



Evaluation of acute and sub-chronic toxicity studies of *Barleria cuspidata* Heyne ex Nees

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Article History:

Received on: 15 Jun 2020
Revised on: 12 Jul 2020
Accepted on: 20 Aug 2020

Keywords:

Acute toxicity study,
Barleria cuspidate Heyne
ex Nees,
Biochemical parameters,
Histopathology,
Sub-chronic toxicity
study

ABSTRACT

The fundamental reason for this examination was to look at the acute and sub-chronic harmfulness investigations of chloroform and methanol extracts of *Barleria cuspidata* Heyne ex Nees (Acanthaceae) on creature models according to the OECD rules 407 and 425 respectively. In acute oral toxicity study a solitary oral dosages of 5000 mg/kg body weight of the individual chloroform and methanol extracts was given to rodents and watched them for two weeks for the discovery of acute changes and for its mortality any. During acute oral toxicity study period no mortality were seen without any denotation of intense changes. Further, it was executed the sub-chronic toxicity of extracts. *Barleria cuspidata* extracts (chloroform and methanol) were independently given every day at dosages of 250 and 500 mg/kg body weight for 90 days to recognize the progressions any at sub-chronic poisonousness levels. Toward the finish of the experimentation by gathering the serum tests of trial creatures and watched for any progressions in hematological, biochemical and histopathological boundaries. All parameters of treated group were shown unaltered changes throughout the study period when compared with that of normal group. The outcomes propose that the oral organization of chloroform and methanol extracts of *Barleria cuspidata* didn't raise any huge poisonous impacts when contrasted with that of control animals. Hence the extracts may be safe for therapeutic use and as an alternative system of medicine.

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ISSN: 0975-7538

DOI: <https://doi.org/10.26452/ijrps.v11i4.3251>

Production and Hosted by

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INTRODUCTION

The genus *Barleria* is a pantropical yet prevalently an old World class, with its most noteworthy focal point of species assortment in tropical East Africa, trailed by South Africa and Asia (Balkwill and Balkwill, 1998). It is the third biggest class in the family Acanthaceae with 300 species among that 32 species are accounted for from India (Balkwill and Balkwill, 1997). The genus *Barleria* is under shrub disseminate in hot areas of the world. Lately the entire plant of genus *Barleria* picked up the significance for treatment of different sicknesses like diabetes, liver issues, neurological issues, immunodeficiency, aggravation, ulcers, HSV-2 viral illnesses, and so on (Preet et al., 2014). Traditionally the

plant species of genus *Barleria* in the form juice prepared by using leaves, roots etc., are used for cough, inflammation, hepatoprotective, wounds and diabetic. Among the genus *Barleria*, the *Barleria buxifolia* is proved for antibacterial activity (Primary et al., 2018), anthelmintic activity (Chander et al., 2014), antifeedant activity (Jeyasankar et al., 2014), anxiolytic activity, antidepressant activity (Purna et al., 2013), Prophylactic and Curative activities (Kapilraj, 2015). *Barleria cristata* is proved for antioxidant activity, thrombolytic activity, membrane stabilizing activity, antimicrobial activity (Tasnuva et al., 2013) and antidiabetic activity (Banu et al., 2012a). *Barleria gibsoni* is proved for anti-inflammatory activity (Lakshman et al., 2014), anthelmintic activity (Firoj and Harinath, 2016), antiulcer and antioxidant activity (Firoj and Harinath, 2016). *Barleria Montana* is proved for antibacterial activity (Natarajan et al., 2012), hepatoprotective activity (Banu et al., 2012a) and antidiabetic activity (Banu et al., 2012b). *Barleria cuspidata* Heyne ex Nees is one of the important species in *Barleria* belongs to the family Acanthaceae. The common vernacular names are Spiny Barleria, Lesser yellow nail-dye, Vellaimuli (Malayalam), Kurantaka (Sanskrit), Kadanculli (Tamil) and others are Narayana Sooli, Manjadhoya mullai. It is a shrub found in waste places, dry plains and rocky hillslopes (Shankar and Yadav, 2010). Experimentally *Barleria cuspidata* Heyne ex Nees has proved for wound healing property (Mazumder et al., 2009) and hepatoprotective activity (Tabassum et al., 2020).

