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ACUTE AND 28-DAY REPEATED DOSE TOXICITY STUDIES WITH POLYHERBAL FORMULATION OF *ISABGOL* HUSK, *SWARNAPATRI* LEAF EXTRACT AND *TRIPHALA* FRUITS EXTRACT (TLPL/AY/01/2008)

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ABSTRACT

The acute and sub-chronic 28-day repeated dose toxicity studies in rodents were performed to assess the safety of polyherbal formulation of *Isabgol* husk (*Plantago ovate), Swarnapatri* leaf extract (*Cassia angustifolia)* and *Triphala* fruits extract (*Emblica officinalis, Terminalia chebula* and *Terminalia belerica)* (TLPL/AY/01/2008). The studies were conducted according to current OECD toxicology guidelines for acute and repeated dose. No mortalities or evidence of adverse effects were observed following acute oral gavage administration up to 2000 mg/kg of TLPL/AY/01/2008 in Sprague dawley rats. The 28-day repeated dose study involving daily oral administration of 70, 175 and 350 mg/kg body weight of TLPL/AY/01/2008, with a post trial 14 day no treatment observation period at high dose level, resulted in no clinical signs and animal deaths. No toxicological significant differences were observed in any of the TLPL/AY/01/2008 treatment groups for body weights, feed consumption, physical appearance, neurological behaviour and urine analysis. Evaluation of haematology and clinical chemistry parameters revealed no toxicological and treatment related effects. No treatment related changes noted in absolute and relative organ weights. Macroscopic and microscopic evaluation of organs revealed no treatment related. Results of this study demonstrate that polyherbal formulation TLPL/AY/01/2008 is not acutely toxic at 2000 mg/kg of body weight/day, with a NOAEL (no-observed-adverse-effect-level) of greater than 350 mg/kg of body weight/day for systemic toxicity from repeated dose 28-day oral gavage administration. The present study demonstrates the non-adverse nature of the polyherbal formulation TLPL/AY/01/2008 on long term administration.

KEY WORDS

Toxicity study, TLPL/AY/01/2008, Constipation, Polyherbal formulation, *Isabgol* husk, Psyllium, *Senna* and *Triphala.*

INTRODUCTION

Herbs are the rich source of minerals, vitamins and many other nutrients as food for humans and animals. Traditional herbs are used as medicines by the practitioners for the treatment of many diseases and are considered the alternative for synthetic medicines not only in rural population, also in the developed countries. It's rising popularity could be attributed to its safety, effectiveness and economy. Constipation is a common human digestive disorder where a person has difficulty passing faeces associated with dry stool. As digested food moves through the colon (large intestine), it absorbs water allowing stooling formation. If too much water is extracted from the stool, it become hard and difficult to pass and leads to constipation. Many plants and their parts were used as laxatives for treating constipation since long, but less research is being conducted on the safety assessment aspect with respect to poly herbal combination of various formulations in spite of other beneficial values.

The present investigation was aimed to assess the safety of polyherbal formulation TLPL/AY/01/2008 for long term administration. The therapeutic indication of the polyherbal formulation TLPL/AY/01/2008 is for chronic constipation and the constipation associated with haemorrhoids, fissure and fistula. TLPL/AY/01/2008 was the combination of Indian traditional herbs. Each 5g of formulation contains the 3.5 g of *Isabgol* husk (*Plantago ovate),* 100 mg of *Swarnapatri* leaf extract (*Cassia angustifolia)* and *Triphala* fruits extract (*Emblica officinalis, Terminalia chebula* and *Terminalia belerica)*. The actives used in the TLPL/AY/01/2008 formulation have many medicinal properties. Only few animal studies and clinical trial have been reported for the above actives individually for their effects and not as combination. Psyllium husk is widely used as a fibre supplement for the treatment of constipation since it contains a high amount of hemicelluloses, composed of arabinose, rhamnose and galacturonic acid units. Many clinical studies indicated that the phyllium husk is also beneficial for haemorrhoids[1]. Psyllium husk is also used as a novel food supplement in drinks and breakfast cereals[2]. Psylium significantly increases the viscosity of an aqueous stool extract, moisture, dry and wet stool weights. The Psyllium husk fibre is not completely fermented in the colon and provides lubrication that facilitates propulsion of colon contents and produces a stool that is bulkier and moist than stools resulted with the use of other bowel regulating fibre sources[3]. The clinical study reported as the treatment with 5.1 g of Psyllium twice daily produces significant reductions in serum total LDL-cholesterol concentrations[4]. Senna is used as a stimulant laxative which increases smooth intestinal motility[5]. A comparative clinical trial reported that the senna preparation has been a safe laxative for pregnant and puerperal women[7, 8]. It is also reported that the ingestion of senna by nursing mothers does not have any observable effect on the infant bowels[9]. The senna-fibre combination was significantly more effective than lactulose at a lower cost and well tolerated for chronic constipation in long stay elderly patients[10]. Another active of the TLPL/AY/01/2008 formulation is Triphala fruit extract. Triphala consists of fruits extracts of *Emblica officinalis, Terminalia chebula* and *Terminalia belerica*. Triphala was found to have good laxative

property, help in management of hyperacidity and also improve appetite and can be used effectively in the treatment of constipation and other gastric problems[11]. It is also used for combating other disorders like high blood pressure, reduction of serum cholesterol, intestinal inflammation, and ulcerative colitis[12]. Triphala contains tannins, phenols and glycosides which are responsible for its strong antioxidant activity apart from its immunomodulatory, anti-inflammatory, analgesic and antimutagenic properties and is effective remedy for geriatric degenerative diseases[13]. Studies in the recent past indicate the immense potential of Triphala in cancer prevention and treatment[14].

