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ARTICLE

Acute and Sub-Acute Toxicity Study of Hydro Alcoholic Extract of Herbal Plant Oorithal Thaamarai Chooranam in Wistar Albino Rats Model

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Abstract

Background: Oorithal thaamarai chooranam is indicated for diabetic patients, it has been screened for toxic effects according to OECD guidelines. The acute toxicity study of Oorithal thaamarai chooranam (OTC) was administered orally in single dose of 5,50,300,1000 and 2000 mg/kg of body weight to the rats. The sub-acute toxicity studies was carried in different doses of Oorithal thaamarai chooranam (OTC)(5, 50, 300, 1000 and 2000mg/kg of body weight) were administered orally to the rats once daily for 28 days. Animals were observed for physiological and behavioural responses, mortality, food and water intake and body weight changes. All the animals were sacrificed on 29^{th} day and changes were noted. No mortality observed up to 2000mg/kg body weight of Oorithal thaamarai chooranam(OTC).. Daily administration of Oorithal thaamarai chooranam (OTC) did not result in any changes in body weight,behaviour and histology of different organs. According to these results Oorithal thaamarai chooranam (OTC) could be concluded as no observed adverse effect level (NOAEL). It also showed the safety of the drug which provided its utility in long time administration without any harm to the human being.

Aim: To carried out the acute and sub-acute toxicity evaluation of hydro alcoholic extract of Oorithalthaamarai chooranam (OTC) in Wistar albino rats.

Study design: Observational in-vivo study

Place and duration of study: Animal house,Dept. of Pharmacology, Arulmigu Kalasalingam College of Pharmacy, Krishnankoil, Srivilliputtur,Tamilnadu.

Materials and methods: Dried parts of Oorithal thaamari whole plant and it was dried under sunshade and then grinded in to fine powder and used for further studies.

Results: It is proved from this evaluation that the herbal siddha drug Oorithal thaamari plant has no adverse effect, mortality and morbidity in wistar rats.

Keywords: Toxicity studies, adverse effect, mortality, morbidity and diabetic.

1 | INTRODUCTION

The Plant medicines are in great demand both in the developed as well as developing countries for primary health care, because of their wide range of biological and medicinal activities ,higher safety margin and low cost toxicity studies reveal some of the risks in case of administration of medicine such as adverse effects, null effects etc. The primary aim of toxicological assessment of any herbal medicine is to identify adverse effect and to determine limits of exposure level at which such effects occur. Oorithal thaamarai chooranam (OTC) is a single herbal siddha formulation contains dried whole plant of Ionidium suffruticosum Linn. The plant powdered has been screened for toxic effects according to OECD guidelines.

Toxicity study: The experimental study was permitted by the Institutional Ethical Committee(IAEC) and CPCSEA (AKCP/IAEC/38/20-21 dated 25.11.2020) of Arulmigu Kalasalingam college of pharmacy, Virudhunagar dt.Tamilnadu.

Acute toxicity study: The test drug Oorithal thaamarai chooranam (OTC) was administered at 5 mg/kg, 50 mg/kg, 300 mg/kg, 1000 mg/kg, and 2000 mg/kg body weight of animal respectively. The results were recorded on day 0, with single oral dosing period of 14 days. A total of 15 Female Wister Rats (100 ± 150) with an approximate age of 6 weeks and purchased from CAP LABS Nagerkovil. The animals were housed in metabolic cages (55 x 32.7 x 19 cm), with sawdust litter, in such a way that each cage contained a maximum of 3 animals of the same sex.

2 | ADMINISTRATION ROUTE AND PROCEDURE

The Female Wister Rats belonging to the control group were treated aqueous extract of OTC. The animals were weighed and then drug was administered orally as single dose using a needle fitted onto a disposable syringe of approximate size at the following different doses (Table no.1).

Table — 1 Animal Dose Level

GROUP	DOSE
GROUP	DOSE
Group-I	5 mg/kg
Group-II	50 mg/kg
Group-III	300 mg/kg
Group-IV	1000 mg/kg
Group-V	2000 mg/kg

The test item was administered as single dose. After single dose administration period, all animals were observed for 14days.