Customary arrangement of medication has drawn colossal consideration for *in vivo* examinations for different exercises from past decades, thus, more investigations are done so as to decide the harmfulness of restorative plants and their items. Toxicity is a condition of unfriendly impacts lead by the connections among poisons and cells. This component of activity may vary and relies upon the cell membrane and compound properties of the toxicants. It might happen inside the cell layer or on the cell surface or tissue underneath just as at the extracellular lattice. In a large portion of the cases essential organs, for example, liver, lungs, heart and kidney and so on, are influenced by the poisons (Das et al., 2015). The organization for economic corporation and development (OECD) board of specialists characterizes acute toxicity as "the unfriendly impact happening inside a brief timeframe of (oral) disposal of a solitary portion of a substance or numerous dosages given inside 24 hrs. Also, sub-chronic toxicity as "the unfavorable impacts happening as consequence of the repetitive every day (oral) dosing of a chemical to test by and large for 1-3 months". Sub-

chronic toxicity testing gives the significant data on the total poisonousness of a compound at low portion delayed presentation and wide assortment of unfavorable impact can be identified. The outcome from such investigations can give data's which will help in choosing dose level (Gandhare et al., 2013). However, there is deficiency in elaborated scientific evaluation for toxicity and adverse effects of *Barleria cuspidata* Heyne ex Nees has been reported in the literatures. Accordingly, the current examination work was to assess the acute and sub-chronic toxicity of chloroform and methanol concentrates of *Barleria cuspidata* Heyne ex Nees in rodents.

MATERIALS AND METHODS

Collection of plant material

Fresh whole plant of *Barleria cuspidata* Heyne ex Nees (Acanthaceae) were pull together from chittoor districts in the areas of Tirumala Hills and Tirupathi surroundings and authenticated by Dr. K. Madava Chetty, Professor, Department of Botany, Sri Venkateswara University, Tirupathi. Andhra Pradesh, India. Voucher specimen (No: BC - 1419) of this plant has been kept in the P. Rami Reddy Memorial College of Pharmacy, Kadapa, Andhra Pradesh, India.

Preparation of plant material

The gathered entire plant of *Barleria cuspidata* was washed with running water, cut into little pieces and shade dried at room temperature to maintain a strategic distance from loss of phytoconstituents of plant. The total shade dried materials pounded for powder and sieved up to 80 meshes. At that point it was homogenized to fine powder and put away in air-tight compartment for additional toxicity considers.

Preparation of plant extracts

Whole plant powder of the *Barleria cuspidata* was extracted successively with two different solvents like chloroform (30-60°C) & methanol (50-70°C) in a Soxhlet apparatus in batches of 500 gm each. The overabundance solvent was expelled from extract utilizing a rotary vacuum evaporator and later on concentrated on a water bath. The rate yield of the extract was determined. At last dried extract was put away in desiccators for toxicity studies (Lachman, 2007).

Procurement of animals and maintenance

Albino rats of either sex, gauging the body weight of 150-250 gms were acquired from Sri Venkateswara Enterprises, Bangalore, India. Animals were kept up according to rules of NIN animal client manual. Ani-

mals are adjusted for 10 days to our creature house, kept up at temperature of 22°C to ± 2 °C. The animal was directed by a 12 hours light, 12 hours dark calendar. Five animals are housed per cage estimated 41 cm length, 28 cm width and height of 14 cm. Paddy husk was utilized for bedding and on elective day bedding was changed and washed altogether with water alongside domex, a disinfectant and detergent. The rats were benefited from a standard pellet diet bought from Suresh organizations, Hyderabad and water not obligatory. The examination convention was investigated and endorsed by the Institutional Animal Ethical Committee (IAEC) and trials were led according to the rules of CPCSEA. Reg. Number: 1423/PO/Re/S/11/CPCSEA, date 30th October 2017.

Acute oral toxicity study

An oral acute toxicity investigation of chloroform and methanol extracts of *Barleria Cuspidata* was performed by the Organization for Economic Cooperation and Development (OECD) rule 425 on rodents (OECD, 2008), where the breaking point test portion of 5000 mg/kg was utilized. All the animals were kept 3 hours fasting before to explore different avenues regarding free abundance to water. A solitary oral dosages of 5000 mg/kg body weight of the individual chloroform and methanol extracts was given utilizing oral gavage for present moment (i.e., 48 hr) and long term (i.e., 14 days) to the rats. Before dose administration, the body weight of every animal was resolved and the dose was determined by the body weight. Animals were seen to distinguish intense changes in morphological and conduct reactions, unconstrained action, touchiness, corneal reflex, tremors, spasms, salivation, loose bowels, torpidity assuming any and furthermore observed for any mortality throughout toxicity study.

