ISSN- 2230-7346
Journal of Global Trends in Pharmaceutical Sciences

EVALUATION OF SUB CHRONIC TOXICITY OF SIDDHA FORMULATION-*THETRAN VIDHAI KUDINEER* IN WISTAR ALBINO RATS

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ARTICLE INFO

Key words:
Thetran vidhai kudineer; Toxicology; Sub chronic toxicity; Biochemical analysis; Histopathology

Access this article online
Website: https://www.jgtps.com
Quick Response Code:

ABSTRACT

Aim: The study is to evaluate the sub chronic toxicity of the Thetran vidhai kudineer in wistar albino rats. **Methods:** Thetran vidhai kudineer was orally administered at three dose levels (360, 1800 and 3600 mg/kg) to the rats repeatedly for 90 days once a day and then the general behavior, adverse effects and mortality was compared to normal group which were recorded for every 24 hours over a period of 90 days. **Results:** At the end of the study period the biochemical, hematological parameters were estimated. The results evidenced no mortalities and no significant difference ($p > 0.05$) in the relative organs weight, body weights, biochemical parameters hematological parameters and gross abnormalities of treated rat groups compared with control rat group. **Conclusion:** They are regarded as safe; the oral administration of the Thetran vidhai kudineer for 90 days does not showed any mortality and no drug toxic reactions were found from histopathological examinations of major organs upto 3600mg/kg b.w.

INTRODUCTION

People from developing nations are more likely to employ alternative medical systems for therapy[1]. Due to the unproven harmful effects of herbs, modern science does not support their usage as a kind of treatment[2,3]. Long-standing folklore, however, holds that specific herbal preparations can be utilised to cure diseases and ailments. To exploit the beneficial properties of herbal preparations and formulations, pre-clinical toxicity tests are done to identify the toxicity of many herbal preparations and formulations. Traditional Based medicines are been used for a long time which doesn't mean to are safe. Traditional medicines are to be used as a placebo owing to their non-toxic nature albeit less efficacy. Albeit lack of toxic evidence never indicate the non-toxic property of the drug, long-term use of traditional medicines in folklore seems to be with hepatotoxicity (DILI) with the notion is not warranted to be safe. This augmented the need for testing the reverse toxicology of the drug *Thetran vidhai kudineer* (TVK)[4]. The present article attempted to

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test the sub-chronic toxicity of TVK in laboratory rats. *Thetran vidhai kudineer* is a polyherbal Siddha formulation that comprises four plants namely thetran vidhai (*Strychnous Potatorum*), *Kadukkai Thol* (*Teminalia chebula*), *Aavarai vidhai* (*Cassia auriculata*) and Vilam pisin (*Limonia acidisimma.Linn*) which is indicated for diabetes mellitus[5]. *Kudineer* is a decoction comprised of herbs/ plants formulated by boiling the herbs with water until it's reduced to 1/16 or 1/8 level of the mixture. TVK is to be used as anti-diabetic in current clinical practice as per ancient textbook literature of siddha system of medicine[6] however, the toxicity profile of TVK for long-time use is not explained. Diabetes is referred to as *Neerizhivunoi* (excess discharge of urine), *Neerperukkal noi* (polyuric condition)[7], and *Madhumegam*, it is classified under *mega noi* i.e. venereal disease. In Siddha system of medicine, *Madhumegam* is also related to lifestyle changes, the heat produced in the body (pitham), stress, and excessive ravenous[8]. The sub-acute toxicity profile of *Thetran vidhai kudineer* was free from toxicity up to a dose level of 3600 mg/kg b.w.[9]. Since there is a shortfall of toxic profile evidence on *Thetran vidhai kudineer* on prolonged administration augmented the need for this study. Hence the drug is used for a prolonged duration; a repeated toxicity study for 90 days was performed to establish the toxicity of the Siddha herbal formulation in experimental Wistar albino rats. Through this study, the safety can be established for the clinical use of this traditional formulation in the diabetes community people.

2. MATERIAL AND METHODS

2.1. Preparation of *thetran vidhai kudineer*

Strychnous potatorum Linn seed and *Terminalia chebula* Retz fruit were procured from local crude drug market, authenticated by the Research Officer (Pharmacognosy) of this Institute. *Cassia auriculata* Linn. Seed and *Limonia accidisima* L.gum were collected and authenticated by Dr. Padma Sorna Subramanium, Research Officer (Botany), Survey of Medicinal Plants Garden, Mettur, Tamil Nadu. The powdered ingredients of Thetran vidhai kudineer were mixed thoroughly and decoctions were prepared by boiling 2 grams of the powdered drug in 240 ml of water for 20 minutes until it reduces up to 60 ml.

2.2. Animal

For the sub-chronic toxicity, male and female Albino rats weighing around (130-250 g) were utilized. Wistar albino adult male and female rats, aged 8 to 12 weeks, were used. Four rats per cage were placed in sterile polypropylene cages with bedding made of sterile maize cob, and the cages were maintained at room temperature of 23 ± 2ºC with a relative humidity of 60% ± 5% during a 12-hour cycle of darkness and light. Rats were given unlimited access to an 18% protein diet and water ad libitum[10].

2.3. STUDY DESIGN

Organization for Economic Cooperation and Development (OECD) guideline 408 and Central Council for Research in Ayurvedic Sciences (CCRAS) guideline was followed in the performance of the oral sub-chronic toxicity investigation[10,11]. Based on their body weights, the animals were randomly divided into four groups, each of which was distinguished by distinctive markings. Before the study started, the investigation team acclimated the animals using restraint and oral gavage. 80 rats received three doses of TVK (360, 1800, and 3600 mg kg-1 b.w.), as well as a control group (group I), at PO for 90 days (10 male and 10 female for each group). Doses were calculated based on the rats' body weights[12]. For 90 days, clinical symptoms, behavioural trends, and writhing were observed daily. Body weight and feed intake of the rats were measured on a weekly basis throughout the research period.

2.4. Estimation of biochemical parameters

Blood was collected from all rats at the end of the study through the retro-orbital route

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by inhalation anaesthesia at a dose equivalent to the body weight of the animals. The blood was allowed to clot and serum was separated by centrifugation, serum was analysed with the BA 400 fully automated analyser, and biosystem laboratory reagent kits were used. Biochemical parameters such as Blood glucose (Glucose oxidase and peroxidise method), Total cholesterol (CHOD-PAP method), Triglyceride (enzymatic method), HDL (direct), LDL, SGOT (UV kinetic method), SGPT (UV kinetic method), ALP (Para- nitro phenyl phosphate kinetic method), GGT (International Federation of Clinical Chemistry), Total protein (Biuret test), Albumin (Bromo cresol green method), LDH(International Federation of Clinical Chemistry), CRP (Turbidometry), Creatinine Kinase (International Federation of Clinical Chemistry), Creatinine Kinase MB levels (International Federation of Clinical Chemistry), Urea (Urease- GLDH), Serum creatinine (Jaffe's kinetic), Total Bilirubin (Diazotized sulfanilic method) and Uric acid levels (Uricase method) are estimated from the serum.

