The Journal of Phytopharmacology 2021; 10(5):298-315
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2021; 10(5): 298-315
September- October
Received: 16-07-2021
Accepted: 09-08-2021

doi: 10.31254/phyto.2021.10505

Kunjan Shah
Department of Toxicology, Jai Research Foundation, Vapi, Gujarat, India

Sudhakar Jadhav
Department of Toxicology, Jai Research Foundation, Vapi, Gujarat, India

Manish Patel
Department of Toxicology, Jai Research Foundation, Vapi, Gujarat, India

Jaydip Mistry
Department of Toxicology, Jai Research Foundation, Vapi, Gujarat, India

Akashrao Shinde
Department of Toxicology, Jai Research Foundation, Vapi, Gujarat, India

Liliya Mito
The Mitomasa SDN BHD, Selangor D.E., Malaysia

Kanchan Khare
Department of Toxicology, Jai Research Foundation, Vapi, Gujarat, India

Correspondence:
Kunjan Shah
Department of Toxicology, Jai Research Foundation, Vapi, Gujarat, India
Email: kunjan.shah@jrfonline.com

Safety Assessment of TGT Primaage using Wistar Rats through Oral Gavage Administration

Kunjan Shah, Sudhakar Jadhav, Manish Patel, Jaydip Mistry, Akashrao Shinde, Liliya Mito, Kanchan Khare

ABSTRACT

TGT Primaage is one of the astonishing extracts, obtained from a combination of *Moringa oleifera* and *Centella asiatica.* At present, there is no finding reported regardin g the repeated exposure of this extract. This study investigates the No Observed Adverse Effect Level (NOAEL) and effects of repeated-dose administration of Astragalin and Isoquercetin (components of interest) present in TGT Primaage on Wistar rats. Rats were divided into 3 treatment and a control group (10 rats/sex/group). To assess reversibility, persistence, or delayed effect, additional control and high dose groups were treated similarly and observed further without any treatment. TGT Primaage was administered orally, through gavage for 90 days, at 0, 250, 500, and 1000 mg/kg body weight/day. No mortality, morbidity, and clinical sign of toxicity was observed. Abnormality related to neurological and functional parameters was not seen. No change in body weight and food consumption was observed. Treatment did not lead to any adverse effect in clinical pathology parameters and organ weights. TGT Primaage did not alter morphological and histopathological characteristics of organs. From these results, it is evident that TGT Primaage appears to be safe and devoid of any toxicity. The No Observed Adverse Effect Level (NOAEL) of TGT Primaage for both sexes were found to be 1000 mg/kg body weight/day.

Keywords: TGT Primaage, Wistar rats, Sub-acute toxicity, Sub-chronic toxicity, Astragalin, Isoquercitrin.

INTRODUCTION

Natural products are infinite treasures of bioactive chemicals which persist as an inexhaustible resource for the discovery of drugs and exploration of their undefined activities. It is estimated that herbal medicines have been traditionally used for the treatment purpose by more than a quarter of the world population as a basis for primary health care. Performing herbal toxicity is a need to dissect favourable from adverse effects, to identify active principles in medicinal plants and to ban poisonous or toxic contents from herbal mixtures. TGT Primaage is a combination of *Moringa oleifera* and *Centella asiatica* leaves extracts [1]. This abstract has been claimed to have high antioxidant and antiaging activity apart from a broad range of biological functions including anti-inflammatory, anti-cancer, hepatoprotective, and neuroprotective function [2]. Besides, many studies have revealed its therapeutic value including anti-diabetes, anti-rheumatoid arthritis, anti-atherosclerosis, anti-infertility, pain relief, anti-depression, diuretic, and thyroid regulation [2].

Moringa oleifera is referred to as the 'drumstick tree', 'miracle tree', 'horseradish tree', and 'kelor tree' [3]. This plant belongs to the family of *Moringaceae* and is widely cultivated in Asian and African countries [4]. The leaves of this plant have been used traditionally in the treatment of constipation, headache, arthritis, diabetes, hypertension, and typhoid fever [3]. Phytochemical screening of this plant contains many constituents such as flavonoid, terpenoids, saponin, and tannins [3].

Centella asiatica also known as 'Pegaga' in Malaysian, belongs to the *Apiaceae* family. It grows widely in Asia (mainly in India, Pakistan), Madagascar, Africa, Central America, and in the tropical region of Oceania [5]. This herb is recommended for the treatment of various skin conditions such as leprosy, lupus, varicose ulcers, eczema, and psoriasis. It is also used in diarrhoea, fever, amenorrhea, anxiety, cognition, and female genitourinary tract diseases [6]. Considering the broad use of these herbs either as a stand alone or in combination, evaluation of safety is the prime force to perform the toxicological study. Here, the study investigates the systemic toxicity and adverse effects of Astragalin and Isoquercetin (components of interest) present in TGT Primaage after repeated-dose administration in Wistar rats according to the criteria mentioned in the EMA guideline [7].

Ethical Consideration: Project proposal for the experimentation was approved by the "Institutional Animal Ethics Committee (IAEC)", Jai Research Foundation. The study was undertaken in compliance with the guidelines of the "Association for Assessment and Accreditation of Laboratory Rat Care

(AAALAC), USA" and "Guidelines for Laboratory Rats Facility" issued by the Committee for the Purpose of Control and Supervision of Experiments on Rats (CPCSEA), India. All the studies were performed in accordance with OECD Principles on Good Laboratory Practice (GLP) and EMA guideline [8].

MATERIALS AND METHODS

TGT Primage details:

Batch Number	MOR/EXT/260216
Analysed Purity	Isoquercetin: 2.06%; Astragalin: 0.74%
Manufactured by	The Mitomasa SDN BHD, Malaysia
Supplied by	The Mitomasa SDN BHD, Malaysia
Date of Manufacture	February 26, 2016
Date of Expiry	February 25, 2019
Appearance	Dark golden-red powder
Storage Condition	In original container as supplied at ambient condition

The phytochemical analysis of leaves extract of *Moringa oleifera* plant signifies the presence of alkaloids, steroids and flavonoids in adequate quantity according to preliminary phytochemical analysis [9] while Phenols and flavonoids are present in hydroalcoholic extract of *Centella asiatica* [5].

Animals

Healthy, young adult male and female rats (*Rattus norvegicus*) of Wistar (RccHan: WIST) strain (5 to 8 weeks of age) were obtained from the Animal Breeding Facility (ABF), JRF, India. Nulliparous and non-pregnant female rats were used for the experiment. Rats were acclimatised for 7 days before randomisation. The body weight variation among the rats was within ±20% of the mean body weight for each sex.

Housing

Rats were maintained in temperature (22 ± 3 °C) and humidity (30 to 70%) controlled room, with the photoperiod of 12 h light/dark cycle (light hours were 06.00 - 18.00 h). Light intensity was maintained between 130 and 325 LUX and air changes were minimum 15 per hour. Rats were housed in groups of 2 rats/cage/sex in sterile prolypropylene cages with bedding material. Feed and water were provided *ad libitum* to rats. Environmental enrichment material was also provided to rats in each cage. Cages were placed on 5 tier racks and cage rotation was performed at weekly intervals to ensure almost similar environmental conditions to different groups.

Experimental design

The dose formulation of test item in vehicle (RO water) was administered through oral gavage at three graduated dose levels (G2, G3, and G4) to male and female Wistar rats for a period of 28 and 90 consecutive days in 28 day and 90 days study, respectively. Rats from a concurrent vehicle control groups (G1) received RO water only.

To assess reversibility, persistence or delayed occurrence of toxic effects, an additional group (G6) was treated at the high dose level for 28 and 90 days and then observed further for a period of 14 and 28 days without any treatment in 28 day and 90 days study, respectively. For comparison purposes, a vehicle control recovery group (G5) was treated with vehicle alone over the equivalent period (28 and 90 days) and observed further for 14 and 28 days. Each group consisted of 10 male and 10 female rats. Rats were treated with three dose levels (low dose at 250, mid dose at 500 and high dose at 1000 mg/kg b. wt./day) in both study (Table 1). A constant dose volume of 10 mL/kg b. wt. was used and individual dose was adjusted according to the most recently recorded body weight of each rat.

Table 1: Distribution of Rats after Randomisation in 28-day and 90-day Toxicity Study: Rats were randomised as per their body weight in such a manner that the variation between group mean body weight and dose-volume of each rat was minimal.

Group N°	Group	Number of Rats		Concentration (mg/mL)	Dose (mg/kg b. wt./day)
		Male	Female		
Main Groups (Treatment was given 28-day and 90-day for respective studies):					
G1	Vehicle Control	10	10	0	0
G2	Low Dose	10	10	25	250
G3	Mid Dose	10	10	50	500
G4	High Dose	10	10	100	1000
Recovery Groups (Recovery period was 14-day and 28-day for respective studies):					
G5	Vehicle Control Recovery	10	10	0	0
G6	High Dose Recovery	10	10	100	1000

Dose levels selection:

The MTD (maximum tolerated dose) single-dose administration and 14-days dose range-finding (DRF) studies were carried out for dose level selection. In MTD study, two dose levels - 2000 and 3000 mg/kg b. wt. - were evaluated in a group of 5 rats/sex/group, orally, through gavage. There was no sign of mortality or morbidity, and abnormal clinical sign during the 14-days observation period.

Based on the MTD study, 14-days DRF study at three doses (250, 500, 1000 mg/kg b. wt.) was evaluated on different groups of 5 rats/sex/group. No mortality, morbidity, and sign of toxicity was observed. The mean body weight, food consumption, and gross examination of treated groups were comparable to that of the control group. In this way, the given doses were confirmed and used to examine a large group of rats (28-day and 90-day repeated oral administration study) along with additional recovery groups to look after any perseverance.

Dose formulation and analysis:

The formulation was freshly prepared on each day of dosing by mixing the extract thoroughly with RO water. The prepared dose formulations were thoroughly mixed using a magnetic stirrer before dosing and using cannula intermittently during dosing. The stability of dose formulation was found up to 24 h. The active ingredient concentration (Astragalin and Isoquercitrin) and homogeneity of formulated doses were analysed using LC/MS-MS. For formulation analysis, samples were collected from the upper, middle, and lower layers of the final prepared formulation before dosing, except in the control group where the sample was collected from the middle layer only (Figure 1).

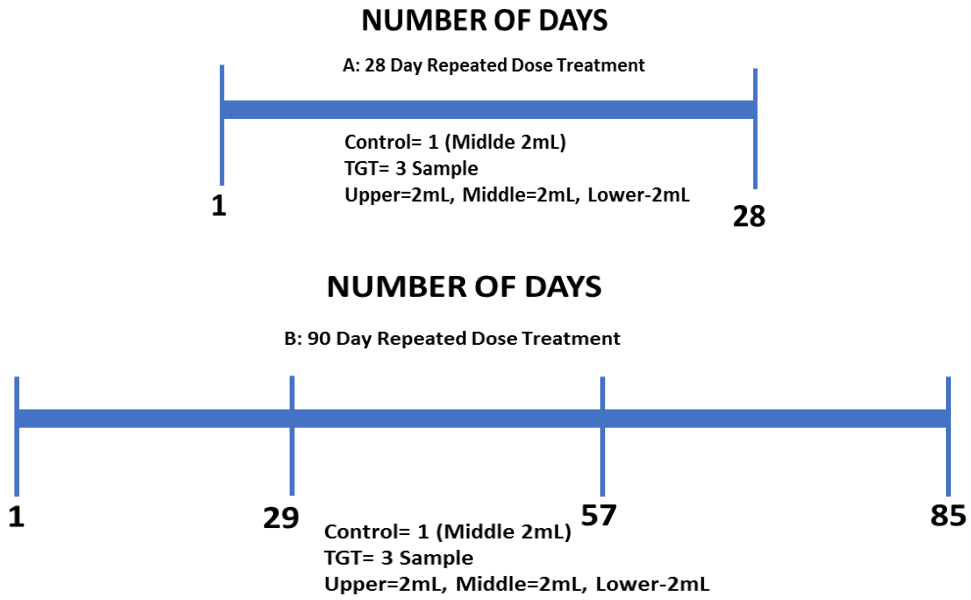


Figure 1: Analysis of Active Ingredient Concentration and Homogeneity of Dose Formulation using LC/MS-MS

The analytical method is sensitive, precise, and accurate enough for the determination of isoquercitrin and astragalin in dose formulation of TGT Primaage in the matrix (RO water) for validating the formulations to be used in these studies [10].

