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Original Article

Safety Assessment of TLPL/AY/03/2008, A Polyherbal Formulation in Sprague Dawley Rats

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ABSTRACT

Objectives: TLPL/AY/03/2008 is a polyherbal formulation intended for treatment of osteoarthritis, rheumatoid arthritis, lumbago, spondylitis etc., Acute and repeated dose 90-days studies were conducted to evaluate the safety profile of TLPL/AY/03/2008 in rats. **Materials and Methods:** In acute study, TLPL/AY/03/2008 was orally administered to Sprague Dawley rats at 2000 mg/kg. In repeated dose study, TLPL/AY/03/2008 was administered to rats at 200, 500 and 1000 mg/kg through oral gavage for 90 days and assessed for treatment related changes in body weight, feed consumption, hematological, biochemical and pathological parameters. Histopathological examination was conducted for tissues from control and the high dose groups and was extended to target organs from the lower dose and recovery groups. **Results:** In acute study, the test item did not produce any mortality or adverse clinical signs. In the 90-days oral toxicity study, animals did not exhibit any toxicity symptoms and no deaths were observed. No significant changes were found in hematological and biochemical endpoints. Also, toxicologically significant alterations in relative organ weights were not observed. Microscopic findings of mild to marked, diffuse hepatocellular degeneration (vacuolar changes with granular of cytoplasm and pyknotic nuclei of hepatocytes) was noticed in males at 1000 mg/kg body weight. Animals of recovery group (1000 mg/kg) did not show any changes when compared with control group animals indicating the complete reversal. **Conclusions:** Based on the findings of the study, the median lethal dose of TLPL/AY/03/2008 was found to be more than 2000 mg/kg. The No Observed Adverse Effect Level (NOAEL) of TLPL/AY/03/2008 can be considered as 1000 mg/kg in both male and female rats, under the experimental conditions and doses employed.

Key words: Acute, polyherbal, subchronic, toxicity, TLPL/AY/03/2008

INTRODUCTION

For several decades, medicinal plants have been enormously benefitting sources of wide therapeutic applications along with their relatively low toxic and less side effects. Despite the availability of advanced medicinal systems, approximately 80% of the world population still depends on herbs and herbal formulations for the treatment of various disease conditions/different ailments.[1] TLPL/AY/03/2008 is a herbal formulation composed of extracts of established medicinal plants such as *Boswellia serrata*,[2] *Commiphora mukul*,[3] *Withania somnifera*,[4] *Vitex negundo*,[5] *Ricinus communis*,[6] *Nyctanthes arbortristis*[7] and *Zingiber officinale*[8,9] and has been indicated for the treatment of osteoarthritis, rheumatoid arthritis, lumbago, spondylitis, etc., Although herbal entities are believed to be relatively safe, the toxicity characteristics of the test materials need to be confirmed prior to human clinical trials. Generally this is accomplished

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by conducting general preclinical safety studies to uncover potential toxic effects of drug in question.[10] Although the herbal formulations are in use for wide variety of clinical applications, the toxicological evaluation of herbal ingredients or combinations is still in infancy. With the above considerations, the present study was aimed to assess the single dose acute oral toxicity and 90-days repeated dose toxicity of novel herbal formulation TLPL/AY/03/2008.

MATERIALS AND METHODS

Animals

Female Sprague Dawley (SD) rats of 8-12 weeks age were used for the acute oral toxicity and SD rats of either sex of 7-8 weeks age were used for the 90-days repeated dose toxicity. All animals were bred and reared at animal house of Bioneeds; Bangalore, India. The females used were nulliparous and non-pregnant. All animals were acclimatized and maintained under standard housing conditions (temperature: 22 ± 3°C, relative humidity: between 40-60% with 12-15 air changes per hour and 12 h light-12 h dark cycle). All animals were provided with purified water and Nutrilab rodent feed (M/s: Provimi Animal Nutrition Private Ltd (Vetcare), Bangalore, India.) *ad libitum*. All experiments were carried out with the approval of Institutional Animal Ethics Committee (IAEC) and in accordance with the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), India.

Preparation of herbal formulation

TLPL/AY/03/2008 was manufactured and supplied in capsule by Tulip Lab Private Ltd, Maharashtra, India in powder form. The composition of the TLPL/AY/03/2008 was as follows: Kunduru extract (*Boswellia serrata)* - 130 mg, Guggulu extract *(Commiphora mukul)* - 100 mg, Ashwagandha extract *(Withania somnifera)* - 75 mg, Nirgundi extract (*Vitex negundo*) - 75 mg, Eranda extract *(Ricinus communis)* - 60 mg, Parijata extract *(Nyctanthes arbortristis)* - 50 mg and Shunthi extract *(Zingiber officinale)* - 15 mg.

The above composition was approved by FDA, Maharashtra State as an Ayurvedic Proprietary Medicine.

