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Research Article

TOXICITY STUDIES OF INDHUPPU BHAVANAI IN RODENTS

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Abstract

The aim and objective of the study was to prepare and evaluate the safety of "INDHUPPU BHAVANAI" in animal models. INDHUPPU BHAVANAI was prepared by standard operative procedure mentioned in the Siddha text. To evaluate its safety, acute and 28-days repeated oral toxicity, 90-days repeated oral toxicity, studies were performed following OECD test guidelines 423, 407,408 respectively.

In acute toxicity study, the animals (female Wistar albino rats) were treated with ***INDHUPPU BHAVANAI*** 2000mg/kg. In repeated dose toxicity study *INDHUPPU BHAVANAI* was administered at the dose of 900,1800 mg/kg body weight daily for 28 days in wistar albino rats. In 90-days repeated oral toxicity INDHUPPU BHAVANAI was administered at the dose of 180mg/kg, 900mg/kg,1800 mg/kg body weight daily for 90 days in Wistar albino rats. In acute oral toxicity study, there were no abnormal signs and behavioral changes in rats upto the dose level of 2000 mg/kg body weight administered orally. No mortality was observed in all groups. No abnormalities were reported by observation and necropsy examination done at the dose levels of 5, 50,300 mg/kg b.w and 2000 mg/kg b.w. All the vital organs were normal.

In 28 days Repeated oral toxicity and 90 days Repeated oral toxicity study there were no significant changes in body weight, food and water intake, hematological parameters, renal parameters, Liver function test , Lipid profile and blood glucose level. The experimental animals were sacrificed by excessive anesthesia and blood samples were collected and sent for investigation. The organs were collected and sent for histopathology study. It revealed the organs such as heart, kidney, liver, spleen, brain was normal in Control, Mid dose and High dose.

Thus the toxicological study of the test drug greatly establishes the safety and gives the justification for long time administration.

Keywords: Indhuppu bhavanai, Acute toxicity, Sub-acute toxicity study.

Introduction

The Siddha system dates back to 5000 B.C. Siddha medicine has 32 types of internal and 32 types of external medicine. Bhavanai is one of the internal medicines which lacks scientific validation. **Bhavanai**[(1)] is a process in which powders are soaked in various fluids, such as the expressed juice of herbs, decoctions etc., and then dried. For this purpose the quantity of juice added to the powder should be sufficient to cover it. The mixture is then allowed to dry in a shaded place. This process is repeated twice or thrice or as many times, as in necessary.

The current research was on ***INDHUPPU BHAVANAI*** to evaluate the safety and pharmacological activity in animal models which was not evaluated so far. The drug *INDHUPPU BHAVANAI* indicated for Kuttam, Kiranthi, Kodiya Viranangal which was selected from the Siddha literature[2].

Materials and Methods

Collection and authentication of raw drug:

All plant materials were freshly collected from Tambaram sanatorium, Tamilnadu. *Indhuppu* was procured from a well reputed country shop in Parrys, Chennai. The raw drugs were procured from raw drug store in Chennai. All the plant meterials were identified and authenticated by the Botanist, National Institute of Siddha, Tambaram Sanatorium, Chennai. Mineral drug was authenticated by the chemist in Central Research Institute of Siddha, Arumbakkam,Chennai. *Indhuppu* was purified and the medicine was prepared in the Gunapadam laboratory of National Institute of Siddha.

Preparation of the medicine[2]:

The Ingredients of *Indhuppu Bhavanai*[2]

Purified Indhuppu *(Sodium chloride impura*)
: 5 palam (175 g)
Vallarai leaf juice *(Centella asiatica)*
: Required volume.
Lemon pulp juice *(Citrus limon)*
: Required volume
Purified fresh Ginger juice *(Zingiber officinale)*
: Required volume
Nellikai pulp juice *(Phyllanthus emblica*
: Required volume.

Purification process:

Indhuppu[3]

Indhuppu was dissolved in vinegar and kept for 3 days and dried in sunlight.

