EVALUATION OF TOXICITY STUDY OF THEHYDROALCOHOLIVEXTRACT OF CLITORIA TERNATEA TO TREAT CNS DISORDER

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Abstract:

Clitoria ternatea commonly known as butterpea belongs to the family Fabaceae.The study of toxicology is to determine the poisonous,harmful and adverse effect produced by drugs and other chemicals constituents isolated from plants sources which increases the chances of mortality or issues in general health.The present study has been pioneered to study the adverse or hazardous effect of hydroalcoholic extract of *Clitoria ternatea* and to ensure the safety of albino wister mice (180-200g) as per (OECD) OrganizationforEconomic CooperationandDevelopment guidelines 423.In the present study ,the oral dose of(50,100,250mg/kg) were administerd for four group of animals in single dose and observed for 14 days to understand the general behaviour,adverse effects and moratlaity were monitored. In acute toxicity, all treated groups revealed neither mortality nor any significant changes were observed. The result indicates that the oral administration of plant hydroalcoholic extract of C*litoria ternatea* did not produce any significant toxic effect in albino rats. Hence, the extract can be utilized safely for therapeutic use in pharmaceutical formulations.

Keywords:*Clitoria ternatea*, Fabaceae, OECD, acute toxicity, Blue Pea

Introduction:

Clitoria ternatea populary known as shankapushpi used traditionally for many ailments,Leaves,atem,Roots of clitoria ternatea are used from traditional system of medicine.The medicinal herb is used from many centurites as treatment of various disorders related to central nervous system,nootropic,antistress,anxiolytic ,antidepressant,anticonvulsant,transquilising and sedative agent.The plant has been eaten as vegetable in from ancient days to till today in India,Asia,Africa,Malaysia and other countries.[1]The bright colour of flowers and leaves has been used as colouring agent in food.Clitoria ternate medicinal her has neuropharmacological potency which is used to cure ailments in Central Nervous system.Hyderoalcoholic extract of this has beenused as main ingredient in Rasayana popularly known as Medhya Rasayana as a receipe to treat neurological disorders and are considered to raise the intellectuality. Plant is considered to generate enormous chemical constituents which produces toxic effect to bacteria and fungi ,many drug discovery process can lead to the development of new drugs plants has efficacy and potential source to play its bioactive compounds.Higher potency of safety is required for safe use of plant extract and hence preclinical and clinical toxicological evaluation of plant extracts and toxicity study of palnt extract are requires with the hydroalcoholic extract.[2]

Materials and methods:

Raw and mature C. terntaea whole plant were collected in around tamil nadu in the year 2019.The leaves were then

washed with water to remove dirt prior to the drying process (40 °C for 3 days). The authencity work was carried out by botanist Dr.Jayaramanfrom School of Biological Sciences, where the herbarium was deposited with a voucher number of ……..

Preparation of plant extracts:
The hydroalcoholic extract was prepared by maceration of dried powdered plant material in dissolved solvent for 3 days. Two hundred g of powdered leaf was macerated in 200 mL water and 200ml ethanol under stirring conditions for 72 h. The macerated extract was then filtered through No. 1 Whatman filter paper. The ethanolic crude extract was vaporized to dryness using the rotary evaporator .
Experimental animals:[3-5]

Swiss albino rats (150-180g) of male were used for the assessment of the acute oral toxicity. Animals were kept in plastic cage top with ventilated stainless steel cover and allowed to adjust to the laboratory
The sighting study was conducted as per OECD-423 guidelinesThe animals were fasted for 7 hr although still allowed free access to water before the commencement of the experiments . Doses of 1000 and 2000 mg/kg of leaf extract were given orally for each animals. The control animals received 0.9% normal saline