2.5. Estimation of haematological parameters

Blood was collected in micro centrifuge tubes with EDTA as an anticoagulant. The blood was analysed with a Haematology analyser (veterinary model). The haematological parameters such as WBC (White Blood Corpuscles), RBC (Red Blood Corpuscles), Lymph (Lymphocytes), Mon (Monocytes), Gran (Granulocytes), Hgb (Haemoglobin), and Hct (Haematocrit) were evaluated.

2.6. Histopathological evaluation

The rats were euthanized by carbon dioxide overdose method and the organs were collected and stored in the 10% neutral buffered formalin for further histopathological examination. The internal organs (lungs, liver, kidney, spleen, heart, brain, stomach, and pancreas) excised from all the rats were fixed in 10% buffered formalin in labelled bottles, and processed for histological examination. Tissues embedded in paraffin wax were sectioned 5 mm and stained with haematoxylin & eosin, mounted on glass slides, and examined under a standard light microscope.

2.7. Statistical analysis

All the values were expressed as Mean ± SEM. Statistical significance between more than two groups was tested using one-way ANOVA (Dunnett's test) using Graph pad prism version-8

3. RESULTS

3.1. CLINICAL OBSERVATIONS

No mortality, no clinical toxicity signs such as physical observations, behavioural changes, and other parameters such as body weight, feed intake, respiration, convulsion, tremor, temperature, changes in eye and skin colours and autonomic changes etc were observed at dose levels 360 mg kg -1b.w., 1800 mg kg -1b.w.and 3600 mg kg -1b.w then compared to the control group (Table 1).

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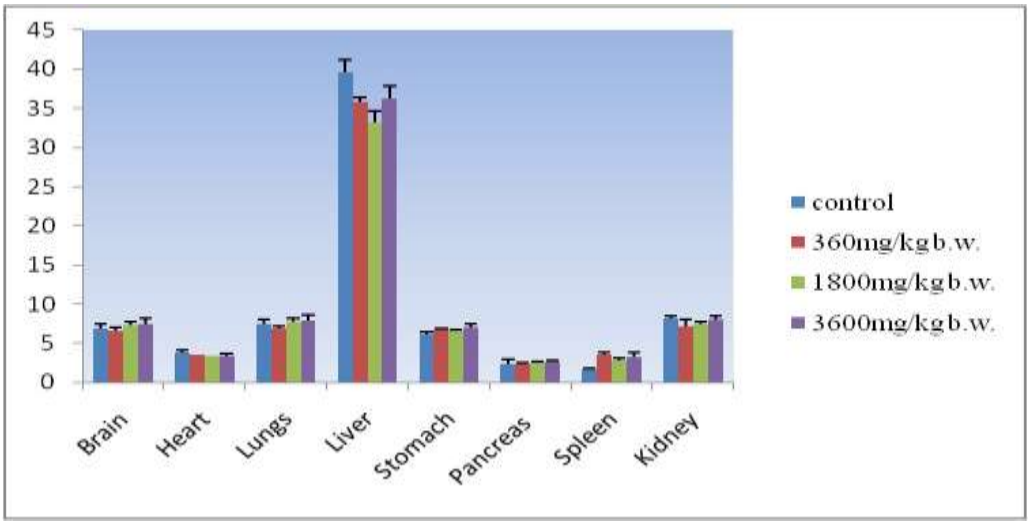

Figure 1: Effect of Thetran vidhai kudineer on Relative organs weight (g) of rats

Table 1: General appearance and behavioral observations for control and treated groups

Response	Group I (Normal)	Group II (360mg/kg b.w)	Group III (1800mg/kg b.w)	Group IV (3600mg/kg b.w)
Colour				
Fur	N	N	N	N
Eyes & Mucous Membrane	N	N	N	N
Urine	N	N	N	N
Behavioral observations				
Mood	N	N	N	N
CNS Excitation	NO	NO	NO	NO
CNS Depression	NO	NO	NO	NO
Motor Indication				
Abnormal gait	NO	NO	NO	NO
Righting reflex	N	N	N	N
Posture	N	N	N	N
Sensory Responses				
Touch & pain response	N	N	N	N
Straube phenomenon	NO	NO	NO	NO
Autonomic effects				
Defecation & Lacrimation	N	N	N	N
Urination & Salivation	N	N	N	N
Piloerection	NO	NO	NO	NO
Miosis & Mydriasis	NO	NO	NO	NO
Diarrhoea	NO	NO	NO	NO
Respiratory effect				
Apnoea & dysponea	NO	NO	NO	NO
Death	NO	NO	NO	NO

N-Normal NO-No Abnormality Observed

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Table 2: Effect of oral administration of Thetran vidhai kudineer on Body weight in male wistar rats

Groups	Week 1	Week 4	Week 9	Week 13
Group- I	180.38±22.01	216.38±20.65	233.63±12.30	257.44±13.38
Group- II	198.33±19.08	237.44±9.57	228.50±11.30	244.86±12.79
Group-III	187.56±17.37	234.67±9.66	222.71±10.90	240.73±13.30
Group-IV	178.00±20.81	237.14±18.86	221.87±16.46	236.27±19.10

All values are expressed as Mean ± SEM

Table 3: Effect of oral administration of Thetran vidhai kudineer on Body weight in female wistar rats

Groups	Week 1	Week 4	Week 9	Week 13
Group-I	160.00±8.48	191.00±8.58	224.69±8.38	240.67±9.43
Group- II	158.36±6.78	194.30±11.82	199.43±11.09	205.00±11.09
Group-III	156.18±4.24	192.45±12.90	193.44±8.51	203.25±10.77
Group-IV	159.92±5.37	182.58±7.67	188.22±12.26	196.00±12.79

All values are expressed as Mean ± SEM

3.2. RELATIVE ORGAN BODY WEIGHT

At the end of 90 days, relative organ weight of *Thetran vidhai kudineer* at dose levels of 360 mg kg -1b.w., 1800 mg kg -1b.w.and 3600 mg kg -1b.w showed statistically no significant differences (P > 0.05) with respect to the control group (Figure 1). It revealed the internal organs of rats were not adversely affected in weight and shape throughout the treatment.