Before the clinical use of the polyherbal formulation TLPL/AY/01/2008 it is essential to determine whether it has any potential toxicity. No evidence has been reported that ingestion of individual actives of this formulation causes any ill effects, and to date, no studies investigating the toxicology of this polyherbal formulation TLPL/AY/01/2008 have been published. The current studies were conducted to assess the acute and repeated dose safety profiles of the polyherbal formulation TLPL/AY/01/2008 in Sprague dawley rats. All studies were conducted in accordance with OECD and GLP guidelines[15, 16].

MATERIAL AND METHODS

1.1 TEST MATERIALS

TLPL/AY/01/2008 (AY0108/001/014) was provided by Tulip Lab Private Limited, as a powder form.

1.2 ANIMALS

All Sprague Dawley rats used in this study were bred in house by Bioneeds Laboratory Animals & Preclinical Services. The experimental protocols were approved by Institutional Animal Ethics Committee. The acute toxicity study used female non-pregnant, 8-10 week old rats of weight 160.6-179.8 g, and the 28 day study used 7-8 week old rats of both sexes, weighing between 132.7-160.0 g for males and 122.6-149.7 g for females.

1.3 HOUSING

The rats were housed under standard laboratory conditions in an air-conditioned environment with adequate fresh air supply (12-16 air changes per hour), at temperature 19-24°C, 52-68% relative humidity, with a 12 hours light and 12 hours dark cycle. For the acute study, the rats were housed singly, and for the repeated dose study, the rats were housed in groups of two of the same sex.

Prior to initiation of treatment, the rats were acclimatised for five days to the laboratory conditions. During this period they were monitored daily for any clinical signs. Veterinary examination of all animals was recorded on the day of receipt and on the day of randomisation and allocation to treatment groups.

Throughout the acclimatisation and experimental period, the animals were fed *ad libitum* on Nutrilab (M/S Provimi Nutrition India Pvt. Ltd., (Vetcare), Bangalore) rodent feed and were provided *ad libitum* with Aquaguard water in plastic water bottles with stainless steel sipper tubes.

1.4 ACUTE ORAL TOXICITY STUDY

The single dose acute oral toxicity study was performed following OECD Guideline 420 (Adopted: 17th December 2001). In an initial step 1 sighting study, one female was administered a dose of polyherbal formulation at 300 mg/kg of body weight by oral gavage, with dose volume of 10 mL/kg body weight. As there was no mortality at 300 mg/kg dose, the step 2 sighting study was conducted in another female rat by administering 2000 mg/kg body weight.

In the main study, 4 females were dosed at 2000 mg/kg of body weight. Body weights of animals were measured weekly. All the animals in the

sighting and main study were observed for mortality at 10 minutes, 30 minutes, 1, 2, 4 and 6 hours following dosing and thereafter once daily during the 14 days observation period. Daily cage side observations included; changes in skin, fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous system function, somatomotor activity and behaviour pattern.

On day 15, all animals in both the sighting and main study were sacrificed humanely by carbon dioxide asphyxiation. The animals were subjected to a complete necropsy and the gross pathological examination was made.

1.5 REPEATED DOSE 28-DAY ORAL TOXICITY STUDY

A 28 day repeated dose toxicity study was performed following OECD Guideline 407 (Adopted: 3 October 2008). The day before the initiation of treatment, the animals procured for the study were weighed and grouped in to body weight ranges. These body weight stratified rats were distributed to all the 6 study groups. Each group consisted of five rats per sex. The body weight variation of animals used in the study was +5.5 to -8.1 % in males and +4.8 to -8.2 % in females of the mean body weight.

The animals in groups G1 and G1R served as vehicle controls and were daily administered normal saline orally by gavage. The animals in groups G2, G3 and G4/G4R were administered daily at the dose of 70, 175 and 350 mg/kg body weight of TLPL/AY/01/2008 by oral gavage for 28 days respectively. Both the test item and normal saline were dosed at 10mL/kg body weight. The animals in vehicle control recovery (G1R) and high dose recovery (G4R) group were maintained for a further 14 days without administration of either vehicle or test item, but water and food was provided *ad libitum* to determine any delayed effects of the treatment.

All the animals were observed once daily for clinical signs of toxicity and twice daily for morbidity/morbidity. Veterinary examination was carried out prior to test item administration on day 1 and weekly thereafter throughout the treatment and recovery periods. Ophthalmological examination was performed prior to initiation of treatment and at the end of treatment and recovery periods for all animals. Neurological/Functional examination was carried out during last week of the treatment and recovery period.