Study design

Acute oral toxicity study

The study was conducted in accordance with OECD guidelines for the testing of chemicals, OECD 420-Fixed dose procedure. Animals were fasted overnight and 3 h after test substance administration but water was provided *ad libitum*. The sighting study was conducted by dosing one animal at 300 mg/kg body weight (p.o.). Since the animal survived, a second animal was treated with 2000 mg/kg. In main study, four rats were administered at 2000 mg/kg body weight. The treated animals were observed carefully for presence of adverse clinical signs and mortality at 10 min, 30 min, 1, 2, 4 and 6 h after dosing and once daily for 14 days.

90-days repeated dose oral toxicity study

The study was conducted in accordance with the OECD guideline 408 (Repeated Dose 90-day Oral Toxicity Study in Rodents).[11] One twenty Sprague Dawley rats of either sex were equally divided into six groups (each consisting of 10 males and 10 females). G1 and G1R (R = Recovery) served as vehicle controls and the animals were daily administered with normal saline by oral gavage. Groups G2, G3 and G4/G4R were administered daily at the dose of 200, 500 and 1000 mg/kg of TLPL/AY/03/2008 by oral gavage once daily for consecutive 90 days, respectively. The dose volume was maintained at 10 mL/kg. Vehicle control recovery (G1R) and high dose recovery (G4R) groups were maintained for a further 28 days period without administration of either vehicle or test item.

Throughout the study period, the animals were observed for clinical signs of toxicity and mortality/morbidity (daily), detailed veterinary examination, body weight and feed consumption (weekly), functional observation test in week 13, ophthalmoscopic examination at pretest and in week 13, hematological, clinical chemistry, urinalysis, gross pathology and organ weighing at termination. Histopathological examination was conducted on the specified list of tissues from the control and the high dose groups and was extended to target organs from the lower dose and recovery groups.

Statistical analysis

The raw data was subjected to statistical analysis. The data on body weight and weight gain, feed intake, organ weights and ratios, hematological and clinical chemistry estimations, urine volume, specific gravity and pH were analyzed statistically. One way ANOVA with Dunnet as *post hoc* test was done for different treatment groups comparing with the control group and the unpaired *t*-test was done for comparing control recovery and high dose recovery group data. All analyses and comparisons were evaluated at the 95% level of confidence ($P < 0.05$).

RESULTS

Acute oral toxicity study

No mortality, abnormal clinical signs, or gross pathology findings were observed in animals treated with 300 and 2000 mg/kg body weight in sighting study and 2000 mg/kg body weight in main study. All animals gained body weights during the experiment period [Table 1].

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90-days repeated dose oral toxicity study

No clinical signs of toxicity or mortality were noted upon subchronic exposure of the test item. All animals gained body weight for 13 weeks and no significant treatment related changes in body weights and percent body weight gain were observed in both the sexes during treatment and recovery period [Tables 2 and 3]. The feed consumption of different groups of rats was found to be comparable to control groups. The feed consumption of recovery groups of control and high dose rats also found to be consistent with the main groups [Figures 1-4]. Functional observational tests (open field observations, locomotor activity, grip strength) and ophthalmoscopic examination revealed no abnormalities. Treatment related changes could not be observed in hematological [Tables 4 and 5], clinical chemistry [Tables 6 and 7] and in urine analysis (data not shown) at all the doses tested.

No treatment related organ weight changes were noted in the animals sacrificed at the end of treatment and recovery periods. A marginal increase in relative weight of liver was observed in male rats of G2 group (200 mg/kg); however, no dose dependent effect was noticed in mid and high dose group animals and corresponding female group did not reveal similar changes [Tables 8 and 9]. Hence, the increase in relative weight of male rats can be considered incidental and non-treatment related. Also, necropsy findings of treated animals (n = 10/sex/group) did not show any evidence of treatment related lesions [Table 10].

Microscopic findings of mild to marked, diffuse hepatocellular degeneration (vacuolar changes with granular of cytoplasm and pyknotic nuclei of hepatocytes) were recorded in males at 1000 mg/kg (G4). However, the high dose recovery (G4R, 1000 mg/kg) rats did not show any changes and the histological details were comparable to the control group animals indicating the complete reversal after 28 days treatment free period. Other lesions such as mono nuclear cell (MNC) infiltration and necrotic foci were comparable to control group animals. The microscopic observations of kidney tissues of both control and high dose group showed almost similar incidence of MNC infiltration, basophilic tubules, pyelitis and dilatation of renal pelvis. In heart and brain, MNC infiltration of high dose group did not vary significantly as compared to control group [Tables 11 and 12].

Table 1: Body weight summary of acute oral toxicity study

Study phase	Dose (mg/kg B.wt.)	No. of animals	Mean	Body weights (g) on day			% Body weight gain with respect to day 1	
				1	7	14	7	14
Sighting study	300	1	Mean	156.8	165.8	174.9	5.7	11.5
	2000	1	Mean	178.9	202.1	213.2	13.0	19.2
Main study	2000	4	Mean	160.0	176.2	191.7	10.1	19.9
			SD	±3.6	±1.2	±7.5	±2.3	±6.7

Table 2: Effect of oral administration of TLPL/AY/03/2008 for 13 weeks on male rat body weights (in grams)

