Short and long-term effects of Trigonelline on behavioural functions, biochemical and haematological parameters in Sprague Dawley rats

Vimalah Devi Gengathurai[1*], Usha Kumari[1], Victor David[1], Subramani Parasuraman[2], Baala Sharmma[3], Sherly Deborah George[4]

[1]Unit of Physiology, Faculty of Medicine, AIMST University, Kedah, Malaysia.
[2]Department of Pharmacology, Faculty of Pharmacy, AIMST University, Kedah, Malaysia.
[3]School of Physiotherapy, Faculty of Allied Health Profession, AIMST University, Kedah, Malaysia.
[4]Department of Physiology, Faculty of Medicine, Manipal University College Malaysia, Melaka, Malaysia.

***Correspondence: Dr. Vimalah Devi Gengathurai**
Unit of Physiology, Faculty of Medicine, AIMST University, Kedah, Malaysia.

Abstract

Objective: The present study was planned to study the short and long-term effect of trigonelline (TGN) on behavioural functions, biochemical and haematological parameters in Sprague Dawley (SD) rats.
Methodology: The male rats were divided into five groups with each of six animals (short and long-term). In short-term study, the rats were treated for 7 days and in long-term study the rats were treated for 45 days. The rats were treated with vitamin C (200 mg/kg) or TGN (12.5/ 25 / 50 mg/kg). The changes in behaviour functions, biochemical and haematological parameters of the experimental animals were monitored at the regular intervals.
Results: In short-term study, the animals treated with vitamin C and TGN did not show any significant changes in locomotor activity, grip strength and Escape Latency Time (ELT) when compared with that of control whereas the animals treated with TGN for 45 days (chronic study), showed a significant increase in locomotor activity and reduction in ELT when compared with control. The animal treated with vitamin C or TGN for 7 days, did not show any significant changes in biochemical and haematological parameters, whereas the animals treated with TGN for 45 days showed a significant change in the levels of AST, ALT, creatinine, and urea when compared with control.
Conclusion: Trigonelline improved the locomotor activity and alertness in long-term administration without affecting liver functions and haematological parameters. Long-term administration of Trigonelline improved the locomotor activity and alertness without affecting liver functions and haematological parameters.

Keywords: Grip strength, Escape latency time, Locomotor activity, Vitamin C.
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Introduction

Plants and its phytoconstituents are the important sources of medicine. It has been estimated that about 75% of the world's population using plants and plant extracts for their medicinal needs.[1] *Coffea arabica* (Rubiaceae) is an important cash crop, source of coffee and has numerous physiological and pharmacological properties. In least 1200 years, coffee taken an important place in human society, and its use has become a regular part of everyday life across the world. *C. arabica* contains many phytoconstituents including carbohydrates, lipids, nitrogenous compounds, vitamins, minerals, alkaloids, and phenolic compounds.[2] Caffeine, trigonelline, chlorogenic acids, mangiferin, and sucrose are the few important phytochemicals present in the *Coffea* species.[3]

Trigonelline (TGN) is a major pyridine alkaloid component present in coffee, where it is present in amounts similar or smaller than those of caffeine.[4] The percentage of trigonelline in aqueous extract of green coffee is found to be 0.83 – 1.13% w/w.[5] TGN is also found in fenugreek seeds (*Trigonella foenum-graecum*), Japanese radish, *Glycine max*, *Allium sepapea*.[6,7] TGN is the active pharmacological constituent of fenugreek and constitutes approximately 0.1-0.15% of the seed weight.[8] TGN is a methylation product of niacin (vitamin B3) and it is also called as 'methylated niacin'.

In preclinical studies, TGN has hypoglycemic, hypolipidemic, neuroprotective, antimigraine, sedative, memory-improving, antibacterial, antiviral, and anti-tumour activities, and is shown to lessen the diabetic auditory neuropathy and platelet aggregation.[9] It acts by affecting β cell regeneration, insulin secretion, activities of enzymes related to glucose metabolism, reactive oxygen species, axonal extension, and neuron excitability.[10] Coffee is known for its physiological effects including effects on behavioural functions, and its individual phytochemicals are not well explored for their physiological effects. Hence, the present study was planned to study the short and long-term effect of TGN on behavioural functions, biochemical and haematological parameters in Sprague Dawley (SD) rats.

Materials and Methods

Animals:

Healthy, adult, male Sprague-Dawley rats (180 ± 20 g body weight [BW]) were used for this study. The rats were obtained from Central animal house, AIMST University, Malaysia. The animals were housed in large, poly acrylic cages at room temperature with 12 h light/12 h dull cycle. Minimum 7 days of acclimatization period were permitted before the inception of the investigation. Food and water were *at libitum*. Prior permission was obtained from the AIMST University Human and Animal Ethics Committee to carry out the animal experiment (AUAEC/FOM/2019/03) All the experiments were carried out as the guideline prescribed by Animal Research Review Panel.

Short-term effect of TGN on behavioural functions:

The animals were equally divided into 5 groups. (6 in a group, for both short-term and long-term).

Group I: Control

Group II: Vitamin C (200 mg/kg) Group III: TGN (12.5 mg/kg) Group IV: TGN (25 mg/kg)

Group V: TGN (50 mg/kg)

Both vitamin C and TGN were suspended in the .5% w/v of carboxymethyl cellulose (CMC). The animals were treated for once daily for seven days through oral route. During the study, the animals body weight and behavioural functions were recorded on pre-study day and 7th day. At the end of the study, the blood samples were collected from the experimental animals for biochemical and haematological analysis.

Long-term effect of TGN on behavioural functions:

The animals were equally divided into 5 groups. (6 in a group, for both short-term and long-term).