Instrumental parameters used for method of analyses:

Column : Waters X-bridge C-18 [150 × 4.6 mm, 3.5 µm particle size]

Mobile Phase : (A) 0.1% Formic acid in Milli-Q Water: (B) Acetonitrile

Flow Rate : 1.5 mL/minute

Column Temperature : 30 °C

Auto Sampler Temperature : 10 ºC

Injection Volume : 5 µL

Elution Mode : Gradient

Run Time : 5 minutes

Timetable :

Time (minute)	Module	Pump B (%)
0.0	Pump	20
2.0	Pump	60
3.0	Pump	60
3.1	Pump	20
5.0	System Controller	Stop

Mass Spectrometer Parameters for Multiple Reaction Monitoring

Instrument Identification	API 4000 LC-MS/MS			
Mass Parameters	Isoquercetin		Astragalin (Kaempferol 3-glucoside)	
MRM Transitions	463.200/ 300.100	463.200/ 271.100	447.400/ 284.200	447.400/ 255.100
Declustering Potential (DP) V	-135	-110	-110	
Collision Energy (CE) eV	-40	-60	-37	-51
Entrance Potential (EP) V	-5			
Collision Cell Exit Potential (CXP) V	-5			
Dwell Time (milli seconds)	200			
Ionisation / Polarity	Negative			
Ionisation Source	Electrospray Ionization (ESI)			
Collision Gas (CAD) psi	8			
Curtain Gas (CUR) psi	10			
Ion Spray Voltage (V)	-4500			
Temperature (°C)	500			
GS1 psi	50			
GS2 psi	60			

General Observations:

Clinical Observations

Rats were observed daily for mortality, morbidity, and clinical sign during acclimatisation, treatment, and recovery periods.

Body Weight

Body weight of rats was recorded at the beginning of the treatment (pre-treatment), at weekly intervals, thereafter, and on the day of necropsy (fasted body weight).

The body weight change compared to the pre-treatment body weight was calculated as per the below-mentioned formula:

$$\text{Body weight change (\%)} = \frac{\text{Body weight on week (g)} - \text{Pre-treatment body weight (g)}}{\text{Pre-treatment body weight}} \times 100$$

Food Consumption

The food consumption was calculated and reported as g/rat/day as per the below-mentioned formula:

$$\text{Food consumption (g/rat/day)} = \frac{\text{Feed input (g)} - \text{Feed leftover (g)}}{\text{Number of rats per cage} \times \text{Number of days}}$$

Ophthalmological Examination

Ophthalmological examination was performed on rats before the commencement of the treatment and the terminal and recovery sacrifices. To facilitate easy examination of the anterior part of the eye, homatropine hydrobromide eye drops were used as a mydriatic solution to dilate the pupil. This mydriatic solution was instilled into each eye before 15 to 20 minutes of the eye examination. Both eyes of each rat were examined by a direct ophthalmoscope.

Functional Observational Battery [11, 12].

Neurobehavioural Observations

To evaluate the occurrence of any neurological toxicity, the behavioural and neurological status of each rat was examined. NBO parameters (Table 2) were evaluated before the initiation of the dosing and at weekly intervals, thereafter.

Table 2: List of parameters included for the evaluation of neurobehavioural toxicity assessment at different surrounding and stimuli exposure:

Neurobehavioural Observations		
Home Cage Observation	**Handling Observation**	**Open Field Observation**
• Posture • Convulsion	• Ease of Removing from the cage • Handling reactivity • Palpebral Closure • Lacrimation • Eye Examination • Piloerection • Skin Examination • Salivation	• Gait • Mobility • Arousal Level • Vocalization • Rears • Respiration • Clonic or Tonic Movement • Urination • Defecation • Stereotypy Behaviour • Bizarre Behaviour

Motor Activity

Motor activity was performed once, during 4th (28-day study) and 12th (90-day study) week of the treatment period and 2nd (28-day study) and 4th (90-day study) week of the recovery period. Motor activity was evaluated for each rat using an automated photobeam activity system (San Diego Instruments, USA). Rats were monitored for three consecutive 10 minutes intervals (total 30 minutes for each rat) allowing for examination of both exploratory and acclimation activity levels. The motor activity parameters including fine, ambulatory, and total activities were evaluated and reported.

Sensory Reactivity Measurements

Sensory measurements were performed once, during 4th (28-day study) and 12th (90-day study) week of the treatment period and 2nd (28-day study) and 4th (90-day study) week of the recovery period. For sensory reactivity measurements, rats were placed in an open arena (size: 495 × 495 × 203 mm) with a flat surface covered with clean absorbent paper. Various parameters, approach, touch, click, pupil, tail-pinch, and air righting reflex total responses were performed and recorded for each rat.

Grip Strength

The grip strength was performed once, during 4th (28-day study) and 12th (90-day study) week of the treatment period and 2nd (28-day study) and 4th (90-day study) week of the recovery period. Grip strength of both forelimb and hindlimb was measured with a grip strength meter (San Diego Instruments, USA) to determine the ability of the rat to grasp and hold on to the mesh platform. The grip strength of each rat was measured for 3 consecutive times and results were averaged separately for the forelimb and hindlimb.

Hindlimb Foot Splay

The hindlimb foot splay was performed once, during 4th (28-day study) and 12th (90-day study) week of the treatment period and 2nd (28-day study) and 4th (90-day study) week of the recovery period. The landing hindlimb feet of each rat were marked with a non-permanent, non-toxic ink just before testing. Each rat was suspended in a prone position and then dropped on to a recording sheet from a height of approximately 30 cm. This procedure was repeated three times. The distance between two footprints was measured, and an average of three feet play values was calculated.

Biochemical Evaluation

At the end of the treatment and recovery periods, rats were fasted overnight (with *ad libitum* supply of drinking water) in metabolic cages for urine collection. Blood was collected from rats for clinical pathology evaluation (approximately 3 mL blood). The blood sample was collected for haematology (in vials containing 4% EDTA anticoagulant for whole blood), coagulation parameters (in vials containing 3.2% sodium citrate anticoagulant for plasma separation), and clinical chemistry analysis (in plain vials for serum separation) under light isoflurane anaesthesia by orbital plexus puncture.

Gross Pathology

At scheduled sacrifices (terminal and recovery), rats were euthanised by carbon dioxide asphyxiation. All rats were subjected to a full gross necropsy under the direct supervision of a veterinary pathologist. Rats were examined carefully for external abnormalities. The cranial, thoracic, and abdominal cavities were cut opened and a thorough examination of organs were carried out to detect abnormalities.

Organ/ Tissue Collection

Organs and tissues from male and female rats of the main and recovery groups were collected, weighed, and preserved. Adherent adipose tissue was trimmed off and the wet weight of the organs was recorded. The paired organs were weighed together, and the combined weight was presented. The organ weight ratios as a percentage of the body weight were determined. All organs were preserved in 10% neutral buffered formalin solution except eyes (in Davidson's) and testes (in modified Davidson's).

Histopathological Examination

Histopathological examination was carried out for the preserved organs and tissues of rats from vehicle control (G1) and high dose (G4) groups. Organ and tissue samples were processed, embedded and cut at a thickness of 3 to 5 micrometres and stained with haematoxylin and eosin. Peer review of histopathology was also performed.

Statistical Analysis

Data processed to get group means and standard deviations with significance among the vehicle control and treatment groups using validated statistical software. Parameters such as body weight, body weight change, food consumption, NBO parameters (urination,

defecation, and rearing), FOB parameters (motor activity, grip strength, and foot splay), organ weight, relative organ weight, and clinical pathology (haematology, clinical chemistry, and some urinalysis) were subjected to Bartlett's test to meet the homogeneity of variance before conducting Analysis of Variance (ANOVA) and Dunnett's test. Where data do not meet the homogeneity of variance, F-test was performed before conducting t-tests to calculate significance [13]. The motor activity counts were subjected to square root transformation to homogenise the data for statistical implementation. All analyses and comparisons were evaluated at the 5% ($P \leq 0.05$) and 1% ($P \leq 0.01$) levels.

RESULTS

The active ingredients (isoquercetin and astragalin) concentration and homogeneity analysis in 28-day and 90-day studies were within an acceptable range of ±15% of the nominal concentration and %CV < 10.

Rats were healthy throughout the study period from the vehicle control and treatment groups. There was no change in body weight and food consumption until the termination of both studies.

Ophthalmological examination conducted during the pre-treatment period and towards sacrifices (main and recovery groups) in both studies did not reveal any abnormality in rats.

Profound functional observation battery was conducted, with manual handling and different surrounding stimuli exposure from less stressful to high traumatic parametric analysis to identify any treatment-related anomaly on behaviour. In a 28-day study, statistically, a significant decrease was observed in foot splay of male rats from high dose group when compared with that of the vehicle control group which could not be considered as a treatment-related effect in the absence of other supporting findings of neuromuscular parameters (grip strength and motor activity). In a 90-day study, forelimb grip strength values of female rats were statistically decreased. These changes could not be considered as the test item related effect due to either lack of dose-dependency or lack of similar effect in main groups.

TGT Primaage treatment did not lead to any significant alteration in haematology, coagulation, clinical chemistry, and urinalysis parameters. In a 28-day study, noted statistically significant alterations [decrease in MCH values in high dose group (1000 mg/kg b. wt.) female rats and increase in MCV and MCH in high dose recovery group (1000 mg/kg b. wt.) female rats] were not treatment related due to an absence of consistency between sexes and lack of effects in other related parameters (haemoglobin, haematocrit, and RBC count). Similarly, significant decrease noted in APTT in high dose recovery group female rats was not related to treatment due to absence of the effect in high dose (at the end of treatment period) and lack of consistency between sexes.

In a 90-day study, a statistically significant increase was observed in lymphocytes of G4 male rats which was not considered related to the test item treatment due to lack of consistency between sexes. Statistically significant increases were observed in PT and APTT (G2 male rats), and basophil (G2 female rats). Statistically, significant decreases were observed in PT of G2 and G4 female rats. These alterations were considered unrelated to the test item treatment due to lack of dose-dependency and inconsistency between sexes. In high dose recovery group, a statistically significant decrease was observed in RBC in males, PT in female rats and a statistically significant increase was observed in monocyte, WBC and lymphocytes in female rats. These alterations were not considered related to the test item treatment due to the absence of effects at the end of treatment and/or consistency between sexes.

In a 28-day study, the statistically significant increase observed in inorganic phosphorus in high dose recovery group (1000 mg/kg b. wt.) in female rats was considered as unrelated to treatment, due to lack of consistency between sexes and absence of similar findings in high dose (at the end of the treatment period. In a 90-day study, a statistically significant increase was observed in albumin in male rats (G4) which was considered unrelated to the test item treatment, due to the lack of consistency between sexes. Statistically, a significant increase was observed in ALT in male rats (G6), which was considered unrelated to test item treatment due to lack of effect at the end of treatment and lack of consistency between sexes.