Week	Group					
	G1	G2	G3	G4	G1R	G4R
0	158.35±2.94	155.41±3.65	154.52±3.65	150.06±4.51	151.05±3.32	158.37±3.31
1	185.08±3.99	187.60±2.84	186.63±4.35	180.03±4.27	183.81±4.12	190.53±3.67
2	214.35±5.14	219.04±4.38	211.34±6.75	207.52±5.93	211.19±4.4	217.35±5.06
3	233.43±8.83	240.02±6.07	228.21±8.37	227.24±9.44	235.01±5.81	234.2±7.69
4	255.45±9.19	260.12±7.07	248.56±9.4	250.87±11.33	250.33±5.86	256.22±7.44
5	270.02±11.27	280.36±10.14	265.9±11.45	253.78±14.34	269.42±7.5	267.73±11.86
6	278.15±12.44	290.47±10.40	277.27±12.31	265.59±14.09	268.99±9.02	264.34±9.22
7	292.70±12.83	304.46±10.25	293.23±13.78	278.84±15.21	290.29±9.57	287.33±12.90
8	305.03±12.83	308.41±8.92	306.05±14.52	289.04±14.85	303.95±9.46	295.73±13.64
9	312.79±13.63	322.73±10.22	318.77±16.18	304.05±15.32	312.7±9.86	306.91±15.21
10	322.42±14.92	326.32±10.48	326.06±16.54	310.00±16.00	321.16±8.77	310.58±15.62
11	322.38±15.20	331.02±11.08	329.16±16.69	310.90±16.14	323.85±9.17	311.19±15.26
12	326.07±15.74	335.18±11.75	332.92±17.24	313.81±16.30	325.58±9.17	313.15±15.62
13	330.86±16.20	337.10±11.37	336.72±17.01	313.82±16.36	328.03±9.32	313.85±15.61
14	-	-	-	-	329.71±10.10	325.52±15.89
15	-	-	-	-	331.94±10.57	337.31±15.42
16	-	-	-	-	337.59±11.18	328.62±15.99
17	-	-	-	-	345.35±10.61	333.06±16.05

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Table 3: Effect of oral administration of TLPL/AY/03/2008 for 13 weeks on female rat body weights (in grams)

Week	Group					
	G1	G2	G3	G4	G1R	G4R
0	142.92±1.80	143.42±2.49	146.56±2.02	143.77±2.07	143.99±1.61	145.27±1.67
1	156.78±1.84	155.70±2.46	157.17±2.08	155.04±2.03	154.97±2.13	156.91±2.29
2	168.34±2.35	171.83±4.18	169.92±3.03	167.53±1.81	167.10±3.46	166.84±3.18
3	177.61±3.78	181.60±3.72	178.00±4.12	174.81±2.91	176.36±4.89	176.84±4.00
4	186.83±5.44	188.29±4.38	186.74±4.80	183.75±4.25	185.26±5.21	185.71±5.15
5	192.78±5.52	197.23±4.89	192.88±5.83	188.31±4.45	190.29±5.42	189.58±5.21
6	195.08±6.89	198.92±4.52	194.16±7.56	189.26±5.46	191.50±6.86	191.12±5.50
7	199.80±6.86	204.51±4.21	201.45±7.80	193.16±5.45	197.50±6.65	195.97±4.27
8	199.22±9.97	211.09±5.44	207.02±8.42	200.39±5.88	201.88±8.15	199.73±5.75
9	209.20±9.78	216.12±5.04	209.38±8.26	204.47±5.69	205.03±7.79	203.84±5.52
10	214.94±9.09	221.89±5.71	212.76±8.95	208.71±6.39	208.80±7.95	207.12±5.32
11	215.86±9.13	221.28±5.4	215.02±9.65	209.69±6.22	211.41±8.45	208.76±5.73
12	218.59±9.63	223.09±5.33	218.41±9.23	211.22±6.62	213.67±9.30	209.45±6.12
13	221.43±9.75	226.19±5.88	221.83±10.07	214.21±6.68	215.27±9.58	211.48±6.17
14	-	-	-	-	218.35±9.84	215.54±6.28
15	-	-	-	-	221.81±10.34	219.11±6.27
16	-	-	-	-	225.42±10.71	222.52±6.33
17	-	-	-	-	229.05±10.79	225.84±6.36