2.1 | Observation period

All animals were observed for any abnormal clinical signs and behavioural changes. The appearance, change and disappearance of these clinical signs, if any, were recorded for approximately 1.0, 3.0 and 4.0 hours post-dose on day of dosing and once daily thereafter for14 days. Animals in pain or showing severe signs of distress were humanely killed. The cage side observation was included changes in skin, fur, eyes and mucous membranes, occurence of secretions and excretions. Autonomic activity like lacrimation, piloerection, pupil size and unusual respiratory pattern, changes in gait, posture, response to handling, presence of clonic or tonic movements, stereotypes like excessive grooming and repetitive circling or bizarre behaviour like self-mutilation, walking backwards etc were observed. At the 14th

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day, sensory reactivity to stimuli of different types (e.g. auditory, visual and proprioceptive stimuli) was conducted. Auditory stimuli responses were measured by clicker sound from approximately 30 cm to the rats; visual stimuli response were measured with the help of shining pen light in the eye of rats and placing a blunt object near to the eye of rats. Response to proprioceptive stimuli was measured by placing anterior/dorsal surface of animals paw to the table edge. The responses of reactions for these three exercises were normal in animals belonging to both the controls as well as drug treatment dose groups.

2.2 | Study Design and Controls

1. Female Wister Rats in controlled age and body weight were selected.

2. The test drug Oorithal thaamarai chooranam (OTC) was administered at 5 mg/kg, 50 mg/kg, 300 mg/kg, 1000 mg/kg, and 2000 mg/kg body weight of animal as suspension along with water.

3. The results were recorded on day 0, with single oral dosing period of 14 days.

A total of 15 Female Wister Rats with an approximate age of 6 weeks and purchased from CAP LABS Nagerkovil. On their arrival a sample of animals was chosen at random and weighed to ensure compliance with the age requested. The mean weights of Female Wister Rats were 100-150 g respectively. The animals were housed in metabolic cages (55 x 32.7 x 19 cm), with sawdust litter, in such a way that each cage contained a maximum of 3 animals of the same sex.

2.3 | Mortality and Morbidity

All animals were observed daily once for mortality and morbidity at approximately 1.0, 3.0 and 4.0 hours post dose on day of dosing and twice daily (morning and afternoon) thereafter for 14 days.

3 | EFFECT OF ACUTE TOXICITY STUDY (14 DAYS) OORITHAL THAAMARAI (OTC)

Table no –2 Physical and behavioural examinations.

Group no.	Dose(mg/kg)	Observation sign	No. of animal affected.
Group-I	5mg/kg	Normal	0 of 3
Group- II	50mg/kg	Normal	0 of 3
Group-III	300mg/kg	Normal	0 of 3
Group-IV	1000mg/kg	Normal	0 of 3
Group-V	2000mg/kg	Normal	0 of 3

As per table no-2Statistical significance (p) calculated by one way ANOVA followed by Dennett's (n=6); ^{ns} p >0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Data obtained in this study indicated p >0.05 no significance physical and behavioral signs of any toxicity due to administration of OTC at the doses of 5mg/kg, 50mg/kg, 300mg/kg, 1000mg/kg and 2000mg/kg to rats.

Table no: 3 Home cage activity

Functional and Behavioural observation	Observation	5mg/kg Group (G-I)	50mg/kg (G-II)	300mg/kg (G-III)	1000mg/kg (G-IV)	2000mg/kg (G-V)
		Female n=3	Female n=3	Female n=3	Female n=3	Female n=3
Body position	Normal	3	3	3	3	3
Respiration	Normal	3	3	3	3	3
Clinic involuntary Movement	Normal	3	3	3	3	3
Tonic involuntary Movement	Normal	3	3	3	3	3
Palpebral closure	Normal	3	3	3	3	3
Approach response	Normal	3	3	3	3	3
Touch response	Normal	3	3	3	3	3
Pinna reflex	Normal	3	3	3	3	3
Tail pinch response	Normal	3	3	3	3	3

As per table no:3 it was found that Statistically significance (p) calculated by one way ANOVA followed by Dennett's (n=6); ^{ns}p >0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Dataobtained in this study indicated ^{ns}p >0.05 05 no significance changes in Home cage activity, signs of any toxicity due to administration of OTC at the doses of 5mg/kg, 50mg/kg , 300mg/kg, 1000mg/kg and 2000mg/kg to rats.