TGT Primaage did not show any treatment-related variation in the terminal body weight. The absolute and relative weight of different organs measured during sacrifices did not show any significant findings or consequence.

An external and internal examination of rats from either sex across various groups did not show any treatment-related abnormality. During the histopathological examination, no change was observed in any organ or tissue which can give any correlation of treatment between treated and control animals (Figure 2 and Figure 3). Histologic evaluation revealed no major difference between the peer review pathologist and the study pathologist. Minor differences of opinion in the histologic findings were discussed, resolved, and mutually agreed upon by each pathologist, and did not make any difference in the interpretation of the result.

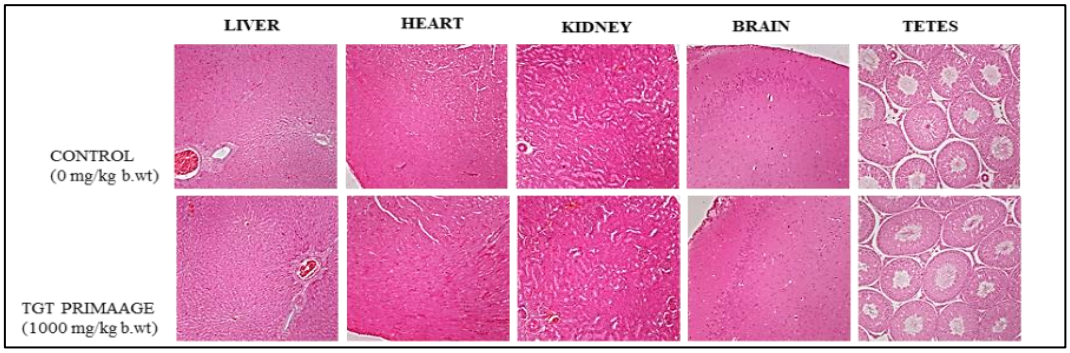
DISCUSSION

The 28-day and 90-day toxicity studies described here was conducted using a well-developed protocol for long term exposure. Based on the various evaluations and parametric data analysis TGT Primaage was found to be safe at given doses for its consumption.

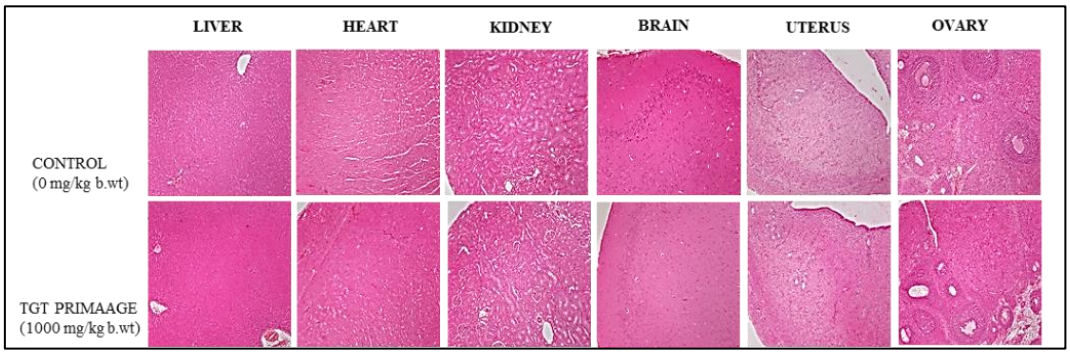
The analytical validated method was developed and successfully applied to evaluate the concentration of isoquercitrin and astragalin in the matrix and analyzed onto LC-MS/MS samples of the toxicology studies for the dose formulation analysis of TGT Primaage [10].

By providing hygienic and sterile micro and macro environment to rats throughout the study period (28 and 90 days), rats remained healthy with the energetic appearance and without showing any visible clinical sign. Overall growth in term of body weight of rats was natural and comparable. The normal body weight gain and food efficiency of the rat during study periods revealed no effect of treatment on daily food and water consumption. Hence, TGT Primaage treatment did not demonstrate any effect on in-life measures after long term administration in male and female rats.

Evaluation of neurological, sensory-motor and behavioural functions of rodent are crucial endpoints in consecutive administration of the compound. Evaluation of neurological and behavioural functions at weekly intervals during both studies by proficient scientist did not reveal any inference related to TGT Primaage treatment. The cluster of parameters related to sensory-motor and neuromuscular function towards the terminations in both studies was assessed by sensitive

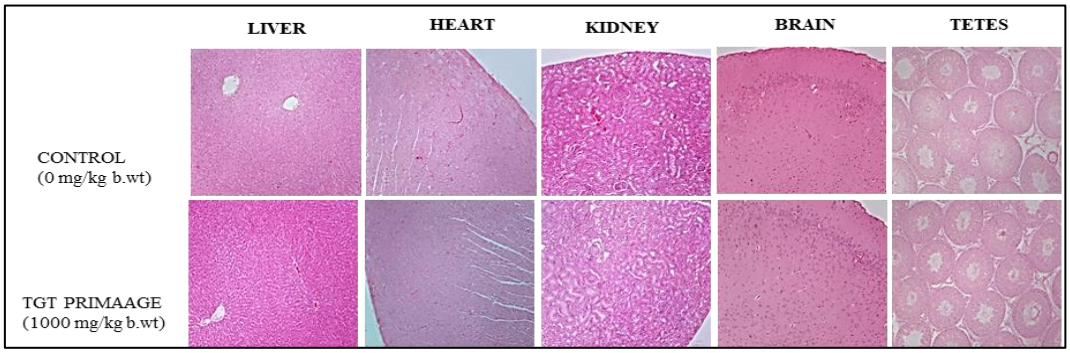


A. Male

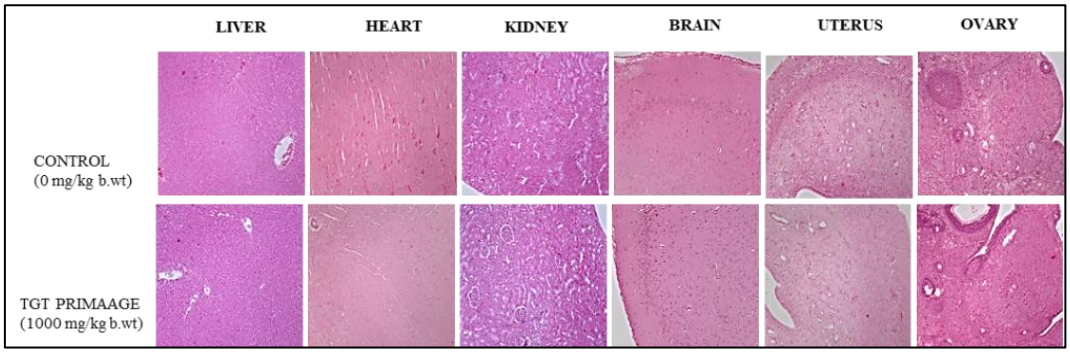


B. Female

Figure 2: The figure represents the normal histological images of vital male and female organs (H and E X 10x) from 28-days toxicity study. No pathological significant lesion was noted in TGT Primaage treated rats at 1000 mg/kg b. wt.



A. Male



B. Female

Figure 3: The figure represents the normal histological images of vital male and female organs (H and E X 10x) from 90-days toxicity study. No pathological significant lesion was noted in TGT Primaage treated rats at 1000 mg/kg b. wt.

instruments with tactic handling shown comparable outcomes of TGT Primaage treatment with control treatment. Hence, TGT Primaage did not alter the CNS related functions after a consecutive period of administration in both sexes.

Numerous parameters, related to the haematological, coagulation functions, and the electrolyte levels, as well as enzyme levels, were evaluated with accuracy, precision, and calibration standards on biological fluid. Urinalysis was also quantified for its physicochemical properties and qualified for various type of cells and molecules. This extensive evaluation of clinical pathology parameters did not reveal any change when compared statistically between the control and treatment group.

The absolute and relative (compared to the terminal body weight) weight of sex-specific and communal organs of rats from the treated groups were neither biologically nor statistically significant, when compared with the control group. Gross and histopathological examinations, with the addition of peer review, were conducted, and no abnormality was detected for any morphological change under the microscopic examination.

In the recovery groups, no sign of toxicity was observed. Rats were normal and healthy, throughout the recovery phase. In-life measures and various sacrifice endpoints were also evaluated in the same manner as for the main groups which had given similar results as that of the main groups in both sexes, in the 28-day and 90-day studies.

In this way, there was no sign of systemic and local toxicity observed in the long-term exposure of TGT Primaage, which included a vast number of parameters with appropriate sample size to evaluate the toxicity from behavioural to microscopical level and, also using both male and female rats to understand any gender variation.

CONCLUSION

These studies conclude that TGT Primaage did not produce any significant toxicity or adverse effect up to the highest dose level of 1000 mg/kg b. wt./day, after the repeated dose (28-day and 90-day), oral administration in Wistar rats. The NOAEL (No Observed Adverse Effect Level) for TGT Primaage of both sexes (male and female rats) was found to be 1000 mg/kg b. wt./day, under conditions and procedures, followed in these studies.

Conflict of Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Acknowledgement

This study was conducted by Jai Research Foundation, Valvada, Gujarat, India and funded by The Mitomasa Sdn Bhd N° 13, Jalan L J 3, Taman Industri Lembah Jaya, 68000 Ampang, Selangor D.E., Malaysia.

Kunjan Shah conducted all studies, reviewing data, and contributed to manuscript writing and reviewing. Sudhakar Jadhav conducted and contributed to the live phase of studies and manuscript reviewing. Dr. Manish Patel aided resource management. Jaydip Mistry contributed to the analysis of clinical pathology. Dr. Akashrao Shinde contributed to the post sacrifice phase of the studies and histopathology examination. Liliya Mito funded all studies and provided the compound of the subject. Kanchan Khare contributed to the literature search and manuscript writing.