Sub chronic toxicity study

The oral sub chronic toxicity study was done by OECD rule 407. The animals were partitioned into 5 groups of 6 rats each were kept in five separate pens. Group I was kept as should be expected normal control and Group II to V were kept as tested groups which got the dosages as follows.

Group I- Normal control animals received normal clean drinking water ad libitum.

Group II- Animal received chloroform extract of *Barleria Cuspidata* (CEBC) 250 mg/kg per orally.

Group III- Animal received chloroform extract of *Barleria Cuspidata* (CEBC) 500 mg/kg per orally.

Group IV- Animal received methanol extract of *Barleria Cuspidata* (MEBC) 250 mg/kg per orally.

Group V- Animal received methanol extract of *Barleria Cuspidata* (MEBC) 500 mg/kg per orally.

All the animals in the above groups get their portions as needs be for the examination time of 90 days. Body weight of every animal in group was recorded at first and for like clockwork span till the most recent day of analysis. Following 90 days blood tests of exploratory rats in each group was acquired by retro orbital with a capillary into ependrof tubes without anticoagulant, centrifuged at 400 x g for 10 min and the serum put away at 4°C for estimation of different serum biochemical boundaries, for example, glucose, creatinine, total protein, albumin, globulin, bilirubin, SGOT, SGPT and ALP and for electrolytes (phosphorus, chloride and calcium). Other blood tests were gathered into isolated tubes previously covered with trisodium citrate for hematological investigation, for example, RBC, WBC, hemoglobin, platelets and mean cell volume. Every trial animal were sacrificed in the wake of gathering of blood tests by infusing the phenobarbital infusion for gathering the internal organs to decide the relative organ loads and for histopathological investigations of brain, liver, kidney, heart and lungs.

Serum biochemical parameters

Serum glucose, creatinine, total protein, albumin, globulin, bilirubin, SGOT, SGPT, ALP and electrolytes were determined using a semi-automated analyzer.

Serum glucose determination

Serum glucose levels were determined by using Trinder method (Glucose, GOD-POD) by the addition of reagents present in reagent kit (AGD Biomedicals Pvt. Ltd.). The absorbance of standard and test against reagent blank were measured at 505 nm (Soon and Tan, 2002).

Serum creatinine determination

Serum creatinine levels were ascertained by reagents present in reagent kit (AGD Biomedicals Pvt. Ltd.). The absorbance of standard and test against reagent blank were measured at 520 nm.

Serum total protein concentration

Serum Total protein levels were ascertained by using End Point Assay method acting by the addition of reagents present in reagent kit (Span Diagnostic Ltd.). The absorbance of standard and test against reagent blank were measured at 578 nm. The values of total proteins present in serum were expressed in g/dL.

Serum albumin concentration

Serum Albumin levels were ascertained by using Bromocresol Green, End Point Assay method by the addition of reagents present in reagent kit (Span

Table 1: Acute oral toxicity study of chloroform and methanol extracts of *Barleria cuspidata* Heyne ex Nees at 5000 mg/kg b.w in rats

S. No.	Observations	Response		
		Control	MEBC 5000 mg/kg b.w	CEBC 5000 mg/kg b.w
01	Consciousness	+	+	+
02	Grooming	-	-	-
03	Touch response	+	+	+
04	Sleeping duration	+	+	+
05	Movement	+	+	+
06	Gripping strength	+	+	+
07	Righting reflex	+	+	+
08	Food intake	+	+	+
09	Water consumption	+	+	+
10	Tremors	-	-	-
11	Diarrhea	-	-	-
12	Hyper activity	-	-	-
13	Pinna reflex	+	+	+
14	Corneal reflex	+	+	+
15	Salivation	+	+	+
16	Skin color	+	+	+
17	Lethargy	-	-	-
18	Convulsions	-	-	-
19	Morbidity	-	-	-
20	Sound response	+	+	+
21	Mortality	Alive	Alive	Alive

Note: + = Normal and - = Absent

Diagnostic Ltd.). The absorbance of standard and test against reagent blank were measured at 630 nm. The values of Albumin present in serum were expressed in g/dL.