Individual animal body weights were recorded at receipt, on day 1, and weekly thereafter and at sacrifice (Fasting body weights). Food consumption was recorded once weekly.

1.5.1 CLINICAL PATHOLOGY

At the end of treatment (day 28) for main groups and end of recovery period (day 42) for recovery groups, all animals were kept on fast overnight. The following day, blood samples were collected from each animal using the retro-orbital plexus puncture method under mild ether anaesthesia. The blood samples were collected in to tubes containing anticoagulants of heparin for the clinical chemistry analyses and K_2-EDTA for the haematology analyses.

1.5.1.1 HAEMATOLOGY

The haematology parameters of haemoglobin (Hb), haematocrit (HCT), erythrocyte count (RBC), total leukocyte count (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelet counts were analysed using a Sysmex, KX-21 (Transasia Bio-Medicals Ltd., India) Haematology Analyser. Blood clotting time (seconds) was estimated by capillary tube method. Terminal bone marrow examination was performed according to standard methodology (Benjamin 2001). Leishman's stain blood smears were examined using standard microscopy to determine the differential leucocytes count (DLC) of neutrophils, lymphocytes, eosinophils and monocytes per 100 cells.

1.5.1.2 CLINICAL CHEMISTRY

Plasma was separated by centrifuging blood samples at 5000 rpm for 10 minutes and was analysed using an EM-360, Fully automated Clinical Chemistry Analyser (Transasia Bio-Medicals Ltd., India) for total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), glucose, total cholesterol, creatinine, blood urea nitrogen, triglycerides, total bilirubin, phosphorous, calcium and chloride. Sodium and potassium were estimated using an Easylyte Na/K Analyser (Medica Corporation, USA).

Urine was collected on completion of the 28 days treatment for the main groups and at the end of the 14 day recovery period for the recovery groups. Prior to the collection of the urine, the animals were fasted overnight with water provided *ad libitum*. The urine was analysed for appearance, colour, volume, pH, specific gravity, occult blood, leucocytes, bilirubin, urobilinogen, ketone bodies, proteins, glucose, and nitrite. Microscopic examination of urine sediments was performed using an H-100, Urine Analyser (DIRUI Industrial Co. Ltd.)

1.5.1.3 PATHOLOGY

On completion of treatment (day 28) for main groups and end of recovery period (day 42) for recovery groups, all surviving animals were kept on fast overnight and sacrificed using carbon dioxide asphyxiation. The animals were weighed before exsanguinations and subsequent gross necropsy procedures. Organs and tissues were collected and were appropriately trimmed of any adherent tissue, before being preserved in 10% neutral buffered formalin. These organs and tissues included; liver, kidneys, adrenals, spleen, heart, thymus, brain, lungs, testes, epididymides, prostate and seminal vesicles with coagulating glands, uterus, ovaries, trachea, pancreas, aorta, spinal cord, stomach, oesophagus, duodenum, jejunum, ileum with payers patches, caecum, rectum, colon, urinary bladder, sciatic nerve, mesenteric lymph nodes, skin with mammary glands, skeletal muscle, thyroid, parathyroid, and eyes.

The following organs were trimmed of adherent tissue and weighed wet prior to any drying; liver, spleen, thymus, heart, brain, kidneys*, adrenals*, testes*, ovaries*, epididymides*, and uterus (* paired organs were weighed together). Relative organ weights were calculated against fasting body weight.

Histopathological examination was performed on organs from the main group control and high dose group treatment animals. The tissues were embedded in paraffin wax, sectioned at five micrometers and stained with haematoxylin and eosin.

1.5.2 STATISTICAL ANALYSIS

Statistical analysis was conducted using Graph Pad Prism version 5.00, Graph Pad Software. The data on body weight and gain, organ weights and ratios, haematological and clinical chemistry estimations, urine volume, specific gravity and pH were analysed statistically. One way ANOVA with Dunnets post test was done for different treatment groups comparing with the control group data and the unpaired 't' test was done for control recovery and high dose recovery group data. All analyses and comparisons were initially evaluated at the 95% level of confidence (p<0.05).

RESULTS AND DISCUSSION

1.6 ACUTE ORAL TOXICITY STUDY

As the animal used in the sighting study appeared healthy, TLPL/AY/01/2008 was administered to a further 4 female rats at the maximum dose of 2000 mg/kg as suggested by OECD guideline. Throughout the study, no animals died or showed any ill effects, with all appearing normal and progressively gaining

weight at similar rates (Table 1). No gross lesions were observed at necroscopy among the rats. These findings indicated that TLPL/AY/01/2008 was not acutely toxic under the conditions of the study. Juan Hancke et al., (2009) reported that the acute toxicity studies of senna did not produce any observable lethal effects and the reported LD_{50} was found to be 10 g/kg.

Table 1

Body weight changes of female Sprague dawley rats administered a single dose of up to 2000mg TLPL/AY/01/2008 per kg of body weight.