Preparation of the drug [2]

Indhuppu was placed in *Kalvam* and powdered. *Vallarai* juice was poured up to the level when *Indhuppu* got immersed. Then it was ground slowly till it loses moisture content and became dry and the same process was repeated for 7 times. Then the above mentioned process was carried out with the following juices respectively. Lemon juice, Ginger juice, Goose berry juice. Finally the powder was dried & preserved.

Preparation of drug and stock solution[4]:

The suspension of siddha drug *INDHUPPU BHAVANAI* in 2% (w/v) CMC was prepared for oral administration by gastric intubation method.

Experimental animals:

Healthy female Wistar Albino rats of either sex weighing (150-200 g) were procured from animal housing facility, K.K College of pharmacy, Gerugambakkam, Chennai. All animals were placed in a polypropylene cages in a controlled room temperature 24°C±1°C and relative humidity of 60-70 % in animal house. The animals were maintained in standard pellet diet and water ad libitum. They were acclimatized to laboratory condition for seven days before commencement of the experiment.

All the protocols and the experiments conducted in strict compliance according to ethical principles and guidelines provided by committee for the purpose of control and Supervision of Experiments on Animals.

Experiment procedure:

Acute toxicity study[5]:

Acute toxicity study was carried out according to the OECD (Organization of Economic Co-operation and Development) guidelines 423.

Administration of the drug:

INDHUPPU BHAVANAI was administered as a single oral dose by gavage using a feeding needle. Animals were fasted prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. After the substance has been administered, food was withheld for further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously observed as per the guideline after the drug administration.

An oral (p.o) dose of 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg was administered step by step according to the guidelines. The general behaviors of the rats were continuously monitored for 1 h after dosing, periodically during the first 24 h (with special attention given during the first 4 hours and then daily thereafter, for a total of 14 days. Changes in the normal psychomotor activity and external morphology and their body weights were monitored periodically before dosing and the time at which signs of toxicity or mortality were recorded.

The visual observations included skin changes, morbidity, aggressiveness, sensitivity to sound and pain, as well as respiratory movements were recorded. They were deprived of food, but not water 12 h prior to the administration of the test substance. Finally, the number of survivors was noted after 24 h and these animals were then maintained for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Repeated dose oral toxicity- 28 days[6]:

Repeated dose oral toxicity- 28 days study was carried out according to OECD GUIDELINES 407.

Randomization, Numbering and Grouping of Animals:

Ten Rats (5 Male and 5 Female) in each group randomly divided into three groups for dosing up to 28 days. Animal's acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was fur marked with picric acid. The female were nulliparous and non-pregnant.

Sub-acute toxicity study was carried out according to OECD 407 and rats were divided into 3 groups of 10 animals (5 male and 5 female). *INDHUPPU BHAVANAI* was administered to rats at the dose of 900mg /kg and 1800mg/kg continuously for 28 days. The animals were observed daily for gross behavioral changes and other signs of toxicity. The weight of the each rat was recorded on day 0 and weekly throughout the course of the study, Food and water consumption per rat was calculated. At the end of 28 days they were fasted overnight ,each animal were anaesthetized with diethyl ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of haematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

Observations:

Experimental animals were kept under observation throughout the course of study for the following:

Body Weight:

Weight of each rat was recorded on day 0 at weekly intervals throughout the course of study and at termination to calculate relative organ weights. From the data, group mean body weights and percent body weight gain were calculated

Food and water Consumption:

The quantity of food consumed by groups consisting of ten animals for different doses was recorded at weekly interval. Food consumed per animal was calculated for control and the treated dose groups.

Clinical signs:

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

Mortality:

All animals were observed twice daily for mortality during entire course of study.

Laboratory investigation:

Following laboratory investigations were carried out on day 29 in animals fasted over-night. On 29th day, the animals were fasted for approximately 18 h, then anesthetized with ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of haematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

Haematological Investigations[7]:

Blood samples of control and experimental rats were analyzed for hemoglobin content, total red blood corpuscles (RBC), white blood corpuscles (WBC) count, Mean corpuscular volume (MCV) and packed cell volume (PCV). From the estimated values of RBC count (millions/mm3) and PCV (volumes percent), mean corpuscular volume (MCV) was calculated.