List of Abbreviations

% - Percentage
°C - Degree centigrade
b. wt. - Body weight
CPMP - Committee for Proprietary Medicinal Products
CV - Coefficient of Variance
FOB - Functional Observational Battery
G - Gram
G1 - Group 1 treated with vehicle (0 mg/kg b. wt./day)
G2 - Group 2 treated with low dose of test item (250 mg/kg b. wt./day)
G3 - Group 3 treated with mid dose of test item (500 mg/kg b. wt./day)
G4 - Group 4 treated with high dose of test item (1000 mg/kg b. wt./day)
G5 - Group 5 treated with vehicle - recovery group (0 mg/kg b. wt./day)
G6 - Group 6 treated with high dose of test item - recovery group (1000 mg/kg b. wt./day)
H - Hour
JRF - Jai Research Foundation
Kg - Kilogram
L - Liter
LC-MS/MS - Liquid Chromatography-Mass Spectrometry/Mass Spectrometry
Mg - Milligram
mL - Milliliter
N° - Numbe
NBO - Neurobehavioural Observations
OECD - Organisation for Economic Co-operation and Development
RO Water - Reverse Osmosis Water

REFERENCES

1. Hisam EEA, Rofiee MS, Khalid AM, Jalaluddin AF, Yusof MIM, Idris MH Ramli, S., James, R.J., Yoeng, W.J., Kek, T.L., Salleh, M.Z., Combined Extract of *Moringa Oleifera* and *Centella Asiatica* Modulates Oxidative Stress and Senescence in Hydrogen Peroxide-Induced Human Dermal Fibroblasts. Turk J. Biol 2018;42:33-44. https://doi.org/10.3906/biy-1708-23.
2. Kou X, Biao L, Julia BO, Justin MD, Ning C. Nutraceutical or Pharmacological Potential of *Moringa oleifera* Lam. Nutrients. 2018;10(3):343. https://doi.org/10.3390/nu10030343.
3. Anwar F, Latif S, Ashraf M, Gilani AH. *Moringa oleifera*: A Food Plant with Multiple Medicinal Uses. Phytother. Res. 2007;21:17-25 https://doi.org/10.1002/ptr.2023.
4. Gopalakrishnan L, Doriya K, Kumar DS. *Moringa oleifera*: A review on nutritive importance and its medicinal application. Food Science and Human Wellness 2016;5:49-56. https://doi.org/10.1016/j.fshw.2016.04.001.
5. Brinkhaus B, Lindner M, Schuppan D, Hahn DE. Chemical, pharmacological and clinical profile of the East Asian medical plant *Centella asiatica*. Phytomedicine 2000;7(5):427-448. https://doi.org/10.1016/S0944-7113(00)80065-3.
6. EMA, The European Agency for the Evaluation of Medicinal Products, CPMP/SWP/1042/99 Rev 1 corr. Evaluation of Medicine for Human Use: Note for Guidance on Repeated Dose Toxicity 2010.
7. OECD, OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1, OECD Principles on Good Laboratory Practice, ENV/MC/CHEM 1998;(98)17.
8. Gohil K, Gajjar A, Patel A. Pharmacological Review on *Centella asiatica*: A Potential Herbal Cure-all. Indian Journal of Pharmaceutical Sciences 2010;72(5):546-556. https://doi: 10.4103/0250-474X.78519.
9. Goswami S, Singhai R. Antioxidant Activity of Moringa oleifera using Successive Solvent Extraction Technique. International Journal of Theoretical and Applied Sciences. Special Issue-NCRTAST 2016;8(1):23-27(2016).

10. Patel M, Prabhu P, Patel A, Trivedi P. Simultaneous Determination of Isoquercitrin and Astragalin in Plant (Leaf) Extract Using Liquid Chromatography with Tandem Mass Spectrometry Method for the Application of Toxicology Studies in Matrix 2020.
11. Irwin S. Comprehensive Observational Assessment: Ia. A Systematic, Quantitative Procedure for Assessing the Behavioral and Physiologic State of the Mouse 1966. https://doi.org/10.1007/bf00401402.
12. Mathiasen J, Moser V. The Irwin Test and Functional Observational Battery (FOB) for Assessing the Effects of Compounds on Behavior, Physiology, and Safety Pharmacology in Rodents 2018. https://doi.org/10.1002/cpph.43.
13. Gad SC, Weil CS. Statistics for Toxicologists In: Principles and Methods of Toxicology, 5th Edition, Hayes, A.W. ed. Raven Press Ltd., New York 2007, 369-452.

HOW TO CITE THIS ARTICLE
Shah K, Jadhav S, Patel M, Mistry J, Shinde A, Mito L, Khare K. Safety Assessment of TGT Primaage using Wistar Rats through Oral Gavage Administration. J Phytopharmacol 2021; 10(5):298-315. doi: 10.31254/phyto.2021.10505

Supplementary data:

Table 1: This table represent the results of the body weight and body weight change from the treatment phase as well as in the recovery phase in 28-day toxicity study.

Duration	Male (Number of animals = 10/group)				Female (Number of animals = 10/group)			
	Control (mg/kg b. wt.)	TGT Primaage (mg/kg b. wt.)			Control (mg/kg b. wt.)	TGT Primaage (mg/kg b. wt.)		
	0	250	500	1000	0	250	500	1000
Mean body weights (g) during treatment and recovery phases								
PT	246.2 ± 8.3	246.7 ± 7.3	248.0 ± 7.2	246.2 ± 9.6	172.8 ± 8.0	173.5 ± 9.3	173.3 ± 7.7	173.5 ± 7.2
Week 1	280.2 ± 11.6	281.0 ± 11.8	283.7 ± 10.1	278.2 ± 10.7	188.4 ± 7.1	189.8 ± 12.2	186.5 ± 7.7	186.6 ± 8.1
Week 2	305.0 ± 14.8	305.9 ± 16.0	309.4 ± 15.3	300.4 ± 11.4	206.9 ± 8.5	205.1 ± 11.8	201.1 ± 8.9	203.2 ± 12.6
Week 3	323.9 ± 18.5	328.0 ± 18.5	332.2 ± 16.4	321.8 ± 14.1	215.5 ± 10.4	212.5 ± 13.9	207.3 ± 9.9	212.5 ± 14.2
Week 4	339.6 ± 19.5	343.4 ± 18.7	348.3 ± 17.2	337.4 ± 16.2	224.7 ± 9.7	221.2 ± 14.4	218.4 ± 12.8	221.3 ± 17.7
Week 5	362.0 ± 19.4			358.7 ± 17.0	234.6 ± 22.0			231.9 ± 18.1
Week 6	374.0 ± 21.7			372.1 ± 21.6	228.2 ± 19.3			230.6 ± 20.1
Mean body weight change (%) during treatment and recovery phases								
PT-week 4	37.90 ± 4.52	39.18 ± 4.68	40.46 ± 5.51	37.02 ± 4.08	30.15 ± 4.33	27.46 ± 4.64	26.04 ± 4.10	27.43 ± 6.83
PT-week 6	53.07 ± 6.90			52.96 ± 7.45	30.14 ± 4.30			32.26 ± 8.90
Mean body weights (g) at necropsy (fasting)								
Week 4	322.3 ± 18.65	324.0 ± 18.99	330.5 ± 17.36	318.4 ± 16.33	214.2 ± 9.29	208.8 ± 13.93	206.2 ± 11.69	209.2 ± 14.65
Week 6	355.9 ± 21.50			355.3 ± 20.37	218.4 ± 18.67			218.7 ± 17.50

Each value shows mean ± SD.

Table 2: This table represent the results of the body weight (g) from the treatment phase of main groups in 90-day toxicity study.

	Male (Number of animals = 10/group)				Female (Number of animals = 10/group)			
	Control (mg/kg b. wt.)	TGT Primaage (mg/kg b. wt.)			Control (mg/kg b. wt.)	TGT Primaage (mg/kg b. wt.)		
	0	250	500	1000	0	250	500	1000
PT	189.41 ± 11.90	189.22 ± 10.43	189.62 ± 10.09	190.49 ± 10.24	149.54 ± 7.01	146.85 ± 6.76	150.11 ± 5.82	150.19 ± 5.80
Week 1	233.49 ± 12.26	231.12 ± 11.64	234.01 ± 11.46	235.98 ± 12.70	166.86 ± 4.87	166.57 ± 9.83	169.02 ± 6.12	170.36 ± 11.27
Week 2	270.43 ± 13.35	268.49 ± 12.86	273.62 ± 14.22	274.54 ± 16.26	184.24 ± 6.73	184.82 ± 8.14	185.57 ± 8.72	184.73 ± 12.04
Week 3	300.76 ± 16.64	297.19 ± 12.68	304.22 ± 16.12	303.40 ± 21.66	199.05 ± 8.40	199.93 ± 7.98	200.08 ± 8.63	200.83 ± 13.02
Week 4	325.34 ± 17.39	319.32 ± 15.14	330.42 ± 18.95	328.24 ± 24.75	212.65 ± 7.98	213.55 ± 9.18	211.31 ± 10.12	211.27 ± 12.22
Week 5	345.19 ± 20.74	337.30 ± 15.80	349.49 ± 21.38	347.93 ± 31.45	222.94 ± 7.57	226.48 ± 10.54	222.10 ± 11.97	223.74 ± 15.32
Week 6	357.05 ± 20.91	353.47 ± 15.70	365.46 ± 21.59	365.09 ± 30.71	230.46 ± 8.56	233.78 ± 10.32	232.13 ± 13.56	225.60 ± 16.31
Week 7	374.46 ± 22.40	366.64 ± 16.20	381.10 ± 23.64	385.12 ± 35.70	236.43 ± 9.77	240.55 ± 10.42	239.18 ± 16.44	236.08 ± 18.27
Week 8	387.92 ± 25.08	379.38 ± 15.68	395.45 ± 25.34	402.57 ± 40.31	236.02 ± 9.18	241.74 ± 11.48	239.01 ± 17.61	235.15 ± 17.75
Week 9	403.61 ± 27.22	392.95 ± 16.58	408.24 ± 26.64	418.48 ± 45.40	242.67 ± 9.55	251.94 ± 11.11	243.74 ± 15.46	243.76 ± 18.97
Week 10	411.47 ± 29.67	399.79 ± 17.70	416.36 ± 27.35	427.53 ± 46.63	250.62 ± 12.32	256.30 ± 14.57	247.63 ± 17.44	249.11 ± 20.50
Week 11	422.66 ± 30.15	410.15 ± 23.41	428.70 ± 26.90	440.85 ± 48.81	252.85 ± 13.21	258.30 ± 11.29	253.17 ± 19.92	249.77 ± 19.60
Week 12	424.82 ± 31.50	415.02 ± 24.30	432.64 ± 31.21	446.74 ± 51.00	252.12 ± 15.47	257.76 ± 13.38	255.29 ± 18.50	247.26 ± 18.09
Week 13	431.16 ± 31.20	422.13 ± 24.53	438.52 ± 31.91	455.99 ± 52.04	251.11 ± 15.27	258.38 ± 11.81	258.86 ± 19.69	250.79 ± 18.77

Each value shows mean ± SD.

Table 3: This table represent the results of the body weight (g) from the treatment and recovery phase of recovery groups in 90-day toxicity study.

	Male (Number of animals = 10/group)		Female (Number of animals = 10/group)	
	TGT Primaage (mg/kg b. wt.)		TGT Primaage (mg/kg b. wt.)	
	Control (mg/kg b. wt.) 0	1000	Control (mg/kg b. wt.) 0	1000
PT	191.17 ± 10.10	186.08 ± 9.22	150.03 ± 5.63	148.83 ± 7.39
Week 1	237.15 ± 10.94	228.32 ± 12.43	167.78 ± 7.67	166.69 ± 9.15
Week 2	276.87 ± 11.67	261.86↓ ± 15.06	185.72 ± 11.47	182.16 ± 9.63
Week 3	306.99 ± 16.14	289.99↓ ± 19.26	202.97 ± 12.28	196.99 ± 10.86
Week 4	330.68 ± 18.30	314.36 ± 21.91	214.82 ± 11.96	208.11 ± 12.47
Week 5	351.18 ± 16.84	333.70 ± 24.30	227.07 ± 12.90	220.81 ± 13.41
Week 6	365.26 ± 17.86	348.72 ± 25.65	238.32 ± 15.93	228.30 ± 10.55
Week 7	380.51 ± 18.84	365.40 ± 27.08	243.84 ± 15.98	234.78 ± 12.55
Week 8	392.27 ± 19.62	380.20 ± 26.14	245.15 ± 16.47	233.23 ± 13.85
Week 9	407.52 ± 19.79	391.94 ± 27.42	252.36 ± 15.59	244.59 ± 13.84
Week 10	414.78 ± 19.62	402.04 ± 29.24	257.04 ± 18.66	245.50 ± 14.12
Week 11	424.22 ± 22.52	412.90 ± 30.38	258.49 ± 20.21	248.52 ± 14.39
Week 12	428.07 ± 22.97	416.88 ± 31.99	257.74 ± 19.68	249.97 ± 13.30
Week 13	432.88 ± 23.99	423.27 ± 34.14	258.33 ± 19.04	249.00 ± 14.46
Week 14	439.62 ± 25.62	429.65 ± 34.75	261.71 ± 20.79	255.50 ± 13.29
Week 15	445.79 ± 26.88	435.33 ± 35.76	267.28 ± 21.83	258.26 ± 14.58
Week 16	449.72 ± 26.62	441.11 ± 37.74	269.10 ± 22.79	258.77 ± 16.06
Week 17	458.53 ± 28.03	448.41 ± 40.84	271.41 ± 25.43	259.18 ± 15.85

Each value shows mean ± SD.
- significantly lower than control; p≥0.05 (ANOVA + Dunnett's test)

Table 4: This table represent the results of the body weight change (%) from the treatment and recovery phase of main groups in 90-day toxicity study.

