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Research Article

ACUTE AND SUB-ACUTE ORAL TOXICITY OF *POLYGONUM MINUS* AQUEOUS EXTRACT (BIOTROPICS®PM101) IN WISTAR RATS

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ABSTRACT

Objective: The present study was designed to determine the acute and subacute oral toxicity of the *Polygonum minus* aqueous extract (biotropics®PM101) in Wistar rats.

Methods: The acute and subacute oral toxicity study was performed according to the OECD guideline. In the acute toxicity test, single dose of 2000 mg/kg of aqueous extract of Polygonus *Minus* (biotropics®PM101) was administered p.o (orally) to wistar rats following a sighting study. The animal was observed for mortality and clinical signs for 14 days post dosing for seven days. In subacute 28-days repeated dose oral toxicity study, three doses of 125, 500 and 1000 mg/kg of biotropics®PM101 were administered orally for a period of 28 days. The animals were examined daily for signs of toxicity, morbidity and mortality upto 28 days. Also, higher dose animals were additionally observed for another 14 days during the post observation period for assessment of reversibility, persistence or delayed occurrence of toxicity. In addition animals were subjected to clinical biochemistry, hematology and Gross pathology.

Results: The acute and subacute 28-days repeated dose oral toxicity of the *Polygonum minus* aqueous extract (biotropics®PM101), a proprietary herbal extract (Malaysia Patent Pending: PI2012003882) was studied extensively in wistar rats. In the acute toxicity test, oral administration of 2000 mg/kg of the biotropics®PM101 produced neither mortality nor changes in behavior or any other physiological activities. In subacute 28-days repeated dose oral toxicity study, no mortality or toxic signs were observed when the three doses of 125, 500 and 1000 mg/kg of biotropics®PM101 were administered orally for a period of 28 days. Also no reversal, persistence or delayed occurrence of toxicity was found, when observed for high dose (1000 mg/kg) for a period of 14 days following 28 day treatment. biotropics®PM101 did not showed any adverse effect on the body weight gain and average daily food consumption in control and treated animals of both sexes upto the dose level of 1000 mg/kg. In the blood chemistry analysis, no significant changes occurred, including total protein, albumin, globulin, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), glucose, creatinine, urea nitrogen, total billirrubin, calcium, phosphorous, cholesterol, triglycerides, sodium and potassium in animals of both sexes upto the dose level of 1000 mg/kg. Hematological analysis (WBC counts, RBC indices, plalete count, reticulocyte count and clotting time) of animals of both the sexes' upto dose level of 1000 mg/kg was found to be comparable to control animals at termination of the treatment and also at the end of the recovery period. The *Polygonum minus* aqueous extract biotropics®PM101 did not affect qualitative and microscopic urinalysis parameters of treated rats. The absolute and relative weights of various organs of animals of both sexes upto dose level of 1000 mg/kg were found to be comparable to control at end of the treatment and recovery. Pathologically, neither gross abnormalities nor histopathological changes were observed. Also, the neurological examination (functional observations) did not reveal any neurotoxic potential of biotropics®PM101 upto its highest level of exposure.

Conclusion: Based on the finding of this study, the no-observed-adverse-effect-level (NOAEL) of *Polygonum minus* aqueous extract (biotropics®PM101) in wistar rats, following oral administration for 28 days was found to be more than 1000 mg/kg body weight.

Keywords: *Polygonum minus* aqueous extract; Toxicity; Acute toxicity; Sub-acute toxicity; 28 days repeated oral toxicity

INTRODUCTION

Polygonum minus is an annual non-woody plant originated from Southeast Asia countries namely Malaysia, Thailand, Vietnam and Indonesia. It is a plant belonging to the family Polygonaceae. It is widely distributed in Malaysia and locally known as kesum[1]. The plant leaves has faintly sour taste, with a delightful clean, lemony aroma reminiscent of turmeric leaves, cilantro, sorrel and lemon basil. The leaves are popularly used as flavoring ingredient in Malaysian delicacies.