Biochemical Investigations:

Serum and Urine was used for the estimation of biochemical parameters. Samples of control and experimental rats were analyzed for protein, bilirubin, urea, uric acid, creatinine, triglyceride, cholesterol and glucose levels by using standard methods. Activities of glutamate oxaloacetate transaminase/ Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/ Alanine amino transferase (GPT/ALT) and alkaline phosphatase were estimated as per the colorimetric procedure.

Necropsy[8]:

All the animals were sacrificed on day 29. Necropsy of all animals were carried out and the weights of the organs including liver, kidneys, adrenals, spleen, brain, heart, uterus and testes/ovaries were recorded. The relative organ weight of each animal was then calculated as followed

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rats on sacrifice day (g)}} \times 100$$

Histopathology:

Histopathological investigation of the vital organs were done.The organ pieces(3-5µm thick) of the highest dose level of 1800mg /kg were preserved and were fixed in 10% formalin for 24 h and washed in running water for 24 h. Samples were dehydrated in an auto technic on and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the “L” moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin.The organs included heart, kidneys, liver ,Spleen, Brain of the animals were preserved they were subjected to histopathological examination.

Statistical analysis[9]:

Findings such as clinical signs of intoxication, body weight changes, food consumption, hematology and blood chemistry were subjected to One-way Anova Followed by Dunnet‟t‟ test using a computer software programme.(Graph Pad Prism 5.0).

Repeated dose oral toxicity-90 days[10]:

Repeated dose oral toxicity- 90 days study was carried out according to OECD GUIDELINES 408.

Randomization, Numbering and Grouping of Animals:

24 Wistar Albino Rats (12M + 12F) were selected and divided into 4 groups. Each group consist of 6 animals (Male -3, and Female-3) (IAEC Approval no NIS/IAEC-I/2016/01).Ist group treated as a control and other three group were treated with test drug (low, mid, high) for 90 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was marked with picric acid. The females were nulliparous and non-pregnant.

As per OECD guideline three dose levels were selected for the study. They were low dose (X), mid dose dose (5X), high dose (10X). X is calculated by multiplying the therapeutic dose and the body surface area of the rat (0.018). i.e X dose is 180 mg/kg, 5X dose is900 mg/kg,10X dose is 1800mg/kg.

Preparation and Administration of Dose:

INDHUPPU BHAVANAI was suspended with distilled water to obtain concentrations of 200mg/ml. It was administered to animals at the dose levels of X, 5X, 10X. The test substance suspensions were freshly prepared every two days once for 90 days. The control animals were administered vehicle only. The drug was administered orally by using oral gavage once daily for 90 consecutive days.

Observations:

Experimental animals were kept under observation throughout the course of study for the following:

Body Weight:

Weight of each rat was recorded on day 0, at weekly intervals throughout the course of study. (Table -20)

Clinical signs:

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

Mortality:

All animals were observed twice daily for mortality during entire course of study.

Laboratory Investigations:

Following laboratory investigations were carried out on day 91 in animals‟fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Bio chemistry and potassium EDTA (1.5 mg/ml) for Haematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes.

Haematological Investigations[8]:

Haematological parameters were determined using Haematology analyzer.

Biochemical Investigations:

Biochemical parameters were determined using auto-analyzer.

Histopathology[7]:

Control and highest dose group animals will be initially subjected to histopathological investigations. If any abnormality found in the highest dose group than the low, then the mid dose group will also be examined. Organs will be collected from all animals and preserved in 10% buffered neutral formalin for 24 h and washed in running water for 24 h. The organ sliced 5 or 6µm sections and were dehydrated in an auto technic on and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the “L” moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin.

Statistical analysis[9]:

Findings such as clinical signs of intoxication, body weight changes, haematology and blood chemistry were subjected to One-way ANOVA followed by dunnet"t"test using computer software programme -INSTAT-V3 version.