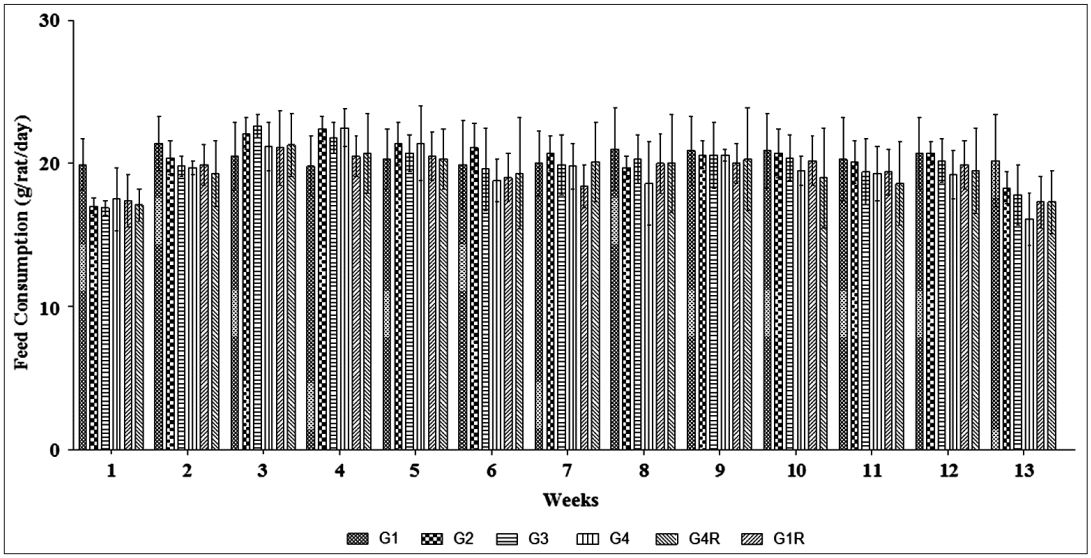


Figure 1: Average weekly feed consumption (g/rat/day) of males during treatment period

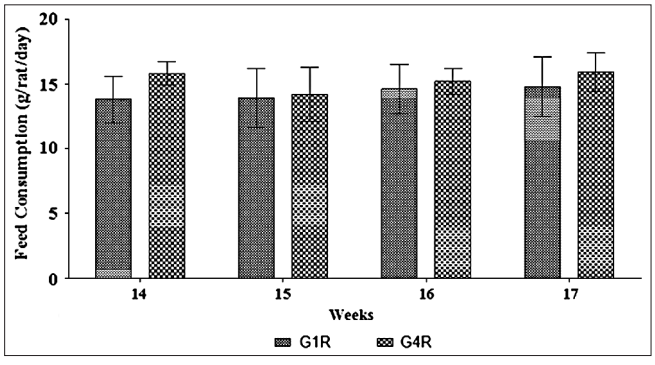


Figure 2: Average weekly feed consumption (g/rat/day) of males during recovery period

DISCUSSION

The interest in use of herbal preparations in different parts of the world has been growing considerably with corresponding developments in the phytomedicinal therapy. Herbal remedies positioned themselves in various forms such as dietary supplements, mono or polyherbal drugs, dietary ingredients, etc., and have become famous and safe commercial commodities. However, the herbal preparations, irrespective of the popular belief that they are safe based on ancient literature, required to be confirmed for their non-toxic/relatively less toxic effects compared to the chemical therapeutic counterparts.[12] This critical

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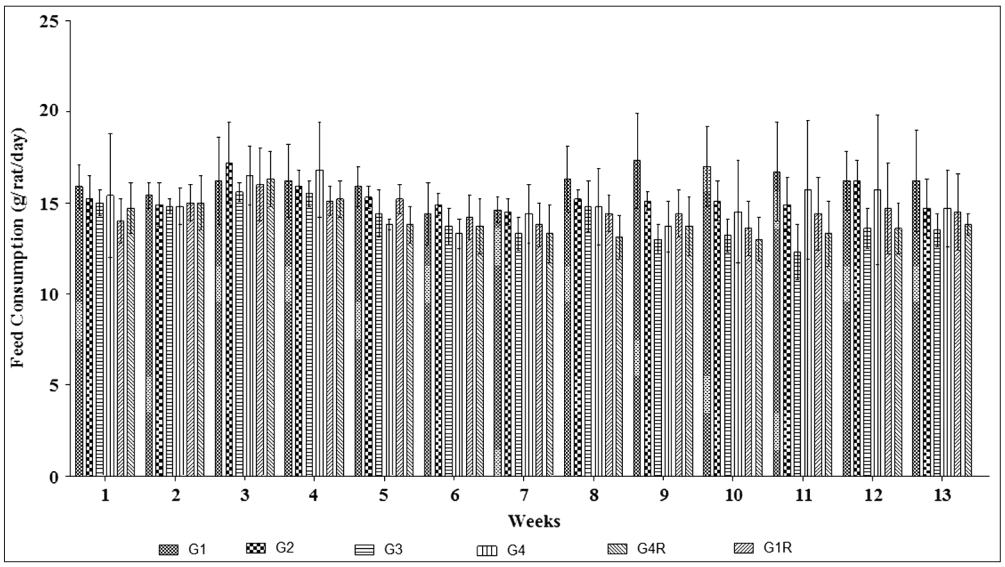


Figure 3: Average weekly feed consumption (g/rat/day) of females during treatment period

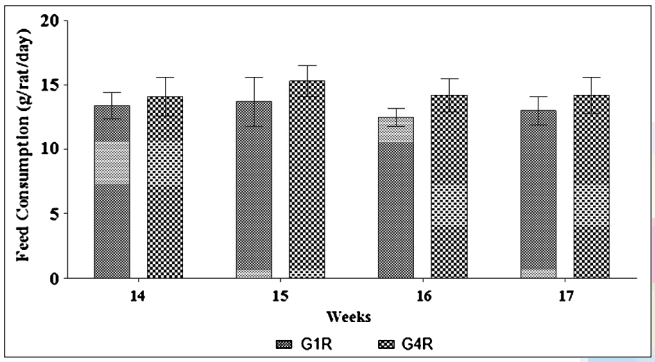


Figure 4: Average weekly feed consumption (g/rat/day) of females during recovery period

prerequisite, especially in the form of acceptance by the western countries, provided the impetus to carry out scientific studies in accordance with the various established regulatory guidelines applicable to the geographic requirements.