Acute oral main study[6-8]
The acute oral study was performed as per OECD 23 guidelines . The animals will be fasted for 7 h although still allowed free access to water before the commencement of the experiment. The animals used for the main study will be divided into 4 groups with each group containing six rats. The control group (I) received 0.9% aqueous normal saline orally in constant volume of 1 mL/kg. While the test group (C) and test group (D) will be treated with 2000 mg/kg of leaf extract. Observation of animals All animal will be observed individually after dosing at least once during the first 30 minutes periodically during the first 24 hours, with special attention given to first 4 hours and daily thereafter for a total of 14 days. Individual weight of animals will be determined shortly before the test substance is administered and at least weekly thereafter. Animal found to show sign of toxicity, severe pain or enduring signs of severe distress will be humanely killed. All test animals (including those that die during the test or removed from the study for animal welfare reasons) will be subjected for gross necropsy. The appropriate dose will then be selected and used for the main study.
Histopathological studies Slices of liver, kidney, spleen and heart were fixed in 4% formaldehyde, embedded in paraffin wax, sectioned at 8μ and stained with haematoxylin and eosin. Detailed microscopic examination was carried out in those organs of both control and treatment groups of both sexes. Data analysis The mean results of brine shrimp mortality against the logarithms of concentrations were plotted using the Microsoft Excel computer program, which also gives the regression equations. The regression equations were used to calculate LC50 value. The data for body weight and organ weight were expressed as the mean ± standard error of the mean (SEM). One way analysis of variance (ANOVA) with subsequent Dunnett's post hoc analysis was used to detect further differences between groups. Values of $p < 0.05$ were considered significant. All statistical analysis was carried out using SPSS version 15 package.

In this work, we evaluated the acute oral toxicity of hydroalcoholic extract of *C. ternatea*, employing the OECD 23 [11] guideline that allows the evaluation of lethality potential and possible toxicant effects developed in a short period of time. Extract with 50, 300, and 2000 mg/kg body weight doses were administered as recommended by OECD guidelines []. Administration of further higher doses was considered physiologically unsound and is not generally recommended. The doses tested did not cause any mortality or any signs of acute toxicity in the tested animals in short term (i.e. 48 h) and long term (i.e. 14 days) observation period. The dose did not cause any appreciable alterations in

water and food intake during 2 weeks is observed. There were no significant differences between the weekly mean body weight between control and the animals treated with extract except on day 1 (Table1). Further, body weight gain throughout the observation period among the treated animals was comparable to their respective controls. No sex-related differences were evident in either species. In addition, there were no significant differences in the mean organ weight between control and animals treated with 50, 100, and 200 mg/kg body weight (Table 2 and 3). Table 4: Water intake ml/day of Wistar albino rats group exposed to Hydroalcoholic Extract of Clitoria ternatea.Table 5: Food intake (gm/day) of Wistar albino rats group exposed to hydroalcoholic extract Clitoria ternatea., Table 6(Grouping of Animals). Table 7: Body weight of wistar albino rats group exposed to hydroalcoholic extract of Clitoria ternatea. Table 8: Water intake (ml/day) of Wistar albino rats group exposed to Hydroalcoholic extract of Clitoria ternatea. Table 9: Food intake (gm/day) of Wistar albino rats group exposed to Hydroalcoholic extract of Clitoria ternatea. Table 10: Haematological parameters of Wistar albino rats group exposed to Hydroalcoholic extract of Clitoria ternatea. Table 11 :Biochemical Parameters of Wistar albino rats group exposed to Hydroaloholic Extract of Clitoria ternatea. Table 12 Renal function test of Wistar albino rats group exposed to hydroalcoholic extract of Clitoria ternatea. Table 13: Liver Function Test of Wistar albino rats group exposed to Hydroalcoholic Clitoria ternatea

The acute toxicity study indicated that 0.9% normal saline of *C. ternatea* crude hydroalcoholic extract is not toxic when administered by the oral route to experimental animals. Therefore, the acute minimum fatal dose of *C. ternatea* hydroalcoholic extract for albino wister rats is higher than 2000 mg/kg body weight which indicates some high level of safety margin in oral formulation. Gross examination at autopsy and histopathological evaluations of the various organs stained with hematoxylin and eosin revealed no significant differences (Fig. 1; Fig. 2.,Fig. 3) for both sexes. In conclusion, the present investigation demonstrates that the hydroalcoholic extract is safe as it did not cause any lethality or changes in general behaviour. This finding justifies its wide application in various communities coupled with lack of any reported serious side effect with traditional use of the hydroalcoholic whole plant extract of clitoria ternatea.