Serum globulins concentration

Serum globulins levels were ascertained by using the equation:

$$\text{Globulins} = \text{Total proteins} - \text{Albumin}$$

And the values are expressed in g/dL.

Serum transaminases (GOT & GPT)

Serum transaminases (GOT & GPT) were determined by the method of Reitman and Frankel (Raja and Ravindranadh, 2017) by the addition of reagents present in reagent kit (Span Diagnostic Ltd). The absorbance of standard and test against reagent blank were measured at 505 nm. Data were communicated as IU L⁻¹.

Serum alkaline phosphatase (ALP)

Serum alkaline phosphatase (ALP) was ascertained by the method of Kind & King by the addition of

reagents present in reagent kit (Span Diagnostic Ltd). The absorbance of standard and test against reagent blank were measured at 640 nm. Data were communicated as UL⁻¹.

Serum bilirubin

Serum bilirubin was evaluated by method of Malloy and Evelyn (Malloy and Evelyn, 1937). The two test tubes were taken and each into was added 0.2 ml of serum test and 1.8 ml of distilled water. To the obscure, 0.5 ml of diazo reagent and to the blank, 0.5 ml of 1.5 % HCl was included.

At last, to each tube, 2.5 ml of methanol was added and afterward permitted to represent 30 min in ice and absorbance was perused at 540nm. For a standard curve, the above standard was weakened 1 out of 5 ml methanol. The measure of direct responding bilirubin was resolved also by subbing 2.5 ml of water for 2.5 ml of methanol. Qualities were communicated as mg/dl.

Hematological analysis

Red blood cells (RBC), white blood cells (WBC),

platelets, haemoglobin (H_g) and mean cell volume (MCV) were ascertained with a semi-automated analyzer.

Statistical analysis

All investigations information were communicated as mean \pm standard error mean (SEM). This statistical analysis was done utilizing one-way ANOVA strategy followed by Dunnet-t test with SPSS statistical programming for correlation with the control group. $p \leq 0.05$ was considered as statistical significant.

RESULTS AND DISCUSSION

In the Indian frameworks of medication, the greater part of the experts detail and apportion their own plans without legitimate proof; thus this require appropriate documentation and research (Stephen and Richard, 2004). India is the biggest maker of therapeutic spices and is appropriately called the botanical garden of the world (Ahmedulla and Nayar, 1999).

Acute oral toxicity study

The acute oral toxicity study was done on albino rats of either sex at single portion of chloroform and methanol extracts of *Barleria cuspidata* at 5000 mg/kg body weight and persistently checked for 14 days according to OECD rules 425. During this investigation period all the animals were watched for its conduct and its mortality.

All the exploratory rats shows ordinary conduct in their consciousness, touch response, sleeping duration, movement, holding quality, righting reflex, food admission, water utilization, pinna reflex, corneal reflex, salivation, skin shading and sound reaction, and there was missing in reactions, for example, grooming, tremors, diarrhea, hyper movement, lethargy, convulsions and morbidity as appeared in Table 1.

Likewise all the tested rats made due till the fulfillment of the investigation time frame (14 days) at all degrees of treatment. Along these lines, this outcome says that there was no aggravation in carbohydrate, protein, fat and some other metabolisms (Klaassen, 2013).

The chloroform and methanol extracts of *Barleria cuspidata* at 5000 mg/kg body weight given orally is by all accounts safe and the LD₅₀ was considered be >5000 mg/kg body weight.

Every one of these outcomes will propose that the chloroform and methanol concentrates of *Barleria cuspidata* at 5000 mg/kg body weight for all intents and purposes non-toxic at single oral dose.

Sub-chronic toxicity study

Sub-chronic toxicity studies was therefore execute with chloroform and methanol extracts of *Barleria cuspidata* at the doses of 250 mg/kg and 500 mg/kg body weight as per the OECD guidelines 407.

Analysis of body weights

The outcomes in of any adjustments in body weight of each experimental rat in group at first and for like clockwork span (30 days) till the most recent day of examination are appeared in Table 2. There were no critical contrasts in mean body weight among the diverse treated groups and the control despite the fact that there will be increment in body weights steadily. These outcomes demonstrated that the chloroform and methanol extracts of *Barleria cuspidata* has immaterial degrees of harmfulness on the development of the animals.

Measurement of organ weights

The results in of any changes in relative organ weights of each experimental rat in group at end of the experimentation are shown in Table 3. The weights of vital organs such as liver, lungs, kidneys and heart were found normal and there were no significant changes in their weight which indicates that nontoxic effect in both control and treated group. The no significance changes in organ weights and it provides support for the safety of *Barleria cuspidata*.