Typically, safety studies on herbal compounds intended for oral use involve acute oral toxicity study in rodents which helps to determine the dose levels for short-term and long-term repeated dose toxicity studies. Despite the alternative views on use of LD_{50} data, acute studies still continue to be considered valuable in establishing target organ toxicity. In the present study, TLPL/AY/03/2008 did not produce mortality/morbidity or adverse clinical signs up to the dose level of 2000 mg/kg which indicates the wide margin of safety level upon exposure of single large dose. It is to be noted that the test substances are generally labeled 'Unclassified (category 5)' according to the Globally Harmonized System (GHS) for classification of chemicals when they have been comparatively safe at limit dose levels (i.e. 2000 mg/kg).

Subacute/subchronic oral toxicity studies are carried out to evaluate the likely adverse effects upon prolonged exposure of a test substance to animals and to gather information about the deleterious health effects due to repeated exposures including target organ toxicity, cumulative effects, and to determine the dose a level at which there is no observed adverse effect.[13] In the current study, treatment with TLPL/AY/03/2008 up to 1000 mg/kg was well tolerated. Absence of treatment related deaths or toxic signs throughout the study are direct indication of relatively harmless nature of the test compound over prolonged exposure. The compounds with toxicity potentials are believed to impact on feed intake, metabolic processes, and consequently on the body weight gain. As is observed from scientific literature, a decrement of more than 10% in body weight gain is considered to be detrimental on long term administration of test materials.[14,15] However, such a trend was not observed in the current study since the body weight gain of different groups remained comparable till the end of the study period with corresponding normal feed consumption clearly demonstrated the normal metabolic process in rats administered with TLPL/AY/03/2008. The body weight gain of both sexes of different groups was found to be continuously increasing over treatment period including the recovery period.

Functional observational tests (open field observations, locomotor activity, grasping strength) and ophthalmoscopic examination of treated rats revealed no considerable alterations. These observations, in general, reveal that the herbal preparation did not interfere with neuromuscular physiology and autonomic activities of treated animals. Although, some of the hematological and biochemical parameters (RBC, Hb, HCT and Clotting time, ALT,

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Table 4: Effect of oral administration of TLPL/AY/03/2008 on hematological parameters (Male)

Group	Hematology													
	Total WBC count (103 cells/μL)	Total RBC count (106 cells/μL)	Hemoglobin (g/dL)	Hematocrit (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Platelet count (103 cells/μL)	Clotting time (Sec)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
G1	11.51±1.01	8.42±0.2	14.3±0.3	45.95±1.03	54.59±0.51	17.01±0.29	31.14±0.3	635.1±40.91	82.4±2.21	13.8±1.41	85.4±1.35	0.7±0.26	0.1±0.1	0±0
G2	11.27±1.62	7.89±0.21	13.73±0.26	42.82±1.02*	54.29±0.36	17.44±0.31	32.12±0.48	624.9±52.7	86.2±4.03	14.8±1.4	84.1±1.22	0.5±0.22	0.6±0.27	0±0
G3	9.61±1.12	7.19±0.13*	12.52±0.17*	39.63±0.6*	55.21±0.86	17.44±0.3	31.6±0.18	665±30.86	81.5±1.85	15.1±1.29	84.1±1.39	0.4±0.22	0.4±0.22	0±0
G4	9.89±0.85	7.52±0.13*	13.09±0.26*	41.32±0.78*	54.98±0.32	17.4±0.16	31.67±0.13	639.4±33.29	84.9±2.25	16.1±1.61	83.2±1.5	0.7±0.26	0±0	0±0
G1R	13.06±0.72	7.38±0.08	13.3±0.22	40.64±0.53	55.04±0.49	18.01±0.22	32.72±0.24	610.7±36.46	89.4±4.06	13.4±1.06	86±1.17	0.5±0.27	0.1±0.1	0±0
G4R	12.82±0.76	7.86±0.41	14.28±0.78	43.52±2.39	55.3±0.47	18.15±0.23	32.84±0.28	647.5±64.14	96.2±5.03	16±0.98	83.1±0.9	0.6±0.27	0.3±0.21	0±0

All values are expressed as Mean±SEM, *n*=10 animals/group, *Significant *P*<0.05 vehicle control vs. TLPL/AY/03/2008 treated groups, RBC= Red Blood Cells; WBC- White Blood Cells; MCH= Mean corpuscular hemoglobin; MCHC=Mean corpuscular hemoglobin concentration

Table 5: Effect of oral administration of TLPL/AY/03/2008 on hematological parameters (Female)