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Results and Discussion:

Table 1: Behavioural Pattern of Wistar Albino Rats in hydroalcoholic extract of Clitoria ternatea treated with Control group treated with vehicle as water and Group-1 1000mg/kg p.o of hydroalcoholic extract of clitoria ternatea and 2000mg/kg p.o of hydroalcoholic extract

Observations of vehicle control and hydroalcoholic extract of clitoria ternatea treated groups

Parameter	**30 Minutes**			**4 Hr**			**24 Hr**			**48Hr**			**7 days**			**14 days**		
	CG	**G-1**	**G-2**	**CG**	**G-1**	**G-2**	**CG**	**G-1**	**G-2**	**CG**	**G-1**	**G-2**	**CG**	**G-1**	**G-2**	**CG**	**G-1**	**G-2**
Body weight	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
Assessments of posture	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
Signs of Convulsion Limb paralysis	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
Body tone	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
Lacrimation	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
Salivation	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
Change in skin color	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
Piloerection	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
Defecation	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
Sensitivity response	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
Locomotion	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**

Muscle gripness	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
Rearing	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑
Urination	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**

CG:Control Group

G-1:Group 1 with1000 mg/kg of ethanolic extract of Clitoria ternatea

G-2: Group 2 with 2000 mg/kg of ethanolic extract of Clitoria ternatea

Behavior:

The animals were observed closely for behaviour in the first four hours which includes abnormal gait, aggressiveness, exophthalmos, ptosis, akinesia, catalepsy, convulsion, excitation, head twitches, lacrimation, loss of corneal reflex, loss of traction, piloerection reactivity of touch, salivation, scratching, sedation, chewing, head movements, sniffing, Straub, tremor and writhes, diarrhea, leathery, sleep and coma.

Body Weight:

Individual weight of animals was determined before the test substance was administered and weights was recorded at day 1, 7, and 14 of the study. Weight changes were calculated and recorded. At the end of the test, surviving animals were weighed.

Food and water Consumption:

Food and water consumed per animal was calculated for control and the treated dose groups.

Mortality:

Animals were observed for mortality throughout the entire period.

Results:

All data were summarized in tabular form, (Table-1-4) showing for each test group the number of animals used, the number of animals displaying signs of toxicity, the number of animals found dead during the test, description of toxic symptoms, weight changes, food and water intake.

No of animals in each group:3Female Wister rats used

Table 2 (Observational study Results)

Parameter	**Control Group**	**Group-1**	**Group-2**

(+ -

		Extract of CT 1000mg/kg	**Extract of CT 2000mg/kg**
Alertness	+	+	-
Aggressiveness	-	-	-
Pile erection			-
Grooming	+	+	-
Gripping			-
Touch Response	+	+	-
Decreased Motor Activity	-	-	-
Tremors	-	-	-
. Convulsions	-	-	-
Muscle Spasm	-	-	-
Catatonia	-	-	-
Muscle relaxant	-	-	-
Hypnosis	-	-	-
Analgesia	-	-	-
Lacrimation	-	-	-
Exophthalmos	-	-	-
Diarrhea	-	-	-
Writhing	-	-	-
Respiration	-	-	-
Mortality	-	-	-

Indicates Present, - - Indicates Absent)

Table 3(Body weight Observation)

DOSE	**DAYS**		
	1	7	14

	Control Group- Normal Saline	176.21± 3.22	177.2± 4.27	179.2 ± 4.82
	Group-1 Extract of CT 1000 mg/kg	210.82±4.21	212.51±2.54	214±6.22*
N.S-	**Group-2 Extract of CT 2000 mg/kg**	172.5± 3.18	174.2± 3.26	175.4 ± 3.27* Not
	P value (p)*	NS	NS	>0.5

Significant,**(p > 0.01), *(p >0.05), n = 10 values are ± SEM (One way ANOVA followed by Dunnett's test)

Table 4: Water intake ml/day of Wistar albino rats group exposed to (Hydroalcoholic Extract of Clitoria ternatea*):*

DOSE	**DAYS**		
	1	6	14
Control Group- Normal Saline	38.7 ± 2.74	32.9± 4.33	33.4± 4.13*
Group-1 Extract of CT 1000 mg/kg	51.2±1.22	54.4±1.24	56.6±2.44*
Group-2 Extract of CT 2000 mg/kg	52.4±1.34	53.5±1.11	55. 9± 4.19*
P value (p)*	NS	NS	>0.05