Results and Discussion

In acute toxicity study, the animals treated with 2000mg/kg were showed tolerance with no toxic signs were shown in the **Table 1A.**

Acute oral toxicity study of *Indhuppu bhavanai* *

Table 1A: Dose finding experiment and its behavioural Signs of Toxicity in wistar albino rats

No	Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	5	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	50	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	300	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	2000	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

1. Alertness 2.Aggressiveness 3.Pile erection 4.Grooming 5.Gripping 6.Touch Response
7. Decreased Motor activity 8. Tremors 9.Convulsions 10.Muscle Spasm 11.catatonia
12. Muscle relaxant 13. Hypnosis 14.Analgesia 15.Lacrimation 16.Exophthalmos
17. Diarrhoea 18. Writhing 19.Respiration 20.Mortality

+Presence of Activity -Absence of Activity

Sub-acute oral toxicity repeated dose of ***INDHUPPU BHAVANAI*** on rats were conducted. All animals from the treated dose survived throughout the dosing period of 28 days. Various parameters were studied and the interpretation of the study result is discussed below. No abnormal behavioural signs were observed during the study period. The test drug "*INDHUPPU BHAVANAI*" did not cause any mortality in mid and high dose levels and were considered as safe dose levels. The result of the body weight of rats exposed to control and the trial drug of different dose groups exhibited overall mild weight gain throughout the dosing period of 28 days. The quantity of food taken by the animals from different dose groups and the control is comparably normal.

Haematological Investigation:

The haematological investigation results of the rats conducted on 28th day after the repeated dose of the drug revealed the values of different parameters. The increase and decrease in the values obtained were all within the normal biological and laboratory limits were shown in the table 1

Table 1: Hematological parameters after 28 days treatment with *Indhuppu Bhavanai* in rats

Parameters	Control	MD dose	HD dose
Total RBC(10^6mm^{-3})	9.08±0.03	9.09±0.02	9.11±0.02
HB (g/dl)	14.40±0.18	14.60±0.32	14.63±0.29
Leukocyte(10^3mm^{-3})	12.33±0.22	12.13±0.12	12.16±0.15
Platelets lakhs/µl	315.81±.4.43	320.83±0.98	325.30±3.18
MCV%ft	51.01±0.76	52.62±1.22	52.21±1.18
PCV%	39.13±0.12	39.24±0.37	39.45±1.22

Values are mean of a 10 animals ± S.E.M (Dunnet's test)* $p<0.05$;**$p<0.01$.N=10

Biochemical Investigation :

The biochemical investigations were conducted on 28th day and the result is produced. The results revealed there is no significant changes in the values of different parameters with that of the control. Urine analysis were also done All the values were within the normal biological and laboratory limits were shown in the table 2,3,4.

Table 2: Effect of treatment with *Indhuppu Bhavanai* on biochemical parameters

Dose(mg/kg)	Control	MD dose	HD dose
Total Bilirubin(mg/dl)	0.3013±0.01	0.3015±0.01	0.27±0.01
Bilirubin direct(mg/dl)	0.2±0.02	0.16±0.02	0.18±0.02
Bilirubin indirect(mg/dl)	0.1±0.02	0.14±0.01	0.1±0.01
ALP(IU/L)	80.0±0.89	82.0±1.17	83.0±1.51
SGOT(IU/L)	52.16±0.75	54.42±0.84	54.07±0.63
SGPT(IU/L)	25.01±0.16	23.41±0.29	24.26±1.60
Total protein(g/dl)	6.24±0.10	6.30±0.10	6.24±0.10
Albumin(g/dl)	3.04±0.04	3.04±0.04	3.06±0.04
Globulin(g/dl)	5.023±0.03	5.29±0.01	5.15±0.04

Values are mean of 10 animals ± S.E.M (Dunnet's test)* p<0.05 ;**p<0.01.N=10

Table 3: Effect of *Indhuppu bhavanai* on Renal function test

Dose (mg/kg)	Control	MD dose	HD dose
Urea(mg/dl)	17.42±0.10	17.23±0.11	17.71±0.11
Creatinine(mg/dl)	0.67±0.01	0.70±0.02	0.72±0.02
Uric acid(mg/dl)	1.4±0.02	1.4±0.02	1.47±0.04
Na m.mol	145.91±0.55	146.50±0.55	146.66±0.52
K m.mol	6.11±0.01	6.16±0.02	6.46±0.20
Cl m.mol	98.20±0.10	100.20±0.10	99.68±1.10