Table no-4 Hand held observation

Functional and Behavioural observation	Observation	Control	5 mg/kg (G-I)	50 mg/kg (G-II)	300mg/kg (G-III)	1000mg/kg (G-IV)	2000mg/kg (G-V)
		Female n=3	Female n=3	Female n=3	Female n=3	Female n=3	Female n=3
Reactivity	Normal	3	3	3	3	3	3
Handling	Normal	3	3	3	3	3	3
Palpebral closure	Normal	3	3	3	3	3	3
Lacrimation	Normal	3	3	3	3	3	3
Salivation	Normal	3	3	3	3	3	3
Piloerection	Normal	3	3	3	3	3	3
Pupillary reflex	Normal	3	3	3	3	3	3
Abdominal tone	Normal	3	3	3	3	3	3
Limb tone	Normal	3	3	3	3	3	3

Statistical significance (p) calculated by one way ANOVA followed by Dennett's (n=6); ^{ns}p >0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Data obtained in this study indicated ^{ns}p >0.05 no significance changes in hand held observation and signs of any toxicity due to administration of OTC at the doses of 5mg/kg, 50mg/kg , 300mg/kg, 1000mg/kg and 2000mg/kg to rats

Table no-5 Effects in Mortality

Group no	Dose no(mg/kg)	Mortality
Group-I	5(mg/kg)	0 of 3
Group-II	50(mg/kg)	0 of 3
Group-III	300(mg/kg)	0 of 3
Group-IV	1000(mg/kg)	0 of 3
Group-V	2000(mg/kg)	0 of 3

Statistical significance (p) calculated by one way ANOVA followed by Dennett's (n=6); ^{ns}p >0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Data obtained in this study indicated ^{ns}p >0.05 no significance changes in hand held observation and signs of any toxicity due to administration of OTC at the doses of 5mg/kg, 50mg/kg, 300mg/kg, 1000mg/kg and 2000mg/kg to rats.

4 | SUB-ACUTE TOXICITY STUDY IN WISTER RATS TO EVALUATE TOXICITY PROFILE OF OORITHAL THAAMARAI CHOORANAM (OTC):

The study was conducted on 5 male 5 female Wister rats for each group. The body weight range was fallen within ± 20% of the mean body weight at the time of Randomization and grouping. The experimental protocol was approved by Institutional Animal Ethical Committee as per the guidance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, government of India.

Table — 6 Dose Level

TEST GROUP	CONCENTRATION/DOSE TO ANIMALS (ml/kg body-weight/day)	NUMBER OFANIMALS
Group-1	1. CONTROL	10 (5MALE and5 FEMALE)
Group-II	2. LOW DOSE OF OTC200mg/kg	10(5MALE and 5 FEMALE)
Group-III	3. MIDDLEDOSE OF OTC400mg/kg	10(5MALE and 5 FEMALE)
Group-IV	4. HIGH DOSE OF OTC600mg/kg	10(5MALE and 5 FEMALE)

The test item was administered as single dose daily. After single dose administration period, all animals were observed for 28 days.

Table: 7 Effect of Sub- Acute Dose (28 Days) of OTC on Body Weight in Gram

GROUP	CONTROL	Low 300mg/kg	Mid 1000mg/kg	High 2000mg/kg
1st day	140.06±0.80	142.15±0.80	143.80±0.80	146.75±0.84
7th day	147.13±0.72	149.21±0.73	150.29±0.82	151.75±0.84
14th day	152.11±0.69	153.17±0.80	154.98±0.84	155.81±0.76
21st day	154.03±0.76	156.09±0.707	157.42±0.83	158.95±0.71
28th day	157.15±0.77*	241.14±0.70*	159.05±0.74*	161.86±0.75*

Values are expressed as mean ± SEM Statistical-significance (p) calculated by one way ANOVA followed by Dennett's (n=6); ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

The effect of OTC was observed, on the body weight changes, significant increase (*p<0.05) in body weight in all the treated animals were observed. The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV.

Table no: 8 Effect of sub-acute dose (28 days) OT on organ weight (physical parameter) in gram

GROUP		CONTROL	LOW	MID	HIGH
HEART		0.47±0.07	0.49±0.08	0.50±0.07	0.56±0.08
LIVER		3.62±0.07	3.75±0.07	4.24±0.14	4.62±0.08
LUNGS		0.81±0.09	0.91±0.09	1.09±0.07	1.20±0.13
KIDNEY	L	0.82±0.07	0.92±0.07	1.08±0.07	1.20±0.07
	R	0.83±0.04	0.93±0.04	1.07±0.044	1.20±0.044

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by Dennett's (n=6); ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

The effects of OTC on kidney, heart, liver and lungs of the rats were recorded. p>0.05changes in the weights of various organs of the animals with higher doses of the extract but macroscopic examinations visualized no changes in colour of the organs of the treated animals compared with the control group.