3.3. BIOCHEMICAL PARAMETERS

The effects of Thetran vidhai kudineer on various biochemical parameters on Total cholesterol, triglycerides, creatinine kinase-MB levels, SGOT, SGPT, albumin, serum creatinine, C- reactive protein, and creatinine kinase levels of treated groups (360 mg kg -1b.w., 1800 mg kg -1b.w.and 3600 mg kg -1b.w) compared with the control group (Figure 2a -2j) showed statistically no-significant differences (p > 0.05).

3.4. HAEMATOLOGICAL PARAMETERS

No significant differences (P > 0.05) in haematological parameters such as White Blood Corpuscles, Red Blood Corpuscles, Lymphocytes, Monocytes, Granulocytes, Haemoglobin, and Hematocrit between treated rat groups as compared with control group rats were found. Further, the values of haematological parameters are in the normal range (Figure 3a-3c).

3.5. HISTOPATHOLOGICAL EXAMINATION

Light microscopic examination sections of various organs like lungs, liver, kidney spleen, heart, brain, stomach, and pancreas of control and treated groups were examined to find any gross pathological lesions. Additionally, macroscopic examination of organs (brain, heart, stomach, spleen, and pancreas) of treated rats revealed no abnormalities when compared with the organs of the control group. Even though some pathological changes have been observed i.e. peribronchiolar and interstitial mononuclear cell infiltration and mild pulmonary congestion, mild mononuclear cell infiltration, and mild hepatocellular degenerations which are all not related to the test compound of *Thetran vidhai kudineer*. The histopathological images are presented in Figures 4-7. Histopathological examination of lungs showed mild pulmonary congestion, mild peribronchiolar, and interstitial mononuclear cell infiltration in both control rats and treated rats groups of *Thetran vidhai*

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kudineer. The findings of the liver mostly showed mild hepatocellular degeneration, sinusoidal congestion, and very mild mononuclear cell infiltration in both control rats and treated rats groups of *Thetran vidhai kudineer*. Histopathological identification of kidney showed congestion with mild tubular epithelial cell degeneration in both control rats and treated rats groups of *thetran vidhai kudineer*. Glandular and non-glandular regions of the stomach showed no abnormality in both control rats and treated rats groups (III and IV) of *Thetran vidhai kudineer*. But Group II showed mild hyperkeratosis only in the non-glandular region at a low dose level of 360mg/kg b.w. which is spurious. The histopathological abnormalities were incidental, congenital, and spurious.

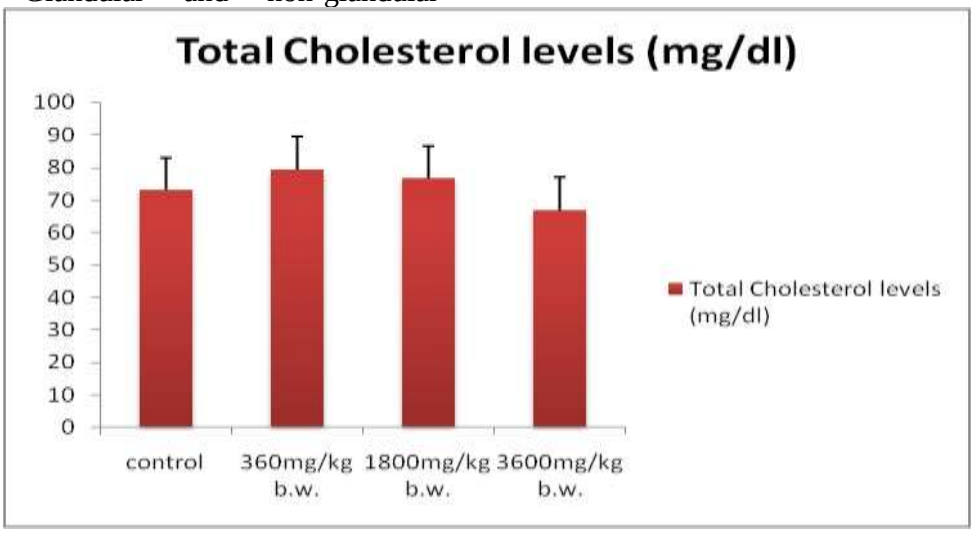

Figure 2a: Effect of oral Thetran vidhai kudineer on Total cholesterol of Wistar rats. All values are expressed as mean ± SEM.

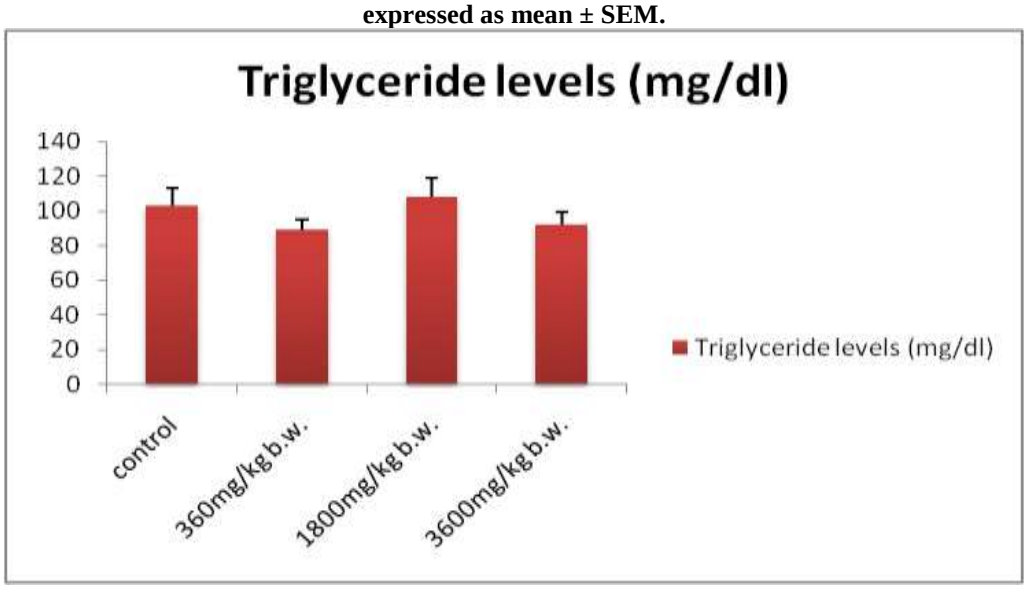

Figure 2b: Effect of oral Thetran vidhai kudineer on Triglycerides on Wistar rats. All values are expressed as mean ± SEM.

