0.43±0.11
Calcium (mg/dL)	Male	12.00±1.53	9.39±0.65	9.74±1.15	8.84±1.10	9.39±0.87	9.29±2.35	10.37±1.43
	Female	11.55±1.01	10.61±1.46	11.29±0.73	10.57±1.35	11.50±0.81	9.87±0.085	9.44±0.76
Phosphorus (mg/dL)	Male	5.99±0.69	5.73±0.62	5.84±0.58	5.83±0.55	5.92±0.63	6.06±1.70	6.54±0.93
	Female	6.06±0.50	5.61±0.66	5.86±0.70	5.70±0.51	6.01±0.43	7.11±1.17	5.99±.25

ALT - Alanine transaminase, AST - Aspartate aminotransferase, ALP - Alkaline phosphatase, BUN – Blood urea nitrogen All values are on Day 93 to 96, except for recovery groups wherein the values are on Day 209. Values are Mean ± SD

Urinalysis

Qualitative and microscopic examination of urine samples collected at the end of three months and at termination of the treatment, from male and female rats of control group and rats treated with CF extract (10% forskolin) at 1000 mg/kg b.wt did not reveal any significant and treatment related differences (Table 6). The values of absolute weights of kidneys, liver, adrenals, testes, ovaries, uterus, epididymides, thymus, spleen, brain and heart of male and female rats treated with CF extract (10% forskolin) at and upto 1000 mg/kg were found to be comparable to those of the control group rats at termination of the treatment. Also, the values of absolute weights of all

these organs treated with CF extract (10% forskolin) at 1000 mg/kg b.wt were found to be comparable with those of control rats at the end of recovery period as well (Table 7).

Table 6. Effect of 180 days oral exposure to CF extract on urine of male & female Wistar rats at necropsy

Group & Dose	Day	Sex	Sp. gravity	pH	Urobilinogen (Ehrlich units /dL)	Microscopy		
						E	L	R
G1 0 mg/kg	95	Male	1.028	6.2	0.2	1	0	0
		Female	1.027	6.3	0.2	1	0	0
	179	Male	0.9735	6.4	0.2	1	1	0
		Female	1.017	7.4	0.2	1	0	0
G4 1000 mg/kg	95	Male	1.026	6.2	0.2	1	1	0
		Female	1.024	6.5	0.2	1	0	0
	179	Male	1.022	7.4	0.2	1	1	0
		Female	1.02	7.2	0.2	1	0	0

E: Epithelial cells L: Polymorphonucleocytes R: Erythrocytes.

0 – none, 1 – few in some fields, 2 – few in all fields, 3 – many in many fields; data represented as mean.

Table 7. Effect of 180 days oral exposure to CF extract on mean absolute organ weight (g) of male & female Wistar rats at necropsy

Organ	Sex	Control 0 G1	Treated		Recovery (R)	
			G3 500	G4 1000	G2 (R) Control 0	G5 (R) 1000
Adrenal	Male	0.042 ± 0.005	0.048 ± 0.008	0.050 ± 0.013	0.044 ± 0.005	0.049 ± 0.004
	Female	0.046 ± 0.004	0.058 ± 0.012	0.066 ± 0.012	0.050 ± 0.005	0.045 ± 0.003
Kidney	Male	3.15 ± 0.41	3.14 ± 0.30	3.23 ± 0.51	3.26 ± 0.34	3.29 ± 0.50
	Female	1.82 ± 0.23	2.11 ± 0.28	1.85 ± 0.20	1.78 ± 0.21	1.86 ± 0.20
Liver	Male	11.85 ± 1.89	12.66 ± 1.95	12.81 ± 2.18	12.60 ± 1.51	12.04 ± 2.12
	Female	7.27 ± 0.97	8.23 ± 1.48	8.40 ± 1.41	7.42 ± 0.98	8.48 ± 0.83
Brain	Male	2.03 ± 0.13	2.17 ± 0.14	2.00 ± 0.14	2.06 ± 0.11	2.10 ± 0.12
	Female	1.92 ± 0.12	2.02 ± 0.10	1.97 ± 0.14	1.91 ± 0.13	2.01 ± 0.08
Thymus	Male	0.310 ± 0.090	0.342 ± 0.090	0.314 ± 0.089	0.273 ± 0.091	0.356 ± 0.137
	Female	0.234 ± 0.057	0.246 ± 0.086	0.242 ± 0.064	0.232 ± 0.088	0.253 ± 0.088
Heart	Male	1.38 ± 0.16	1.44 ± 0.17	1.40 ± 0.22	1.35 ± 0.13	1.36 ± 0.19
	Female	0.90 ± 0.11	1.07 ± 0.15	1.05 ± 0.11	0.95 ± 0.15	1.01 ± 0.22
Spleen	Male	1.14 ± 0.17	1.34 ± 0.31	1.09 ± 0.28	1.26 ± 0.26	1.14 ± 0.37
	Female	0.84 ± 0.14	0.88 ± 0.31	0.88 ± 0.17	0.79 ± 0.14	0.90 ± 0.24
Testes	Male	3.41 ± 0.36	3.32 ± 0.54	3.12 ± 0.31	3.16 ± 0.15	3.34 ± 0.41
Epididymis	Male	1.333 ± 0.140	1.613 ± 0.370	1.287 ± 0.218	1.260 ± 0.043	1.302 ± 0.213
Ovary	Female	0.089 ± 0.009	0.129 ± 0.017	0.119 ± 0.029	0.087 ± 0.009	0.087 ± 0.008
Uterus	Female	0.781 ± 0.261	0.782 ± 0.228	0.741 ± 0.226	0.875 ± 0.245	0.925 ± 0.351