Polygonum minus was found to have very high anti-oxidant properties and a good source for natural anti-oxidants [2]. Nutritionally, *Polygonum minus* leaves is rich in fiber, beta carotene, Vitamin A and vitamin C, as well as minerals such as potassium, calcium and phosphorus. It also contains flavonoids, a fraction of pholyphenol, which give beneficial effects on cardiovascular risk on consumption [3-6]. It has been assessed against normal lung fibroblast cell line Hs888Lu and the results did not present any inhibition percentage of cell viability in both ethanol and aqueous extract solvent [7]. In other study, *Polygonum minus* was evaluated for antioxidant activity and genotoxic effects on human lymphocytes and they proved that aqueous extract of *Polygonum minus* is harmless [8].

The present study was designed to determine the acute and subacute oral toxicity of the *Polygonum minus* aqueous extract (biotropics®PM101) in Wistar rats.

MATERIALS AND METHODS

Preparation of the extract

The extract was obtained from a commercial batch of biotropics®PM101 from Phytes Biotek Sdn Bhd, Malaysia. The standardized extract was prepared by a water extraction of *Polygonum minus* using the unique best-available-method of aqueous extraction technology (Malaysia Patent Pending: PI2012003882), comprising the steps of a) subjecting the plant materials to hot water extraction by percolation; b) filtering; c) followed by concentration; d) freeze drying without any carrier such as maltodextrin or lactose; and e) size reduction obtaining the dry extract powder.

Acute toxicity

The acute toxicity of the biotropics®PM101 was evaluated in wistar rat as per the OECD guideline [9,10].

In sighting study, one single female rat received biotropics®PM101 starting at 300 mg/kg orally by gavage. The animal was observed for

mortality and clinical signs for 14 days post dosing. As no toxic sign observed till 14 days, another female rat recieved highest dose of 2000 mg/kg of biotropics®PM101, orally by gavage and observed for 14 days. As there was no clinical sign even at highest dose, the main study was carried out in female wistar rats (four female rats, weight: 173 - 184 g, age 8-9 weeks, overnight fasted) received biotropics®PM101at 2000 mg/kg orally by gavage. The animals were observed for mortality and toxic symptoms continuously for the first 6 h after dosing. The rats were then observed for incidence of mortality and signs of intoxication for 14 days.

Observation

The animals were examined perticularly for changes in skin, fur, eyes, nucous membrane, occourance of secreations, excretions and autonomic activity. Changes in gait, posture, response to handling, presence of clonic or tonic movement, sterotypies or bizzare behaviour were also recorded. Individual animals body weights were recorded at one day before dosing (day 0),1,7 and at termination. Mean body weight, individual and group mean percent gain in body weight over basal values were calculated (Table 2). All animals were euthanized at end of the observation period and subjected to a complete necroscopy. As no gross pathological findings were encountered in any of the organs, histopathological examination was not conducted (Table 3).

Subacute toxicity

The sub acute toxicity of the biotropics®PM101 was evaluated in wistar rat as per the OECD guideline [11].

Experimental animals

Sixty wistar rats (weight; male 115–146 g, female 109-150 g; age: 6–8 weeks old) were randomly assigned into six groups (n = 10), five females and five males in each group. Groups of five rats were housed together in polypropylene cages (males separated from females) with 12 h light/dark cycle in a temperature and humidity controlled environment. Treatments were administered orally by gavage once a day for 28 days. The first and second groups of animals, serving as control and control recovery, received analytical grade water alone. The third, fourth, fifth and sixth group received the biotropics®PM101 at doses of 125, 500, 1000 and 1000 mg/kg respectively. The sixth group of animals, served as high dose reversal. All animals were supplied with Rodent extruded pelleted feed (Provimi brand, Tetragon Chemie Pvt. Ltd. Bangalore) and Aquaguard water *ad libitum* during the testing periods.

All rats were examined daily for signs of toxicity, morbidity and mortality upto 28 days. Animals belonging to second and sixth group were additionally observed for another 14 days during the post observation period for assessment of reversibility, persistence or delayed occurrence of toxicity. Gross pathology for group third, fourth, and fifth was performed on day 29.

For selection of dose levels a 7-day dose range finding was also conducted separately on twenty four wistar rats divided in groups of three male and three female were administered biotropics®PM101 by oral gavage daily at the doses of 125, 250, 500 and 1000 mg/kg body weight for 7 days and were sacrificed on day 8 for detailed necropsy and organ weight.