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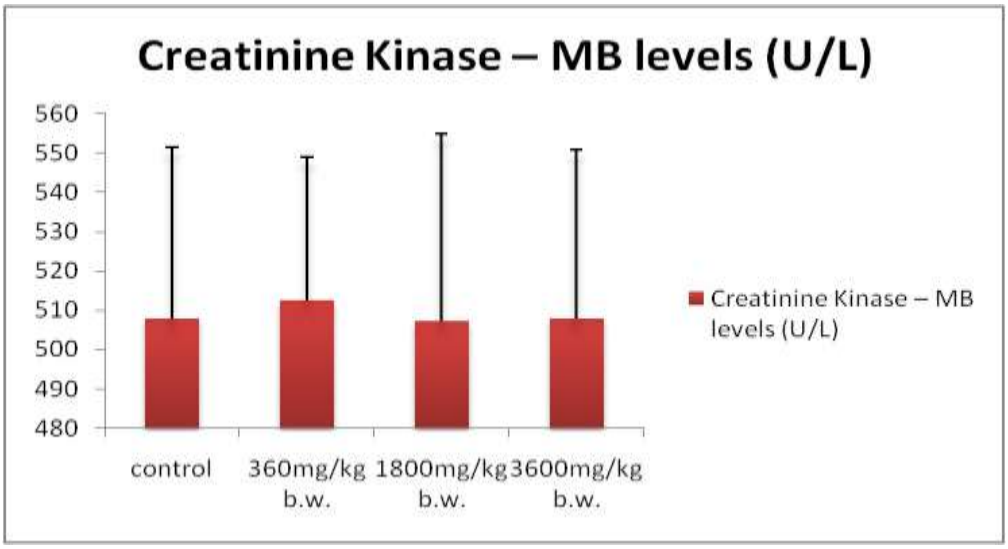

Figure 2c: Effect of oral Thetran vidhai kudineer on Creatinine kinase- MB of Wistar rats. All values are expressed as mean ± SEM.

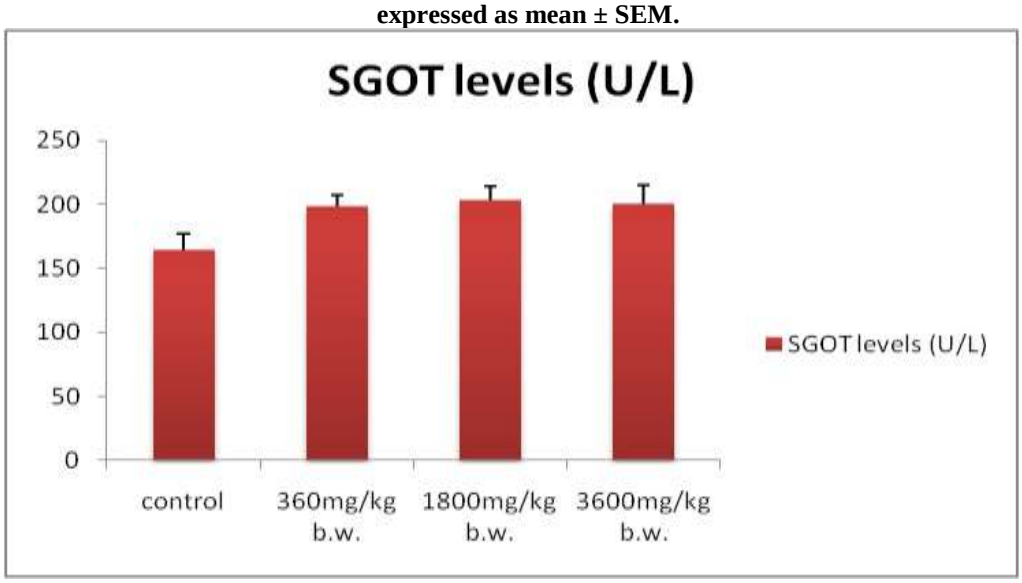

Figure 2d: Effect of oral Thetran vidhai kudineer on SGOT of Wistar rats. All values are expressed as mean ± SEM.

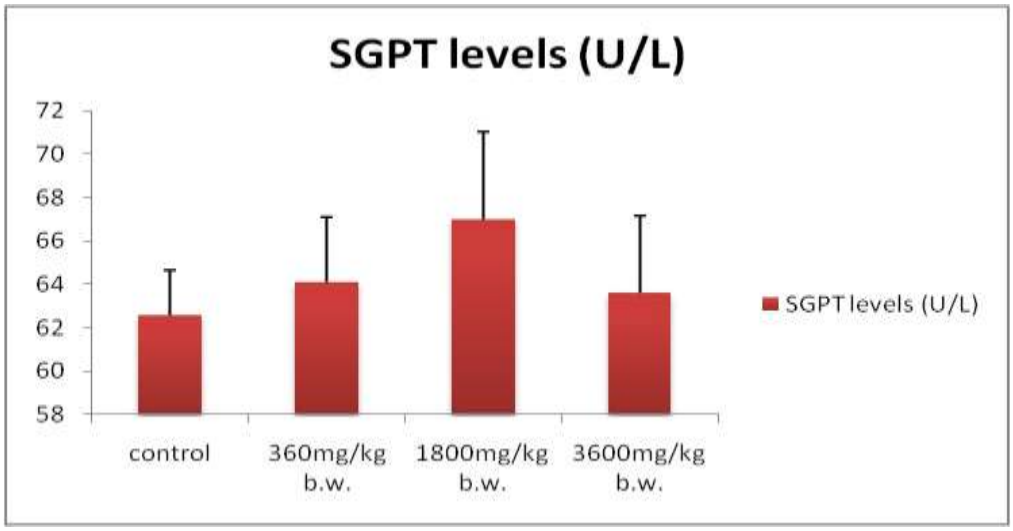

Figure 2e: Effect of oral Thetran vidhai kudineer on SGPT. All values are expressed as mean ± SEM.