Values as on Day 182-185 for G1, G3 and G4, whereas for G2 (R) and G5 (R) groups, values as on Day 209

Bacterial reverse independent repeat assay

The maximum dose tested was 5000 µg per plate; this dose was achieved using a concentration of 100 mg/mL and 50 µL plating aliquot. Neither precipitate nor appreciable toxicity was observed. Based on the findings of the toxicity assay, the maximum dose plated in the mutagenicity assay was 5000 µg per plate. In the initial mutagenicity assay, no positive responses were observed with any of the tester strains in the presence and absence of S9 activation. Due to inconsistent toxicity profiles as compared to the initial toxicity assay, tester strains TA100 and TA1535 in the absence of S9 activation were retested. In the repeat assay, no positive responses were observed with same tester strains. Furthermore, in the independent repeat assay, no positive responses were observed with tester strains TA98, TA1535, TA1537 and WP2 *uvr*A in the presence of S9 activation and with any of the tester strains in the absence of S9 activation (Table 8).

Table 8 Effect of CF extract on bacterial reverse mutation assay with (+S9) mix (Ames)

Dose µg/Plate	TA -98		TA 100		TA 1535		TA 1537	
	Without S9	With S9	Without S9	WithS9	Without S9	With S9	Without S9	With S9
DMSO	16 ± 1	28 ± 2	81 ± 2	100 ± 3	10 ± 4	10 ± 1	8 ± 2	12 ± 4
25	18 ± 3	27 ± 5	91 ± 8	103±17	12 ± 3	9 ± 2	10 ± 4	16 ± 3
75	19 ± 6	29 ± 2	89 ± 1	86 ± 9	11 ± 2	10 ± 3	9 ± 2	14 ± 4
200	18 ± 2	27 ± 4	92 ± 14	97± 4	13 ± 5	7 ± 2	13 ± 3	13 ± 7
600	21 ± 4	28 ± 4	86 ± 13	Precipitate	11 ± 4	10 ± 2	9 ± 3	19 ± 4
1800	17 ± 6	24 ± 5	91 ± 5	Precipitate	10 ± 4	9 ± 3	9 ± 4	13 ± 3
5000	9 ± 3	22 ± 6	66 ± 21	Precipitate	7 ± 2	9 ± 3	6 ± 2	9 ± 2
Positive Control	115 ± 24[a]	270±42[b]	306 ± 51[a]	355±26[c]	123 ± 10[a]	35 ± 6[d]	137 ± 4[a]	38 ± 5[e]

Values are average revertants per plate ± standard deviation

a- Methyl methane sulphonate, **b** – 2-Nitrofluorene, **c** –Sodium Azide, d– 2 -Amino anthracene, e – 9-Aminoacridine

DISCUSSION

The wide usage of universally popular herbal medicine for self medication is result of the fact that general public, believes them to be safe and free from any compromising health effects [14].