Group I: Control

Group II: Vitamin C (200mg/kg) Group III: TGN (12.5mg/kg)

Group IV: TGN (25mg/kg)

Group V: TGN (50mg/kg)

Both vitamin C and TGN were suspended in the

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.5% w/v of carboxymethyl cellulose (CMC). The animals were treated for once daily for 45 days through oral route. During the study, the animals body weight and behavioural functions were recorded on pre-study day, 15th, 30th and 45th day of the study. The blood samples were collected from the experimental animals on day 15th, 30th and 45th of the experiment and used for biochemical and haematological analysis.

Experimental Parameters:

Body weight analysis: The body weight of each rat in each group were recorded at regular intervals.

Behaviour study: Locomotor motor activity, grip strength and escape latency time were measured at regular intervals. The rat's behaviour was tested using actophotometer, 30 minutes after the treatment with varying doses of TGN.

Locomotor Activity: The locomotor behaviour of the animal was monitored using actophotometer, furnished with acrylic cage and encompassed by a stainless-steel frame, with 8 beams of infrared light on x axis. The individual rat's movement was checked at room temperature for 10 min.[11]

Rota-rod Test: The muscle grip and strength of the animal was monitored using a rota-rod apparatus to measure the time a rat remains on a rotating rod. The starting speed was adjusted to 4 rpm followed by the acceleration rate to 40 rpm. Rats were pre-trained on rota-rod approximately for 1 to 3 hours prior to testing. During the study fall-on time was recorded.[11,12]

Morris Water Maze Test (MWM): The escape latency time of the animals was observed using the MWM test described by Chowdhury *et al*.[11] Water navigation test was utilized as a technique to assess spatial learning and memory capacities. Training was conducted for 3 sequential days, with a 4 consecutive trials/day for each experimental rat at the inter-trial interval of 30 min. The MWM consists of a round pool, filled with tap water, which is close to 23-26°C, to the depth of 0.3–0.4 m. The pool was partitioned into four theoretical quadrants, with a getaway stage set hidden 1cm beneath the water surface on the inside four distinctive beginning stages for rats were set around the edge of the pool. On every one of the 3 preparing days, each of the four beginning focuses was utilized once in a pseudorandom arrangement. The water maze was situated on a huge stay with various additional maze viewable signs. The trail began by placing the animal in the water facing the wall of the pool at one of the starting points. If the animal failed to escape on the platform within 180sec, they will be excluded from the study. Each animal was subjected to a daily session of three to four trials for 3 consecutive days. Escape latency time (ELT) is to find the hidden stage in the water maze was noted as a file of learning.

Biochemical analysis: Blood samples (1 ml) were collected on pre-study day and end of the experiment through retro-orbital plexus in plain and disodium EDTA tubes. The serum sample was used for the analysis of biochemical parameters including glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), cretinine and urea. Haematological analyses were carried out, including the haemoglobin, red blood cell count (RBC), white cell count (WCC), platelets, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW) and platelet. The samples were tested using biochemical analyzer (Cobas 6000 biochemical analyzer, Roche Diagnostics) and automated haematology analyser in Clinipath Malaysia Sdn Bhd laboratory, Malaysia.

Statistical Analysis

The mean ± standard error of the mean (SEM) values was calculated for each group. Statistical differences among the groups were determined using repeated ANOVA followed by Tukey's *post-hoc* test. $P < 0.05$ was considered significant.

Results:

Effect of TGN on body weight:

TGN did not show any significant change in the body weight of animals in both short and long-term study, when compared with that of control (Figure 1 and Figure 2).

Effect of TGN on behavioural functions:

In short-term study, TGN administered animals did not show any significant changes in the behavioural functions when compared with that of control group (Figure 3). In long-term study, significant increased locomotor activity in TGN administered animals on 30th day onwards at 50

mg/kg dose level when compared with that of control group. The animals administered with TGN 25 mg/kg, also showed significant increase in locomotor on 45th day when compared with that of control group (Table 1). In grip strength test, TGN administered animals did not show any significant changes on day 15th, 30th and 45th day of the experiment when compared with that of control group whereas vitamin C administered animals showed significant increase in grip strength on 45th day of the experiment when compared with that of control group (Table 2). In Morris water test, both vitamin C and TGN 12.5, 25 and 50 mg/kg administered showed significant decreases in ELT compared with that of control group from 30th day onwards.

Effect of TGN on biochemical parameters:

In short-term study, TGN administered animals did not show any significant changes in the biochemical parameters when compared with that of control group (Figure 4). In long-term study, on 15th day analysis of biochemical parameters in TGN administered animals showed significant increases in the levels of creatinine (at 25 mg/kg) and urea (at 50 mg/kg) when compared with that of control group (Figure 5). In long-term study, on 30th and 45th day analysis of biochemical parameters in TGN administered animals did not show any significant changes (Figure 6 & 7).

Effect of TGN on haematological parameters:

In short and long-term study, TGN administered animals did not show any significant changes in the haematological parameters when compared with that of control group (Figure – 8 to 11).

Discussion

TGN is a phytochemical in coffee and is the second most abundant alkaloid present in coffee beans. TGN has been reported to have several biological activities including neuroprotective, antimicrobial, antioxidant, and anticancer effects.[13] In this present study, TGN did not show any significant changes in behaviour and biochemical parameters in short-term study.