N.S- Not Significant,**(p > 0.01), *(p >0.05), n = 3 values are ± SEM (One way ANOVA followed by Dunnett's test)

Table 5: Food intake (gm/day) of Wistar albino rats group exposed to hydroalcoholic extract Clitoria ternatea

DOSE	**DAYS**		
	1	7	14
Control Group-	32.56±2.16	32.92±3.26	30.92±3.26*

Normal Saline			
Group-1 Extract of CT 1000 mg/kg	42.4±7.54	44.2±6.82	48.6±8.22*
Group-2 Extract of CT 2000 mg/kg	44.12±8.64	44.31±1.22	47.22±2.24*
P value (p)*	NS	NS	>0.05

N.S- Not Significant,**(p > 0.01), *(p >0.05), n = 3 values are ± SEM (One way ANOVA followed by Dunnett's test)

Repeated dose 28-day oral toxicity study of hydroalcoholic clitoria ternatea

Test Substance : **Hydroalcoholic extract of Clitoria ternatea**

Animal Source :Faculty of Pharmacy, Dr.M.G.R.Educational and Research Institute, Velappanchavadi,Chennai-77

Animals : Wistar Albino Rats (Male -12, and Female-12)

Age **:** >6 weeks

Body Weight **:** 160-180 gm.

Acclimatization **:** Seven days prior to dose.

Veterinary examination **:** Prior and at the end of the acclimatization period.

Identification of animals **:** By cage number, animal number and individual marking by using Picric acid

Diet **:** Pellet feed supplied by Ultra scope , Biotech ,Chennai

Water **:** Aqua guard portable water in polypropylene bottles.

Housing & Environment **:** The animals were housed in Polypropylene cages provided with bedding of husk.

Housing temperature **:** between 22ºC $\pm$ 3ºC.

Relative humidity **:** between 30% and 70%,

Air changes **:** 10 to 15 per hour

Dark and light cycle **:** 12**:**12 hours.

Duration of the study **: 28 Days.**

Table 6(Grouping of Animals)

Groups	**No of Rats**
Group I control (Vehicle: Normal Saline)	6 Rats
Group II (LD) Extract of CT 50mg/kg	6 Rats
Group III (MD)Extract of CT 100 mg/kg	6 Rats
Group IV (HD) Extract of CT 200 mg/kg	6 Rats

Methodology

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. First group treated as a control and other three group were treated with test drug (low, moderate, high) for 28 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.

Justification for Dose Selection:

As per OECD guideline 407 three dose levels were selected for the study. They are low dose (50 mg/kg), moderate dose (100 mg/kg), high dose (200 mg/kg). X is calculated from 1000mg (X=50mg) , 2X mid dose is 100 mg/kg, 4X high dose is 200 mg/kg.

Preparation and Administration of Dose:

Hydroalcoholic extract of Clitoria ternatea is suspended in water, It was administered to animals at the dose levels of 50, 100 and 200 mg/kg. The test substance suspensions were freshly prepared every two days once for 28 days. The control animals were administered vehicle only. The drug was administered orally by using oral gavage once daily for 28 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.

Food and water Consumption:

Food and water 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.

Necropsy:

All the animals were sacrificed by excessive anaesthesia on day 29. Necropsy of all animals was carried out.

Laboratory Investigations:

Following laboratory investigations were carried out on day 29 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 Hematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes.

Haematological Investigations:

Haematological parameters were determined using **Haematology analyzer**.

Biochemical Investigations:

Biochemical parameters were determined using **auto-analyzer.**

Histopathology:

Control and highest dose group animals were 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 were 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 technicon 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 Hematoxylin-eosin red.