Values are mean of 10 animals ± S.E.M (Dunnet's test) *p<0.05 ;**p<0.01.N=10

Table 4: Lipid Profile

Parameters	Control	MD dose	HD dose
Total cholesterol (mg/kg)	34.86±0.39	34.50±0.38	35.01±.0.35
HDL(mg/dl)	12.05±0.02	12.2±0.05	12.17±0.09
LDL(mg/dl)	35.83±0.42	36.73±0.36	37.18±0.70
VLDL (mg/dl)	15.80±0.03	15.81±0.02	15.75±0.04
Triglycerides(mg/kg)	78.33±0.52	79.0±1.26	81 ±0.82
TC/HDL ratio (g/dl)	3.41±0.04	3.36±0.04	3.22±0.03
Blood glucose (mg/dl)	121.90±0.66	121.84±0.82	121.98±0.36

Values are mean of 10 animals ± S.E.M (Dunnet's test) *p<0.05 ;**p<0.01.N=10

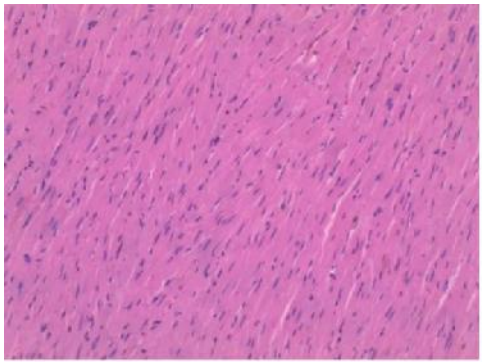
Histopathology:

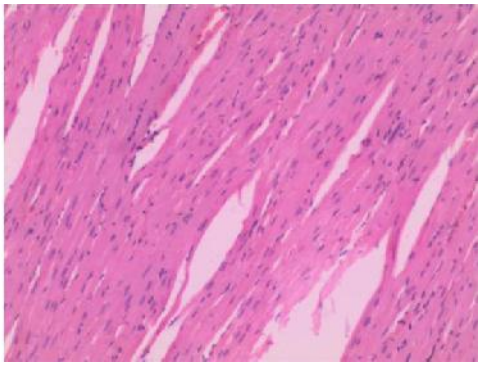
The histopathological study of the organs such as heart, liver, spleen, kidney and Brain were normal in control, and all test groups were shown in the figure (1)

Histopathological studies of various organs after the repeated dose 28 days oral toxicity study of *Indhuppu Bhavanai* in wistar albino rats

Heart

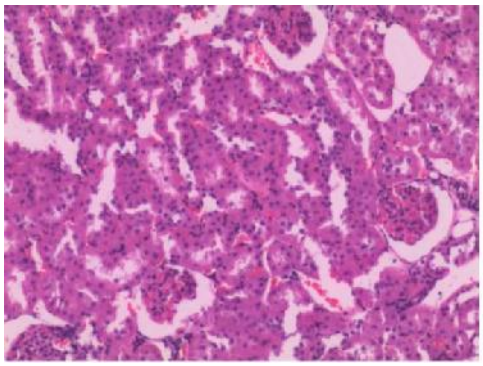
Control **High dose(1800mg/kg)**



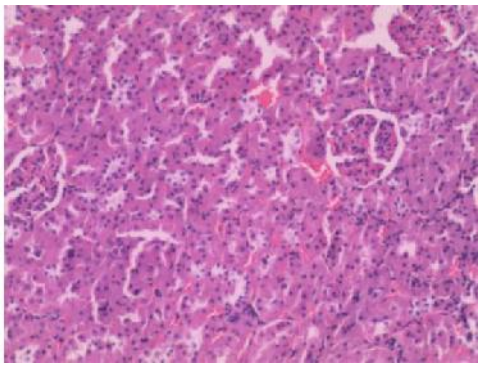


Kidney

Control

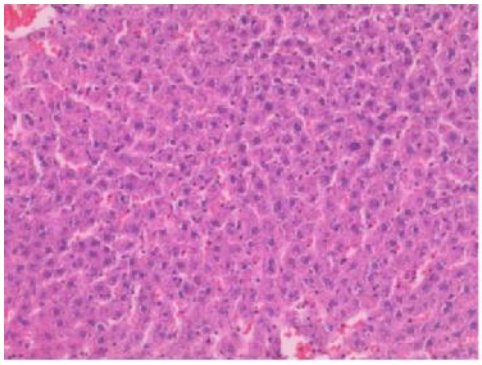


High dose (1800mg/kg)