Table no: 9 Effect of Sub- Acute Dose (28 Days) Of OTC on Haematological Parameters

Drug treatment	RBC 10^{12}/litre	WBC 10^{9}/litre	Haemoglobin /litre	Differential count %			
				Neutrophils	Eosinophil's	Monocyte	Lymphocyte
Control	5.10±0.07	6.86±3.40	10.22±0.07	58.02±0.07	1.80±0.08	2.17±0.07	39.25±0.08
LOW	5.20±0.06	9.90±3.23	11.20±0.08	59.72±0.16	2.20±0.07	2.19±0.02	37.41±0.07
MID	5.30±0.07	9.99±3.15	11.29±0.07	59.47±0.14	2.59±0.07	2.59±0.08	37.88±0.08
HIGH	5.70±0.07	11.43±3.45	12.22±0.07	58.28±0.65	3.2±0.06	2.60±0.07	37.04±0.06

Values are expressed as mean ± SEM Statistical-significance (p) calculated by one way ANOVA followed by Dennett's (n=6); ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groupswith control group.

The effects of **OTC** were observed for its effect on hematological parameters in experimental rat. Final study, not significant (p<0.05) in the values of treated groups. The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV.

Table: 10 Effect of Sub- Acute Dose (28 Days) of OTC on Biochemical Parameters

Drug Treatment	SGPT (U/L)	SGOT(U/L)	ALP(U/L)	Urea (mg/dl)	Creatinine(mg/dl)
Control	26.20±0.08	49.19±0.08	119.29±0.07	28.59±0.04	0.41±0.077
LOW	27.51±0.25	58.71±0.06	120.77±0.06	29.62±0.07	0.47±0.06
MID	29.62±0.55	60.84±0.67	122.16±0.72	29.74±0.49	0.61±0.08
HIGH	30.10±0.74	63.17±0.75	129.89±0.67	30.86±0.66	0.80±0.08

Values are expressed as mean ± SEM Statistical significance (p) calculated by one-way ANOVA followed by Dennett's (n=6); ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Table no: 11Effect of Sub- Acute Dose (28 Days) Of OTC on Biochemical Parameters

GROUP	CONTROL	low(300mg/kg)	mid(1000mg/kg)	high(2000mg/kg)
TOTAL BILIRUBIN (mg/dl)	0.38±0.07	0.49±0.07	0.50±0.07	0.65±0.06

Values are expressed as mean ± SEM Statistical-significance (p) calculated by one-way ANOVA followed by Dennett's (n=6); ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

The Bilirubin values in experimental rat. Final study, not significant (p<0.05) in the bilirubin values are. The values are expressed as mean ± S.E.M. n=6 was compared in Group I and other groups II, III, IV, and V.

Table: 12 Effect of Sub- Acute Dose (28 Days) of on Food Intake in Gram

GROUP	CONTROL	Low 300g	Mid 1000g	High 2000g
1st DAY	13.49±0.07	14.10±0.07	16.18±0.08	17.60±0.07
7th DAY	14.66±0.07	15.90±0.07	17.78±0.07	18.20±0.08
14th DAY	16.78±0.06	16.89±0.08	18.90±0.08	19.77±0.08
21st DAY	17.90±0.07	17.80±0.07	19.89±0.071	21.21±0.06
28th DAY	18.06±0.08	18.41±0.06	20.05±0.05	22.05±0.06

Values are expressed as mean ± SEM Statistical significance (p) calculated by one-way ANOVA followed by Dennett's (n=6); ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

The Food intake values was compared in Group I to other groups II, III, IV, and V in experimental rats. Final study, shows significantly increase in the Food intake values . The values are expressed as mean ± S.E.M. n=6.

5 | DISCUSSION

All animals from control and all the treated dose groups survived throughout the dosing period of

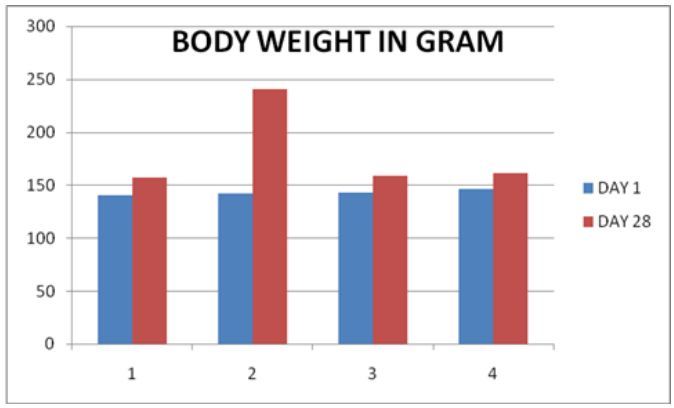


FIGURE 1:

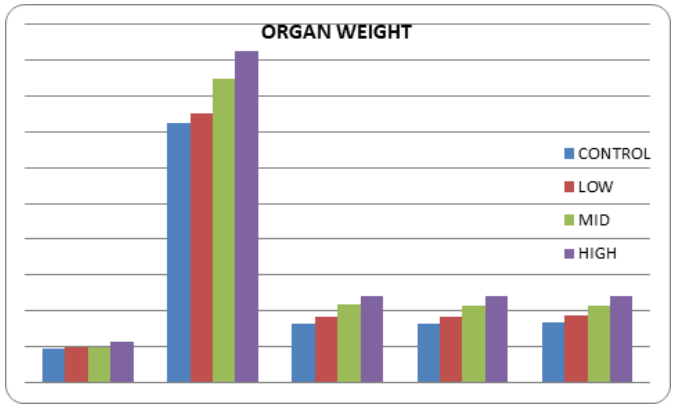


FIGURE 2:

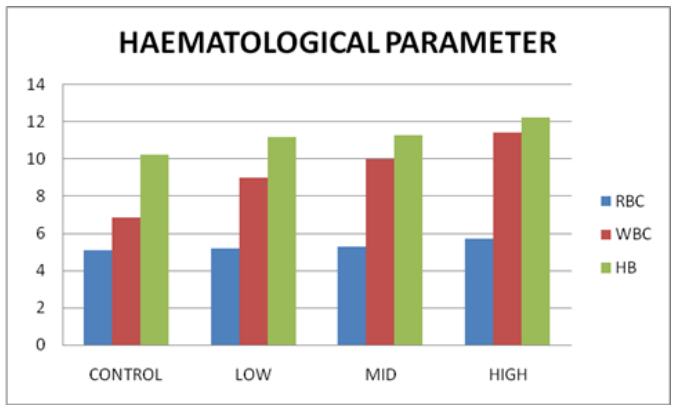
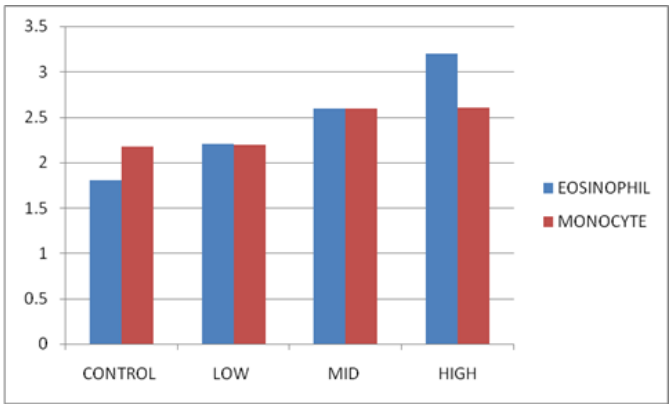


FIGURE 3:

28 days. The results of hematological investigations conducted on day 29th day revealed no significant changes in the hematological values when compared with those of respective controls. The results of Biochemical investigations conducted on days 29 and recorded in revealed the no significant changes in the values of different parameters studied when compared with those of respective controls;



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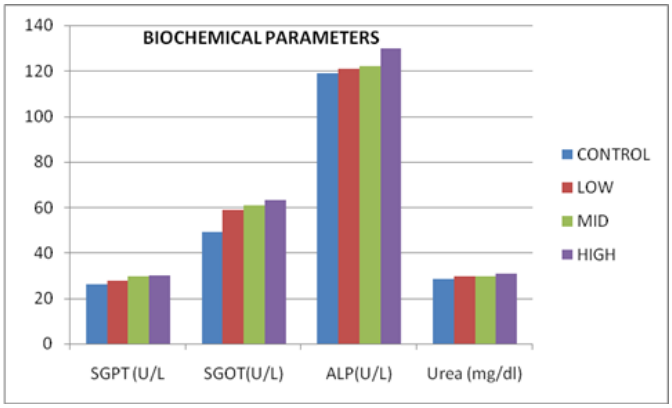