Study Type	Dose (mg/kg)	No. of Animals		Body weights on day		
				1	7	14
Sighting Study – Step 1	300	1	Mean	163.1	172.3	179.8
Sighting Study – Step 2	2000	1	Mean	176.8	190.1	201.3
Main Study	2000	4	Mean±SD	168.5±7.1	192.2±10.8	198.8±12.8

REPEATED DOSE 28-DAY ORAL TOXICITY STUDY

1.6.1 OBSERVATIONS

No animals died and no clinical signs of toxicity or neurological abnormalities were noted in cage side and veterinary examinations in all test groups. Functional responses of all animals to visual, audio, proprioceptive stimuli and ophthalmoscopy examination reveal no alterations. Administration of TLPL/AY/01/2008 up to 350 mg/kg/day was well tolerated. Mean body weight changes (Table 2 male, Table 3 female) and feed consumption were not affected by administration of TLPL/AY/01/2008 by oral gavage. Animals in the recovery groups continued to gain body weights during the 14-day post treatment period.

Table 2

Body weight changes of male Sprague dawley rats following a 28 day repeated dose treatment of TLPL/AY/01/2008. Values are means±standard deviation of 5 rats per group.

Study Days	Body weights (g) during Treatment period				Body weights (g) during Treatment period + 14-day Recovery period	
	Control	70 mg/kg	175 mg/kg	350 mg/kg	Control	350 mg/kg
1	151.68±4.11	150.84±5.03	155.76±4.90	157.40±5.43	152.78±9.02	150.96±5.48
8	182.42±9.72	178.26±5.65	179.76±7.82	176.78±5.54	174.24±12.81	165.82±5.69
5	204.18±14.33	196.18±11.92	194.84±10.78	199.40±6.40	193.78±14.91	184.82±8.21
22	233.90±15.04	230.54±11.78	225.02±14.86	225.54±5.74	224.70±14.79	216.34±6.02
28	248.08±16.82	246.04±16.46	246.10±17.86	233.30±5.82	245.16±22.79	224.54±8.00
35					260.32±29.39	246.70±15.11
42					268.78±32.28	258.88±18.46

Table 3

Body weight changes of female Sprague dawley rats following a 28 day repeated dose treatment of TLPL/AY/01/2008. Values are means±standard deviation of 5 rats per group.

Study Days	Body weights (g) during Treatment period				Body weights (g) during Treatment period + 14-day Recovery period	
	Control	70 mg/kg	175 mg/kg	350 mg/kg	Control	350 mg/kg
1	144.34±4.84	144.20±5.04	144.52±7.85	146.50±6.28	145.52±4.42	144.40±5.30
8	154.14±7.43	157.30±8.36	158.82±10.68	152.42±6.14	155.16±5.40	148.52±3.50
5	166.46±8.81	167.58±8.19	174.82±13.96	168.48±5.27	166.96±10.32	159.34±4.80
22	177.66±10.60	175.84±8.72	187.64±14.48	180.18±8.61	176.40±14.01	169.06±9.38
28	182.14±15.77	179.26±12.10	195.32±19.72	190.48±5.96	180.60±14.85	171.58±14.34
35					186.46±16.54	183.62±15.34
42					189.76±16.70	187.90±15.63

1.6.2 HAEMATOLOGY

No toxicologically significant changes in haematology parameters (Table 4 male, Table 5 female) were noted in any of the TLPL/AY/01/2008 administered groups of both the sexes at the end of treatment and recovery period as compared to the vehicle controls.

The statistically significant ($p<0.05$) increase in monocytes levels in the females of mid dose group was considered to be incidental, due to lack of dose dependency.

Table 4

Haematology data for male Sprague dawley rats following a 28 day repeated dose treatment of TLPL/AY/01/2008. Values are means ± standard deviation of 5 rats per group.

	End of treatment period				End of 14-day recovery period	
Parameters	**Control**	**70 mg/kg**	**175 mg/kg**	**350 mg/kg**	**Control**	**350 mg/kg**
WBC (10^3 cells/µL)	8.44±3.25	8.58±1.86	12.40±3.62	15.22±10.06	12.60±3.74	13.32±2.89
RBC (10^6 cells/µL)	7.19±0.77	8.85±1.25	8.65±1.49	6.57±1.17	8.82±1.11	8.29±1.18
Haemoglobin (g/dL)	14.40±1.55	17.26±2.13	16.86±2.77	13.10±2.15	15.94±1.60	15.40±2.13
Hematocrit (%)	43.58±4.20	54.22±9.13	52.04±8.94	40.00±6.11	40.62±14.70	47.92±6.98
MCV (fL)	60.70±1.51	61.12±3.13	60.20±0.23	61.14±2.42	55.86±1.94	57.76±0.88
MCH (pg)	20.04±0.60	19.58±1.02	19.52±0.69	19.98±0.66	18.14±0.97	18.58±0.43
MCHC (g/dL)	33.02±1.21	32.06±1.87	32.46±1.18	32.70±0.62	32.46±0.88	32.16±0.52
Platelet count (10^3 cells/µL)	614.60±147.30	367.20±208.81	481.20±212.38	700.20±51.43	511.20±132.11	456.60±70.55
Clotting time (Sec)	85. 4±7.1	89.0±11.0	85.0±11.6	80.0±10.1	85.0±15.5	87.8±7.9
Neutrophils (%)	11.4±3.4	14.6±3.5	13.0±5.8	13.2±6.1	20.8±2.2	21.4±3.6
Lymphocytes (%)	88.2±2.9	84.4±4.6	86.4±6.2	85.8±5.3	77.6±1.7	77.0±3.2