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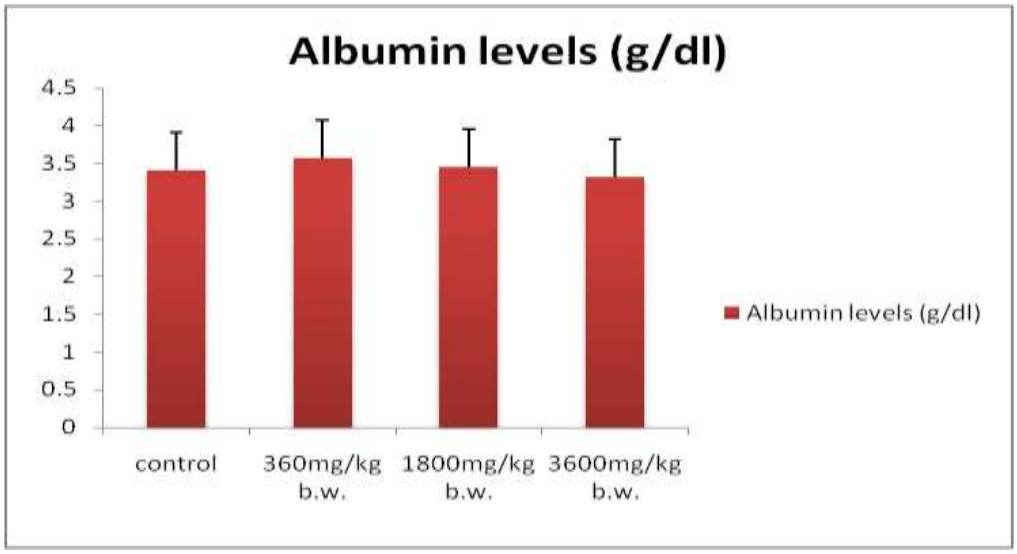

Figure 2f: Effect of oral Thetran vidhai kudineer on Albumin. All values are expressed as mean ± SEM.

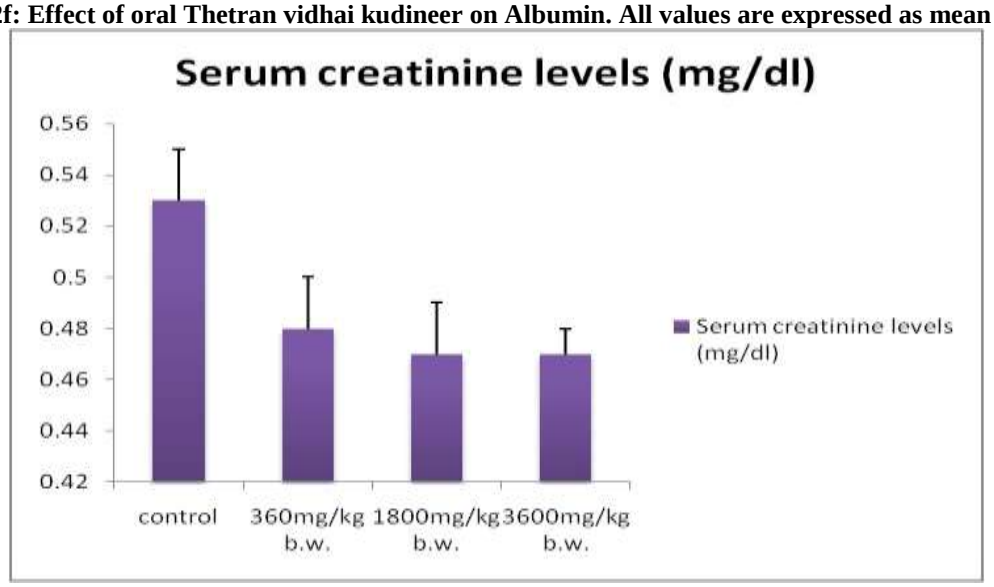

Figure 2g: Effect of oral Thetran vidhai kudineer on Serum creatinine. All values are expressed as mean ± SEM.

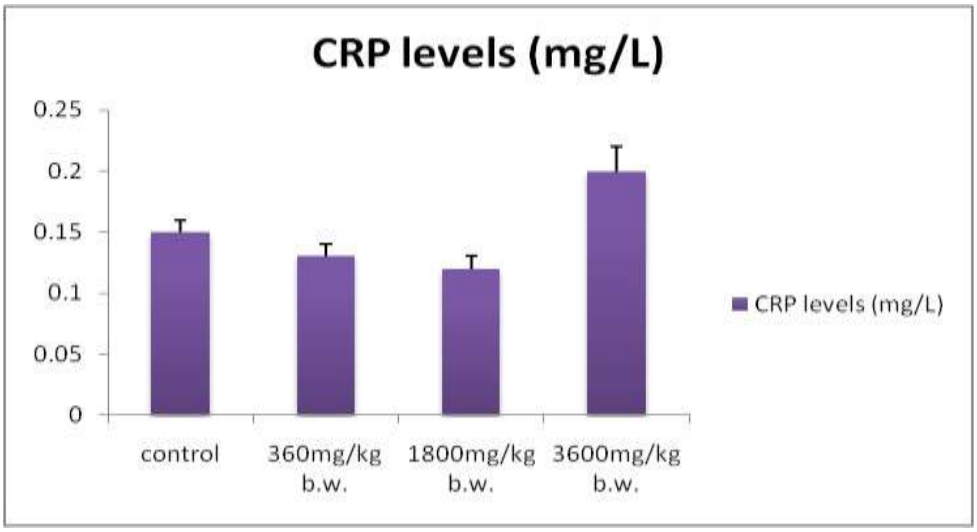

Figure 2h: Effect of oral Thetran vidhai kudineer on C - reactive protein. All values are expressed as mean ± SEM.

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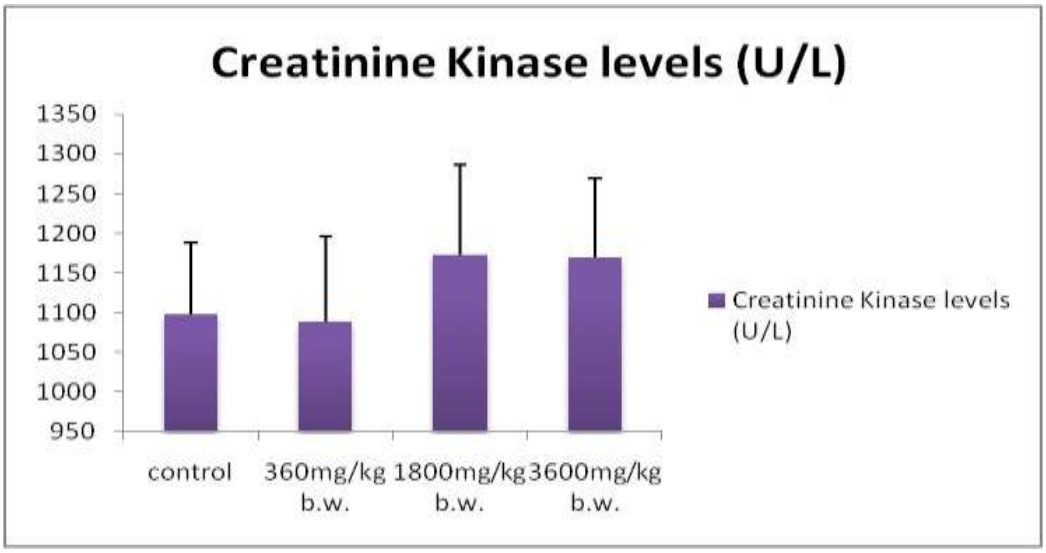

Figure2i: Effect of oral Thetran vidhai kudineer on Creatinine kinase. All values are expressed as mean ± SEM.