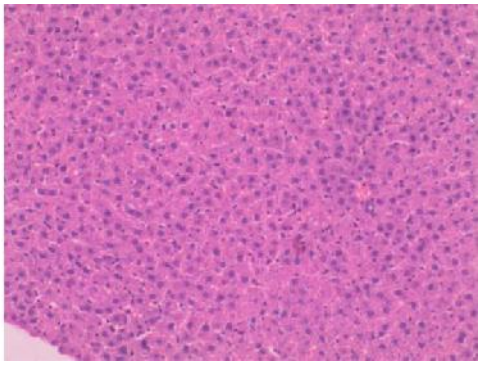


Liver

Control



High dose (1800mg/kg)



Repeated dose 90 days oral toxicity study :

Repeated dose 90 days oral toxicity study of *INDHUPPU BHAVANAI* on rats were conducted. All animals from the treated dose survived throughout the dosing period of 90 days. No abnormal behavioural signs were observed during the study period. The result of the body weight of rats exposed to control and the trial drug of different dose groups exhibited over all mild weight gain throughout the dosing period of 90 days were shown in the table 5.

Table 5: Body wt of wistar albino rats exposed to *Indhuppu bhavanai* for 90 days

DAYS	Weight(gms)/Days				P value (p)*
	Control	Low dose	Mid dose	High dose	
1	161.6±33.68	148.1 ± 1.11	156.6± 13.57	159.5±28.75	NS
15	172.8 ± 28.87	162.5 ± 21.71	166.7 ± 29.01	166.8 ± 32.13	NS
30	181.8 ± 28.31	175.71 ± 14.88	181.8 ± 32.11	181± 28.94	NS
45	204.5± 27.73	194.5± 29.76	204.7± 19.75	208.3± 22.75	NS
60	218.6±33.68	214.6±23.68	226.6±33.68	215.6±23.78	NS
75	236.8 ± 26.85	226.8 ± 28.87	241.8± 28.87	224.8 ±26.87	NS
90	248.5± 27.32	239.5± 27.68	251.4± 27.32	236.3±36.32	NS

NS- Not Significant, **(p < 0.01),*(p <0.05), n = 6 values are mean ± S.D (One way ANOVA followed by Dunnett's test

Haematological investigation interpretation

The haematological investigation results of the rats conducted on 90 th day after the repeated dose of the drug revealed the values of different parameters. The increase and decrease in the values obtained were all within the normal biological and laboratory limits were shown in the table 6.

Table 6: Haematological parameters of Wistar albino rats group exposed to *Indhuppu bhavanai* for 90 days

Category	Control	Low dose	Mid dose	High dose	P value (p)*
Haemoglobin(g/dl)	12.53±0.31	12.7±0.59	12.63±0.45	12.78±0.67	N.S
Total WBC (cells/cu.mm)	11.2±4.02	11±0.54	11.15±5.12	11.4±6.37	N.S
Platelets lkhs/µl	351±.21	367±25	387.05±26	36±33	N.S
Total RBC (cells/cu.mm)	7.25±0.3	7.08±0.3	7.13±0.2	7.16±0.3	N.S
PCV%	37.59±0.95	38.29±0.18	37.9±1.34	38.36±2.05	N.S
MCHC g/dl	30±3.03	33.66±1.86	31±1.22	30.5±0.07	N.S
MCV%ft	92.16±2.62	91.3±3.75	92.3±1.98	91.5±1.89	N.S
MCH pg	30± 3.03	32.75±1.86	31±1.22	30.5±0.07	N.S

N.S- Not Significant, **(p < 0.01), *(p <0.05), n = 6 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Biochemical investigation interpretation

The biochemical investigations were conducted on 91st day and the result is produced. The results revealed there is no significant changes in the values of different parameters with that of the control. All the values were within the normal biological and laboratory limits were shown in the table 7,8,9,10.