Group	Hematology													
	Total WBC count (103 cells/μL)	Total RBC count (106 cells/μL)	Hemoglobin (g/dL)	Hematocrit (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Platelet count (103 cells/μL)	Clotting time (Sec)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
G1	7.75±0.69	6.43±0.04	12.57±0.18	36.74±0.46	57.1±0.44	19.54±0.23	34.21±0.25	589.7 ± 33.23	77.5±0.99	13.1±1.05	85.6±1.13	1±0.33	0.3±0.15	0±0
G2	8.06±0.73	6.65±0.13	12.78±0.11	37.72±0.45	56.84±0.64	19.26±0.26	33.9±0.16	688.8 ± 25.19	72.9±1.43	14.3±1.39*	84.7±1.57	0.7±0.26	0.3±0.15	0±0
G3	7.94±0.79	6.87±0.06*	13.06±0.07	38.78±0.32*	56.44±0.51	19.01±0.22	33.68±0.2	665.6 ± 44.2	74.6±1.24	15±1.55	84.3±1.64	0.6±0.22	0.1±0.1	0±0
G4	7.7±0.39	6.59±0.11	12.75±0.18	37.19±0.61	56.48±0.53	19.38±0.18	34.3±0.15	665.5 ± 34.01	73.4±0.76	17±1.69*	82.1±1.68	0.7±0.26	0.3±0.15	0±0
G1R	11.17±0.85	6.64±0.34	13.06±0.54	38.07±1.84	57.38±0.37	19.74±0.34	34.43±0.51	579.7 ± 49.43	89.4 ± 4.06	14.7±1.26	84.6±1.31	0.5±0.27	0.2±0.13	0±0
G4R	11.19±0.88	6.89±0.24	13.64±0.51	39.87±1.42	57.98±0.81	19.82±0.31	34.2±0.32	559.2 ± 56.51	96.2 ± 5.03	14.6±1.13	84.8±1.07	0.5±0.22	0.1±0.1	0±0

All values are expressed as Mean±SEM, *n*=10 animals/group *Significant *P*<0.05 vehicle control vs. TLPL/AY/03/2008 treated groups, RBC= Red Blood Cells; WBC- White Blood Cells; MCH= Mean corpuscular hemoglobin; MCHC=Mean corpuscular hemoglobin concentratio

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Table 6: Effect of oral administration of TLPL/AY/03/2008 on clinical chemistry parameters (Male)

Group	Clinical chemistry															
	Fasting glucose (mg/dL)	Total cholesterol (mg/dL)	Creatinine (mg/dL)	Total protein (g/dL)	Albumin (g/dL)	Triglycerides (mg/dL)	Urea nitrogen (mg/dL)	Total bilirubin (mg/dL)	Chloride (mmol/L)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Phosphorus (mg/dL)	Calcium (mg/dL)	Sodium (mmol/L)	Potassium (mmol/L)
G1	93.2±2.25	47.6±2.25	0.78±0.03	6.37±0.07	3.61±0.09	70±4.65	22.7±0.8	0.49±0.03	101.5±0.7	54.1±2.81	101.2±2.41	115.5±3.74	5.81±0.14	9.4±0.07	9.44±0.04	4.08±0.08
G2	99±2.24	44.8±1.24	0.74±0.04	6.08±0.08	3.9±0.08	66.5±4.29	22±0.75	0.47±0.04	102.6±0.54	52.9±2.67	98.4±1.34	109.4±3.91	5.72±0.07	9.45±0.09	147±0.41	4.22±0.14
G3	102.9±2.16*	48.9±2.09	0.85±0.05	6.21±0.1	3.84±0.11	77.3±4.82	22.5±0.98	0.53±0.04	105.2±0.96*	43.8±2.28	94.1±2.16*	121.7±6.14	5.61±0.07	9.44±0.1	148.27±0.27	4.17±0.05
G4	102.3±3.13*	46.8±1.42	0.87±0.04	6.33±0.12	3.99±0.19	69.7±3.08	23.9±0.98	0.56±0.05	103.10±0.82	52.6±3.65	105.4±2.05	131.6±7.3	5.30±0.1*	9.48±0.08	147.86±0.32	4.09±0.05
G1R	92.4±4.15	46.4±3.02	0.8±0.01	6.4±0.07	3.32±0.18	55.1±6.64	18.5±0.56	0.82±0.07	106.7±0.73	45.5±3.11	99.7±4.99	84.5±6.67	5.6±0.23	7.61±0.04	151.29±0.68	4.24±0.19
G4R	83.6±4.38	49.7±2.24	0.75±0.02	6.53±0.06	3.44±0.11	64.3±5.42	16.9±0.72	0.87±0.07	109±0.99	45.8±2.63	111.9±5.61	96.2±12.71	5.11±0.1	7.52±0.04	151.93±0.34	3.94±0.07

All values are expressed as Mean±SEM, *n*=10 animals/group, *Significant *P*<0.05 vehicle control vs. TLPL/AY/03/2008 treated groups, ALT= Alanine Aminotransferase; AST= Aspartate Aminotransferase, ALP= Alkaline Phosphatase

Table 7: Effect of oral administration of TLPL/AY/03/2008 on clinical chemistry parameters (Female)