Monocytes (%)	0.4±0.5	0.6±0.9	0.2±0.4	1.0±1.4	1.6±1.1	0.8±0.8
Eosinophils (%)	0.0±0.0	0.4±0.5	0.4±0.5	0.0±0.0	0.0±0.0	0.4±0.5
Basophils (%)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

Table 5

Haematology data for female Sprague dawley rats following a 28 day repeated dose treatment of TLPL/AY/01/2008. Values are means ± standard deviation of 5 rats per group.

Parameters	End of treatment period				End of 14-day recovery period	
	Control	70 mg/kg	175 mg/kg	350 mg/kg	Control	350 mg/kg
WBC (10^3 cells/µL)	9.98±2.66	9.14±4.09	13.50±10.53	11.76±6.47	8.14±1.68	9.20±2.47
RBC (10^6 cells/µL)	6.45±0.66	5.93±0.70	6.07±0.63	6.36±0.74	7.67±0.92	6.83±0.67
Haemoglobin (g/dL)	13.22±1.77	12.32±1.16	12.36±0.84	13.52±1.75	14.46±2.06	13.34±1.20
Hematocrit (%)	38.12±4.11	35.64±3.61	36.02±3.14	38.22±3.83	43.38±6.15	40.32±3.22
MCV (fL)	59.12±1.31	60.28±2.16	59.40±1.51	60.26±2.95	56.42±1.97	59.18±2.44
MCH (pg)	20.48±1.01	20.86±1.14	20.42±0.83	21.26±1.18	18.82±0.78	19.58±1.32
MCHC (g/dL)	34.64±1.94	34.60±0.79	34.38±1.19	35.30±1.19	33.32±0.41	33.06±1.10
Platelet count (10^3 cells/µL)	634.00±144.01	661.60±76.21	641.40±61.08	603.60±148.92	705.60±182.09	751.40±105.33
Clotting time (Sec)	77.8±10.0	82.6±10.7	86.4±9.1	79.0±9.1	80.6±11.7	79.4±9.8
Neutrophils (%)	18.0±3.4	16.6±5.9	16.4±3.6	16.8±3.6	24.0±3.2	25.6±2.9
Lymphocytes (%)	81.4±2.6	83.2±6.1	82.4±4.1	82.8±3.8	74.6±4.2	73.0±3.1
Monocytes (%)	0.2±0.4	0.0±	0.8±0.4*	0.0±	0.8±0.8	0.6±0.5
Eosinophils (%)	0.4±0.5	0.2±	0.4±0.9	0.4±	0.8±0.8	0.8±0.4
Basophils (%)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

: Statistically significant ($p<0.05$) than control group.

1.6.3 CLINICAL CHEMISTRY

No toxicologically significant changes in clinical chemistry (Table 6 male, Table 7 female) parameters were noted in treated groups of both the sexes at the end of treatment and recovery period. The statistically significant elevated triglycerides levels and decreased blood urea nitrogen and glucose levels in males and decreased calcium levels in females of high dose group and potassium levels of mid and high dose group at the end of treatment noted was resolved at the end of recovery period. The significant increase in aspartate aminotransferase (AST) level in females of high dose group recovery group noted was absent at the end of treatment. Therefore, these alterations were considered to be of no toxicological relevance. The statistically significant increase in albumin, creatinine and cholesterol levels in the males of mid dose group was considered to be incidental, due to lack of dose dependency. All values for the various test parameters were within the normal physiological ranges routinely observed in historical control animals from the testing facility (unpublished data). Mitchell JM et al., (2006) reported that the changes noted in electrolytes at 300 mg/kg/day for up to 104 consecutive days administration are most likely

physiologic adaptations to the laxative effect of senna. Mengs U et al., (2004) reported that the biochemical and morphological changes seen following 13 weeks of treatment of senna at the high doses of 750 and/or 1500 mg/kg per day significantly reversed following 8 weeks of recovery. The significant changes in potassium levels and other biochemical parameters are could be due to the presence of senna in the formulation. However, these changes were resolved by the end of recovery period and hence considered to be no toxicological relevance.

Table 6

Clinical chemistry data for male Sprague dawley rats following a 28 day repeated dose treatment of TLPL/AY/01/2008. Values are means ± standard deviation of 5 rats per treatment group.