Locomotor action is utilized to survey the psychostimulant or narcotic action utilizing movement confine or actophotometer.[14] Grasp quality examination utilizing rota-rod was intended to assess depressant consequences for the focal sensory system in CNS and conduct with impacts of TGN. Muscle quality estimations could be helpful as a feature of clinical assessment in figuring out which patients are most in danger of a quickened decrease in cognizance.[15] Rota-rod test was utilized to assess motor coordination and muscle strength in Sprague Dawley rats which was under energizers or depressants for focal sensory system treatment. Results of the preset study indicated that the animal treated with TGN at 25 and 50 mg/kg for 45 days significantly increased in locomotor activity and reduced ELT. Increase of the locomotor activity may be due to the CNS stimulant effect of TGN.[16] When the locomotor activity increases, it will be mediated through continuous release of calcium current which may affect the muscular system and animal may develop muscle fatigue. Excitation– contraction coupling in skeletal muscle triggered by opening of the dihydropyridine receptor or voltage-gated sarcolemmal l-type Ca^{2+} channel which leads to a substantial and fast activating Ca^{2+} inward current and this increased calcium current triggers Ca^{2+}. Ca^{2+} release channels will open and release massive amounts of Ca^{2+} ions from sarcoplasmic reticulum stores into the cytoplasm and induces muscle contraction.[17] The CNS stimulant effect of TGN may induce hyperactivity and this effect may cause muscle fatigue which may reduce the muscular strength. The animals treated with vitamin C and TGN showed significant reduction in ELT. This result indicates that, both vitamin C and TGN have beneficial effect on memory functions. The increasing attention by both vitamin C and TGN is a positive outcome antagonize the oxidative stress and Alzheimer's disease.[18]

Fahanik-Babaei *et al.*, 2019 reported the ameliorative effects of TGN against Alzheimer's disease and this is mediated through suppressing astrocyte activity, oxidative stress, and inflammation and over the salvation of mitochondrial integrity.[19] TGN has an effective antioxidant property and strong free-radical foraging activity which is important for the therapeutic treatment of neurodegenerative diseases. TGN is known to have the capability of

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reversing the motor dysfunction in rats. For this to be the main reason, TGN would be regarded as a forthcoming effective defence against Parkinson's disease.[20]

It has been recognized that CNS development is a classic case of events involving the growth and maturation of cells and their organization in functional structures or nuclei, leading to physiological functions. Each region of the brain has its own characteristic growth rate and maturational sequence.[21] Reasonable relationships between physical activity and cognitive functions best defines psychomotor behaviours. Expanded social tests can be utilized in animal models, for the most part, mice and rats, to investigate psychomotor execution however not constrained to an open field test, raised in addition to labyrinth test, quickened rota rod test, and a constrained swimming test.[22]

In the present study, TGN did not show any significant effect on liver enzyme profile and blood glucose level. In general, TGN was established to be individually effective and has shown several pharmacological activities including hepatoprotective.[23] TGN showed anticarcinogenic especially in cervix and liver, antimigraine, antiseptic, hypocholesterolaemia, and hypoglycaemic activities.[24] The degenerative changes in kidney tissue and fibrosis were improved with TGN.[25] But in long-term study, TGN treated animals showed an increase in the level of creatinine and urea when compared with that of control. In the haematological investigation, no significant changes in TGN administered animals were observed in haemoglobin, RBC, WCC, platelet, RDW, PCV, MCV, MCH and MCHC. The results indicates that, TGN at up to 50 mg/kg did not cause any abnormality in haematological parameter.

TGN has demonstrated a few pharmacological exercises including against hyperglycemic and hostile to hyperlipidemic, neuroprotective, against headache, and memory-improving possibilities.[9,26] TGN has beneficial effects in several human diseases, notably diabetes and CNS disease. In the present study, TGN improved the behavioural functions (alertness) without affecting the liver enzymes and haematological parameters.

Conclusion

Trigonelline improved the locomotor activity and alertness in long-term administration without affecting liver functions and haematological parameters. In long-term administration, trigonelline affected renal functions. In short-term study, trigonelline did not affect the behavioural functions, biochemical and haematological parameters.

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Table 1: Long-term effect of Trigonelline on locomotor activity of SD rats

	Pre-study Day	**15th day**	**30th day**	**45th day**
Control	243.83 ± 12.43	240.17 ± 9.78	247.83 ± 11.07	235.83 ± 7.44
Vitamin C	261.67 ± 7.89	255.17 ± 13.66	258.33 ± 14.92	246.33 ± 12.25
TGN 12.5mg/kg	251.83 ± 10.57	266.00 ± 6.88	267.50 ± 8.82	274.83 ± 11.34
TGN 25 mg/kg	256.50 ± 7.34	270.50 ± 8.50	279.33 ± 8.78	299.50 ± 10.68*
TGN 50 mg/kg	255.83 ± 10.12	289.00 ± 9.69	303.33 ± 11.56*	352.00 ± 18.75***

Values are expressed as mean ± SEM (n = 6).
*P<0.05 and ***P<0.001 compared with that of control (One-way ANOVA followed by Turkey's *post-hoc* test).

Table 2: Long-term effect of Trigonelline on grip strength of SD rats

	Pre-study Day	**15th day**	**30th day**	**45th day**
Control	24.67 ± 0.88	20.33 ± 1.05	21.67 ± 1.15	21.33 ± 2.25
Vitamin C	27.00 ± 1.26	28.67 ± 2.08	25.50 ± 2.38	28.83 ± 1.22*
TGN 12.5mg/kg	26.00 ± 0.58	23.83 ± 2.09	23.33 ± 1.48	23.67 ± 0.84
TGN 25 mg/kg	28.17 ± 0.79	23.17 ± 1.68	19.17 ± 0.95	19.83 ± 1.54
TGN 50 mg/kg	27.33 ± 1.17	25.17 ± 3.13	20.17 ± 1.01	17.83 ± 1.08

Values are expressed as mean ± SEM (n = 6).
*P<0.05 compared with that of control
(One-way ANOVA followed by Turkey's *post-hoc* test).