As a consequence of metabolism, animals show slight changes in behaviour when high doses of plant extracts or chemicals were given but the signs are quickly reversible [15]. Invariably, increase or decrease in the body weights of animals can be used as a sign of adverse effects of drugs and chemicals [16]. However, increase in the body weights of animals is more closely related to body fat accumulation rather than to the toxic effects of drugs or chemicals [17]. In the present toxicity studies, increase in body weights of all the animal groups during the study period should be interpreted as normal growth but not related to the CF extract *per se*. Blood parameters are relevant indicators for risk evaluation. Changes in the hematological system have a higher predictive value for human toxicity when the data are extrapolated from animal studies [18]. In our studies here, no significant changes in hematology values were reported in the treatment groups when compared to their respective control animal groups. In the serum biochemistry assessments, a number of cases of renal and hepatic toxicity have been reported following the use of phytotherapeutic products [19]. In cases of acute or chronic renal toxicity, creatine and urea are usually increased to four or five times higher than the normal values in control animals. In the present toxicity studies, all of the rats that had been orally administered with various doses of CF extract did not show any anomalies.

In necropsy, CF extract (10% forskolin), at and upto to dose level of 1000 mg/kg in 180 day repeated oral toxicity study, did not induce any remarkable and treatment related gross pathological alterations in any of the tissues of exposed rats, as evident at the detailed necropsy examination carried out at termination of the study and also at the end of recovery period. There were isolated instances of necropsy findings such as reddening, consolidation, hepatisation, abscess & pleural adhesions in lungs; reddening of liver, red patches & while nodular growth on surface of liver; hydronephrosis, red & white patches on surface of kidney, enlarged & rudimentary kidneys; distension of uterus and fibrous thick material on heart. However, the incidence being extremely small and not dose dependent, was therefore not considered to be of toxicological significance. Higher incidence of lung abscess in treated rats does not indicate any toxic potential of the test article as these changes were due to infectious cause. This anomaly may also be explained as either it may have been due to inhalation of inspired air by these animals or these types of incidences have been reported to occur spontaneously in rats [20]. In the histopathology, study of various microscopic changes noticed in several organs showed all the microscopic changes noticed were incidental in nature as their frequency was more in control

animals as compared to treated rats. Certain microscopic changes like suppurative pyelonephritis; granuloma and granulomatus pneumonia in lungs; abscess in heart, chronic tracheitis; suppurative inflammation in prostate and hemorrhage in spinal cord were again considered as incidental since these were solitary cases and not dose dependent. Result of this study revealed lymphoid hyperplasia in the rectum of treated rats more frequently as compared to controls. Lymphoid hyperplasia in gastrointestinal tract of rodents is frequent and recording of marginally high frequency appears to be of no significance. Abscess in the lungs recorded more frequently in G4 males could again be incidental as it is not dose related since all the groups showed lung abscess with varying frequencies.

There was no incidence of any treatment-related mortality amongst the rats treated with CF extract (10% forskolin) at 500 mg and 1000 mg/kg b.wt. No treatment related clinical signs, gross or histopathological findings were observed in all animals during the study period. All other animals survived throughout the treatment period of 180 days and also during recovery period. Clinical signs: The daily clinical examinations and weekly detailed clinical examination did not reveal any changes, which could be attributed to test article. Some of the clinical signs observed included abdominal breathing, wet perineum, diarrhea and circling disorder. However, the incidence being small and not dose dependent, is not considered to be of any toxicological significance. The ophthalmoscopic examination conducted at the end of 3 months and at termination of the treatment did not reveal any remarkable changes. The neurological examination (functional observational battery) conducted during the 26th week of the study did not reveal any neurotoxic potential of the test substance at any of the doses tested. It should be noted that only significant findings were discussed here and complete data is not shown for necropsy, histopathology, mortality, clinical and ophthalmic signs of all animal groups due to space constraints.

To the best of our knowledge, this is the first time an attempt has been made to study the acute, sub-acute, chronic toxicities and mutagenic potency of forskolin. The NOAEL in the present study was 1000 mg/kg/d and based on body surface area normalization, the human equivalent dose (HED) is ~9600 mg for a 60 kg adult [21]. The Trichigadi tribes consume CF roots twice a day at a quantity far less than this amount. Also, it should be noted that generally pharmacologically active phyto-constituents are non-toxic at the pharmacological dose [22].

CONCLUSION

Coleus forskohlii (CF extract containing 10% forskolin) in the repeated dose 28 day oral toxicity study and in chronic 180 oral toxicity study, showed no signs of toxicity and hence it could be concluded that a repeated oral exposure to this extract up to 1000 mg/kg b.wt/day does not produce any toxic effects and may be treated as 'No Observed Adverse Effect Level' (NOAEL) under the test conditions employed. Furthermore, as CF extract exhibited a negative response in the presence and absence of Aroclor 1254 induced rat liver S9 in the assay up to 5000 μg/plate, it was concluded that *Coleus forskohlii* extract was not mutagenic.

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CONFLICT OF INTEREST

Declared none

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