Parameters	End of treatment period				End of 14-day recovery period	
	Control	70 mg/kg	175 mg/kg	350 mg/kg	Control	350 mg/kg
AST (U/L)	124.00±26.92	125.00±19.51	117.00±8.15	114.20±16.57	113.00±28.10	119.80±11.54*
ALT(U/L)	46.40±9.40	42.60±10.64	42.20±7.26	40.20±10.62	46.00±16.39	40.60±5.46
ALP (U/L)	164.00±37.62	185.00±28.57	157.20±34.01	179.00±77.51	123.00±12.55	138.80±15.85
Total Biluribin (mg/dL)	0.68±0.19	0.72±0.19	0.76±0.24	0.70±0.16	1.00±0.12	0.90±0.16
Triglycerides (mg/dL)	65.20±16.71	75.20±16.84	77.40±24.50	101.20±9.31*	62.20±7.16	71.20±7.46
Total Cholesterol (mg/dL)	48.20±4.82	48.60±6.19	59.20±4.60*	43.20±5.50	53.00±12.21	53.00±11.81
BUN (mg/dL)	17.78±0.77	16.08±2.85	15.64±2.67	13.80±2.50*	14.28±2.25	15.12±1.58
Creatinine (mg/dL)	0.62±0.04	0.66±0.05	0.70±0.00*	0.68±0.04	0.60±0.00	0.60±0.00
Glucose (mg/dL)	104.20±15.96	88.80±11.99	98.60±19.65	76.20±16.66*	87.40±30.56	70.80±11.90
Phosphorous (mg/dL)	6.56±0.50	6.28±0.57	6.28±0.26	6.06±0.42	5.74±0.42	6.04±0.38
Calcium (mg/dL)	9.94±0.15	9.96±0.09	10.02±0.08	9.96±0.05	9.88±0.23	9.92±0.08
Chloride (mmol/L)	106.40±1.67	105.40±0.89	105.80±0.84	106.00±0.00	108.80±3.27	109.20±0.84
Total Protein (g/dL)	5.96±0.48	6.20±0.29	6.18±0.30	6.42±0.32	6.80±0.27	7.00±0.22
Albumin (g/dL)	3.02±0.11	3.18±0.24	3.40±0.25*	3.12±0.27	3.02±0.23	3.00±0.16
Sodium (mmol/L)	147.60±1.75	147.24±0.68	148.32±1.33	148.88±1.69	150.78±1.03	149.22±1.37
Potassium (mmol/L)	4.03±0.47	4.0±20.24	3.99±0.26	3.67±0.17	3.90±0.28	4.13±0.17

: Statistically significant (p<0.05) than control group.

Table 7

Clinical chemistry data for female Sprague dawley rats following a 28 day repeated dose treatment of TLPL/AY/01/2008. Values are means ± standard deviation of 5 rats per treatment group.

Parameters	End of treatment period				End of 14-day recovery period	
	Control	70 mg/kg	175 mg/kg	350 mg/kg	Control	350 mg/kg
AST (U/L)	125.00±23.38	115.60±23.18	116.00±23.89	109.00±8.92	100.80±6.46	90.20±4.76
ALT(U/L)	44.40±8.79	35.00±6.16	36.60±6.23	34.40±4.62	36.20±13.39	29.80±5.36
ALP (U/L)	93.40±21.08	101.40±27.69	116.80±29.10	98.80±12.91	127.00±13.10	128.20±15.19
Total Biluribin (mg/dL)	0.92±0.13	0.92±0.38	0.60±0.57	0.70±0.27	0.78±0.13	0.88±0.13
Triglycerides (mg/dL)	66.20±19.46	60.60±9.96	66.20±14.22	59.00±27.85	66.20±14.96	54.40±6.02
Total Cholesterol (mg/dL)	53.20±8.41	58.20±17.91	48.60±9.10	53.60±9.40	63.80±15.47	58.80±10.16
BUN (mg/dL)	18.90±1.31	16.90±3.13	19.38±4.55	17.26±2.90	16.10±2.59	16.88±1.98
Creatinine (mg/dL)	0.64±0.05	0.62±0.04	0.60±0.00	0.62±0.04	0.64±0.05	0.58±0.04
Glucose (mg/dL)	93.40±12.22	95.00±12.96	95.60±19.86	100.60±10.31	77.20±6.83	88.00±9.92
Phosphorous (mg/dL)	5.90±0.14	5.90±0.29	5.64±0.18	5.72±0.56	5.32±0.43	5.26±0.83
Calcium (mg/dL)	10.02±0.11	9.98±0.08	9.96±0.11	9.82±0.08*	9.96±0.21	10.06±0.05
Chloride (mmol/L)	105.60±1.34	106.20±0.84	106.80±0.45	106.00±0.71	108.60±1.34	110.20±2.17
Total Protein (g/dL)	6.24±0.31	6.52±0.19	6.44±0.35	6.20±0.23	7.60±0.67	7.68±0.30
Albumin (g/dL)	3.36±0.19	3.36±0.15	3.40±0.19	3.32±0.18	3.26±0.26	3.48±0.18
Sodium (mmol/L)	145.82±1.50	146.64±1.41	146.26±0.74	145.78±0.99	149.70±1.11	149.82±1.56
Potassium (mmol/L)	4.18±0.26	3.85±0.18	3.78±0.18*	3.77±0.32*	3.95±0.32	4.04±0.49

: Statistically significant ($p<0.05$) than control group.

1.6.4 URINE ANALYSIS

No toxicologically significant differences were observed in the urine analysis between any groups.