Table 3: Long-term effect of Trigonelline on escape latency time of SD rats

	Pre-study Day	**15th day**	**30th day**	**45th day**
Control	20.83 ± 2.89	17.50 ± 2.88	17.33 ± 1.15	16.33 ± 1.52
Vitamin C	18.17 ± 0.79	14.17 ± 2.04	11.83 ± 1.22**	11.50 ± 0.92*
TGN 12.5mg/kg	17.33 ± 1.33	9.33 ± 0.76	9.17 ± 0.54***	7.50 ± 0.89***
TGN 25 mg/kg	18.17 ± 1.76	10.17 ± 1.40	9.17 ± 0.65***	6.50 ± 1.09***
TGN 50 mg/kg	19.33 ± 2.23	13.67 ± 2.50	10.17 ± 0.60***	10.17 ± 1.05**

Values are expressed as mean ± SEM (n = 6).
*P<0.05, **P<0.01 and ***P<0.001 compared with that of control (One-way ANOVA followed by Turkey's *post-hoc* test).

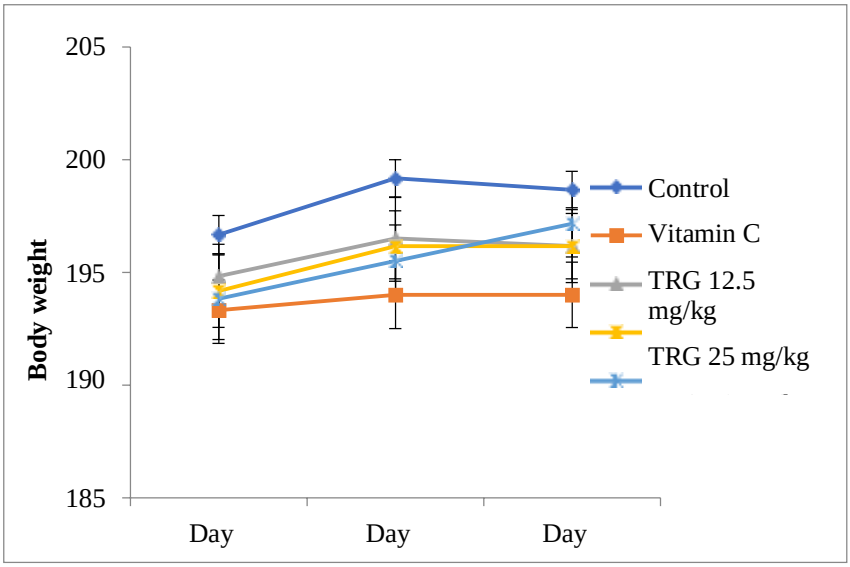

Figure 1: Short-term effect of trigonelline on body weight of SD rats