Observation

Clinical signs of ill health, behavioral changes, reaction to treatment and mortality were observed at least once a day through the 28 days of dosing. A general and detailed clinical examination was done before initiation of the treatment and weekly thereafter during the treatment and recovery period. Body weight, food intake and weight gain were measured once a week. Ophthalmological examination of each animal was carried out using ophthalmoscope prior to the first treatment and sacrifice. Sensory and motor activity was observed during the 4th week of treatment for all groups. These included Functional observational battery (FOB) [12].

Blood analysis

Samples of blood were drawn at termination of treatment (day 29) and end of the recovery period (day 43). The animals were fasted overnight, and anesthetized afterwards for blood collection from the orbital sinus. The samples were collected in tubes containing Heparin (for clinical chemistry) and K-EDTA (for hematology) as an anticoagulant. The hematology was carried out by using 'Coulter A^c.T diff' Hematology Analyser (Beckman Coulter, Inc., Miami, Florida, USA), which included estimation of hemoglobin concentration (Hb), haematocrit, red blood cell count (RBC), White blood cells (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), and reticulocyte. Blood clotting time was performed manually.

The heparinized blood was allowed to coagulate before being centrifuged and the plasma separated. Plasma samples were analysed individually for total protein, albumin, globulin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, urea nitrogen, urea, creatinine, total billirrubin, calcium, phosphorus, total cholesterol, triglyceride, sodium and potassium. The clinical biochemistry was performed using Synchron CX-5 Pro random access analyser (Beckman Coulter, Inc., Miami, Florida) and commercially available diagnostic kits (Beckman Coulter, Inc., USA).

Urinalysis

Urinalysis was performed on all animals, at termination of the treatment period (day 27/28) and at the end of the reversal period (day 42). The urine was subjected to qualitative and microscopic examination using Multistix®SG multiple reagent diagnostic strips (Bayer Diagnostics India Ltd., Baroda, India).

Tissue analysis

At termination of treatment period (day 29) and at the end of reversal period (day 43), all rats were sacrificed under anesthesia and complete necropsies were carried out. The tissues were collected and preserved using 10% neutral buffered formalin solution. Testes and eyes were collected in modified davidson's fixative. The organs such as Brain, Heart, Thymus, Spleen, Liver, Kidney, Adrenals, Testes, and Epididymides were removed and weighted immediately for subsequent analysis. Spinal cord, intestines, stomach, thyroid, trachea, seminal vesicles, ovary, lymph nodes, bone marrow, lungs, prostate, uterus, urinary bladder and the sciatic nerve were also extracted. Tissues were subjected to dehydration process, embedded in paraffin, sectioned at 3 to 5 micron and followed by hematoxillin-eosin staining. The pathological observations of all tissues were performed on gross and microscopic bases. Histological plates of the preserved tissues were encrypted for analysis by a pathologist.

Statistical analysis

Statistical evaluation was performed using Graph Pad Prism Ver.5.0, for Windows XP. All results are presented as Mean ± SEM. The body weight, hematology, clinical chemistry and organ weight data was compared by Bartlett's test for homogeneity. The data with homogeneous intra-group variances was subjected to one-way analysis of variance (ANOVA-Snedecor and Cochran, 1980). When 'F' values were significant, Dunnett's pair wise comparison (Scheffe, 1953) of means of treated groups with control mean was done individually. All analysis and comparison were evaluated at 5% ($P<0.05$) level.

RESULTS

Acute toxicity

No death was recorded in the 14 days of observation period in the male and female animals given 300 mg /kg and 2000 mg/kg of the biotropics®PM101orally in sighting study. No mortality and clinical signs of intoxication was recorded in the 14 days of observation period in male and female animals given 2000 mg/kg of biotropics®PM101orally in the main study. The animals did not show any changes in the general appearance during the observation period (Table 1).