1.6.5 ORGAN WEIGHTS

No significant differences were observed in absolute (Table 8 male, Table 9 female) and relative organ weights (Table 10 male, Table 11 female) in all the groups. A significant difference in absolute and relative kidneys weight, at $p<0.05$ confidence, was observed in females for the recovery groups. However, there were no indications in urinary laboratory parameters of any renal dysfunction and also not correlated with the histological findings of kidneys at the end of 28 days treatment period. In addition, the above change was not observed at the end of treatment, hence considered to be incidental finding.

Table 8

Absolute organ weight data for male Sprague dawley rats following a 28 day repeated dose treatment of TLPL/AY/01/2008. Values are means ± standard deviation of 5 rats per treatment group

Organs	End of treatment period				End of 14-day recovery period	
	Control	70 mg/kg	175 mg/kg	350 mg/kg	Control	350 mg/kg
Liver	7.3564±0.8810	7.8762±0.9961	7.3587±0.2895	7.0229±1.1268	7.2421±1.8514	7.1493±0.5810
Spleen	0.9954±0.1921	1.6159±0.6371	1.2398±0.1101	1.1521±0.6475	0.9651±0.3227	1.0697±0.2059
Heart	0.8634±0.0686	0.9026±0.0564	0.8436±0.0153	0.8250±0.0561	0.9029±0.1257	0.8134±0.0899
Kidneys	1.7896±0.2471	1.7236±0.2025	1.7880±0.0751	1.7317±0.2077	1.7254±0.3115	1.7217±0.1410
Brain	1.8068±0.0876	1.7979±0.0287	1.8780±0.0646	1.7945±0.0539	1.8123±0.1457	1.7409±0.1016
Thymus	0.3220±0.0839	0.4081±0.0690	0.4314±0.1561	0.3379±0.0843	0.2573±0.0825	0.3330±0.0699
Adrenals	0.0507±0.0127	0.0392±0.0028	0.0498±0.0065	0.0489±0.0056	0.0407±0.0073	0.0413±0.0097
Testes	3.0369±0.3569	2.7590±0.3237	2.9110±0.5110	2.8814±0.1131	3.0036±0.2913	2.8678±0.1145
Epididymides	0.8848±0.0947	0.9704±0.0496	0.8577±0.0931	0.8839±0.0956	1.0471±0.0847	0.9835±0.0548
Prostate & Seminal vesicles	1.5095±0.4499	1.6841±0.4398	1.2592±0.1759	1.2547±0.4074	1.6495±0.1017	1.8719±0.3256

Table 9

Absolute organ weight data for female Sprague dawley rats following a 28 day repeated dose treatment of TLPL/AY/01/2008. Values are means ± standard deviation of 5 rats per treatment group.

Organs	End of treatment period				End of 14-day recovery period	
	Control	70 mg/kg	175 mg/kg	350 mg/kg	Control	350 mg/kg
Liver	6.4531±1.1002	6.1645±0.6317	6.6000±0.5641	6.9704±1.0254	5.2278±0.7948	5.7145±0.7846
Spleen	0.9519±0.3152	1.0388±0.1600	1.1980±0.2553	1.1422±0.4640	0.6657±0.2022	0.7537±0.1849
Heart	0.8601±0.2811	0.6970±0.0743	0.7427±0.0876	0.7164±0.0665	0.6687±0.0617	0.6739±0.0290
Kidneys	1.4382±0.1897	1.2877±0.1047	1.4881±0.1803	1.5483±0.2270	1.1485±0.1493	1.3945±0.1804*
Brain	1.7936±0.0545	1.6653±0.0825	1.7261±0.0707	1.7429±0.1176	1.6065±0.0878	1.7368±0.1924
Thymus	0.3226±0.0273	0.3209±0.0558	0.3551±0.0756	0.2822±0.0756	0.2876±0.0782	0.2758±0.0822
Adrenals	0.0622±0.0130	0.0649±0.0104	0.0624±0.0086	0.0610±0.0103	0.0501±0.0108	0.0569±0.0131
Ovaries	0.1036±0.0108	0.1107±0.0315	0.1159±0.0261	0.1203±0.0255	0.0753±0.0312	0.0847±0.0082
Uterus	0.6339±0.1188	0.5574±0.0873	0.4884±0.1485	0.5323±0.1418	0.6046±0.1529	0.5492±0.0498

: Statistically significant ($p<0.05$) than control group.

Table 10

Relative organ weight data for male Sprague dawley rats following a 28 day repeated dose treatment of TLPL/AY/01/2008. Values are means ± standard deviation of 5 rats per treatment group.