All the values are mean ± SEM (n = 6). TGN: Trigonelline

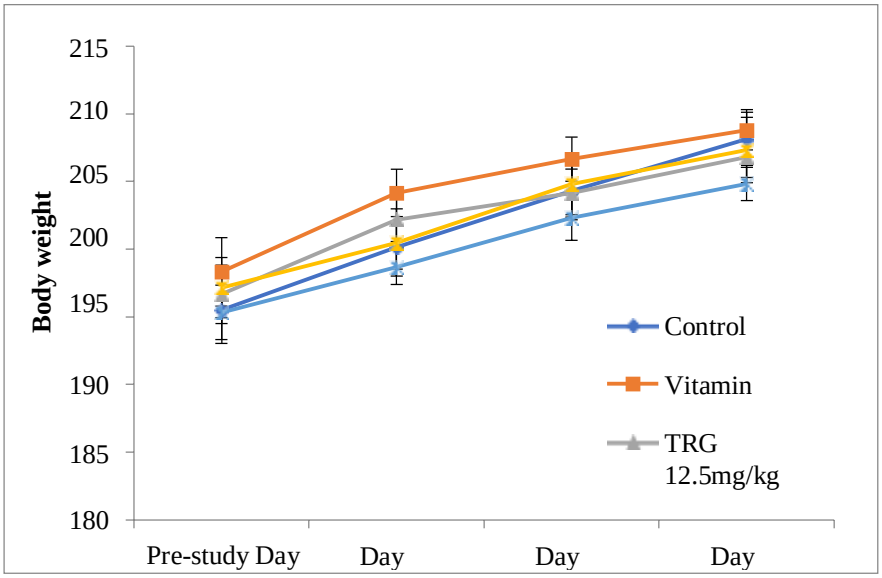

Figure 2: Long-term effect of trigonelline on body weight of SD rats

All the values are mean ± SEM (n = 6). TGN: Trigonelline

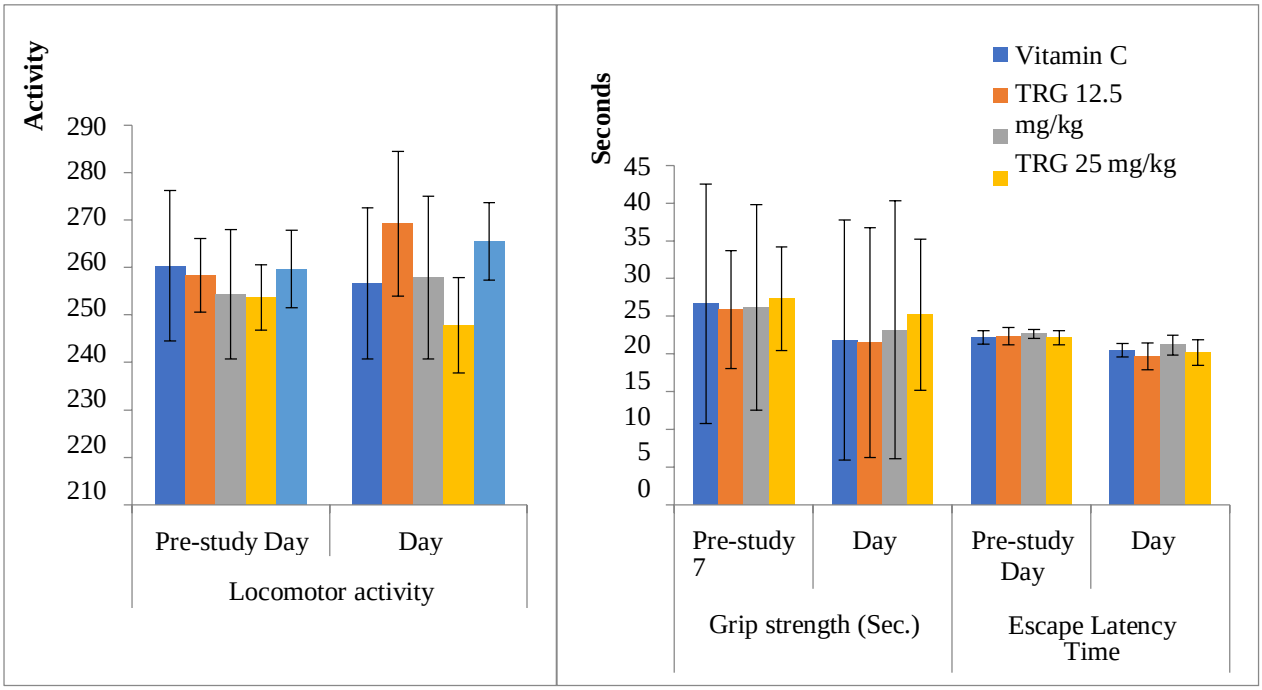

Figure 3: Short-term effect of trigonelline on behavioural functions of SD rats from short-term study

All the values are mean ± SEM (n = 6).

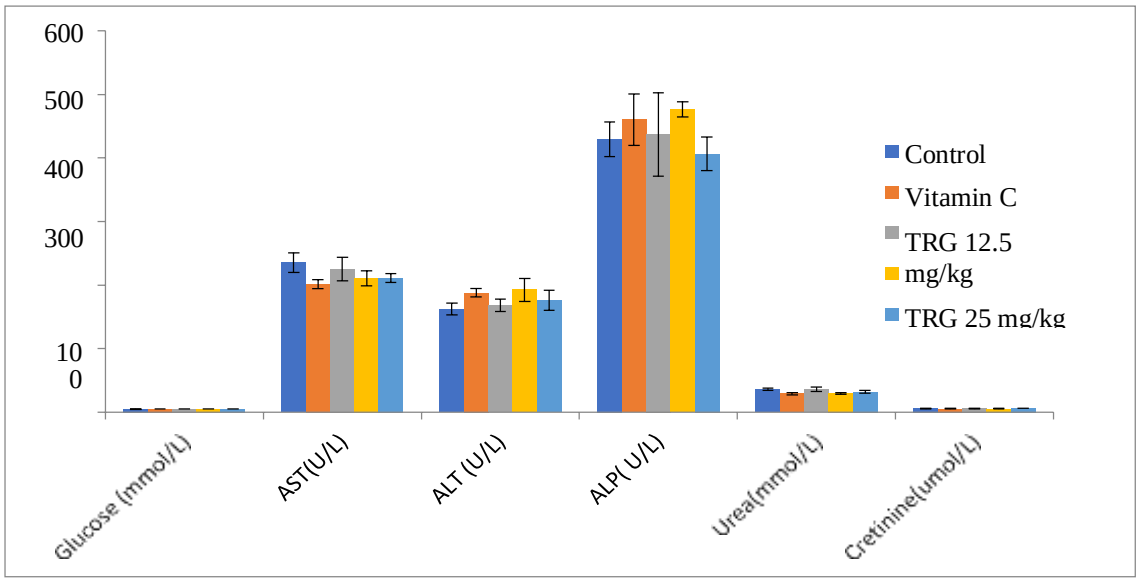

Figure 4: Short-term effect of trigonelline on biochemical parameters of SD rats

Values are expressed as mean ± SEM (n = 6).
AST: Aspartate aminotransferase; ALT: Alanine aminotransferase, ALP: Alkaline phosphatase.