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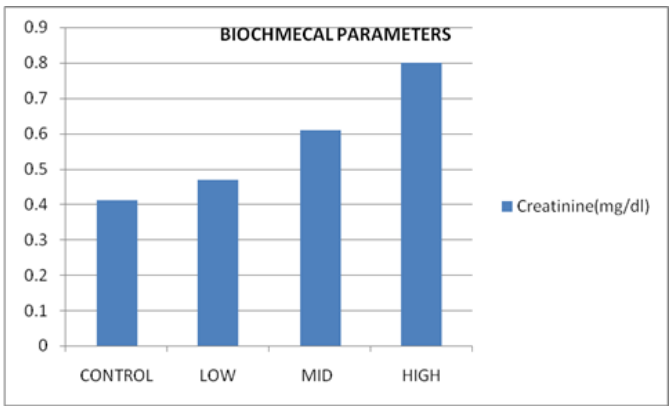
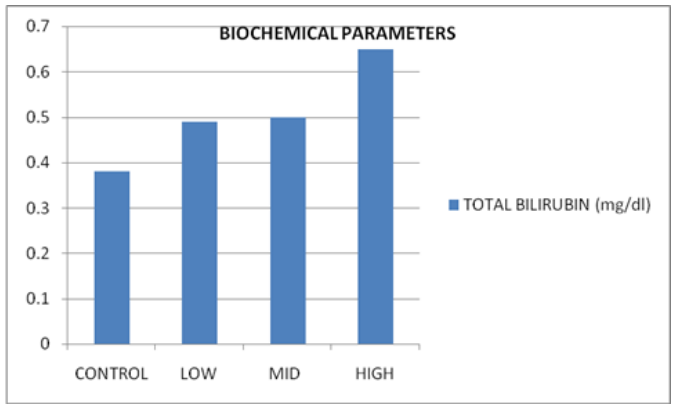


FIGURE 6:

Urea, SGOT,SGPT, Bilirubin were within the limits.. Group Mean Relative Organ Weights are recorded Comparison of organ weights of treated animals with respective control animals on day 29 was found to be normal comparable with respective control group.



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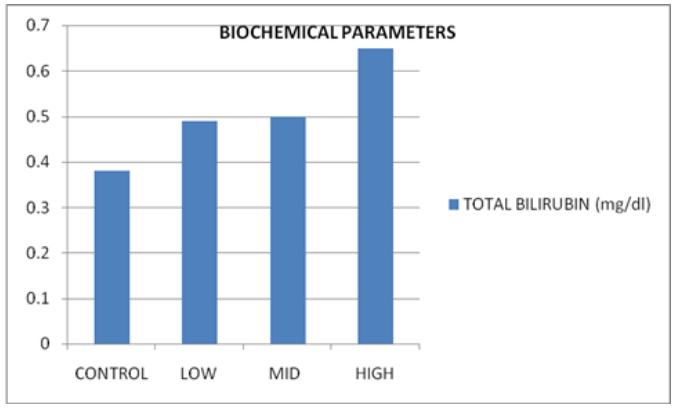

FIGURE 8:

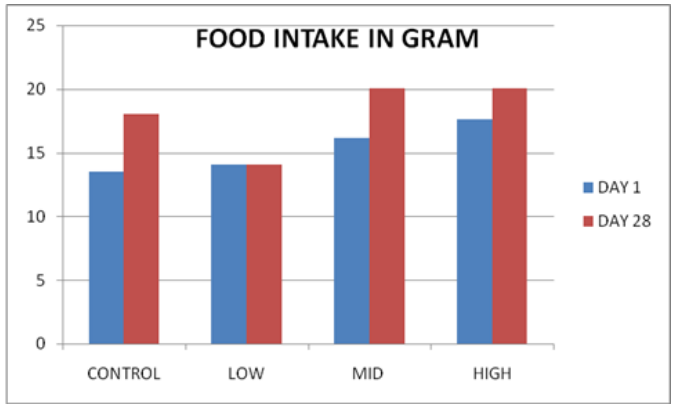

FIGURE 9:

6 | CONCLUSION

Acute and sub-acute toxicity were carried out in wister albino rats according to OECD guidelines (423) This drug has no acute toxicity as there was no mortality seen. Sub acute toxicity is carried by repeated dose of test drug for 28 days. Mortality, the functional observation, haemotological and bio-chemical investigations were done. There were no

significant changes in the biochemical and haematological profile. So the toxicological study of these test drug, OTC establishes the safety of the drug for long time administration. The study concluded that OTC is suitable for clinical use in human based on body weight.OTC is safe and can be used for the management of patients with diabetes.

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