Statistical analysis:

Findings such as body weight changes, water and food consumption,hematology and blood chemistry were subjected to One-way ANOVA followed by dunnett test using a computer software programme – Graph pad version 9.3 All data were summarized in tabular form, (Table-7 to 13)

Result

Table 7: Body weight of wistar albino rats group exposed to hydroalcoholic extract of Clitoria ternatea

DOSE	**DAYS**				
	1	7	14	21	28
Group I control (Vehicle:Normal	165.6± 2.76	166.4 ± 3.42	167.7 ± 3.26	169.2 ± 3.73	170.7 ± 1.31*

Saline)					
Group II (LD) Extract of CT 50mg/kg	165.2 ± 4.12	166.7 ± 2.64	166.9± 1.51	172.9 ± 1.66	174.42± 2.76*
Group III (MD) Extract of CT 100 mg/kg	168.6± 1.24	168.9 ± 4.74	170.4 ± 8.92	171.1 ± 6.36	174.7 ± 9.12*
Group IV (HD) Extract of CT 200 mg/kg	171.4± 3.74	173.6 ± 6.32	174.6 ± 2.86	175.1± 8.82	175.92 ± 6.42*
P value (p)*	NS	NS	NS	NS	>0.05*

NS- Not Significant, **(p > 0.01),*(p >0.05), n = 6 values are ± SEM (One way ANOVA followed by Dunnett's test)

Table 8: Water intake (ml/day) of Wistar albino rats group exposed to Hydroalcoholic extract of Clitoria ternatea

DOSE	**DAYS**				
	1	6	14	21	28
Group I control (Vehicle:Normal Saline)	31.5 ± 8.95	32.0 ±6.23	28.5±6.23	29.12±8.19	31.5±3.96
Group II (LD) Extract of CT 50mg/kg	29.5±3.31	29.9±6.62	31.7±4.02	32.2±4.29	34.9±3.13
Group III (MD) Extract of CT 100 mg/kg	31.7±3.93	32.3±3.11	34.1±2.83	32.4±4.11	34.4±2.14*
Group IV (HD) Extract of CT 200 mg/kg	32.1±1.12	33.2±2.43	34.7±2.53	35.2±1.89	36.4±2.45*

P value (p)*	NS	NS	NS	NS	>0.05*

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

LD:Low Dose,MD:Moderate Dose, HD:High Dose

Table 9: Food intake (gm/day) of Wistar albino rats group exposed to Hydroalcoholic extract of Clitoria ternatea

DOSE	**DAYS**				
	1	7	14	21	28
Group I control (Vehicle:Normal Saline)	37.12 ±5.37	38.5±3.22	39.5±3.37	38.5±3.37	37.12±3.12
Group II (LD) Extract of CT 50mg/kg	63.7±2.12	65.3±1.42	65.9±1.68	66.4±2.62	65.9±8.42
Group III (MD) Extract of CT 100 mg/kg	64.2±3.64	65.9±3.64	66.2±6.15	67.4±2.18	65.2±2.64
Group IV (HD) Extract of CT 200 mg/kg	68.2±2.14	69.2±2.18	70.6±2.14	71.2±4.28	72.2±2.18*
P value (p)*	NS	NS	NS	NS	>0.05*

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

LD:Low Dose,MD:Moderate Dose, HD:High Dose

Table 10: Haematological parameters of Wistar albino rats group exposed to Hydroalcoholic extract of Clitoria ternatea

Parameter	unit	Group I control (Vehicle: Normal Saline)	Group II (LD) Extract of CT 50mg/kg	Group III (MD) Extract of CT 100 mg/kg	Group IV (HD) Extract of CT 200 mg/kg	Pvalue (p)*
Haemoglobin	(g/dl)	14.8±1.88	12.98±1.28	13.01±1.26	14.18±3.96	N.S
Total WBC	($\times10^3$ l)	10.91±2.59	12.25±3.53	12.18±3.61	12.96±3.47	N.S
Neutrophils	(%)	32.65±1.06	34.23±2.54	34.91±1.36	33.40±2.80	N.S
lymphocyte	(%)	69.34±2.48	70.22±3.42	71.48±2.66	71.20±3.96	N.S
Monocyte	(%)	0.78±0.17	0.81±0.12	0.84±0.11	0.95±0.16	N.S
Eosinohil	(%)	0.64±0.09	0.19±0.12	0.78±0.06	0.42±0.04	N.S
Platelets cells	10^3/µl	687.17±8.76	698.71±8.16	705.18±4.0	712.16±4.64	N.S
Total RBC	10^6/µl	7.99±0.12	6.82±1.87	6.92±0.59	6.18±0.72	N.S
PC	%	37.79±0.6	36.35±1.53	38.2±1.18	36.82±2.14	N.S
MCHC	g/dL	33.6±2.23	34.19±1.19	35.18±1.92	34.13±1.94	N.S
MCV	fL(µm^3)	49.17±3.64	48.20±1.24	49.28±1.24	49.99±1.84	N.S