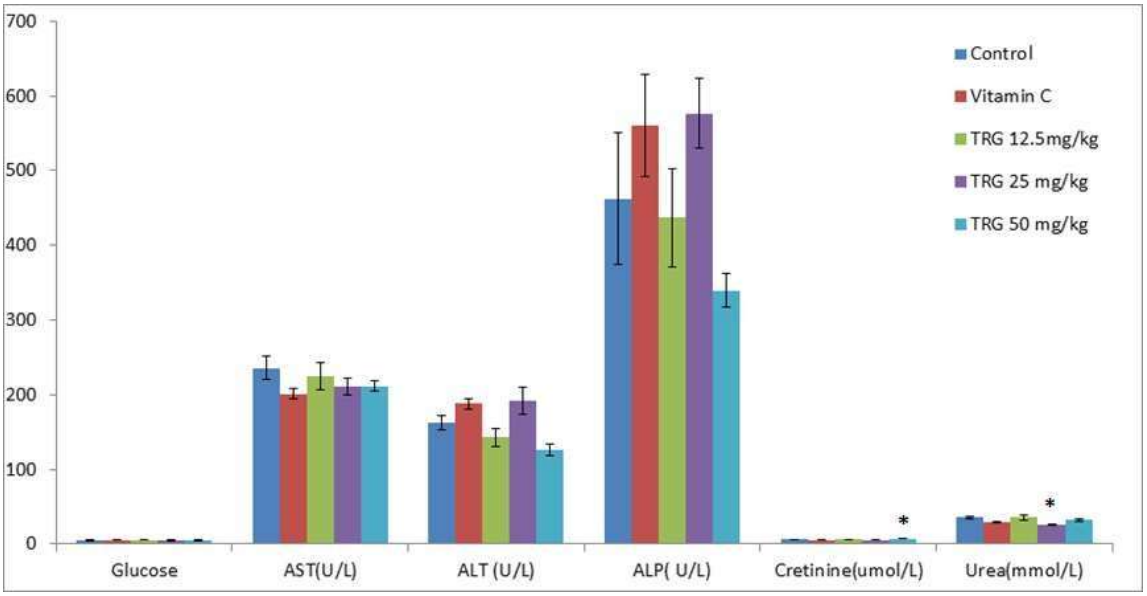

Figure 5: Long-term effect of trigonelline on biochemical parameters (15th day)

Values are expressed as mean ± SEM (n = 6).
*P<0.05 compared to control.
(One-way ANOVA followed by Turkey's *post-hoc* test).

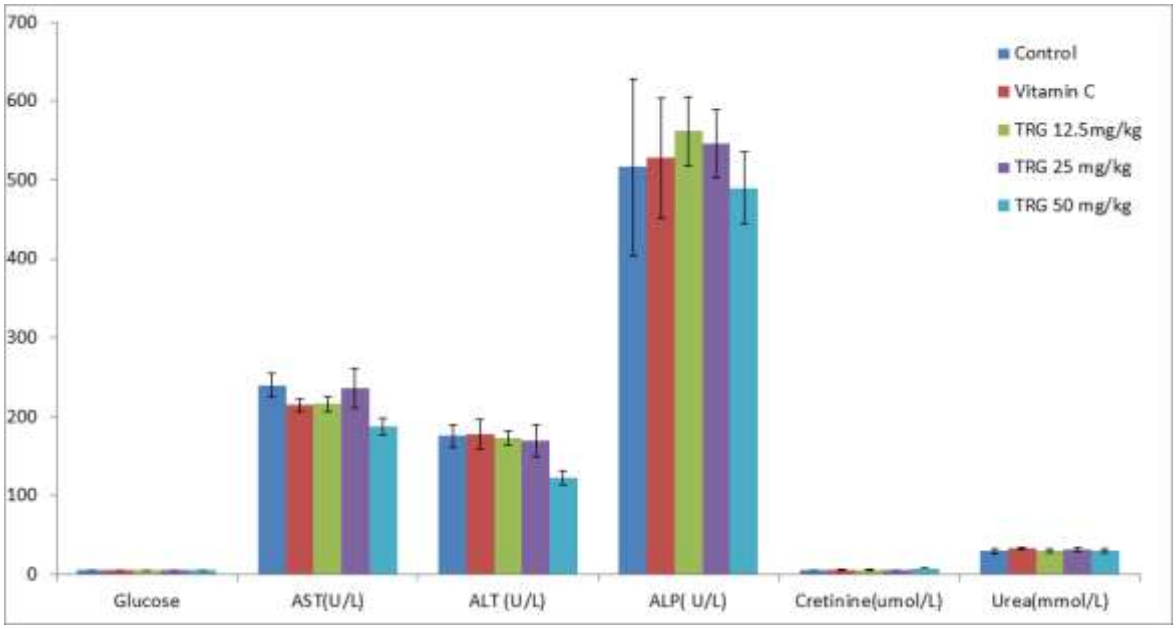

Figure 6: Long-term effect of trigonelline on biochemical parameters (30th day)

Values are expressed as mean ± SEM (n = 6).