Group	Clinical chemistry															
	Fasting glucose (mg/dL)	Total cholesterol (mg/dL)	Creatinine (mg/dL)	Total protein (g/dL)	Albumin (g/dL)	Triglycerides (mg/dL)	Urea nitrogen (mg/dL)	Total bilirubin (mg/dL)	Chloride (mmol/L)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Phosphorus (mg/dL)	Calcium (mg/dL)	Sodium (mmol/L)	Potassium (mmol/L)
G1	91.1±3.73	49.7±3.32	0.78±0.04	6.7±0.13	3.7±0.1	68.6±4.17	22.6±0.96	0.41±0.03	103.1±0.59	41.2±2.97	87.3±3.8	107.2±3.38	5.9±0.19	9.76±0.1	9.85±0.04	4.12±0.11
G2	103.1±3.42	53±3.44	0.86±0.06	7.02±0.08	3.59±0.12	61.4±2.75	23.3±1.01	0.39±0.04	102.3±0.62	37.3±2.86	88±3.45	104.7±3.7	5.13±0.13*	9.98±0.06	148.81±0.47	3.96±0.11
G3	104.2±4.64	52.2±2.57	0.81±0.05	6.8±0.07	3.7±0.1	62.6±2.34	22.4±0.96	0.48±0.04	102.4±0.73	32.6±1.03*	85±3.02	109.5±6.57	5.55±0.14	9.81±0.09	149.7±0.47	4.09±0.17
G4	97.0±3.56	52.9±2.54	0.71±0.03	6.85±0.07	3.75±0.07	67±2.78	21.2±0.73	0.45±0.05	102.3±0.84	53.1±2.34*	95.7±2.53	127.3±6.01*	5.65±0.12	9.84±0.07	149.93±0.61	4.13±0.15
G1R	88.6±4.24	55.5±4.12	0.74±0.02	6.47±0.11	3.18±0.13	45.4±4.74	16.4±0.73	0.91±0.06	112.4±0.43	44.8±2.58	108.9±2.44	154.9±7.78	5.25±0.1	7.52±0.03	147.29±0.3	3.83±0.08
G4R	96.9±4.34	52.2±2.91	0.74±0.02	6.52±0.11	3.19±0.09	38±3	15.3±0.7	0.98±0.06	113.2±0.49	47.1±3.66	113.9±6.46	175.3±6.18	4.88±0.13	7.45±0.02	147.93±0.44	3.7±0.09

All values are expressed as Mean±SEM, *n*=10 animals/group, *Significant *P*<0.05 vehicle control vs. TLPL/AY/03/2008 treated groups, ALT= Alanine Aminotransferase; AST= Aspartate Aminotransferase; ALP= Alkaline Phosphatase

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Table 8: Effect of oral administration of TLPL/AY/03/2008 on relative organ weights of male rats

Group	Relative organ weights (mg/100 g body weight)								
	Liver	Spleen	Heart	Kidneys	Brain	Thymus	Adrenals	Testes	Epididymis
G1	2.8±0.1	0.4±0.04	0.3±0.01	0.65±0.02	0.57±0.01	0.1±0.01	0.01±0	0.96±0.03	0.36±0.01
G2	3.2±0.07*	0.4±0.02	0.34±0.01	0.74±0.02	0.59±0.02	0.12±0.01	0.01±0	1.04±0.04	0.4±0.01
G3	3.17±0.12	0.42±0.03	0.35±0.01	0.77±0.04	0.58±0.02	0.11±0.01	0.01±0	0.99±0.04	0.39±0.02
G4	2.8±0.16	0.38±0.03	0.32±0.02	0.72±0.04	0.6±0.02	0.11±0.01	0.01±0	1.03±0.04	0.38±0.01
G1R	2.92±0.09	0.39±0.02	0.36±0.01	0.72±0.03	0.57±0.02	0.1±0.01	0.02±0	0.95±0.04	0.38±0.02
G4R	3.07±0.13	0.43±0.03	0.37±0.01	0.77±0.02	0.55±0.04	0.11±0.01	0.02±0	0.98±0.06	0.4±0.02

All values are expressed as Mean±SEM, n=10 animals/group, *Significant P<0.05 vehicle control vs. TLPL/AY/03/2008 treated groups

Table 9: Effect of oral administration of TLPL/AY/03/2008 on relative organ weights of female rats

Group	Relative organ weights (mg/100 g body weight)								
	Liver	Spleen	Heart	Kidneys	Brain	Thymus	Adrenals	Ovaries	Uterus
G1	3.34±0.05	0.47±0.02	0.4±0.02	0.81±0.02	0.88±0.03	0.15±0.01	0.03±0	0.04±0	0.3±0.03
G2	3.11±0.08	0.42±0.02	0.42±0.03	0.74±0.01	0.87±0.02	0.16±0.01	0.03±0	0.04±0	0.23±0.02
G3	3.27±0.15	0.42±0.02	0.37±0.01	0.78±0.04	0.82±0.02	0.16±0.01	0.03±0	0.03±0	0.3±0.04
G4	2.99±0.13	0.42±0.02	0.36±0.02	0.71±0.07	0.82±0.03	0.13±0.01	0.03±0	0.04±0	0.26±0.04
G1R	3.09±0.12	0.44±0.02	0.41±0.02	0.68±0.02	0.82±0.03	0.14±0.01	0.03±0	0.05±0	0.34±0.03
G4R	3.07±0.06	0.46±0.03	0.39±0.02	0.73±0.01	0.86±0.04	0.14±0.01	0.03±0	0.05±0	0.3±0.01