	Male (Number of animals = 10/group)				Female (Number of animals = 10/group)			
	Control (mg/kg b. wt.) 0	TGT Primaage (mg/kg b. wt.) 250	500	1000	Control (mg/kg b. wt.) 0	TGT Primaage (mg/kg b. wt.) 250	500	1000
	Mean body weights (g) during treatment and recovery phases							
Week 1	23.35 ± 1.58	22.18 ± 1.72	23.45 ± 1.77	23.89 ± 1.84	11.67 ± 2.27	13.42 ± 3.95	12.65 ± 3.36	13.35 ± 3.87
Week 2	42.92 ± 3.78	41.98 ± 3.36	44.35 ± 3.44	44.12 ± 3.73	23.31 ± 3.93	25.93 ± 4.21	23.73 ± 6.34	22.93 ± 4.79
Week 3	58.92 ± 4.97	57.20 ± 4.26	60.52 ± 5.41	59.23 ± 6.30	33.28 ± 6.65	36.26 ± 5.00	33.47 ± 7.62	33.62 ± 4.26
Week 4	71.94 ± 5.87	68.89 ± 5.12	74.35 ± 7.12	72.27 ± 8.14	42.40 ± 6.86	45.53 ± 5.39	40.99 ± 9.00	40.59 ± 3.55
Week 5	82.42 ± 7.91	78.41 ± 5.61	84.40 ± 8.20	82.51 ± 10.83	49.33 ± 7.68	54.33 ± 5.94	48.21 ± 10.47	48.85 ± 5.11
Week 6	88.75 ± 8.94	87.06 ± 8.11	92.81 ± 7.52	91.60 ± 11.36	54.34 ± 7.59	59.36 ± 7.16	54.90 ± 11.61	50.07 ± 5.89
Week 7	97.91 ± 8.83	94.08 ± 9.62	101.06 ± 8.81	102.05 ± 13.49	58.39 ± 9.40	64.01 ± 8.27	59.58 ± 12.97	57.01 ± 6.59
Week 8	105.03 ± 10.52	100.85 ± 10.01	108.61 ± 9.11	111.17 ± 15.64	58.14 ± 9.59	64.80 ± 8.31	59.56 ± 14.69	56.41 ± 6.67
Week 9	113.34 ± 12.08	108.02 ± 10.28	115.36 ± 9.91	119.43 ± 17.61	62.57 ± 9.51	71.82 ± 9.73	62.66 ± 12.96	62.13 ± 7.29
Week 10	117.41 ± 11.98	111.64 ± 10.83	119.60 ± 9.26	124.19 ± 18.34	67.79 ± 8.78	74.85 ± 12.97	65.30 ± 14.63	65.68 ± 8.63
Week 11	123.30 ± 11.70	117.10 ± 13.04	126.14 ± 9.48	131.16 ± 19.27	69.26 ± 8.98	76.16 ± 10.08	68.97 ± 15.88	66.13 ± 7.65
Week 12	124.47 ± 13.15	119.67 ± 13.23	128.14 ± 11.03	134.17 ± 19.60	68.73 ± 9.88	75.74 ± 10.01	70.46 ± 15.88	64.48 ± 6.79
Week 13	127.85 ± 13.29	123.47 ± 14.07	131.25 ± 11.73	139.01 ± 19.88	68.02 ± 8.97	76.19 ± 9.53	72.88 ± 16.79	66.82 ± 7.10

Each value shows mean ± SD.

Table 5: This table represent the results of the body weight change (%) from the treatment and recovery phase of recovery groups in 90-day toxicity study.

	Male (Number of animals = 10/group)		Female (Number of animals = 10/group)	
	TGT Primaage (mg/kg b. wt.)		TGT Primaage (mg/kg b. wt.)	
	Control (mg/kg b. wt.) 0	1000	Control (mg/kg b. wt.) 0	1000
Week 1	24.11 ± 2.13	22.70 ± 2.63	11.81 ± 1.87	12.01 ± 2.80
Week 2	44.91 ± 2.49	40.74↓ ± 4.53	23.75 ± 4.91	22.41 ± 2.79
Week 3	60.62 ± 3.74	55.85 ± 7.12	35.29 ± 6.46	32.39 ± 4.28
Week 4	73.00 ± 4.65	68.96 ± 8.67	43.22 ± 6.73	39.88 ± 5.88
Week 5	83.82 ± 6.04	79.37 ± 10.42	51.41 ± 7.84	48.43 ± 6.77
Week 6	91.17 ± 5.56	87.46 ± 11.31	58.95 ± 10.73	53.55 ± 6.44
Week 7	99.16 ± 6.13	96.46 ± 12.57	62.67 ± 11.37	57.89 ± 7.50
Week 8	105.36 ± 7.90	104.43 ± 12.04	63.63 ± 12.91	56.82 ± 7.85
Week 9	113.38 ± 8.88	110.75 ± 12.85	68.44 ± 12.43	64.45 ± 7.57
Week 10	117.26 ± 10.60	116.21 ± 14.39	71.52 ± 13.69	65.08 ± 8.16
Week 11	122.13 ± 10.56	122.06 ± 15.18	72.49 ± 14.68	67.11 ± 8.17
Week 12	124.18 ± 11.54	124.22 ± 16.36	71.92 ± 13.32	68.10 ± 7.63
Week 13	126.67 ± 11.49	127.60 ± 16.73	72.32 ± 12.95	67.41 ± 7.85
Week 14	130.14 ± 11.28	131.07 ± 17.68	74.61 ± 14.61	71.87 ± 8.71
Week 15	133.38 ± 12.20	134.12 ± 18.26	78.32 ± 15.23	73.70 ± 9.03
Week 16	135.44 ± 11.97	137.22 ± 19.20	79.53 ± 15.79	74.01 ± 9.45
Week 17	140.05 ± 12.69	141.12 ± 20.66	81.05 ± 17.314	74.35 ± 10.48

Each value shows mean ± SD.
- significantly lower than control; p≥0.05 (ANOVA + Dunnett's test)

Table 6: This table represent the results of the grip strength and foot splay parameters from the treatment phase as well as in the recovery phase in 28-day toxicity study.

Parameters	Male (Number of animals = 10/group)				Female (Number of animals = 10/group)			
	Control (mg/kg b. wt.)	TGT Primaage (mg/kg b. wt.)			Control (mg/kg b. wt.)	TGT Primaage (mg/kg b. wt.)		
	0	250	500	1000	0	250	500	1000
Mean Forelimb Grip Strength (kg)								
Main	1.119 ± 0.044	1.130 ± 0.053	1.102 ± 0.074	1.093 ± 0.042	0..941 ± 0.097	0.946 ± 0.070	0.963 ± 0.085	0.962 ± 0.066
Recovery	1.185 ± 0.068			1.208 ± 0.091	1.008 ± 0.044			1.038 ± 0.055
Mean Hindlimb Grip Strength (kg)								
Main	0.423 ± 0.028	0.421 ± 0.030	0.416 ± 0.029	0.439 ± 0.025	0.339 ± 0.033	0.333 ± 0.021	0.343 ± 0.027	0.336 ± 0.013
Recovery	0.471 ± 0.048			0.460 ± 0.044	0.420 ± 0.047			0.456 ± 0.048
Mean Hindlimb Foot Splay (mm)								
Main	114.20 ± 9.90	107.70 ± 10.73	101.80 ± 15.05	95.80↓ ± 18.50	87.70 ± 18.98	87.50 ± 17.17	90.80 ± 13.27	86.90 ± 10.83
Recovery	89.50 ± 18.39			90.60 ± 10.56	86.20 ± 10.32			87.80 ± 12.89

Each value shows mean ± SD.
- significantly lower than control; p≥0.05 (ANOVA + Dunnett's test)

Table 7: This table represent the results of the grip strength and foot splay parameters from the treatment phase as well as in the recovery phase in 90-day toxicity study

Parameters	Male (Number of animals = 10/group)				Female (Number of animals = 10/group)			
	Control (mg/kg b. wt.)	TGT Primaage (mg/kg b. wt.)			Control (mg/kg b. wt.)	TGT Primaage (mg/kg b. wt.)		
	0	250	500	1000	0	250	500	1000
Mean Forelimb Grip Strength (kg)								
Main	1.199 ± 0.054	1.165 ± 0.048	1.205 ± 0.061	1.173 ± 0.082	1.152 ± 0.067	1.095 ± 0.051	1.077↓ ± 0.050	1.102 ± 0.038
Recovery	1.141 ± 0.066			1.142 ± 0.076	1.175 ±0.041			1.127↓ ± 0.042
Mean Hindlimb Grip Strength (kg)								
Main	0.439 ± 0.039	0.464 ± 0.026	0.435 ± 0.043	0.452 ± 0.040	0.389 ± 0.029	0.385 ± 0.018	0.387 ± 0.026	0.406 ± 0.030
Recovery	0.633 ± 0.062			0.582 ± 0.080	0.554 ± 0.050			0.521 ± 0.050
Mean Hindlimb Foot Splay (mm)								
Main	107.60 ± 14.28	92.90 ± 16.76	90.90 ± 18.14	90.80 ± 15.91	83.70 ± 15.25	87.70 ± 21.18	72.90 ± 10.04	77.50 ± 23.42
Recovery	91.20 ± 17.50			92.00 ± 17.82	70.90 ± 22.21			69.60 ± 14.35

Each value shows mean ± SD.
- significantly lower than control; p≥0.05 (ANOVA + Dunnett's test)

Table 8: This table represent the hematology data evaluated after the termination of the treatment phase in control group and treatment groups of 28-day toxicity study.