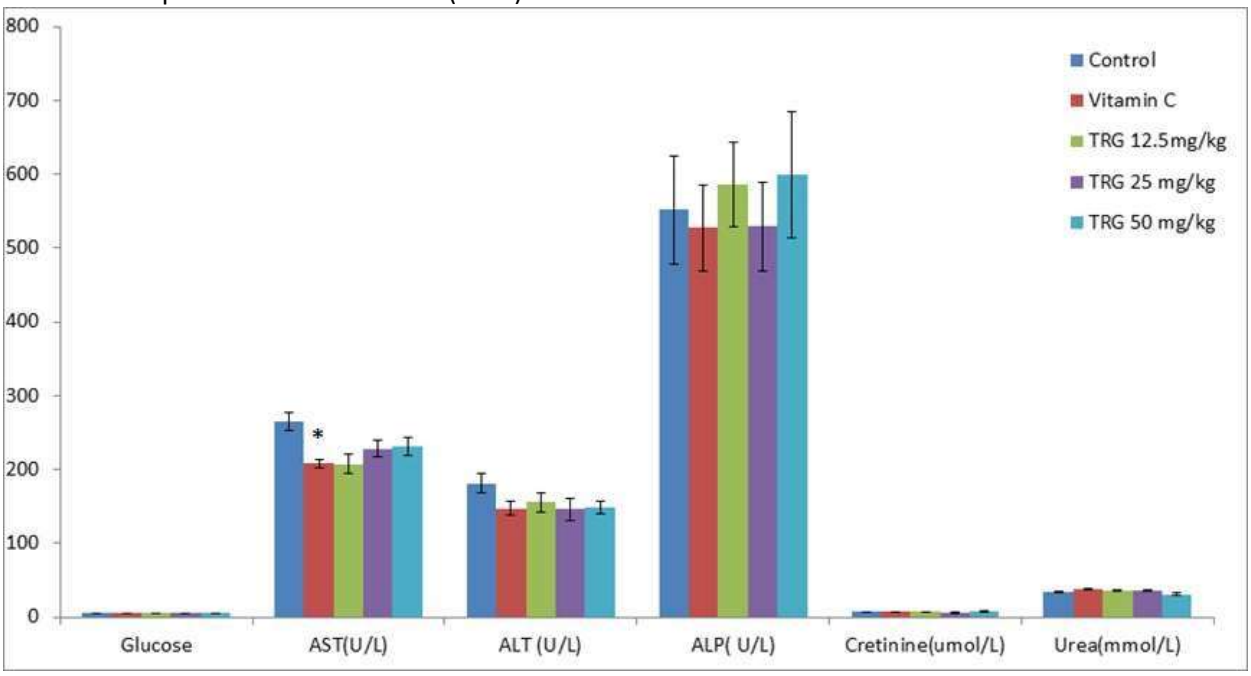

Figure 7: Long-term effect of trigonelline on biochemical parameters (45th day)

Values are expressed as mean ± SEM (n = 6).

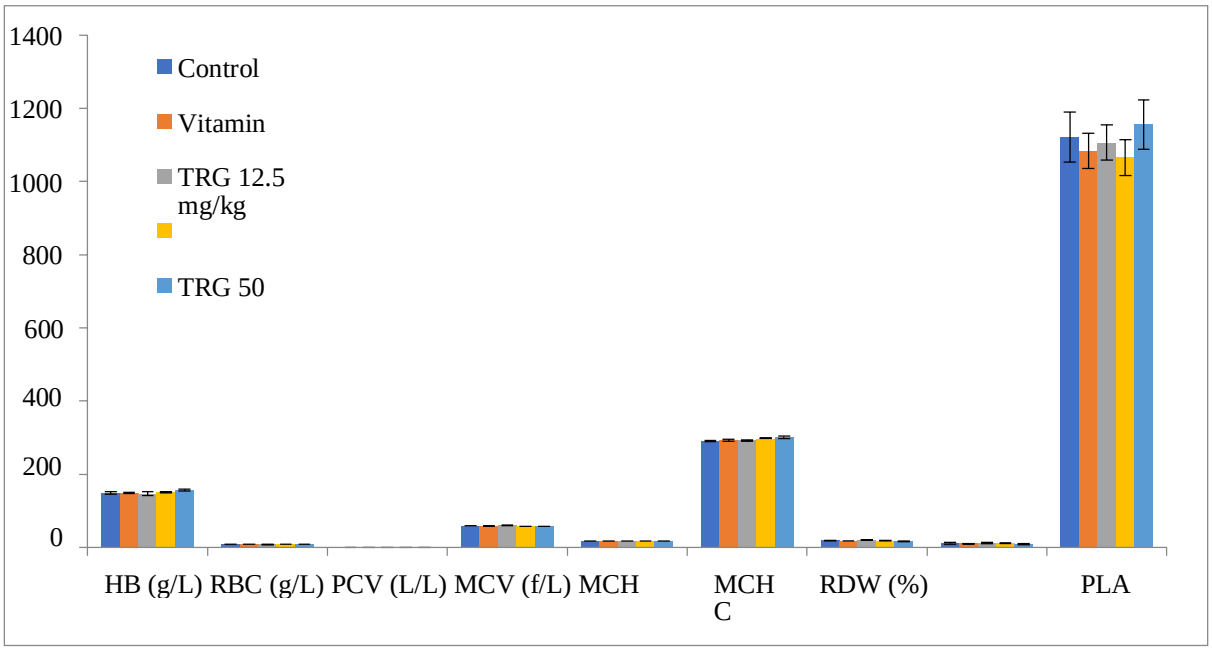

Figure 8: Short-term effect of trigonelline on haematological parameters of SD rats

Values are expressed as mean ± SEM (n = 6).

HB: Haemoglobin; RBC: Red cell count, PCV: Packed cell volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; RDW: Red cell distribution width; WCC: White cell count; PLAT: Platelet.

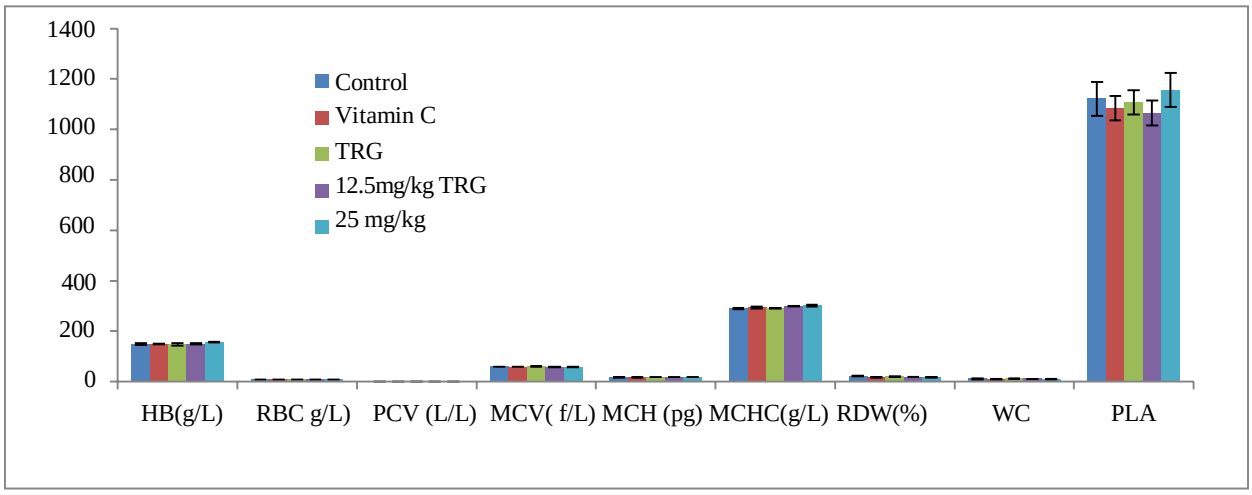

Figure 9: Long-term effect of trigonelline on haematological parameters of SD rats (15th day)

HB: Haemoglobin; RBC: Red cell count, PCV: Packed cell volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; RDW: Red cell distribution width; WCC: White cell count; PLAT: Platelet.