Table 1: Toxic signs assessment of rats in acute toxicity of the formulations from biotropics®PM101 (300 and 2000 mg/kg)

Study	Animal no.	Incidences of clinical signs observed after dosing on																		Mortality*
		Day 1																		
		Min		Hour				Days												
		10	30	1	2	4	6	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Sighting	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0/1
	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
Main	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0/4
	4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	5	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	6	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	

*Number of animals died/number of animals treated, N – Normal

Table 2: Individual animal body weights (g)

Dose (mg/kg)	Animal No.	Day 0	Day 1	Day 7	Day 0-7 (% Gain)	Day 15	Day 0-15 (% Gain)
300	1	173	162	186	7.51	196	13.29
2000	2	184	181	201	9.24	213	15.76
	3	175	170	191	9.14	198	13.14
	4	174	169	193	10.92	201	15.52
	5	173	168	190	9.83	200	15.61
	6	179	171	194	8.38	203	13.41
Mean		177.00	171.80	193.80	9.49	203.00	14.69
± SD		± 4.53	± 5.26	± 4.32	-	± 5.87	-

Table 3: Individual animal fate and necropsy findings

Animal No.	Fate	Day	Necropsy findings
1	TS		NAD
2	TS	15	NAD
3	TS		NAD
4	TS		NAD
5	TS		NAD
6	TS		NAD

TS – Terminal sacrifice, NAD – No abnormality detected.

Table 4: Hematological values of rats in subacute toxicity of the biotropics®PM101

	Male						Female					
	Control		*P. minus* aqueous extract [mg/kg]				Control		*P. minus* aqueous extract [mg/kg]			
	G1	G2*	125	500	1000	1000#	G1	G2*	125	500	1000	1000#
WBC[a]	7.52 ±2.60	5.40 ±1.72	7.54 ±2.13	8.80 ±2.37	7.64 ±1.03	7.14 ±1.05	6.96 ±0.97	8.04 ±4.65	6.66 ±2.08	7.72 ±1.12	6.80 ±0.99	7.94 ±1.43
RBC[b]	7.75 ±0.40	8.06 ±0.47	7.61 ±0.45	7.89 ±0.27	7.37 ±0.53	7.85 ±0.44	7.40 ±0.34	7.34 ±0.35	7.74 ±0.46	7.74 ±0.33	7.67 ±0.30	7.75[S+] ±0.17
Hb[c]	14.34 ±0.44	14.82 ±0.76	14.16 ±1.05	14.22 ±0.65	13.36 ±0.72	14.20 ±1.96	14.40 ±1.18	13.46 ±0.37	14.46 ±1.06	14.34 ±0.56	13.94 ±0.68	14.20[S+] ±0.56
PCV[d]	38.24 ±1.03	40.00 ±2.46	37.86 ±2.80	38.86 ±1.38	35.90 ±1.82	39.30 ±1.96	37.58 ±2.88	36.34 ±1.01	38.24 ±2.74	38.40 ±1.35	37.44 ±1.67	38.00[S+] ±1.19
MCV[e]	49.42 ±2.94	49.62 ±0.49	49.75 ±1.50	49.25 ±1.32	48.81 ±1.58	50.11 ±1.61	50.74 ±1.81	49.54 ±1.83	49.42 ±1.47	49.64 ±0.46	48.82 ±1.64	49.05 ±1.30
MCH[f]	18.54 ±1.24	18.39 ±0.23	18.61 ±0.55	18.02 ±0.61	18.16 ±0.63	18.09 ±0.74	19.44 ±0.86	18.35 ±0.84	18.69 ±0.69	18.54 ±0.64	18.18 ±0.58	18.33 ±0.61
MCHC[g]	37.50 ±0.28	37.07 ±0.42	37.40 ±0.22	36.58[S-] ±0.38	37.21 ±0.43	36.11[S-] ±0.79	38.31 ±0.56	37.04 ±0.41	37.81 ±0.66	37.35 ±1.03	37.22 ±0.29	37.36 ±0.39
PLT[h]	911.60 ±32.70	723.80 ±159.66	808.60 ±145.65	996.40 ±84.37	1123.80 ±202.69	879.60 ±64.38	896.80 ±63.83	869.80 ±161.51	975.20 ±127.82	939.00 ±88.05	911.80 ±56.56	924.60 ±77.26
N[i]	17.40 ±4.72	18.00 ±6.75	26.80 ±8.29	23.60 ±8.08	20.00 ±8.37	18.40 ±3.78	26.20 ±7.22	31.20 ±13.61	31.80 ±6.38	26.40 ±5.68	23.80 ±7.26	30.20 ±9.20
L[j]	81.40 ±4.45	81.00 ±6.89	71.20 ±9.18	76.00 ±7.38	78.60 ±9.02	79.40 ±3.36	72.20 ±7.73	67.80 ±13.81	67.60 ±5.73	72.00 ±6.60	75.60 ±7.47	68.40 ±8.62
E[k]	0.00 ±0.00	0.40 ±0.55	0.20 ±0.45	0.00 ±0.00	0.00 ±0.00	1.00[S+] ±0.00	0.40 ±0.89	0.40 ±0.55	0.20 ±0.45	0.80 ±1.10	0.40 ±0.55	1.00 ±0.71
M[l]	1.20 ±0.45	0.60 ±0.55	1.80 ±1.30	0.40 ±0.89	1.40 ±1.14	1.20 ±0.84	1.20 ±1.30	0.60 ±0.55	0.40 ±0.55	0.80 ±1.10	0.20 ±0.45	0.40 ±0.55
CT[m]	113.60 ±32.70	85.20 ±16.63	124.40 ±17.56	101.40 ±9.86	116.20 ±14.20	89.40 ±25.97	102.40 ±28.55	75.40 ±14.59	105.60 ±24.99	106.60 ±5.94	107.40 ±19.40	101.20 ±24.88
RT[n]	3.00 ±0.41	2.66 ±0.52	2.125 ±0.33	2.22 ±0.43	2.465 ±0.11	2.08 ±0.54	2.26 ±0.29	2.26 ±0.38	2.28 ±0.59	2.02 ±0.33	2.58 ±0.39	2.10 ±0.44