Table 7: Blood sugar test of Wistar albino rats group exposed to *Indhuppu bhavanai* for 90 days

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
Bl.sugar (mg/dl)	118.30±8	126.5±6.28	112.6±16.1	121±20.9	N.S.

N.S- Not Significant, **(p < 0.01), *(p <0.05), n = 6 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Table 8: Lipid profile test of Wistar albino rats group exposed to *Indhuppu bhavanai* for 90 days

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
Sr.TC(mg/dl)	108.±8.3	114.83±12.93	115.6±8.28	112.83±6.76	N.S
Sr.TG(mg/dl)	129.83±2.4	136.55±9.35	4132.5±9.73	136.5±9.31	N.S
HDL (mg/dl)	38.5±1.64	41.16±4.40	42.69±2.04	42.83±4.02	N.S
LDL(mg/dl)	44.66±9.30	50.83±12.54	44.16±8.68	43.66±7.33	N.S
VLDL(mg/dl)	25.66±1.25	27.3±1.87	26.4±2.01	27.3±1.86	N.S

N.S- Not Significant, **(p < 0.01), *(p <0.05), n = 6 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Table 9: Liver Function Test of Wistar albino rats group *Indhuppu bhavanai* for 90 days

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
T.BILIRUBIN(mg/dl)	0.86±0.04	0.79±0.1	0.75±0.1	0.80±0.13	N.S
SGOT(IU/L)	21.6±5.08	22.5±9.97	27.6±9.09	26.15±2.04	N.S
SGPT(IU/L)	27±5.9	30.8±5.45	28.5±3.72	31.57±10.9	N.S
ALP (IU/L)	82.83±17.56	79.8±12.09	79±15.7	75.3±17.84	N.S

NS- Not Significant, **(p < 0.01), * (p <0.05), n = 6 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Table 10: Renal Function Test of Wistar albino rats group *Indhuppu bhavanai* for 90 days

Parameters	Control	Low dose	Mid dose	High dose
Urea (mg/dl)	32	30.16	28.3	26.5
Creatinine (mg/dl)	0.48	0.5	0.51	0.53

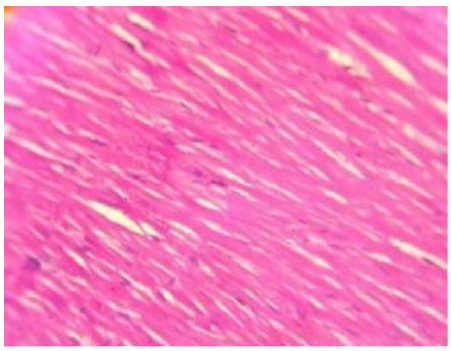
NS- Not Significant, **($p < 0.01$), * ($p < 0.05$), n = 6 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Histopathology interpretation:

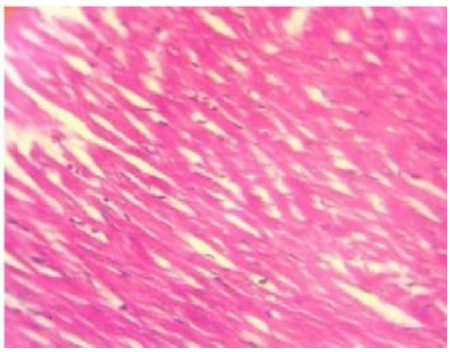
The histopathological study of the organs such as heart, liver, spleen,kidney and Brain were normal in control, and all test groups were shown in figure 2.

Histopathological of various organs after the repeated dose 28 day oral toxicity study of *indhuppu bhavanai* in wistar albino rats

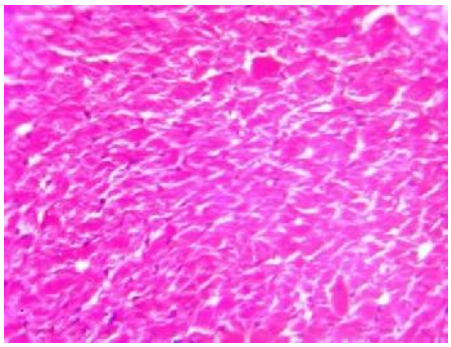
Heart



Control

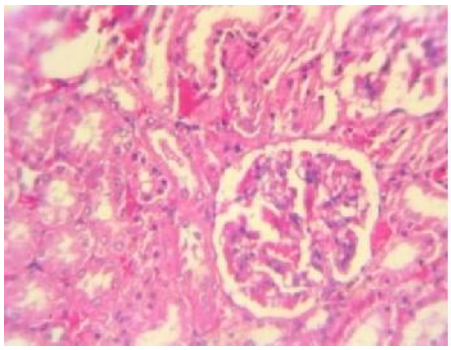


Mid dose group(900mg/kg)