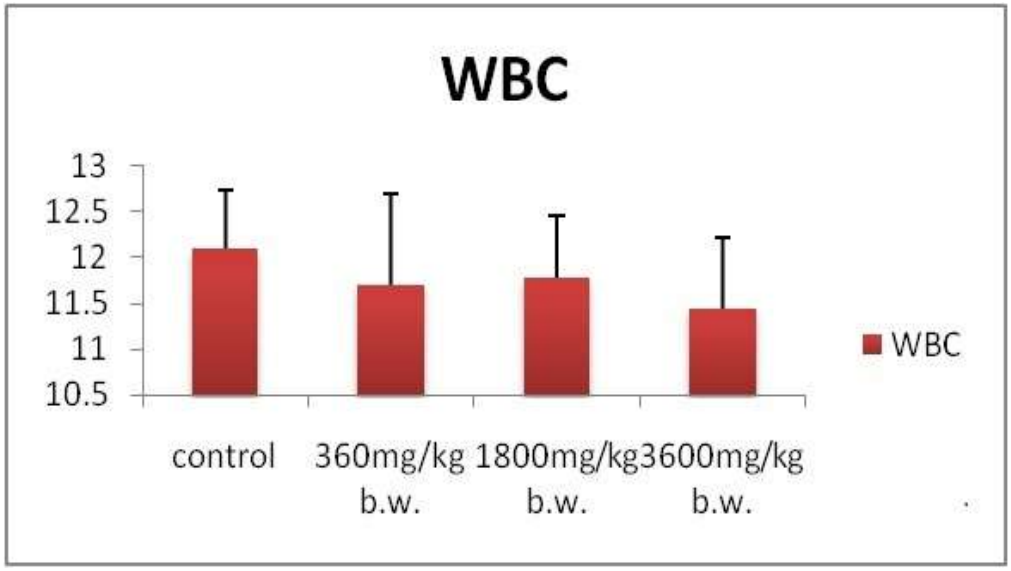

Figure 3a: Effect of oral Thetran vidhai kudineer on White Blood Corpuscles. All values are expressed as mean ± SEM.

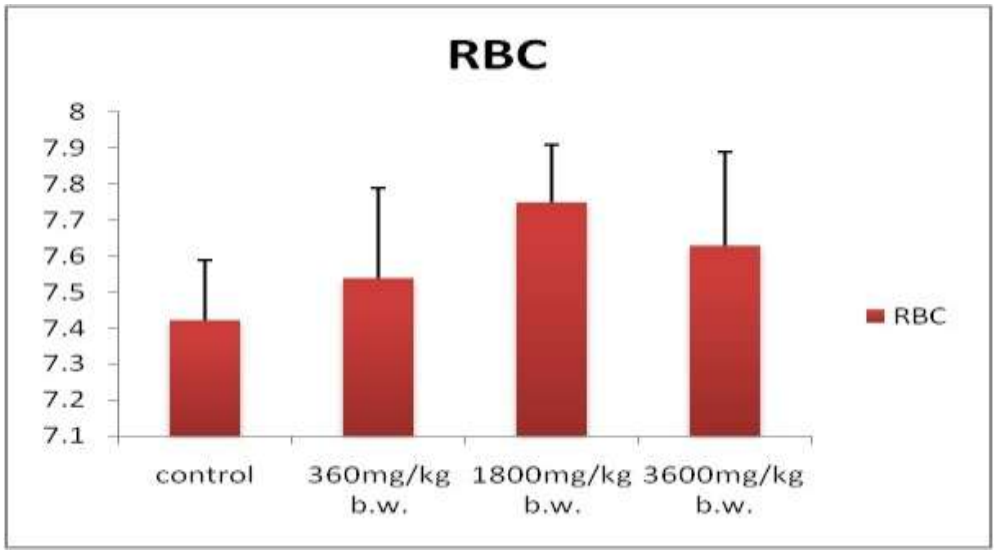

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Figure 3b: Effect of oral Thetran vidhai kudineer on Red Blood Corpuscles. All values are expressed as mean ± SEM.

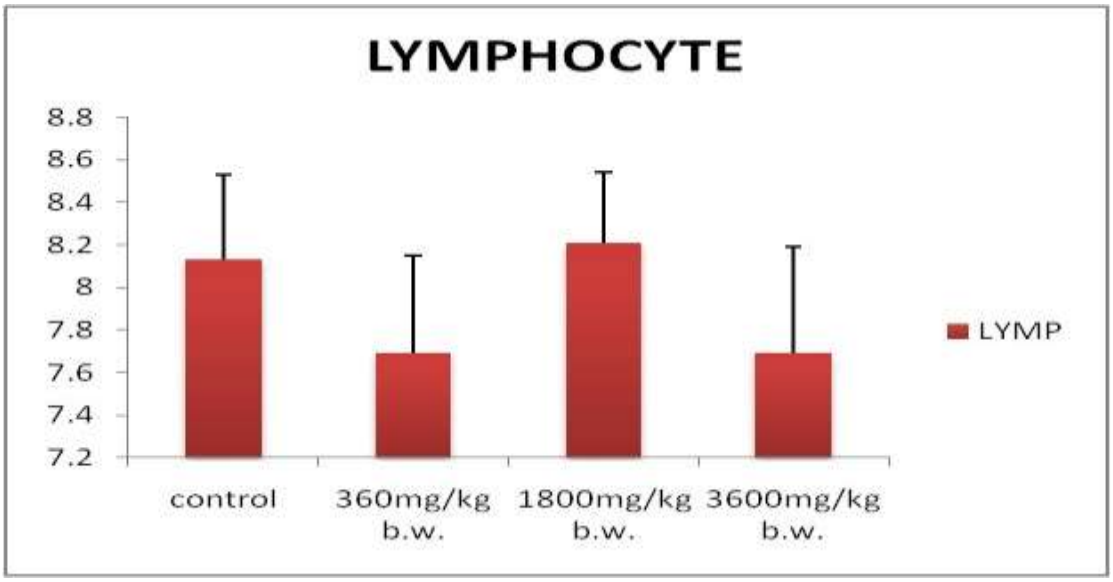

Figure 3c: Effect of oral Thetran vidhai kudineer on Lymphocyte. All values are expressed as mean ± SEM