Values are expressed as mean ±SEM for *n* = 10 rats per group, [a] White blood cell (×103 mm−3), [b] Red blood cell (×106 mm−3), [c] Hemoglobin concentration (g/dl), [d] Packed cell volume (%), [e] Mean corpuscular value (FL), [f] Mean corpuscular hemoglobin (pg), [g] Mean corpuscular hemoglobin concentration (g/dl), [h] Platelet Count (×103 mm−3), [i]Neutrophil (%), [j] Lymphocyte (%), [k]Eosinophil [l]Monocytes (%), [m] Clotting time, [n]Reticulocyte,* Control reversal, # High dose reversal.

Sub acute toxicity

General signs

No deaths or significant changes in general behavior or other physiological activities were observed in control and treated animals throughout the treatment period of 28 days and also during recovery period.

Body weight, food, and water intake

Body weight gain by male and female rats treated at 125, 500 and 1000 mg/kg body weight was found to be comparable to control throughout the treatment period. A lowered weight gain was observed for low dose and intermediate dose male animals but further found to be not related to treatment. Average feed intake by male and female rats treated at 125, 500 and 1000 mg/kg body weight was found to be comparable to control throughout the treatment period. Sensory, motor and grip strength were reported normal and no findings, indicative of a neurotoxic potential of biotropics®PM101, were encountered during functional observation (FOB).

Hematological and plasma biochemical data

The hematological analysis (Table 4), showed no significant changes of hematological parameters in the male and female animals of treatment group compared to the control group.

The biochemical analysis (Table 5), showed no significant differences in any of the parameters examined in either the control or treated group of the male and female rats, at termination of the treatment and at the end of recovery period. Although a statistically significant change was seen in few parameters, but it was not considered to be the treatment related change due to lack of dose related effect.

Urinalysis

The data on urinalysis evaluated at termination of treatment and also at the end of recovery period did not indicate any abnormality due to treatment with biotropics®PM101. The data in treated animals and control was found to be comparable.

Tissue analysis

The values of absolute and relative organ weights of male and female rats treated with biotropics®PM101 at and upto 1000 mg/kg were found to be comparable to control at termination of the treatment and at the end of recovery period (Table 6). Pathological examinations of the tissues on a gross basis did not indicated pathological alteration. No alterations were seen in the microscopic examination of the internal organs. Few microscopic changes noticed appeared to be incidental as their frequency were similar to control group animals and not dose dependent.