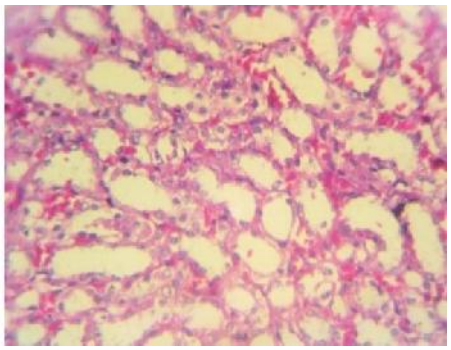


High dose group (1800mg/kg)

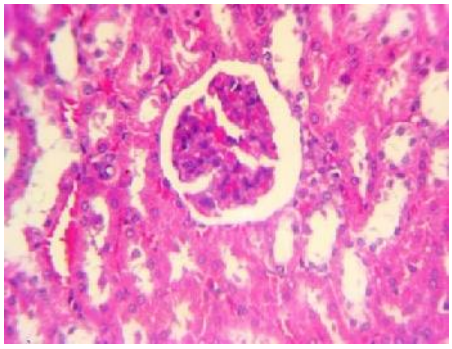
Kidney



Control

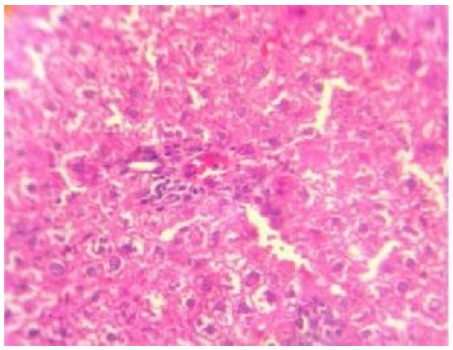


Mid dose (900mg/kg)

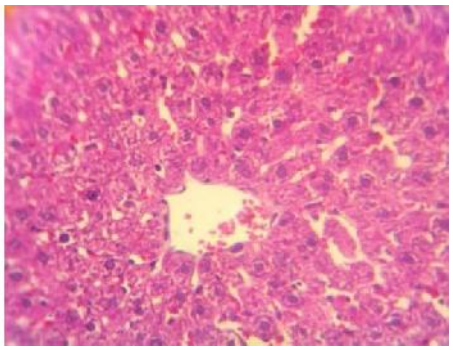


High dose (1800mg/kg)

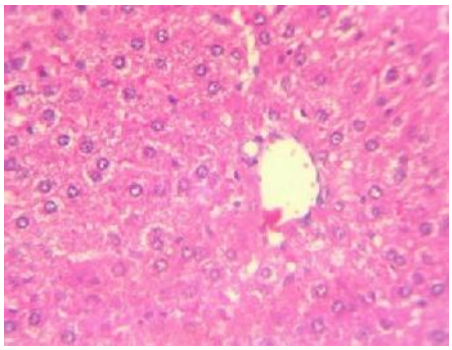
Liver



Control



Mid dose (900mg/kg)



High dose (1800mg/kg)

Conclusion:

Acute toxicity studies revealed no abnormal signs and behavioral changes in rats upto the dose level of 2000 mg/kg body weight administered orally. The haematological and bio chemical investigations were conducted after repeated oral toxicity 28 and 90 days of the drug Indhuppu Bhavanai revealed there were no significant changes in the values of different parameters with that of the control.Histopathological study of the organ such as heart, kidney, liver, spleen and Brain were normal in control and test groups. The above studies showed that the drug *INDHUPPU BHAVANAI* was safe in animal models and may be tried for further studies to establish for the clinical use.

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