Serum biochemical parameters

The consequences of serum biochemical parameters, for example, glucose, creatinine, total protein, albumin, globulin, bilirubin, SGOT, SGPT, ALP and electrolytes are recorded in Tables 4, 5 and 6. Following 90 days of study period, the serum biochemical parameters, for example, SGOT, SGPT, ALP and Bilirubin of rewarded group don't show significance when contrasted and that of control group. The estimations of these biochemical parameters typically quantifies as markers of the best possible liver capacity (Yu *et al.*, 2012). In addition, levels of serum total protein, albumin and globulin demonstrated no significant when contrasted the rewarded group animals and that of control group animals. Hypoproteinaemia, a typical finding in liver damage (Larrey, 2002), was additionally not seen in present examination. Additionally, the degrees of creatinine were not essentially extraordinary between the control and test group of rats. The estimations of serum creatinine will gauges the best possible capacity of urinary framework (Jia *et al.*, 2014), thus with no criticalness in serum creatinine levels of experimental animals shows the correct working of urinary framework in animals rewarded with *Barleria cuspidata*.

Table 2: Body weights of experimental rats during sub-chronic study of chloroform and methanol extracts of *Barleria cuspidata* Heyne ex Nees

S. No.	Group	Body Weight (gms)			
		Initial Day	30th Day	60th Day	90th Day
01	I	181.5 ± 0.42	202.83 ± 0.47	252.16 ± 0.47	286.6 ± 0.42
02	II	181.66 ± 0.76	198.16 ± 0.3	245.16 ± 0.4	274.33 ± 0.42
03	III	183.16 ± 0.4	191 ± 0.36	236 ± 0.51	282 ± 0.44
04	IV	182 ± 0.57	198.66 ± 0.33	243.16 ± 0.6	276.16 ± 0.65
05	V	181.66 ± 0.55	191 ± 0.44	237.83 ± 0.65	281.16 ± 0.4

The values are communicated as mean ± SEM of six animals in each group shows no significance by utilizing SPSS. Statistical significant test for correlation was done by ANOVA, trailed by Dunnet's -'t' test.

Table 3: Effect of chloroform and methanol extracts of *Barleria cuspidata* Heyne ex Nees on Organ weights

S. No	Group	Organ Weights (gms)			
		Liver	Lungs	Heart	Kidneys
01	I	3.23 ± 0.01	0.58 ± 0.03	0.38 ± 0.01	0.62 ± 0.02
02	II	3.5 ± 0.01	0.56 ± 0.03	0.36 ± 0.01	0.68 ± 0.03
03	III	3.6 ± 0.01	0.49 ± 0.04	0.36 ± 0.02	0.67 ± 0.04
04	IV	3.46 ± 0.01	0.59 ± 0.02	0.35 ± 0.02	0.64 ± 0.03
05	V	3.6 ± 0.01	0.49 ± 0.03	0.37 ± 0.02	0.63 ± 0.03

The values are communicated as mean ± SEM of six animals in each group shows no significance by utilizing SPSS. Statistical significant test for correlation was done by ANOVA, trailed by Dunnet's -'t' test.

Table 4: Effect of chloroform and methanol extracts of *Barleria cuspidata* Heyne ex Nees on Serum Biochemical Parameters

S. No.	Group	Serum Biochemical Parameters				
		Bilirubin (mg/dl)	Creatinine (mg/dl)	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
01	I	0.87 ± 0.01	0.4 ± 0.01	8.06 ± 0.04	4.4 ± 0.04	3.65 ± 0.06
02	II	0.86 ± 0.01	0.39 ± 0.01	8.09 ± 0.05	4.45 ± 0.02	3.64 ± 0.07
03	III	0.86 ± 0.01	0.4 ± 0.02	8.1 ± 0.03	4.41 ± 0.03	3.69 ± 0.06
04	IV	0.84 ± 0.02	0.39 ± 0.01	8.11 ± 0.04	4.42 ± 0.05	3.68 ± 0.09
05	V	0.87 ± 0.01	0.41 ± 0.01	8.16 ± 0.02	4.39 ± 0.03	3.78 ± 0.04

The values are communicated as mean ± SEM of six animals in each group shows no significance by utilizing SPSS. Statistical significant test for correlation was done by ANOVA, trailed by Dunnet's -'t' test.