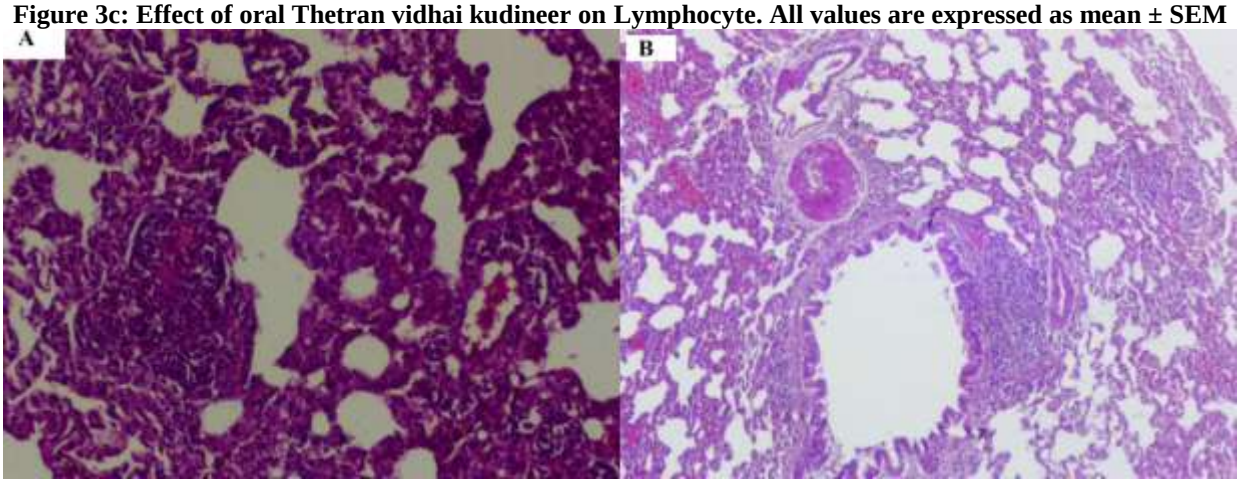

Figure 4: Effect of thetran vidhai kudineer on major organs. A, B- Lungs of Group-I and Group-IV

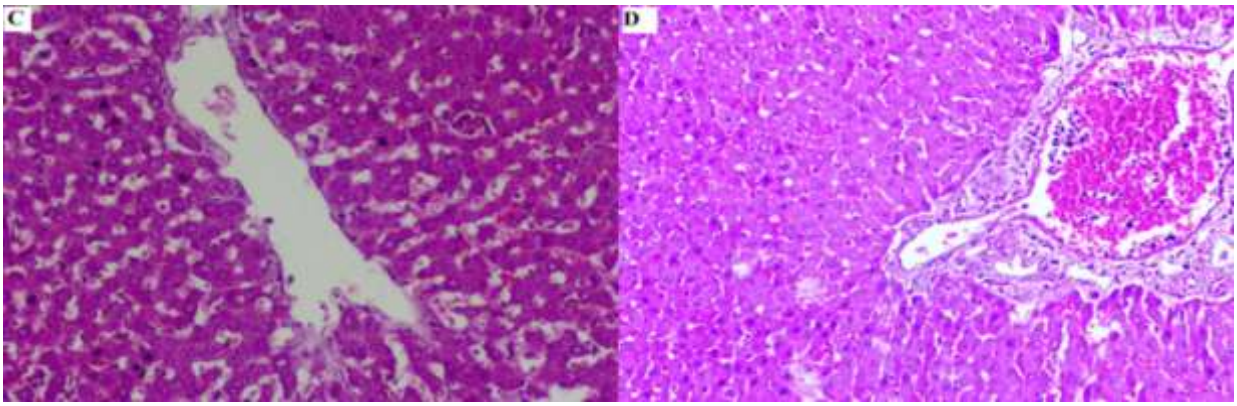

Figure 5: Effect of thetran vidhai kudineer on major organs- C, D- Liver of Group-I and Group-IV

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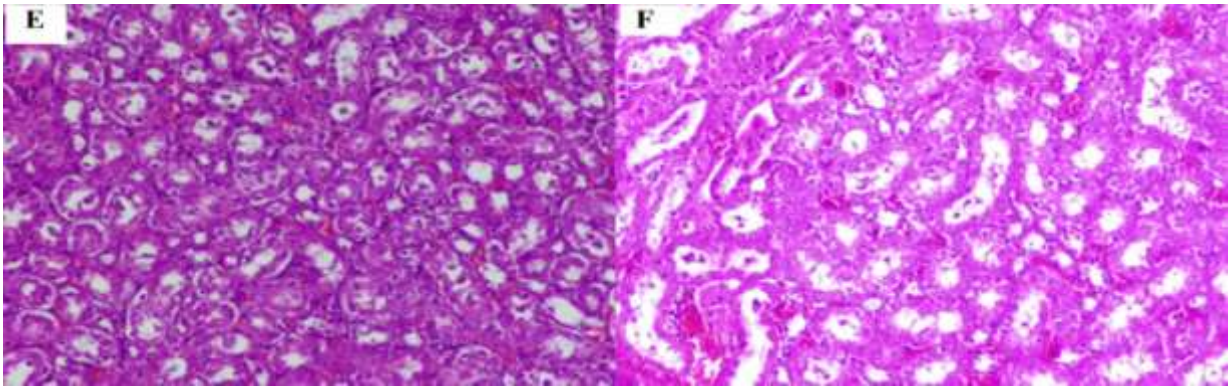

Figure 6: Effect of thetran vidhai kudineer on major organs- E, F- Kidney of Group-I and Group-IV

