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Letter to the Editor

Safety evaluation of a polyherbal formulation containing hydroalcoholic extracts of *Hippophae salicifolia*, *Nyctanthes arbor-tristis*, *Ocimum tenuiflorum*, and *Reinwardtia indica* in rodents

Dear Editor,

Herbal medicines have been used for prevention and treatment of human diseases and promotion of healthy living. These medicines, often self medicated and consumed as concentrated extracts, are not strictly regulated. Considering their popular use and increasing safety concerns, thorough evaluations of their efficacy and safety are warranted to protect consumers from potential adverse effects. Toxicity tests (acute, sub-acute and chronic) using animals are widely applied to evaulate adverse effects of a drug and thereby determine its "No Observed Adverse Effect Level" (NOAEL). Conducting such studies for herbal medicines will be valuable to determine potential toxic effects as well as safe dose ranges of herbal medicines just as pre-clinical and non-clinical toxicological studies are important for determining the therapeutic index of drugs.

We evaluated the acute and sub-acute toxicity of a polyherbal formulation containing hydro-alcoholic (70% ethanol) extracts of four medicinal plants, namely *Nyctanthes arbor-tristis* (75 mg from leaves), *Ocimum tenuiflorum* (50 mg from whole plant), *Hippophae salicifolia* (40 mg from seeds, fruits and leaves) and *Reinwardtia indica* (35 mg from roots) in rodents. The notable medicinal properties of these plants include the serotonergic properties of *Nyctanthes arbor-tristis*, whose leaves contain bioactive molecules including mannitol, glucose, essential oil, carotene, β-amyrin, β-sitosterol, hentriacontane, benzoic acid, triterpenoid (oleanolic acid, nyctanthic acid, friedeline, lupeol tannic acid, ascorbic acid, methyl salicylate) and iridoid glycosides (arborsides A, B, C)[1–2]. *Ocimum tenuiflorum* (family Lamiaceae) notably possesses cholinergic properties and contains eugenol, which has been reported to have acetylcholinesterase inhibitory activity[3–4]. *Hippophae salicifolia* (family Elaeagnaceae) is nutrient rich and possesses potent anti-oxidant properties[5]. *Reinwardtia indica* (family Linaceae) contains saponins, which could potentially help in the management of hyperglycemia[6–7].

We conducted acute and sub-acute oral dose toxicity studies of the test formulation in Swiss albino mice and albino Wistar rats, respectively. Single doses (10 to 5,000 mg/kg) were administered orally to mice. No treatment related deaths or clinical signs of toxicity were recorded at any of the doses at two weeks after drug administration and the lethal dose 50% of the test drug was greater than 5,000 mg/kg. For the sub-acute toxicity assessment, the doses employed ranged from 100 to 800 mg/kg·day (and vehicle as the control), which, in most cases, is acceptable as the limit dose for toxicity studies[8]. The formulation was administered orally to rats for either 14 or 28 days during which food intake and body weight were monitored. At the end of the treatment period, organ weights and haematological and biochemical parameters were measured along with a histopathologic examination. No treatment-emergent toxicities or mortality was observed. Additionally, no treatment related changes in the behaviour of the rats were observed. There was a small and insignificant reduction in body weight and food consumption of the rats in the treatment groups compared with the control group (***Table 1***), suggesting that sub chronic administration of the test formulation did not affect the normal growth of rats. Similarly, there were no significant changes in the weight of the organs (brain, liver, kidney, heart) following either 14 or 28 days of treatment at any of the doses compared to the controls.

Haematological parameters including haemoglobin, red blood cell count, white blood cell count, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were found to be all within the normal range in both the control and treatment groups (***Table 2***), with

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Table 1 **Effect of the test formulation on the body weight and food intake of rats in the sub-acute toxicity study**

	Dose (mg/kg·day)	Day 0 (*n*=6)	Day 7 (*n*=6)	Day 14 (*n*=6)	Day 21 (*n*=3)	Day 28 (*n*=3)
Body weight (g)[#]	0	180.62±8.13	189.73±9.66	195.62±7.65	206.65±8.85	230.62±10.93
	100	176.93±8.62	184.45±10.42	190.15±12.73	199.73±10.17	202.60±11.62
	200	190.62±9.06	198.13±10.53	206.33±9.25	210.83±10.60	212.62±8.55
	400	184.53±8.62	196.62±7.44	201.65±11.63	209.76±7.86	216.59±8.90
	800	181.41±6.34	192.54±4.71	199.54±9.47	204.19±4.12	211.15±5.36
Food intake (g)[#]	0	14.16±3.81	15.59±3.50	16.20±2.32	17.35±1.90	16.86±1.83
	100	12.93±2.73	14.69±0.39	15.51±3.12	14.67±2.98	15.80±3.14
	200	13.80±0.92	13.93±0.76	14.16±0.23	14.86±0.91	15.10±1.32
	400	13.12±1.29	14.28±1.62	15.06±0.90	15.18±1.34	14.42±0.77
	800	13.36±1.30	14.93±0.77	15.16±0.24	15.86±0.92	16.1±1.33

[#] Values are expressed as mean±SD.