Table 5: Blood chemistry values of rats in subacute toxicity of the biotropics®PM101

	Male						**Female**					
	Control		***P. minus* aqueous extract [mg/kg]**				**Control**		***P. minus* aqueous extract [mg/kg]**			
	G1	**G2***	**125**	**500**	**1000**	**1000#**	**G1**	**G2***	**125**	**500**	**1000**	**1000#**
TP[a]	6.88 ±0.34	7.04 ±0.33	6.98 ±0.22	6.82 ±0.40	6.92 ±0.19	7.20 ±0.17	7.00 ±0.19	7.44 ±0.36	7.22 ±0.13	7.12 ±0.08	6.88 ±0.37	7.62 ±0.28
ALB[b]	1.80 ±0.16	1.74 ±0.09	1.60 ±0.47	1.70 ±0.12	1.64 ±0.23	1.80 ±0.24	1.80 ±0.35	1.78 ±0.43	1.76 ±0.30	1.94 ±0.09	1.92 ±0.13	1.94 ±0.33
GLB[c]	5.08 ±0.47	5.30 ±0.38	5.38 ±0.16	5.12 ±0.36	5.28 ±0.36	5.40 ±0.30	5.20 ±0.35	5.66 ±0.53	5.46 ±0.36	5.18 ±0.08	4.96 ±0.32	5.68 ±0.35
ALP[d]	153.60 ±28.35	119.40 ±23.23	159.60 ±15.50	158.80 ±35.60	145.20 ±14.87	126.40 ±19.96	113.80 ±43.50	75.80 ±19.08	73.80 ±16.98	90.80 ±20.62	121.60 ±26.53	69.60 ±21.09
AST[e]	90.80 ±7.56	105.20 ±25.35	92.80 ±14.82	86.80 ±2.77	93.20 ±17.04	101.40 ±23.06	134.40 ±35.19	101.40 ±26.20	91.60[S-] ±6.88	90.60[S-] ±11.46	94.60[S-] ±13.97	86.80 ±11.30
ALT[f]	30.00 ±4.30	29.20 ±2.77	34.80 ±5.40	27.80 ±5.63	28.80 ±4.27	31.40 ±3.71	30.40 ±5.59	28.40 ±6.11	24.40 ±3.91	28.00 ±6.04	27.40 ±9.91	20.60[S-] ±3.36
GLU[g]	108.60 ±18.61	138.20 ±31.33	82.40[S-] ±5.55	78.40[S-] ±18.15	79.60[S-] ±6.23	96.40[S-] ±14.17	83.80 ±19.51	91.60 ±7.83	94.00 ±10.05	91.40 ±7.86	81.80 ±6.98	89.00 ±5.43
UN[h]	19.60 ±2.79	16.80 ±2.49	18.20 ±2.28	20.40 ±4.67	17.40 ±1.95	15.00 ±1.73	22.60 ±8.35	16.60 ±3.78	22.20 ±4.21	22.00 ±4.58	23.60 ±2.51	17.60 ±1.52
UREA[i]	41.94 ±5.98	35.95 ±5.33	38.95 ±4.88	43.66 ±9.99	37.24 ±4.17	32.10 ±3.71	48.36 ±17.88	35.52 ±8.09	47.51 ±9.00	47.08 ±9.81	50.50 ±5.37	37.66 ±3.25
CRT[j]	0.32 ±0.04	0.33 ±0.05	0.39 ±0.05	0.34 ±0.06	0.37 ±0.08	0.33 ±0.06	0.14 ±0.09	0.39 ±0.06	0.46 ±0.11	0.41 ±0.07	0.38 ±0.01	0.40 ±0.04
TBN[k]	0.16 ±0.05	0.16 ±0.05	0.16 ±0.05	0.14 ±0.05	0.16 ±0.09	0.26 ±0.26	0.28 ±0.15	0.16 ±0.09	0.16 ±0.05	0.18 ±0.08	0.28 ±0.08	0.10 ±0.00
CA[l]	10.96 ±0.17	11.12 ±0.46	11.12 ±0.18	11.08 ±0.25	11.14 ±0.13	11.26 ±0.30	11.12 ±0.16	11.26 ±0.04	11.06 ±0.22	11.22 ±0.33	11.42 ±0.50	11.08 ±0.11
PH[m]	8.86 ±0.93	8.82 ±0.40	7.98 ±0.65	9.26 ±1.02	8.88 ±1.02	9.06 ±0.61	9.00 ±1.09	8.64 ±1.86	8.08 ±0.51	8.38 ±0.79	9.20 ±1.64	7.00 ±0.67
CHL[n]	54.00 ±8.28	50.60 ±8.93	49.80 ±10.35	51.40 ±9.21	47.20 ±8.14	44.40 ±6.66	38.20 ±25.48	35.60 ±6.23	35.40 ±8.05	42.60 ±12.14	38.40 ±3.97	39.80 ±7.16
TG[o]	100.40 ±54.56	87.00 ±20.00	70.60 ±33.22	53.00 ±10.44	62.00 ±14.09	104.40 ±50.06	39.80 ±25.48	33.00 ±15.60	36.00 ±9.51	42.60 ±7.40	41.80 ±13.22	48.80 ±14.65
NA[p]	145.06 ±1.25	148.30 ±2.61	144.90 ±3.22	146.40 ±1.94	145.72 ±0.98	147.62 ±0.77	143.54 ±2.61	145.44 ±2.62	145.66 ±1.81	145.72 ±0.89	144.76 ±1.40	146.36 ±1.07
K[q]	5.25 ±0.18	5.21 ±0.58	5.12 ±0.34	5.43 ±0.71	5.30 ±0.53	5.34 ±0.56	6.05 ±1.34	6.58 ±3.32	5.01 ±0.25	5.06 ±0.68	6.26 ±2.16	4.45 ±0.34