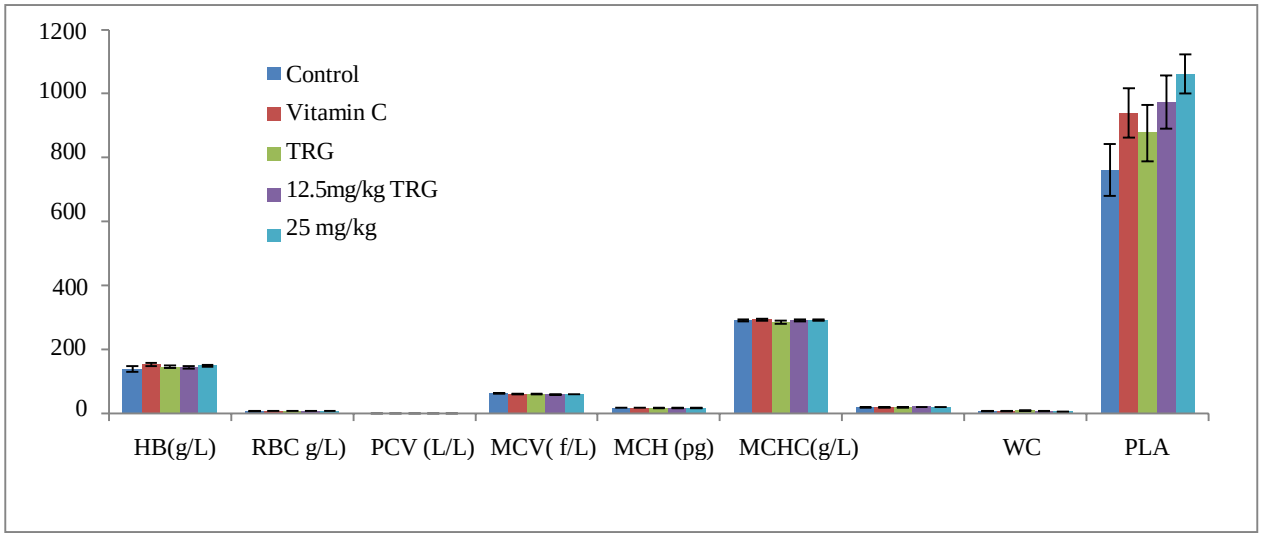

Figure 10: Long-term effect of trigonelline on haematological parameters of SD rats (30th day)

HB: Haemoglobin; RBC: Red cell count, PCV: Packed cell volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; RDW: Red cell distribution width; WCC: White cell count; PLAT: Platelet.

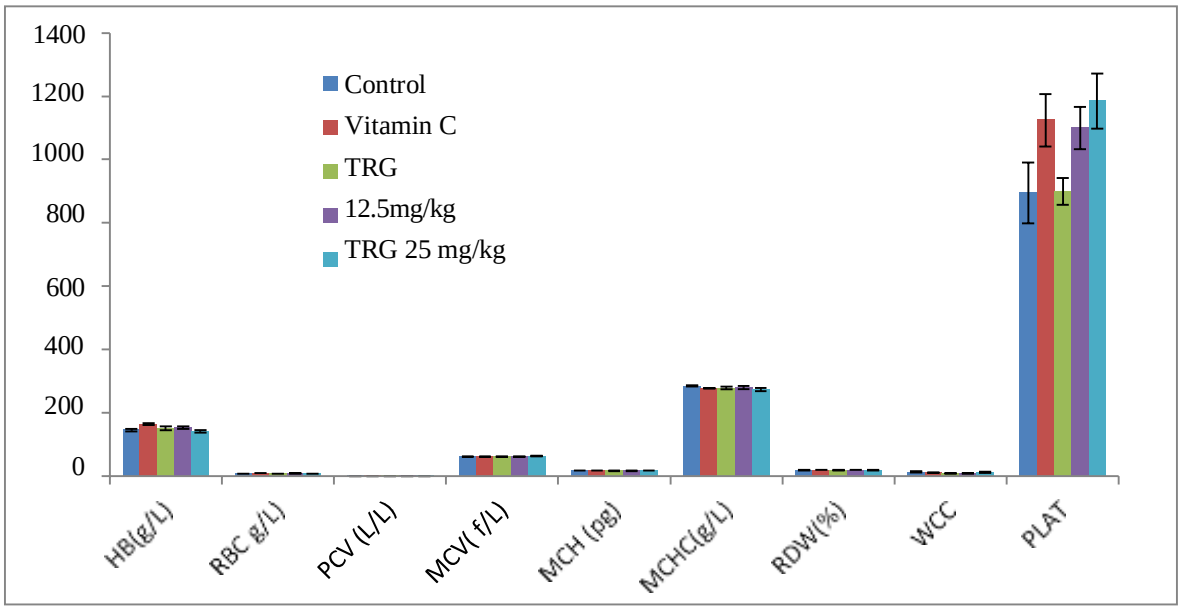

Figure 11: Long-term effect of trigonelline on haematological parameters of SD rats (45th day)

HB: Haemoglobin; RBC: Red cell count, PCV: Packed cell volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; RDW: Red cell distribution width; WCC: White cell count; PLAT: Platelet.