Organs	End of treatment period				End of 14-day recovery period	
	Control	70 mg/kg	175 mg/kg	350 mg/kg	Control	350 mg/kg
Liver	3.2972±0.3342	3.6154±0.4746	3.3399±0.2779	3.2609±0.5847	3.0054±0.6321	3.0144±0.1538
Spleen	0.4512±0.1126	0.7454±0.3043	0.5635±0.0744	0.5318±0.2880	0.4008±0.1252	0.4531±0.0953
Heart	0.3869±0.0169	0.4137±0.0128	0.3827±0.0238	0.3813±0.0179	0.3771±0.0485	0.3422±0.0216
Kidneys	0.8009±0.0852	0.7899±0.0822	0.8113±0.0636	0.8033±0.1143	0.7189±0.1119	0.7255±0.0275
Brain	0.8115±0.0465	0.8252±0.0257	0.8507±0.0318	0.8305±0.0311	0.7604±0.0921	0.7353±0.0454
Thymus	0.1433±0.0324	0.1874±0.0325	0.1933±0.0621	0.1564±0.0390	0.1086±0.0384	0.1414±0.0330
Adrenals	0.0228±0.0056	0.0180±0.0012	0.0227±0.0040	0.0227±0.0032	0.0169±0.0020	0.0175±0.0046
Testes	1.3597±0.1235	1.2659±0.1479	1.3112±0.1711	1.3357±0.1021	1.2589±0.1564	1.2123±0.0810
Epididymides	0.3960±0.0235	0.4458±0.0327	0.3900±0.0567	0.4099±0.0524	0.4375±0.0300	0.4151±0.0186
Prostate & Seminal vesicles	0.6540±0.1950	0.7297±0.1906	0.5456±0.0762	0.5436±0.1765	0.7147±0.0441	0.8110±0.1411

Table 11

Relative organ weight data for female Sprague dawley rats following a 28 day repeated dose treatment of TLPL/AY/01/2008. Values are means ± standard deviation of 5 rats per treatment group.

Organs	End of treatment period				End of 14-day recovery period	
	Control	70 mg/kg	175 mg/kg	350 mg/kg	Control	350 mg/kg
Liver	3.6056±0.8177	3.6428±0.7142	3.5795±0.4490	3.8012±0.8720	2.8339±0.1732	3.0922±0.6732
Spleen	0.5405±0.2202	0.6110±0.1274	0.6521±0.1621	0.6257±0.2907	0.3543±0.0540	0.4103±0.1315
Heart	0.4623±0.0717	0.4084±0.0576	0.4041±0.0673	0.3892±0.0679	0.3669±0.0468	0.3625±0.0531
Kidneys	0.8059±0.1708	0.7587±0.1267	0.8093±0.1367	0.8393±0.1766	0.6238±0.0225	0.7550±0.1615*
Brain	1.0000±0.1555	0.9814±0.1491	0.9377±0.1153	0.9396±0.1059	0.8910±0.1689	0.9386±0.1912
Thymus	0.1805±0.0360	0.1870±0.0311	0.1898±0.0315	0.1536±0.0500	0.1558±0.0385	0.1476±0.0460
Adrenals	0.0343±0.0072	0.0384±0.0089	0.0336±0.0037	0.0327±0.0052	0.0273±0.0057	0.0309±0.0091
Ovaries	0.0580±0.0124	0.0634±0.0108	0.0616±0.0090	0.0651±0.0160	0.0412±0.0170	0.0454±0.0064
Uterus	0.3490±0.0644	0.3347±0.0906	0.2636±0.0805	0.2912±0.0919	0.3332±0.0929	0.2943±0.0403

****: Statistically significant (p<0.05) than control group.***

1.6.6 PATHOLOGY

In the post-mortem pathology examinations, some minor and isolated incidence abnormalities including peticheal haemorrhage of the lungs and enlarged spleen were occurred at similar incidences and severity in both control and treatment groups.

Histopathology of both the test item and control non recovery groups showed some isolated incidences of irregularities in the lungs and spleen, these findings are not uncommon in this type of laboratory animals with occasional abnormalities and outliers being observed in every repeated dose study, hence considered not related to the treatment. Lydén-Sokolowski et al., (1993) reported that the results of two year carcinogenicity study investigation at daily dose of 25 mg/kg do not indicate any relationship

between long-term administration of purified senna extract and gastrointestinal, liver, kidney or adrenal tumours in the rat.

CONCLUSIONS

No animals died and all animals continued to gain weight when administered up to 2000 mg/kg TLPL/AY/01/2008 as a single dose and considered to be nontoxic. In the 28 day repeated dose toxicity study, no deaths occurred. No abnormalities were found in cage side behaviour, body weight, food consumption, neurological, ophthalmological or urine analyses. No toxicologically significant and treatment related findings observed in clinical pathology parameters, organ weights and organ weight ratios. Macroscopic and microscopic findings revealed no treatment related effects in any of the organs examined.

The Maximum tolerated dose, No Observed Adverse Effect Level (NOAEL) of polyherbal formulation TLPL/AY/01/2008 in Sprague dawley rats is greater than 2000 mg/kg when administered as a single acute dose, and greater than 350 mg/kg when administered as repeat dose for 28 consecutive days. Hence the polyherbal formulation TLPL/AY/01/2008 can be considered to be safe for long term use without producing physiological dependence and devoid of adverse effects as revealed by safety assessment studies.

DISCLOSURE

The studies described herein were performed by the contract research organisation Bioneeds Laboratory Animals and Preclinical Services and sponsored by Tulip Lab Private Limited, which manufactures TLPL/AY/01/2008.

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