Values are expressed as mean ±SEM for *n* = 10 rats per group. [a]Total Protein, [b]Albumin, [c]Globulin, [d]Alkaline phosphatase, [e]Aspartate aminotransferase , [f] Alanine aminotransferase, [g]Glucose, [h]Urea nitrogen, [I]Urea, [j]Creatinine, [k] Total billirubin, [l]Calcium, [m]Phosphorous, [n]Cholesterol, [o]Triglycerides, [p]Sodium, [q]Potassium, * Control reversal, [#]High dose reversal.

Table 6: Relative organ weight of rats in subacute toxicity of the biotropics®PM101

	Male						Female					
	Control		*P. minus aqueous extract* [mg/kg]				Control		*P. minus* aqueous extract [mg/kg]			
	G1	G2*	125	500	1000	1000#	G1	G2*	125	500	1000	1000#
FBW	246.80 ±15.07	279.80 ±19.29	217.60^{S-} ±16.79	234.20 ±6.69	241.20 ±9.26	276.20 ±35.01	176.20 ±14.55	192.40 ±13.69	176.20 ±12.21	181.40 ±13.58	181.00 ±13.38	189.60 ±17.21
ADRENALS	0.052 ±0.008	0.055 ±0.008	0.057 ±0.018	0.051 ±0.004	0.054 ±0.005	0.041 ±0.011	0.070 ±0.003	0.076 ±0.008	0.075 ±0.008	0.071 ±0.006	0.067 ±0.012	0.078 ±0.007
TESTES	3.17 ±0.23	3.33 ±0.24	3.27 ±0.33	3.18 ±0.21	3.04 ±0.06	3.28 ±0.22	---	---	---	---	---	---
KIDNEYS	2.07 ±0.18	2.15 ±0.26	1.85 ±0.23	1.96 ±0.07	2.14 ±0.22	2.43 ±0.22	1.61 ±0.17	1.72 ±0.20	1.50 ±0.08	1.55 ±0.18	1.61 ±0.22	1.81 ±0.24
LIVER	8.77 ±0.99	8.93 ±0.88	7.59 ±1.24	7.65 ±0.57	8.56 ±1.14	9.63 ±1.24	6.76 ±1.03	7.36 ±0.83	6.00 ±0.71	6.45 ±0.55	6.28 ±1.07	6.87 ±0.63
BRAIN	1.84 ±0.05	1.87 ±0.04	1.85 ±0.10	1.86 ±0.08	1.83 ±0.08	1.92 ±0.08	1.77 ±0.05	1.85 ±0.10	1.77 ±0.07	1.80 ±0.08	1.83 ±0.08	1.88 ±0.06
THYMUS	0.53 ±0.09	0.53 ±0.09	0.43 ±0.14	0.47 ±0.11	0.56 ±0.06	0.47 ±0.09	0.47 ±0.13	0.53 ±0.08	0.47 ±0.09	0.49 ±0.06	0.43 ±0.09	0.51 ±0.08
HEART	0.95 ±0.09	1.03 ±0.10	0.80^{S-} ±0.07	0.91 ±0.05	0.91 ±0.05	1.07 ±0.14	0.71 ±0.06	0.74 ±0.06	0.70 ±0.08	0.69 ±0.10	0.75 ±0.11	0.77 ±0.08
SPLEEN	0.53 ±0.02	0.51 ±0.06	0.57 ±0.19	0.49 ±0.04	0.59 ±0.14	0.57 ±0.07	0.45 ±0.13	0.54 ±0.08	0.41 ±0.08	0.45 ±0.06	0.43 ±0.07	0.49 ±0.07
EPIDIDYMES	0.79 ±0.11	1.05 ±0.03	0.82 ±0.09	0.88 ±0.12	0.78 ±0.10	1.06 ±0.07	---	---	---	---	---	---
PROSTATE & SEMINAL VESICLE	0.89 ±0.13	1.24 ±0.12	0.72 ±0.21	0.98 ±0.04	0.98 ±0.21	1.37 ±0.18	---	---	---	---	---	---
OVARIES	---	---	---	---	---	---	0.123 ±0.014	0.104 ±0.026	0.120 ±0.018	0.117 ±0.015	0.127 ±0.027	0.096 ±0.017
UTERUS	---	---	---	---	---	---	0.49 ±0.22	0.58 ±0.11	0.42 ±0.11	0.47 ±0.11	0.56 ±0.13	0.50 ±0.10