N.S- Not Significant, **($p > 0.01$), *($p > 0.05$), n = 6values are± SEM (One way ANOVA followed by Dunnett's test)
LD:Low Dose,MD:Moderate Dose, HD:High Dose

Table 11 :Biochemical Parameters of Wistar albino rats group exposed to Hydroaloholic Extract of Clitoria ternatea

Parameters	Units	Group I control (Vehicle: Normal Saline)	Group II (LD) Extract of CT 50mg/kg	Group III (MD) Extract of CT 100 mg/kg	Group IV (HD) Extract of CT 200 mg/kg	**P Value (p)***
GLUCOSE (R)	mg/dl)	76.45±13.4	76.16±8.54	79.64±9.20	77.42±11.6	N.S
T. CHOLOSTEROL	mg/dl	115.26±1.83	112.45±1.13	112.42±1.98	115.22±1.83	N.S
TRIGLY	mg/dl	46.35±1.48	45.32±1.48	45.58±1.26	46.66±1.45	N.S
LDL	mg/dl	72.81±2.13	70.14±2.34	71.8±2.94	72.64±6.12	NS
VLDL	mg/dl	15.2±2.44	14.42±4.63	14.44±6.64	14.94±5.14	NS
HDL	mg/dl	26.66±6.88	27.96±2.34	27.88±5. 66	29.78±6.22	NS
Ratio 1(T.CHO/HDL)	mg/dl	4.42±2.44	4.36±1.44	4.84±2.44	4.86±1.92	NS
Ratio 2(LDL/HDL)	mg/dl	2.83±4.22	3.02±1.52	2.96±4.80	2.86±3.82	NS
Albumin(g/dL)	g/dL	3.63±0.17	3.13±1.12	3.10±1.92	2.94±3.86	NS

NS- Not Significant, **(p > 0.01), * (p >0.05), n = 6 values are ± SEM (One way ANOVA followed by Dunnett's test)
LD: Low Dose,MD:Moderate Dose, HD:High Dose

Parameters	Units	Group I control (Vehicle: Normal saline)	Group II (LD) Extract of CT 50mg/kg	Group III (MD) Extract of CT 100 mg/kg	Group IV (HD) Extract of CT 200 mg/kg	P Value (p)*
UREA	mg/dl	13.35±0.99	12.91±1.86	13.16±1.98	13.18±3.92	N.S

CREATININE	mg/dl	0.28±0.08	0.16±1.16	0.12±0.14	0.18±1.22	N.S
BUN	mg/dL	15.02±0.10	14.80±1.20	14.66±0.44	15.10±2.32	NS
URIC ACID	mg/dl	5.17±0.35	5.25±1.43	5.02±1.35	5.18±1.08	NS

Table 12 Renal function test of Wistar albino rats group exposed to hydroalcoholic extract of Clitoria ternatea

NS- Not Significant, **(p > 0.01), * (p >0.05) , n = 10 values are ± SEM (One way ANOVA followed by Dunnett's test)

LD:Low Dose,MD:Moderate Dose, HD:High Dose

Table 13: Liver Function Test of Wistar albino rats group exposed to Hydroalcoholic Clitoria ternatea

Parameters	Units	GroupI control (Vehicle: Normal saline)	Group II (LD) Extract of CT 50mg/kg	Group III (MD) Extract of CT 100 mg/kg	Group IV (HD) Extract of CT 200 mg/kg	**P Value (p)***
T BILIRUBIN	mg/dl	0.48±0.07	0.43±1.26	0.64±1.28	0.68±1.25	N.S
SGOT/AST	U/L	79.95±1.39	77.15±1.31	78.71±1.83	80.35±3.03	N.S
SGPT/ALT	U/L	31.23±1.28	31.81±3.52	30.14±3.18	31.9±1.88	N.S
ALP(U/L)	U/L	143.25±8.70	141.9±8.17	142.16±4.10	144.33±4.25	NS
T.PROTEIN	g/dL)	5.32±0.38	5.28±0.34	5.21±1.33	5.13±1.06	N.S