Table 5: Effect of chloroform and methanol extracts of *Barleria cuspidata* Heyne ex Nees on Serum Biochemical Parameters

S. No.	Group	Serum Biochemical Parameters			
		Glucose (mg/dl)	SGOT (IU/L)	SGPT (IU/L)	ALP (U/L)
01	I	86.5 ± 2.06	162.83 ± 1.3	36.5 ± 1.057	31.16 ± 1.046
02	II	86 ± 1.86	161 ± 1.506	38.5 ± 0.88	33.33 ± 0.8
03	III	86.33 ± 1.05	159.33 ± 0.98	41.33 ± 1.46	36.33 ± 1.05
04	IV	85.66 ± 2.4	162 ± 1.065	38.16 ± 0.98	31.83 ± 0.79
05	V	86 ± 2	161.33 ± 1.66	41 ± 1.31	36.16 ± 0.65

The values are communicated as mean ± SEM of six animals in each group shows no significance by utilizing SPSS. Statistical significant test for correlation was done by ANOVA, trailed by Dunnet's -'t' test.

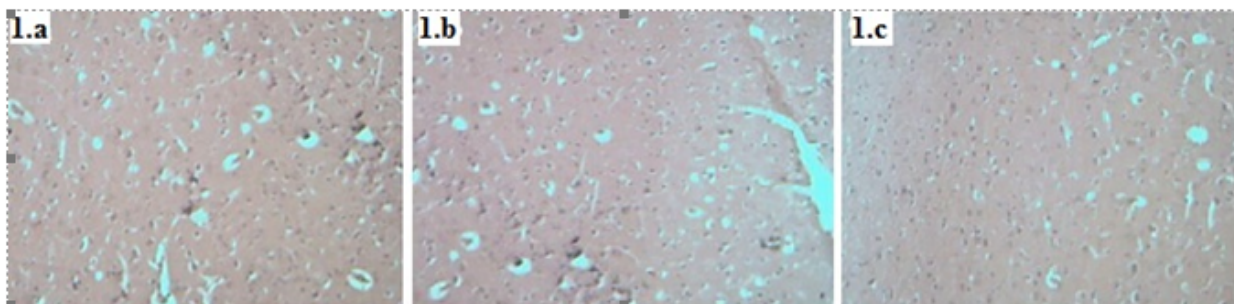


Figure 1: Histology of rats' brain treated with different doses of *Barleria cuspidata* (a) Control group (b) Methanol extract and (c) Chloroform extract at 500 mg/kg per day

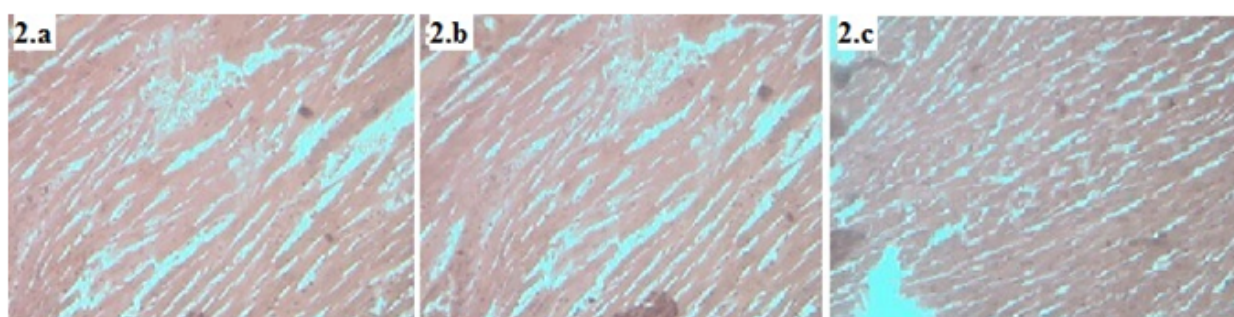


Figure 2: Histology of rats' heart treated with different doses of *Barleria cuspidata* (a) Control group (b) Methanol extract and (c) Chloroform extract at 500 mg/kg per day