Values are expressed as mean ±SEM for *n* = 10 rats per group. FBW: Fasting body weight, * Control reversal, #High dose reversal.

DISCUSSION AND CONCLUSION

The acute toxicity study does not show any toxic symptoms, changes in behavior or mortality at 2000 mg/kg doses. On the basis of above observations, the acute oral LD50 of biotropics®PM101 was determined as > 2000 mg/kg body weight and it was classified according to the Globally Harmonized System (GHS) Category 5 or Unclassified. The subacute 28-days toxicity guideline appliedconsiders a full study with three dose levels when at least 1000 mg/kg day does not show toxicity effects, as was observed in the acute toxicity experiment. The objective was to evaluate toxicity profile, arising from repeated oral administration of biotropics®PM101 to rats for a period of 28 days and to find out the reversibility of any observed toxic effects or withdrawal syndrome. The high dose of biotropics®PM101 (1000 mg/kg day) in the subacute 28-days study was applied because human exposure indicates the use of a high dose level in accord with the subacute 28-days guideline. A lower dose of 125 mg/kg day was used to determine dose related toxic effects. In a subacute toxicity study, it appeared that biotropics®PM101at the doses used did not produce any marked changes in both male and female rats, as evidenced by the absence of toxic symptoms, no changes in water/food ingestion, or weight gain. All animals survived until the scheduled euthanasia and no gross pathological alteration was found in the internal organs. Organ weight revealed that biotropics®PM101, at the doses used, did not produce organ swelling, atrophy or hypertrophy. Moreover, the microscopic evaluation did not find any abnormalities in the 1000 mg/kg biotropics®PM101group compared to the control group. Microscopic evaluation in the 125 mg/kg group was not performed in accordance with the subacute 28-days guideline that considers unnecessary the microscopic examination of organs in the low dose group when no histopathological abnormalities are found in the high dose group. The comparable biochemical results in the control group and biotropics®PM101treated groups were consistent with the morphological analysis.

In summary the *Polygonum minus* aqueous extract (biotropics®PM101) was found to be nontoxic when oral acute and subacute 28-days toxicities in rats were performed. Mutagenicity and carcinogenicity studies are necessary to further support the safe use of this plant product. Based on the above investigation, the NOAEL of *Polygonum minus* aqueous extract (biotropics®PM101) for Wistar rats can be considered as greater than 1000 mg/kg body weight under the condition of this investigation.

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