All values are expressed as Mean±SEM, n=10 animals/group

Table 10: Summary of gross pathology findings

Group	G1		G1R		G2		G3		G4		G4R	
Dose (mg/kg)	0		0		200		500		1000		1000	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
No. of animals	10	10	10	10	10	10	10	10	10	10	10	10
Organs/ lesions	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD

M=Male, F=Female, NAD=No abnormality detected

AST, ALP, Chloride and Phosphorus) are found to be significantly increased or decreased as compared to vehicle control group, the values are within the normal reference ranges specified for the species.[16] Since, no consistent dose dependent changes have been observed in the blood parameters, the variations can be considered spontaneous, incidental and non-treatment related in the tested species.

Repeated dose safety studies provide information on target organ toxicity upon continuous exposure of test substance intended for prolonged use in target species.[13] The present investigation did not record any treatment related gross pathological lesions. A statistical significance was observed in the relative weight of liver alone in male rats at 200 mg/kg dose which could be due to the individual animal variation. Neither dose dependent effect was noticed in other dose groups nor was any significant change recorded in the relative weights of other vital organs. No toxicologically significant or treatment related changes in urine analysis parameters (data not shown) were observed at all the dose levels. The normal values of majority of hematological and clinical chemistry end points revealed no organ damage related effects.

With respect to target organ toxicity, microscopic findings of mild to marked, diffuse hepatocellular degeneration (vacuolar changes with granular of cytoplasm and pyknotic nuclei of hepatocytes) were noticed in males at 1000 mg/kg; however, G4R (1000 mg/kg) rats did not show any such lesions and the hepatic cellular details were comparable to control group animals indicating the complete reversal after 28 days treatment free period. No significant test item related microscopic observations were found in other organs. The microscopic lesions observed in other organs of both sexes of different groups were found to be in the normal laboratory incidence of baseline values of control animals.

CONCLUSION

Based on the results of the study, it can be concluded that TLPL/AY/03/2008 is non toxic up to 2000 mg/kg when administered as a single dose by oral gavage to Sprague Dawley rats and can be categorized 'Unclassified (category 5)' according to the Globally Harmonized System (GHS) for classification of chemicals. The No Observed Adverse Effect Level (NOAEL) of

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Table 11: Summary of histopathological findings

Group No.	G1		G4	
Dose (mg/kg)	0		1000	
Sex	Male	Female	Male	Female
No. of Animals	10	10	10	10
Organs and observations				
Liver				
MNC infiltration	8	5	5	6
Necrotic foci	1	1	0	1
Hepatocellular degeneration	0	0	10	0
Kidneys				
MNC infiltration	7	7	7	5
Basophilic tubules	1	2	1	0
Pyelitis	0	1	0	0
Renal pelvis dilated, unilateral	0	1	1	0
Lungs				
MNC infiltration	7	5	7	3
Foam cell infiltration	0	1	0	0
Heart				
MNC infiltration	4	4	5	2
Thymus				
Epithelial cyst (s)	4	7	2	4
Trachea				
MNC infiltration	0	0	1	0
Stomach				
Glandular, cystic gland	1	0	0	0
Colon				
Parasite/s	1	0	1	2
Cystic gland	0	0	1	0
Rectum				
Parasite/s	1	0	0	1
Adrenals				
Accessory cortical tissue, unilateral	2	0	1	1
Prostate		X		X
MNC infiltration	2		0	
Uterus	X		X	
Luminal dilatation, unilateral		0		2
Epididymides		X		X
MNC infiltration	1		1	
Urinary bladder				
Parasite/s	3	2	2	3
MNC infiltration	0	0	1	1
Brain				
MNC infiltration	0	0	1	0
Eyes with optic nerve				
Peri-orbital inflammation, unilateral	1	1	3	1
Peri-orbital inflammation, bilateral	1	0	1	0
Keratitis, unilateral	0	1	1	0
Skeletal muscle				
MNC infiltration	0	0	1	1
Mesenteric lymph nodes				
Sinusoidal dilatation	0	1	0	0
Pituitary				
Rathke's cleft	0	2	1	1
Cyst	0	0	1	1

X=Not applicable, MNC=Mono nuclear cell

Table 12: Summary of histopathological findings

Groups	G2	G3	G1R	G4R
Dose (mg/kg)	200	500	0	1000
Sex	Male	Male	Male	Male
No. of Animals	10	10	10	10
Liver				
MNC infiltration	7	6	8	4
Necrotic foci	0	0	1	1

TLPL/AY/03/2008 can be considered as 1000 mg/kg in both male and female rats, under the experimental conditions and doses employed.

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