Parameters	Male (Number of animals = 10/group)				Female (Number of animals = 10/group)			
	Control (mg/kg b. wt.)	TGT Primaage (mg/kg b. wt.)			Control (mg/kg b. wt.)	TGT Primaage (mg/kg b. wt.)		
	0	250	500	1000	0	250	500	1000
WBC ($x10^3$/µL)	7.35 ± 1.64	7.24 ± 1.93	7.05 ± 1.04	6.31 ± 0.97	3.83 ± 0.72	4.30 ± 0.84	4.21 ± 0.65	4.23 ± 0.95
RBC ($x10^6$/µL)	9.01 ± 0.45	9.39 ± 0.43	9.36 ± 0.44	9.21 ± 0.44	8.61 ± 0.22	8.55 ± 0.53	8.80 ± 0.33	8.95 ± 0.30
Hb (g/dL)	15.89 ± 0.50	16.09 ± 0.50	16.39 ± 0.59	15.88 ± 0.79	15.61 ± 0.33	15.10 ± 0.84	15.42 ± 0.58	15.57 ± 0.24
HCT (%)	48.30 ± 2.26	49.39 ± 1.75	49.47 ± 1.70	48.61 ± 2.37	47.27 ± 1.36	46.55 ± 2.74	47.30 ± 1.74	47.90 ± 1.10
MCV (fL)	53.64 ± 1.49	52.69 ± 2.35	52.93 ± 1.63	52.83 ± 1.20	54.92 ± 1.34	54.46 ± 0.97	53.79 ± 1.56	53.58 ± 1.42
MCH (pg)	17.65 ± 0.67	17.18 ± 0.84	17.52 ± 0.64	17.24 ± 0.50	18.12 ± 0.55	17.69 ± 0.44	17.54 ± 0.69	17.42↓ ± 0.56
MCHC (g/dL)	32.95 ± 0.91	32.62 ± 0.31	33.11 ± 0.54	32.61 ± 0.54	33.02 ± 0.74	32.46 ± 0.42	32.61 ± 0.72	32.51 ± 0.55
Platelet ($x10^3$/µL)	879.90 ± 81.70	925.70 ± 58.62	915.30 ± 76.18	867.50 ± 115.52	870.60 ± 55.19	915.20 ± 121.56	952.90 ± 126.04	868.80 ± 151.00
Neutrophil ($x10^3$/µL)	1.37 ± 0.47	1.40 ± 0.49	1.52 ± 0.48	1.24 ± 0.28	0.75 ± 0.20	0.78 ± 0.32	0.67 ± 0.16	0.69 ± 0.22
Lymphocyte ($x10^3$/µL)	5.42 ± 1.26	5.21 ± 1.49	4.97 ± 0.64	4.60 ± 0.68	2.82 ± 0.70	3.23 ± 0.69	3.22 ± 0.63	3.20 ± 0.81
Monocyte ($x10^3$/µL)	0.24 ± 0.09	0.27 ± 0.08	0.23 ± 0.05	0.17 ± 0.04	0.11 ± 0.04	0.10 ± 0.04	0.12 ± 0.05	0.11 ± 0.03
Eosinophil ($x10^3$/µL)	0.10 ± 0.05	0.11 ± 0.05	0.10 ± 0.03	0.10 ± 0.07	0.06 ± 0.03	0.07 ± 0.03	0.06 ± 0.03	0.11 ± 0.11
Basophil ($x10^3$/µL)	0.16 ± 0.05	0.18 ± 0.10	0.16 ± 0.04	0.15 ± 0.07	0.08 ± 0.03	0.10 ± 0.03	0.10 ± 0.04	0.09 ± 0.02
Reticulocyte Count ($x10^9$/L)	209.71 ± 20.25	214.35 ± 20.77	216.14 ± 26.43	220.80 ± 26.34	247.03 ± 46.81	261.73 ± 32.85	257.69 ± 36.17	247.68 ± 47.95
PT (Seconds)	10.65 ± 0.34	11.01 ± 0.95	10.49 ± 0.54	10.41 ± 0.55	9.78 ± 1.01	9.80 ± 0.55	9.49 ± 0.65	9.98 ± 0.94
APTT (Seconds)	24.21 ± 1.62	25.45 ± 1.18	24.47 ± 2.44	25.13 ± 3.18	25.88 ± 3.27	24.66 ± 4.59	27.40 ± 3.34	23.79 ± 3.73

Each value shows mean ± SD.
- significantly lower than control; p≥0.05 (ANOVA + Dunnett's test)

Table 9 : This table represent the Hematology data evaluated after the termination of the control group and treatment group in the recovery phase of 28-day toxicity study.

Parameters	Male (Number of animals = 10/group)		Female (Number of animals = 10/group)	
	Control (mg/kg b. wt.)	TGT Primaage (mg/kg b. wt.)	Control (mg/kg b. wt.)	TGT Primaage (mg/kg b. wt.)
	0	1000	0	1000
WBC ($x10^3$/µL)	6.77 ± 0.89	7.10 ± 2.33	3.11 ± 0.55	3.25 ± 1.15
RBC ($x10^6$/µL)	9.59 ± 0.46	9.79 ± 0.30	8.68 ± 0.56	8.71 ± 0.82
Hb (g/dL)	16.22 ± 0.50	16.43 ± 0.51	14.77 ± 0.79	15.47 ± 1.20
HCT (%)	50.20 ± 1.48	50.39 ± 1.30	46.61 ± 2.63	48.17 ± 3.52
MCV (fL)	52.42 ± 1.88	51.52 ± 1.37	53.76 ± 0.95	55.41↑ ± 1.82
MCH (pg)	16.94 ± 0.69	16.79 ± 0.63	17.03 ± 0.43	17.79↑↑ ± 0.64
MCHC (g/dL)	32.30 ± 0.31	32.61 ± 0.43	31.71 ± 0.48	32.12 ± 0.79
Platelet ($x10^3$/µL)	900.00 ± 109.29	913.90 ± 92.67	871.80 ± 150.78	875.50 ± 102.74
Neutrophil ($x10^3$/µL)	1.37 ± 0.31	1.43 ± 0.44	0.59 ± 0.08	0.59 ± 0.14
Lymphocyte ($x10^3$/µL)	4.83 ± 0.64	5.06 ± 1.76	2.31 ± 0.55	2.41 ± 1.09
Monocyte ($x10^3$/µL)	0.24 ± 0.06	0.26 ± 0.09	0.08 ± 0.02	0.08 ± 0.03
Eosinophil ($x10^3$/µL)	0.12 ± 0.08	0.12 ± 0.07	0.06 ± 0.03	0.05 ± 0.02
Basophil ($x10^3$/µL)	0.17 ± 0.02	0.17 ± 0.06	0.09 ± 0.04	0.10 ± 0.05
Reticulocyte Count ($x10^9$/L)	248.25 ± 33.55	242.17 ± 31.91	209.09 ± 34.87	214.09 ± 45.14
PT (Seconds)	10.62 ± 0.66	10.60 ± 088	10.19 ± 0.35	10.24 ± 0.41
APTT (Seconds)	22.05 ± 7.32	20.40 ± 5.47	21.17 ± 4.52	17.36↓ ± 2.61

Each value shows mean ± SD.

↓ - significantly lower than control; p≥0.05 (ANOVA + Dunnett's test)

↑ - significantly higher than control; p≥0.05 (ANOVA + Dunnett's test)

↑ - significantly higher than control; p≥0.01 (ANOVA + Dunnett's test)

Table 10: This table represent the Hematology data evaluated after the termination of the control group and treatment group in the treatment phase in 90-day study.

	Male (Number of animals = 10/group)				Female (Number of animals = 10/group)			
	Control (mg/kg b. wt.) 0	TGT Primaage (mg/kg b. wt.) 250	500	1000	Control (mg/kg b. wt.) 0	TGT Primaage (mg/kg b. wt.) 250	500	1000
WBC ($x10^3$/µL)	5.46 ± 1.29	6.07 ± 1.50	5.45 ± 0.85	6.57 ± 1.04	2.90 ± 0.70	3.71 ± 1.10	3.25 ± 0.90	3.19 ± 0.71
RBC ($x10^6$/µL)	8.81 ± 0.22	8.91 ± 0.52	8.82 ± 0.43	8.86 ± 0.44	8.14 ± 0.28	8.11 ± 0.22	7.94 ± 0.58	8.05 ± 0.22
Hb (g/dL)	15.03 ± 0.29	14.91 ± 0.90	14.84 ± 0.74	14.99 ± 0.60	14.32 ± 0.37	14.35 ± 0.52	14.19 ± 1.03	14.46 ± 0.44
HCT (%)	44.28 ± 1.10	44.47 ± 2.53	44.19 ± 1.91	44.85 ± 1.58	42.72 ± 1.17	43.01 ± 1.34	42.37 ± 2.95	43.03 ± 0.41
MCV (fL)	50.24 ± 0.78	49.94 ± 0.83	50.16 ± 1.50	50.66 ± 1.93	52.53 ± 1.39	53.04 ± 0.97	53.38 ± 0.86	53.51 ± 1.13
MCH (pg)	17.06 ± 0.38	16.76 ± 0.50	16.85 ± 0.54	16.95 ± 0.94	17.62 ± 0.52	17.70 ± 0.52	17.89 ± 0.35	17.99 ± 0.47
MCHC (g/dL)	33.98 ± 0.78	33.56 ± 0.58	33.58 ± 0.44	33.45 ± 0.63	33.53 ± 0.43	33.37 ± 0.65	33.51 ± 0.58	33.61 ± .76
Platelets ($x10^3$/µL)	853.30 ± 56.88	888.50 ± 99.99	831.60 ± 92.59	917.20 ± 95.82	886.50 ± 90.35	879.20 ± 106.55	856.90 ± 134.48	992.70 ± 12.75
Neutrophil ($x10^3$/µL)	1.28 ± 0.30	1.39 ± 0.66	1.28 ± 0.46	1.50 ± 0.33	0.58 ± 0.26	0.78 ± 0.58	0.61 ± 0.18	0.63 ± 0.17
Lymphocyte ($x10^3$/µL)	3.86 ± 0.98	4.30 ± 0.77	3.87 ± 0.43	4.72↑ ± 0.70	2.15 ± 0.55	2.72 ± 0.76	2.45 ± 0.96	2.38 ± 0.67
Monocyte ($x10^3$/µL)	0.14 ± .08	0.15 ± 0.06	0.12 ± 0.04	0.17 ± 0.06	0.06 ± 0.03	0.09 ± 0.07	0.08 ± 0.04	0.08 ± 0.04
Eosinophil ($x10^3$/µL)	0.10 ± 0.03	0.14 ± 0.07	0.11 ± 0.03	0.10 ± 0.04	0.08 ± 0.05	0.06 ± 0.02	0.07 ± 0.05	0.06 ± 0.02
Basophil ($x10^3$/µL)	0.05 ± 0.01	0.06 ± 0.02	0.05 ± 0.01	0.06 ± 0.02	0.03 ± 0.01	0.04↑ ± 0.02	0.03 ± 0.03	0.03 ± 0.01
Reticulocytes Count ($x10^9$/L)	171.39 ± 24.98	169.64 ± 16.11	172.41 ± 28.42	173.96 ± 30.23	204.38 ± 44.68	190.32 ± 25.18	193.40 ± 52.53	190.21 ± 38.38
PT (Seconds)	10.99 ± 0.63	11.90↑ ± 0.64	10.31 ± 0.92	11.32 ± 0.75	11.52 ± 0.75	10.77↓↓ ± 0.32	11.03 ± 0.50	10.65↓↓ ± 0.28
APTT (Seconds)	17.45 ± 1.44	19.12↑↑ ± 0.85	17.59 ± 1.70	18.85 ± 2.70	17.24 ± 2.94	18.20 ± 1.28	16.75 ± 2.48	17.23 ± 1.26

Each value shows mean ± SD.