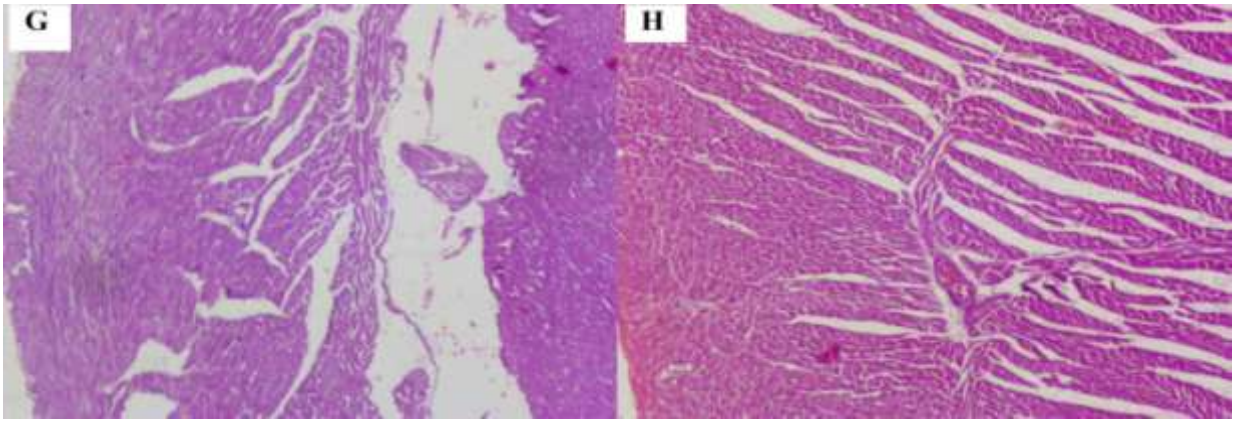

Figure 7: Effect of thetran vidhai kudineer on major organs- G, H- Heart, of Group-I and Group-IV

DISCUSSION

Nowadays toxicologist reveals that all compounds are relatively found to produce some toxicity under sufficient exposure which may vary[13]. The present study attempted to test the toxicity of one of the Siddha formulations used in the treatment of diabetes mellitus. Diabetes mellitus has long been known to be a metabolic disorder that can only be tamed with a certain specific class of drugs however since time immemorial the traditional systems of medicines have acknowledged the existence of this disorder and have been professing cures for this disorder by employing herbal formulations. Siddha system of medicine, a traditional medicinal system unique to Tamil Nadu, India has been adept in treating various metabolic disorders, diabetes in particular. Modern science considers herbal alternatives risky owing to the lack of standardization, quality control, and misidentification of herbs leading to vast damage to humans[14,15]. In our present study, the TVK was authenticated by the botanist and standardized by a chemist who Ensures the scientific evidence; hence the risk factors are considered to be absent. The formulation of TVK consists of Cassia auriculata seeds containing flavonoids, alkaloids, and glycosides. S. potatorum seeds are proclaimed to contain diaboline and brucine. T. Chebula consists of phytoconstitutents rich in tannins predominantly phenolics like ellagic acid, gallic acid (a major bioactive compound), and chebulic acid. The standardization of TVK was reported with gallic acid, chrysophanol, and emodin[16], these compounds probably exhibit the anti-diabetic property. As evidence of it, a study by Elankani et al., 2020 supported the anti-diabetic property at a dose level of 1200 mg/kg b.w showed a significant decrease in blood glucose levels of diabetes-induced rats. The body weight of the animals showed no significant gain which corroborates the good health signs. The relative organ weight is the ratio of the absolute organ weight to the fasted body weight, relative organ weight correlates with

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the normal health signs i.e. if the increase in organ weight then it would be the signs of edema, or the decrease in weight would be the alarming sign for atrophy or necrosis. In our present study, the relative organ weight of the major organs is normal between the treated groups as compared with the control group. Increased use of herbal medicines and a lack of more scientific evidence on their safety, a comprehensive evaluation of sub-chronic toxicity (90 days) need was raised[17]. Various organs such as the liver, lungs, and kidney are more commonly affected by herbal medicines. The drug-induced liver injury was found to be increasing in herbal drugs and the cause was due to the variable composition of herbs in a formulation[18]. In the present study, TVK was free from liver profile risk factors supported by the normal levels of SGOT, and SGPT in the presence of gallic acid[19, 20]. The albumin is a transport protein; levels of TVK treated groups showed a normal range compared with control rats, as evidence that the liver is free from liver damage. Histopathological findings state that the liver is free from toxicity caused by the drug and it is the major organ for metabolism and excretion[21]. TVK didn't harm the serum profile of Creatinine kinase MB levels which were further added on by the normal morphology of the heart[22, 23]. Cardiac function is also associated with lipid profile levels, an increase that leads to many etiological conditions like atherosclerosis, myocardial infarction, and arterial diseases. The evident results of serum profiles of Total cholesterol and triglycerides are free from significant changes which corroborate the healthy functioning of the heart[24]. Gallic acid plays an important role in preventing oxidative damage to RBC[25]. The hematopoietic system was free from adverse effects with supportive normal values in all analysed parameters of treated groups as compared with the control group. The kidney's role is major in the elimination, filtration, and excretion of by-products. Various renal diseases associated with traditional medicines will promote or increase the risk including acute kidney injury, tubular functional defects, chronic kidney disease, and systemic hypertension[26]. In the present study, we observed the serum levels of creatinine, urea, total protein and albumin were normal which helps in the homeostasis of the kidney. In support of these findings, the histopathology of the kidney was free from drug-related pathogenesis. Therefore, the drug had no noticeable adverse effects on renal function. We the authors believe that adverse effects of allopathic medicines are not found in TVK, and the chemical structure, and pharmacological action of gallic acid (antioxidant), chrysophanol, and emodin adhere to be free from toxic signs. The conclusive results surmised that the TVK was clear from toxic effects up to a dose of 3600 mg/kg b.w. per day on 90 days of repeated administration and no significant changes were found in clinical observations, body weight, biochemical and haematological analysis, relative organ weight, and histopathology examination. Therefore, the findings manifest that the No Observed Adverse Effect Level (NOAEL) of TVK was found to be higher than 3600 mg/kg b.w. on 90 days of repeated toxicity. In addition to it, this is the first study to evaluate the *Thetran vidhai kudineer* formulation for repeated 90 days of oral toxicity. This optimistic approach supports the non-toxic nature which can evidently act as an alternative system of medicine.

6. ACKNOWLEDGEMENT

We take this opportunity to thank all pharmacology, biochemical department staffs and administrative staffs of Siddha Central Research Institute, Chennai for their support and timely completion of studies.

7. AVAILABILITY OF DATA

All data and materials are archived in the Department of Pharmacology SCRI, Chennai

8. ETHICS COMMITTEE

All experimental procedures compliance with the Animal Ethical Committee and were approved by Institutional Animal Ethical Committee of Siddha Central Research Institute with an approval number 163/Pharma/SCRI/2017.

9. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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