Table 2 **Effect of the test formulation on haematological and biochemical parameters of rats in the sub-acute toxicity study**

	14 days (*n*=6)				
Hematological parameters[#]	0	100	200	400	800
Hb(gm/dL)	10.65±0.51	11.54±0.72	13.06±0.47	13.18±0.26	13.04±0.32
RBC(106/mm^3)	3.91±0.23	4.41±0.31	3.34±0.57	4.94±0.23	4.57±0.11
WBC(/mm^3)	9400±120	10100±230	11200±210	10500±230	10100±200
PCV(%)	34.05±5.12	36.78±5.14	39.65±5.06	35.56±4.30	38.26±4.50
MCV(mm^3)	69.54±3.48	70.36±3.53	64.09±5.51	71.10±5.25	70.09±5.54
MCH(pg)	25.92±2.46	26.19±3.10	28.65±2.46	28.97±7.13	28.97±7.13
MCHC(%)	23.64±2.88	24.45±3.23	25.11±3.70	24.36±3.48	26.80±3.21
Biochemical parameters[#]	0	100	200	400	800
Glucose(mg/dL)	115.52±8.85	113.65±6.75	111.54±4.25	108.81±6.20	105.11±5.34
Urea(mg/dL)	35.12±5.58	40.54±5.12	39.56±3.44	41.03±4.21	38.45±4.34
Creatinine(mg/dL)	0.85±0.04	0.79±0.01	0.90±0.03	0.74±0.03	0.89±0.04
Cholesterol(mg/dL)	62.19±5.80	63.54±8.31	59.19±6.91	60.57±6.31	63.28±7.54
Protein(mg/dL)	7.98±0.65	8.94±0.53	8.23±0.98	8.56±0.84	7.86±0.59
SGOT(U/L)	34.79±6.13	35.86±7.68	46.41±5.86	39.54±9.55	34.59±5.14
SGPT(U/L)	30.11±9.23	31.86±5.10	36.19±4.54	29.80±5.53	30.14±8.75
	28 days (*n*=3)				
Hematological parameters[#]	0	100	200	400	800
Hb(gm/dL)	11.54±0.69	12.73±0.45	9.95±0.27	13.08±0.26	13.09±0.44
RBC(106/mm^3)	4.21±0.33	4.64±0.45	3.42±0.62	5.09±0.95	5.06±0.54
WBC(/mm^3)	9800±170	10600±250	11500±260	10900±250	10300±210
PCV(%)	37.65±7.05	38.84±7.34	40.64±6.46	39.66±4.37	38.29±4.56
MCV(mm^3)	72.81±3.98	73.62±4.93	66.15±8.53	73.19±7.67	70.12±5.24
MCH(pg)	25.92±2.46	26.19±3.10	28.65±2.46	28.97±7.13	28.98±7.15
MCHC(%)	24.68±2.95	25.32±3.25	26.13±3.82	25.93±3.51	25.98±3.56
Biochemical parameters[#]	0	100	200	400	800
Glucose(mg/dL)	117.63±9.95	116.79±8.76	113.68±6.25	110.93±8.24	106.75±5.21
Urea(mg/dL)	39.65±4.38	41.65±5.02	40.69±3.84	42.65±4.51	40.01±3.19
Creatinine(mg/dL)	0.87±0.05	0.76±0.09	0.92±0.06	0.62±0.08	0.73±0.02
Cholesterol(mg/dL)	60.18±7.83	62.64±9.30	58.15±6.93	57.65±6.32	59.64±5.20
Protein(mg/dL)	7.68±0.55	8.09±0.36	7.90±0.58	8.19±0.64	8.54±0.78
SGOT(U/L)	33.69±5.13	35.69±7.38	41.41±6.20	36.68±8.35	38.51±8.50
SGPT(U/L)	29.53±8.53	30.68±5.12	35.13±4.19	28.89±5.13	28.74±4.51

[#] Values are expressed as mean±SD.

Hb, haemoglobin; RBC, red blood cell count; WBC, white blood cell count; PCV, packed cell volume;MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamate pyruvate transaminase.

no significant differences between the treatment and control groups. There were also no significant treatment related effects on liver and kidney functions as determined by serum levels of cholesterol, creatinine, glucose, urea, protein, serum glutamic oxaloacetic transaminase, and serum glutamate pyruvate transaminase (***Table 2***). This was further confirmed by histological assessment of these organs. Additionally, there was no damage or defect in the architecture of the brain and heart in the treated or control rats. Further findings from the histological analyses of the treated rats are consistent with normal background lesions observed in clinically normal control rats. Based on these results, the NOAEL for the test formulation was established as 800 mg/kg for 28 days.

In conclusion, no adverse effects of the polyherbal formulation following acute (up to 5,000 mg/kg) and sub acute (up to the maximum tested dose of 800 mg/kg/day for 28 days) oral administration in rodents were observed, which thereby demonstrates a favourable safety profile of the test formulation. This study provides valuable data on the toxicity profile of hydro alcoholic extracts of the medicinal plants *Nyctanthes arbor-tristis, Ocimum tenuiflorum, Hippophae salicifolia* and *Reinwardtia indica,* with results supporting their safe longer term use in combination.

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