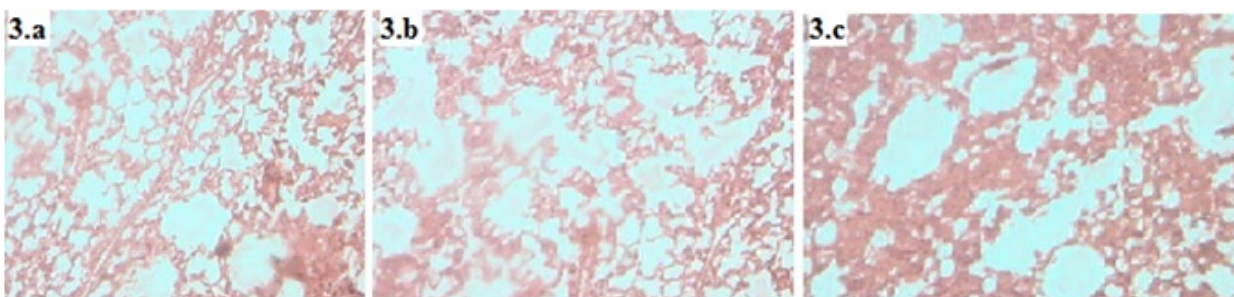


Figure 3: Histology of rats' lungs treated with different doses of *Barleria cuspidata* (a) Control group (b) Methanol extract and (c) Chloroform extract at 500 mg/kg per day

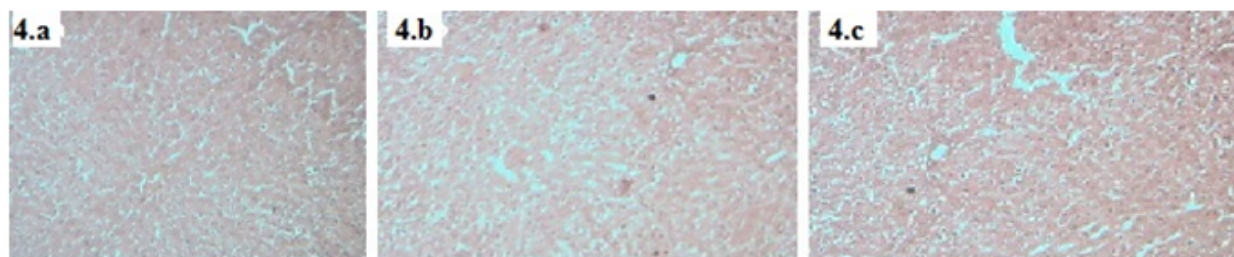


Figure 4: Histology of rats' liver treated with different doses of *Barleria cuspidata* (a) Control group (b) Methanol extract and (c) Chloroform extract at 500 mg/kg per day

Table 6: Effect of chloroform and methanol extracts of *Barleria cuspidata* Heyne ex Nees on Serum Electrolyte levels

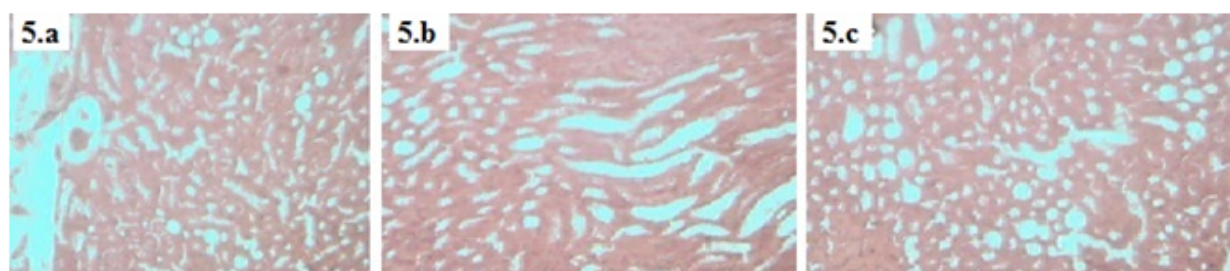
S. No.	Group	Serum Electrolytes Levels		
		Phosphorus (mg/dl)	Chloride (mmol/lit)	Calcium (mg/dl)
01	I	5.65 ± 0.108	104.83 ± 1.04	10.4 ± 0.2
02	II	5.45 ± 0.08	104.5 ± 0.99	10.1 ± 0.13
03	III	5.48 ± 0.08	103.66 ± 0.66	10.25 ± 0.12
04	IV	5.16 ± 0.24	103.66 ± 1.05	10.13 ± 0.11
05	V	5.4 ± 0.12	103.83 ± 0.79	10.11 ± 0.16