↓ - significantly lower than control; p≥0.05 (ANOVA + Dunnett's test)

↑ - significantly higher than control; p≥0.05 (ANOVA + Dunnett's test)
↑ - significantly higher than control; p≥0.01 (ANOVA + Dunnett's test)

Table 11: This table represent the Hematology data evaluated after the termination of the control group and treatment group in the recovery phase in 90-day study

	Male (Number of animals = 10/group)		Female (Number of animals = 10/group)	
	Control (mg/kg b. wt.) 0	TGT Primaage (mg/kg b. wt.) 1000	Control (mg/kg b. wt.) 0	TGT Primaage (mg/kg b. wt.) 1000
WBC ($x10^3/\mu L$)	5.83 ± 0.98	5.69 ± 1.19	3.09 ± 0.73	4.02↑ ± 0.72
RBC ($x10^6/\mu L$)	9.08 ± 0.18	8.73↓ ± 0.52	8.02 ± 0.20	7.82 ± 0.96
Hb (g/dL)	15.50 ± 0.26	15.20 ± 0.92	14.64 ± 0.49	13.95 ± 1.56
HCT (%)	45.77 ± 0.77	44.26 ± 2.46	43.28 ± 1.20	41.65 ± 4.48
MCV (fL)	50.43 ± 0.81	50.71 ± 0.85	53.97 ± 1.12	53.36 ± 2.06
MCH (pg)	17.08 ± 0.38	17.44 ± 0.53	18.25 ± 0.49	17.87 ± 0.61
MCHC (g/dL)	33.88 ± 0.41	34.39 ± 0.93	33.82 ± 0.51	33.49 ± 0.34
Platelets ($x10^3/\mu L$)	797.60 ± 95.19	758.30 ± 120.22	826.60 ± 74.83	835.10 ± 108.64
Neutrophil ($x10^3/\mu L$)	1.50 ± 0.58	1.10 ± 0.37	0.55 ± 0.19	0.73 ± 0.25
Lymphocyte ($x10^3/\mu L$)	3.99 ± 0.72	4.33 ± 0.99	2.40 ± 0.65	3.14↑ ± 0.72
Monocyte ($x10^3/\mu L$)	0.15 ± 0.06	0.11 ± 0.04	0.05 ± 0.02	0.07↑ ± 0.04
Eosinophil ($x10^3/\mu L$)	0.12 ± 0.04	0.10 ± 0.04	0.06 ± 0.02	0.06 ± 0.01
Basophil ($x10^3/\mu L$)	0.06 ± 0.04	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
Reticulocytes Count ($x10^9/L$)	180.82 ± 25.74	164.12 ± 23.22	200.46 ± 39.18	168.65 ± 32.10
PT (Seconds)	11.58 ± 0.45	11.29 ± 1.12	11.24 ± 0.74	10.00↓↓ ± 0.91
APTT (Seconds)	5.83 ± 1.91	17.69 ± 2.24	16.80 ± 3.02	16.04 ± 1.58

Each value shows mean ± SD.
↓ - significantly lower than control; p≥0.05 (ANOVA + Dunnett's test)
↑ - significantly higher than control; p≥0.05 (ANOVA + Dunnett's test)
↑↑ - significantly higher than control; p≥0.01 (ANOVA + Dunnett's test)

Table 12: This table represent the clinical chemistry data evaluated after the termination of the control group and treatment group in the recovery phase in 28-day study.

Parameters	Male (Number of animals = 10/group)		Female (Number of animals = 10/group)	
	Control (mg/kg b. wt.)	TGT Primaage (mg/kg b. wt.)	Control (mg/kg b. wt.)	TGT Primaage (mg/kg b. wt.)
	0	1000	0	1000
Glucose (mg/dL)	137.01 ± 18.82	140.76 ± 12.50	132.93 ± 20.66	121.28 ± 17.40
Total Cholesterol (mg/dL)	83.38 ± 16.25	76.04 ± 13.62	77.60 ± 13.34	83.37 ± 13.18
Triglycerides (mg/dL)	75.77 ± 26.02	93.28 ± 46.82	34.97 ± 14.70	35.96 ± 15.14
Creatinine (mg/dL)	0.55 ± 0.02	0.54 ± 0.02	0.59 ± 0.04	0.59 ± 0.03
Lactate Dehydrogenase (U/L)	989.00 ± 237.93	851.20 ± 227.18	845.50 ± 252.52	822.60 ± 261.47
Creatinine Kinase (U/L)	349.00 ± 88.74	303.40 ± 84.89	337.07 ± 154.30	453.23 ± 507.85
GGT (U/L)	0.43 ± 0.25	0.44 ± 0.14	0.46 ± 0.25	0.83 ± 0.94
ALP (U/L)	108.11 ± 30.24	108.40 ± 27.61	46.17 ± 9.58	50.97 ± 15.80
ALT (U/L)	48.91 ± 8.84	51.91 ± 6.14	41.57 ± 6.45	43.81 ± 15.52
AST (U/L)	125.50 ± 23.07	113.68 ± 21.10	137.15 ± 47.48	138.08 ± 34.64
Calcium (mg/dL)	10.73 ± 0.35	10.63 ± 0.25	10.67 ± 0.29	10.62 ± 0.24
Inorganic Phosphorus (mg/dL)	6.71 ± 0.34	6.79 ± 0.70	4.97 ± 0.64	5.55↑ ± 0.52

Total Protein (g/dL)	6.64 ± 0.30	6.66 ± 0.15	7.05 ± 0.42	6.82 ± 0.42
Albumin (g/dL)	3.81 ± 0.12	3.84 ± 0.08	4.20 ± 0.23	4.04 ± 0.26
GLB (g/dL)	2.83 ± 0.21	2.83 ± 0.11	2.85 ± 0.21	2.78 ± 0.18
ALB:GLB	1.35 ± 0.07	1.36 ± 0.06	1.48 ± 0.06	1.45 ± 0.05
Urea (mg/dL)	40.72 ± 0.05	40.31 ± 4.84	46.83 ± 7.09	44.16 ± 4.81
BUN (mg/dL)	19.02 ± 2.36	18.82 ± 2.26	21.87 ± 3.31	20.62 ± 2.25
Total Bilirubin (µmol/L)	6.60 ± 2.80	4.97 ± 2.68	7.40 ± 1.84	6.84 ± 2.08
Sodium (mmol/L)	143.04 ± 0.77	142.44 ± 1.11	141.11 ± 0.73	141.13 ± 0.64
Potassium (mmol/L)	4.50 ± 0.28	4.42 ± 0.23	3.95 ± 0.27	3.90 ± 0.30
Chloride (mmol/L)	107.35 ± 1.14	106.75 ± 1.92	106.42 ± 1.13	106.48 ± 1.27

Each value shows mean ± SD.
- significantly higher than control; p≥0.05 (ANOVA + Dunnett's test)

Table 13: This table represent the clinical chemistry data evaluated after the termination of the control group and treatment group in the treatment phase in 90-day study.

	Male (Number of animals = 10/group)				Female (Number of animals = 10/group)			
	Control (mg/kg b. wt.)	TGT Primaage (mg/kg b. wt.)			Control (mg/kg b. wt.)	TGT Primaage (mg/kg b. wt.)		
	0	250	500	1000	0	250	500	1000
Glucose (mg/dL)	155.31 ± 16.93	156.09 ± 13.89	151.21 ± 13.71	155.29 ± 16.77	147.00 ± 12.20	149.32 ± 17.15	147.51 ± 15.63	144.28 ± 14.78
Total Cholesterol (mg/dL)	90.07 ± 23.31	93.80 ± 18.84	90.25 ± 14.60	85.39 ± 19.37	97.88 ± 28.70	110.49 ± 19.34	87.83 ± 17.02	103.00 ± 17.97
Triglycerides (mg/dL)	61.59 ± 14.37	66.23 ± 20.95	59.77 ± 22.28	67.29 ± 21.72	42.01 ± 11.24	41.19 ± 9.30	43.09 ± 10.11	43.75 ± 14.18
Creatinine (mg/dL)	0.58 ± 0.03	0.57 ± 0.03	0.58 ± 0.03	0.59 ± 0.04	0.72 ± 0.03	0.73 ± 0.04	0.71 ± 0.05	0.72 ± 0.03
LDH (U/L)	1111.40 ± 499.69	974.10 ± 353.94	1245.10 ± 482.87	832.60 ± 337.80	645.60 ± 274.48	629.40 ± 317.86	938.10 ± 238.55	717.30 ± 204.63
CK (U/L)	446.28 ± 177.84	377.04 ± 102.06	459.47 ± 173.51	348.24 ± 120.86	417.05 ± 523.61	272.00 ± 131.82	666.42 ± 925.81	366.49 ± 175.31
GGT (U/L)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
ALP (U/L)	91.10 ± 19.84	89.37 ± 12.06	92.27 ± 26.60	98.74 ± 17.77	32.27 ± 7.57	34.68 ± 10.98	38.95 ± 14.32	33.88 ± 11.41
ALT (U/L)	45.90 ± 9.76	45.25 ± 9.54	49.25 ± 10.83	48.75 ± 4.31	46.83 ± 18.80	48.70 ± 26.28	46.98 ± 9.35	44.80 ± 16.20
AST (U/L)	121.60 ± 19.50	121.12 ± 25.40	131.38 ± 42.92	121.52 ± 25.62	125.44 ± 43.07	150.40 ± 92.22	134.38 ± 46.67	117.36 ± 36.93
Calcium (mg/dL)	10.29 ± 0.24	10.34 ± 0.13	10.25 ± 0.18	10.48 ± 0.28	10.83 ± 0.42	11.07 ± 0.41	10.66 ± 0.35	11.19 ± 0.26
Inorganic Phosphorus (mg/dL)	5.37 ± 0.72	5.49 ± 0.67	5.28 ± 0.42	5.41 ± 0.60	4.44 ± 0.90	4.89 ± 0.85	5.05 ± 0.84	5.30 ± 1.02
Total Protein (g/dL)	6.53 ± 0.20	6.64 ± 0.18	6.62 ± 0.17	6.78 ± 0.29	7.45 ± 0.58	7.67 ± 0.45	7.21 ± 0.37	7.61 ± 0.43
ALB (g/dL)	3.77 ± 0.09	3.78 ± 0.08	3.79 ± 0.07	3.90↑ ± 0.11	4.40 ± 0.35	4.48 ± 0.25	4.23 ± 0.23	4.50 ± 0.24
GLB (g/dL)	2.76 ± 0.12	2.86 ± 0.11	2.83 ± 0.12	2.88 ± 0.21	3.05 ± 0.24	3.19 ± 0.21	2.97 ± 0.18	3.11 ± 0.23
ALB:GLB	1.37 ± 0.04	1.32 ± 0.03	1.34 ± 0.05	1.36 ± 0.08	1.44 ± 0.05	1.41 ± 0.04	1.43 ± 0.08	1.45 ± 0.07
Urea (mg/dL)	42.77 ± 5.85	40.03 ± 4.67	44.37 ± 4.90	44.58 ± 6.82	44.26 ± 9.10	44.96 ± 6.39	45.88 ± 5.05	45.22 ± 4.56
BUN (mg/dL)	19.98 ± 2.73	18.69 ± 2.18	20.72 ± 2.29	20.82 ± 3.19	20.67 ± 4.25	21.00 ± 2.98	21.42 ± 2.36	21.12 ± 2.13
Total Bilirubin (µmol/L)	4.54 ± 2.01	4.37 ± 1.83	4.52 ± 2.40	5.63 ± 1.47	5.73 ± 2.01	5.52 ± 1.75	5.80 ± 1.47	5.79 ± 1.20
Bile acids (µmol/L)	5.98 ± 2.46	7.32 ± 4.14	7.29 ± 4.71	6.95 ± 2.05	11.80 ± 6.11	10.86 ± 10.29	8.96 ± 4.13	11.33 ± 6.01
Sodium (mmol/L)	143.28 ± 0.82	143.70 ± 0.88	143.26 ± 0.86	143.88 ± 1.22	142.01 ± 1.35	143.30 ± 1.44	142.21 ± 1.53	143.32 ± 1.77
Potassium (mmol/L)	4.39 ± 0.26	4.46 ± 0.17	4.33 ± 0.22	4.31 ± 0.19	4.02 ± 0.38	3.86 ± 0.43	4.06 ± 0.22	4.06 ± 0.38
Chloride (mmol/L)	106.83 ± 0.82	107.09 ± 1.25	106.41 ±	107.23 ± 1.16	105.94 ± 1.48	106.28 ± 2.12	106.47 ± 1.54	106.24 ± 1.97