NS- Not Significant, **(p > 0.01), * (p >0.05), n = 10 values are mean ± SEM (One way ANOVA followed by Dunnett's test)

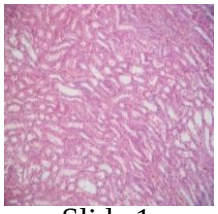
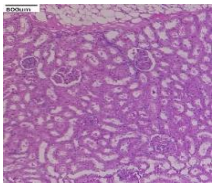
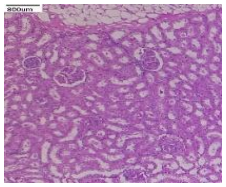
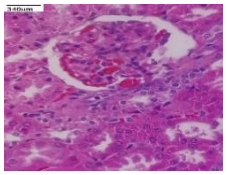
LD:Low Dose,MD:Moderate Dose, HD:High Dose

Histo pathological study

Histopathological study was done for control, high dose,moderate dose and low dose animals were randomly selected from each dose Based on the results of acute oral toxicity studies, it was concluded that a dose of 2000 mg/kg body weight of the extracts of C. ternatea C. ternatea hydroalcoholic extract given orally appeared to be non-toxic. However, there must be a point at which it can be concluded that a test substance is practically non-toxic or non-lethal after an acute exposure. The dose of 2000 mg/kg body weight for acute oral toxicity is generally considered to be very high. Thus C. ternatea hydroalcoholic extracts have a high margin of safety. Histopathological report also revealed that there is no change in cellur,tissues and pathological change on Vital organs such as Kidney,Liver and Spleen.However, further invivo study to be carried out to predict and complete the safety profile of hydroalacoholic extract.

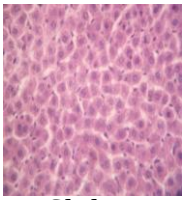
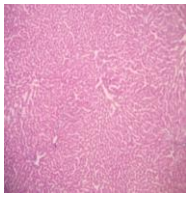
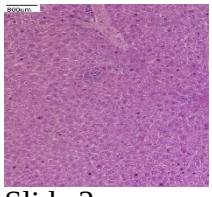
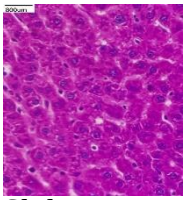
Histopathological Report

KIDNEY Fig:1

 Slide 1	 Slide 2	 Slide 3	 Slide 4
Control	Low dose	Moderate Dose	High dose

- Slide 1:Control Group showed no abnormal changes in tissues and cells
- Slide 2:Low dose 50mg/kg of Extract of CT showed no abnormal changes in tissues and cells
- Slide 3: Low dose 100mg/kg of Extract of CT showed no abnormal changes in tissues and cells
- Slide 4: Low dose 200mg/kg of Extract of CT showed no abnormal changes in tissues and cells

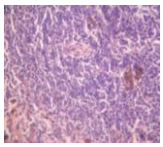
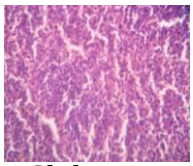
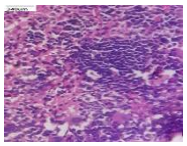
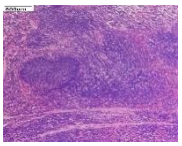
LIVERFig:2

 Slide 1	 Slide 2	 Slide 3	 Slide 4

Control	Low dose	Moderate Dose	High dose

- Slide 1:Control Group showed no abnormal changes in tissues and cells
- Slide 2:Low dose 50mg/kg of Extract of CT showed no abnormal changes in tissues and cells
- Slide 3: Low dose 100mg/kg of Extract of CT showed no abnormal changes in tissues and cells
- Slide 4: Low dose 200mg/kg of Extract of CT showed no abnormal changes in tissues and cells

SPLEENFig:3

 Slide 1	 Slide 2	 Slide 3	 Slide 4
Control	Low dose	Moderate Dose	High dose

- Slide 1:Control Group showed no abnormal changes in tissues and cells
- Slide 2:Low dose 50mg/kg of Extract of CT showed no abnormal changes in tissues and cells
- Slide 3: Low dose 100mg/kg of Extract of CT showed no abnormal changes in tissues and cells
- Slide 4: Low dose 200mg/kg of Extract of CT showed no abnormal changes in tissues and cells

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