The values are communicated as mean ± SEM of six animals in each group shows no significance by utilizing SPSS. Statistical significant test for correlation was done by ANOVA, trailed by Dunnet's -'t' test

Table 7: Effect of chloroform and methanol extracts of *Barleria cuspidata* Heyne ex Nees on Haematological Parameters

S. No.	Group	Blood Haematological Parameters				
		RBC (10 ² /L)	WBC (10 ⁹ /L)	Platelets (10 ² /L)	Haemoglobin (g/l)	MCV (fL)
01	I	8.48 ± 0.19	11.7 ± 0.23	277 ± 8.43	14.3 ± 0.15	45 ± 1.21
02	II	8.71 ± 0.15	9.25 ± 0.22	276.16 ± 8.75	13.86 ± 0.17	45.83 ± 0.87
03	III	8.76 ± 0.18	10.4 ± 0.19	281.5 ± 8.02	13.7 ± 0.29	41.66 ± 1.33
04	IV	8.43 ± 0.15	8.96 ± 0.19	275.5 ± 7.38	13.96 ± 0.26	44.5 ± 1.17
05	V	8.9 ± 0.21	10.13 ± 0.21	270.33 ± 6.92	14.1 ± 0.15	47 ± 0.63

The values are communicated as mean ± SEM of six animals in each group shows no significance by utilizing SPSS. Statistical significant test for correlation was done by ANOVA, trailed by Dunnet's -'t' test.

**Figure 5: Histology of rats' kidney treated with different doses of *Barleria cuspidata* (a) Control group (b) Methanol extract and (c) Chloroform extract at 500 mg/kg per day**

Further, there was no significant in glucose levels in the treated groups when contrasted with the control group which likewise demonstrates for typical working of liver.

Likewise the serum levels of electrolytes, for example, phosphorus, chloride and calcium indicated no importance contrast when contrasted the rewarded group animals and that of control group animals. Which shows that chloroform and methanol extracts of *Barleria cuspidata* was protected concerning that

of the electrolyte levels.

Hematological parameters

The consequences of hematological parameters, for example, RBC, WBC, platelets, hemoglobin and MCV are recorded in Table 7. The hematological parameters, for example, RBC, WBC, platelets, hemoglobin and mean cell volume indicated no centrality when rewarded group animals are contrasted and that of control group animals. Administration of any compound or medication or plant extracts can bring

about loss of blood cells and/or hindrance of blood cell synthesis and diminishing in such hematological parameters in experimental animals has been related with anemia (Onyeyilli *et al.*, 1998). The bone marrow is liable for the creation of the blood cell and a few phytochemicals disconnected from plant extracts will influence blood cell levels (Kofi *et al.*, 2014). As the tested plant extracts didn't show any of unsafe impacts on bone marrow work, with that we may legitimize for all the portions of chloroform and methanol extracts of *Barleria cuspidata* didn't initiate anemia and making it ok for use in this regard.

Histology study

Histological investigations of liver, kidney, lungs, heart and cerebrum tissues in chloroform and methanol extracts of *Barleria cuspidata* rewarded rats are appeared from Figures 1, 2, 3, 4 and 5, which shows no variations from the norm when contrasted the treated and that of control. In this way, Histopathological assessment of chloroform and methanol extracts of *Barleria cuspidata* didn't show any unfavorable impact on morphology of tissues which bolsters the biochemical outcomes referenced previously. Hence, it is inferred that *Barleria cuspidata* didn't create any harmful impact in albino rats of either sex.

CONCLUSIONS

The aftereffects of acute oral toxicity study and sub-chronic toxicity study of chloroform and methanol extracts of *Barleria cuspidata* shows no noteworthy contrasts in hematological and biochemical tests in the rewarded groups on contrasted and that of control and it was proposed the degree of wellbeing of *Barleria cuspidata* on animals. Accordingly, the outcome shows that the chloroform and methanol extracts of *Barleria cuspidata* might be protected and furthermore gives the data to the utilization of *Barleria cuspidata* for additional examinations and as an elective arrangement of medication.

ACKNOWLEDGEMENT

The authors are sincerely thankful to the administration of GITAM (Deemed to be University), Visakhapatnam and management of P. Rami Reddy Memorial College of Pharmacy, Kadapa, Andhra Pradesh, India, for giving vital offices to complete the research work.

Financial Support

The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that there was no conflict of interest in this research.

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