Each value shows mean ± SD.
- significantly higher than control; p≥0.05 (ANOVA + Dunnett's test)

Table 14: This table represent the clinical chemistry data evaluated after the termination of the control group and treatment group in the recovery phase in 90-day study

	Male (Number of animals = 10/group)		Female (Number of animals = 10/group)	
	Control (mg/kg b. wt.)	TGT Primaage (mg/kg b. wt.)	Control (mg/kg b. wt.)	TGT Primaage (mg/kg b. wt.)
	0	1000	0	1000
Glucose (mg/dL)	149.27 ± 9.00	150.76 ± 23.44	136.32 ± 17.25	136.61 ± 14.10
Total Cholesterol (mg/dL)	93.37 ± 11.61	88.80 ± 14.83	88.65 ± 19.29	99.20 ± 28.31
Triglycerides (mg/dL)	86.21 ± 25.62	84.37 ± 21.88	52.91 ± 18.48	47.13 ± 15.83
Creatinine (mg/dL)	0.64 ± 0.04	0.64 ± 0.03	0.69 ± 0.07	0.67 ± 0.05
LDH (U/L)	1352.00 ± 498.46	1272.90 ± 644.40	963.10 ± 216.97	931.80 ± 205.32
CK (U/L)	527.20 ± 183.52	510.80 ± 230.69	393.99 ± 104.53	436.39 ± 318.96
GGT (U/L)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
ALP (U/L)	89.31 ± 12.25	86.54 ± 19.09	32.59 ± 8.14	33.38 ± 7.95
ALT (U/L)	53.25 ± 6.92	62.08↑ ± 11.21	45.19 ± 18.71	49.97 ± 14.93
AST (U/L)	150.55 ± 45.33	132.07 ± 22.46	132.18 ± 37.61	124.66 ± 28.65
Calcium (mg/dL)	10.22 ± 0.21	10.16 ± 0.23	10.63 ± 0.32	10.58 ± 0.47
Inorganic Phosphorus (mg/dL)	5.49 ± 0.57	5.70 ± 0.78	4.32 ± 0.48	4.70 ± 0.79
Total Protein (g/dL)	6.64 ± 0.15	6.61 ± 0.18	7.35 ± 0.34	7.22 ± 0.66
ALB (g/dL)	3.78 ± 0.09	3.79 ± 0.07	4.35 ± 0.21	4.26 ± 0.38
GLB (g/dL)	2.86 ± 0.09	2.83 ± 0.11	3.00 ± 0.17	2.96 ± 0.29
ALB:GLB	1.32 ± 0.04	1.34 ± 0.04	1.46 ± 0.08	1.44 ± 0.04
Urea (mg/dL)	42.14 ± 3.99	41.27 ± 5.29	48.14 ± 4.54	47.53 ± 6.32
BUN (mg/dL)	19.68 ± 1.86	19.27 ± 2.47	22.48 ± 2.12	22.20 ± 2.95
Total Bilirubin (µmol/L)	5.36 ± 1.79	5.43 ± 1.83	5.09 ± 1.94	3.60 ± 1.88
Bile acids (µmol/L)	11.30 ± 9.18	10.76 ± 4.83	7.05 ± 2.03	16.94 ± 19.05
Sodium (mmol/L)	142.44 ± 0.78	142.65 ± 0.67	142.15 ± 0.83	142.13 ± 1.37
Potassium (mmol/L)	4.52 ± 0.21	4.47 ± 0.47	4.22 ± 0.39	4.15 ± 0.47
Chloride (mmol/L)	105.43 ± 1.14	105.68 ± 1.63	105.42 ± 1.20	105.30 ± 2.81

Each value shows mean ± SD.

↑ - significantly higher than control; p≥0.05 (ANOVA + Dunnett's test)

Table 15: This table represent the organ weight data evaluated after the termination of the control group and treatment group in 28-day study

Organs	Male (Number of animals = 10/group)				Female (Number of animals = 10/group)			
	Control (mg/kg b. wt.)	TGT Primaage (mg/kg b. wt.)			Control (mg/kg b. wt.)	TGT Primaage (mg/kg b. wt.)		
	0	250	500	1000	0	250	500	1000
Liver	10.201 ± 1.060	10.084 ± 0.947	10.540 ± 0.601	9.930 ± 0.713	6.742 ± 0.497	6.566 ± 0.532	6.282 ± 0.729	6.695 ± 0.649
Heart	1.032 ± 0.087	1.000 ± 0.083	1.028 ± 0.069	0.997 ± 0.055	0.746 ± 0.067	0.763 ± 0.058	0.723 ± 0.057	0.760 ± 0.060
Spleen	0.602 ± 0.088	0.586 ± 0.092	0.587 ± 0.064	0.547 ± 0.030	0.427 ± 0.055	0.444 ± 0.049	0.415 ± 0.075	0.403 ± 0.044
Brain	2.055 ± 0.133	2.023 ± 0.092	1.986 ± 0.057	1.969 ± 0.056	1.868 ± 0.042	1.888 ± 0.049	1.854 ± 0.071	1.863 ± 0.074
Thymus	0.503 ± 0.139	0.439 ± 0.081	0.465 ± 0.071	0.459 ± 0.063	0.366 ± 0.053	0.390 ± 0.054	0.379 ± 0.064	0.377 ± 0.046
Kidneys	2.195 ± 0.201	2.096 ± 0.153	2.169 ± 0.180	2.085 ± 0.155	1.412 ± 0.105	1.398 ± 0.117	1.311 ± 0.101	1.414 ± 0.113
Adrenals	0.088 ±0.013	0.078 ± 0.013	0.085 ± 0.008	0.081 ± 0.011	0.090 ± 0.010	0.098 ± 0.011	0.086 ± 0.014	0.091 ± 0.006
Testes	3.491 ± 0.369	3.656 ± 0.281	3.515 ± 0.208	3.411 ± 0.355	-	-	-	-
Seminal vesicle with coagulating glands	1.070 ± 0.192	1.054 ± 0.195	0.986 ± 0.173	0.960 ± 0.159	-	-	-	-
Epididymides	1.149 ± 0.144	1.141 ± 0.080	1.128 ± 0.108	1.103 ± 0.099	-	-	-	-
Ovaries	-	-	-	-	0.097 ± 0.008	0.106 ± 0.016	0.096 ± 0.014	0.104 ± 0.015
Uterus with cervix	-	-	-	-	0.499 ± 0.139	0.562 ± 0.160	0.602 ± 0.211	0.462 ± 0.109

Table 16: This table represent the organ weight data evaluated after the termination of the control group and treatment group in treatment phase of 90-day study

	Male (Number of animals = 10/group)				Female (Number of animals = 10/group)			
	Control (mg/kg b. wt.)	TGT Primaage (mg/kg b. wt.)			Control (mg/kg b. wt.)	TGT Primaage (mg/kg b. wt.)		
	0	250	500	1000	0	250	500	1000
B. wt. (TS)	414.44 ± 31.17	407.63 ± 24.74	423.18 ± 30.54	436.64 ± 50.42	241.88 ± 14.21	247.91 ± 9.35	246.78 ± 17.93	240.54 ± 18.59
Liver	10.40 ± 1.06	10.43 ± 0.73	10.51 ± 0.90	11.37 ± 1.81	6.70 ± 0.56	7.09 ± 0.53	7.34 ± 1.11	7.26 ± 0.72
Heart	1.08 ± 0.05	1.09 ± 0.10	1.14↑ ± 0.07	1.17↑ ± 0.13	0.75 ± 0.06	0.82 ± 0.06	0.81 ± 0.09	0.77 ± 0.08
Spleen	0.65 ± 0.08	0.61 ± 0.07	0.66 ± 0.10	0.64 ± 0.10	0.43 ± 0.06	0.40 ± 0.05	0.41 ± 0.07	0.41 ± 0.07
Brain	2.14 ± 0.09	2.11 ± 0.08	2.14 ± 0.12	2.10 ± 0.06	1.93 ± 0.07	1.92 ± 0.08	1.90 ± 0.08	1.92 ± 0.08
Thymus	0.40 ± 0.07	0.40 ± 0.06	0.38 ± 0.06	0.40 ± 0.04	0.28 ± 0.06	0.29 ± 0.05	0.31 ± 0.05	0.31 ± 0.04
Kidneys	2.26 ± 0.17	2.25 ± 0.14	2.38 ± 0.20	2.45 ± 0.30	1.47 ± 0.09	1.52 ± 0.08	1.55 ± 0.14	1.54 ± 0.17
Adrenals	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.02	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.01
Testes	3.95 ± 0.38	4.03 ± 0.25	4.01 ± 0.14	3.93 ± 0.32	-	-	-	-
Epididymides	1.47 ± 0.10	1.52 ± 0.10	1.53 ± 0.10	1.55 ± 0.14	-	-	-	-
Prostate + Seminal vesicles with coagulating glands	2.30 ± 0.20	2.27 ± 0.26	2.43 ± 0.22	2.35 ± 0.35	-	-	-	-
Uterus with cervix	-	-	-	-	0.66 ± 0.12	0.68 ± 0.16	0.64 ± 0.08	0.71 ± 0.19
Ovaries with Oviduct	-	-	-	-	0.12 ± 0.03	0.12 ± 0.01	0.13 ± 0.02	0.12 ± 0.03
Thyroid with parathyroid	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00

Each value shows mean ± SD.
- significantly higher than control; p≥0.05 (ANOVA + Dunnett's test)

Table 17: This table represent the organ weight data evaluated after the termination of the recovery groups of 90-day study

	Male (Number of animals = 10/group)		Female (Number of animals = 10/group)	
	TGT Primaage (mg/kg b. wt.)		TGT Primaage (mg/kg b. wt.)	
	Control (mg/kg b. wt.) 0	1000	Control (mg/kg b. wt.) 0	1000
B. wt. (TS)	441.49 ± 25.92	433.32 ± 38.78	260.61 ± 24.5	249.91 ± 15.55
Liver	11.09 ± 0.92	10.76 ± 1.35	7.25 ± 0.63	6.91 ± 0.65
Heart	1.16 ± 0.06	1.15 ± 0.11	0.91 ± 0.08	0.84↓ ± 0.04
Spleen	0.62 ± 0.08	0.60 ± 0.10	0.48 ± 0.08	0.46 ± 0.09
Brain	2.13 ± 0.08	2.13 ± 0.08	1.96 ± 0.07	1.97 ± 0.10
Thymus	0.26 ± 0.04	0.28 ± 0.03	0.28 ± 0.05	0.27 ± 0.05
Kidneys	2.49 ± 0.25	2.35 ± 0.17	1.67 ± 0.20	1.55 ± 0.13
Adrenals	0.07 ± 0.01	0.06 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
Testes	3.99 ± 0.33	3.77 ± 0.17	-	-
Epididymides	1.49 ± 0.14	1.41 ± 0.10	-	-
Prostate + Seminal vesicles with coagulating glands	2.50 ± 0.36	2.41 ± 0.30	-	-
Uterus with cervix	-	-	0.78 ± 0.19	0.66 ± 0.12
Ovaries with Oviduct	-	-	0.14 ± 0.02	0.13 ± 0.02
Thyroid with parathyroid	0.03 ± 0.00	0.03↑ ± 0.00	0.02 ± 0.00	0.02 ± 0.00

Each value shows mean ± SD.
- significantly higher than control; p≥0.05 (ANOVA + Dunnett's test)