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Research Article

ACUTE ORAL TOXICITY STUDY OF AQUEOUS AND METHANOLIC EXTRACT OF *BLUMEA VIRENS* IN SPRAGUE DAWLEY RATS

Reni John[1], Krishnaprasad. G. Koorse[2], Surya. S[3], Bibu John K.[4*], Dhanush K.B.[5], Usha P.T.A.[6]

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ABSTRACT: *Blumea virens*, belonging to family Asteraceae, is found abundantly in tropical and subtropical zones of Asia, especially in India. The consumption of whole plant has been reported to produce mortality in animals in Kerala. Thus, the present study was undertaken to evaluate the *in vivo* acute oral toxicity of aqueous and methanolic extract of *Blumea virens* in Sprague Dawley rats as per OECD guidelines 425. Limit test was conducted at a dose of 2000 mg/Kg. The animals administered with the aqueous and methanolic extract did not produce any related signs of toxicity or mortality during the 14-day observation period. Gross and histopathology was performed at the end of the study. Methanolic extract evinced hepatotoxicity and pulmonary toxicity to the animal whereas no abnormalities were detected with aqueous extract. Phytochemical analysis of the plant methanolic extract revealed presence of steroids, alkaloids, flavonoids, tannins, diterpenes, triterpenes and glycosides whereas aqueous extract revealed presence of steroids, alkaloids, flavonoids, diterpenes and glycosides.

Key words: Acute oral toxicity, Aqueous and methanolic extract, *Blumea virens*, Sprague Dawley rats, Phytochemical analysis.

INTRODUCTION

Blumea virens DC. belonging to the family Asteraceae, is found abundantly in tropical and subtropical zones of Asia, especially India. There have been some incidences of mortality due to the consumption of whole plant of *B. virens* causing sudden death in goats of Malappuram district of Kerala. As toxicity report of this plant is not found and toxicity of *Blumea lacera* (Burm.F.) DC., a closely related plant of same genus was reported to be of lesser aptitude (Nusrat Zahan *et al.* 2015), the necessity for analysis of toxicological profile of this plant was felt. Hence the objectives of the study were to evaluate the acute toxicity of aqueous and methanolic extract of whole plant of *B. virens* and to identify the active principles present in the plant. The results of acute toxicity study would be useful for selection of doses for repeated dose toxicity studies and may also provide preliminary information on the target organs of toxicity.

MATERIALS AND METHODS

The study was performed as per the Organisation for Economic Cooperation and Development (OECD) Guidelines for the Testing of Chemicals No.425. The study was approved by Institutional Animal Ethics Committee of College of Veterinary and Animal Sciences, Mannuthy vide Order No. IAEC/CVASMTY8/16-17 held on 28/9/16.

Procurement and Preparation of Plant Material

The whole plant of *B. virens* (Fig. 1) was collected from Thirunavaya, Malappuram district of Kerala in the month of January, 2016 and was authenticated by plant taxonomist and plant specimen (HERB/VPT/CVASMTY/5/2016) was preserved in the Department of Veterinary Pharmacology & Toxicology, College of Veterinary and Animal Sciences, Mannuthy. The plant materials were cleaned, shade dried and ground to coarse powder using an electric pulverizer. Powdered samples were collected and stored in a closed container until required for extraction.

Preparation of aqueous crude extract

Ninety grams of the pulverized sample was extracted with distilled water in the ratio 1:4 by decoction method

[1,2]P. G Scholar, [4]Assistant Professor, [6] Professor and Head, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy-680651, Kerala, India.
[3]Project Fellow, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy-680651, Kerala, India.
[5]Assistant Professor, Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy-680651, Kerala, India.
**Corresponding author. e - mail: bibujohn@kvasu.ac.in*

Table 1. Survival Record - acute oral toxicity study of aqueous extract of whole plant of *Blumea virens* (n = 5/group).

Animal ID (aqueous)	Animal ID (methanolic)	Dose (mg/kg)	Short term Result (48 h.)	Long term Result (14 days)
1FNM	2FNM	2000	Survived	Survived
1FH	2FH	2000	Survived	Survived
1FB	2FB	2000	Survived	Survived
1FT	2FT	2000	Survived	Survived
1FFL	2FFL	2000	Survived	Survived

Table 2. Clinical Signs observed - acute oral toxicity study of aqueous extract of whole plant of *Blumea virens* (n = 5/group).

Animal ID (aqueous)	Animal ID (methanolic)	Dose(mg/kg)	Observed Signs	Period of Signs in days (From-To)
1FNM	2FNM	2000	Nil	0-14
1FH	2FH	2000	Nil	0-14
1FB	2FB	2000	Nil	0 -14
1FT	2FT	2000	Nil	0 -14
1FFL	2FFL	2000	Nil	0 -14

(Handa *et al*. 2008) using open pan type steam extractor. Briefly, pulverized sample tied in a muslin cloth was immersed in an open pan containing boiling water and concentrated until the volume of mixture is reduced to 1/4th of original mixture. The extraction was completed within 5 hours to ensure complete extraction. The liquid extract obtained was then concentrated to dryness using flash evaporator followed by lyophilization and the solid residue was then stored in refrigerator at 4°C. The yield of the extract was 9.2 %.

Preparation of methanolic crude extract

Thirty grams of the pulverized sample was extracted using Soxhlet apparatus with methanol. The methanolic extract was then concentrated in a flash evaporator and kept under refrigeration for the complete evaporation of the solvent. The yield of the extract was 30 %.

Test System

A total of ten adult female rats five each for each extract, of the Sprague-Dawley strain, 6 to 8 weeks old and weighing between 100 to 200 gm obtained from the Small Animal Breeding Station of College of Veterinary and Animal Sciences, Mannuthy, Kerala were used for the study.

The rats were kept in polypropylene cages in the animal house with an ambient temperature of 25^{0}C, 12h light and 12h dark periodicity. The rats were fed with standard diet and water ad libitum and allowed to acclimatize for seven days before the procedure. They were randomly assigned to the cages and the individual animal was fur marked with felt tip marker pen. Veterinary examination was done before allocation of animals to groups and after the completion of acclimatization period.

Fig. 1. *Blumea virens* DC. plant.

Table 3. Body weight gain and Per cent body weight gain - acute oral toxicity study of aqueous extract of whole plant of *Blumea virens* (n = 5).

Dose (mg/ kg)	Animal ID kdldkld	Body weight day 0 (gm)	Body weight day 7(gm)	Body weight gain (%) 0-7	Bodyweight on day 14 (gm)	Body weight gain (%) 0-14
2000	1FNM	200	200	0	210	5
2000	1FH	200	210	5	200	0
2000	1FB	160	210	25	190	15
2000	1FT	200	230	15	220	10
2000	1FFL	180	220	20	200	10

Animal ID - Animal Identification number.

Table 4. Body weight gain and Per cent body weight gain - acute oral toxicity study of methanolic extract of whole plant of *Blumea virens* (n = 5).

Dose (mg/kg)	Animal ID	Body weight day 0 (gm)	Body weight day 7 (gm)	Body weight gain (%) 0-7	Body weight on day14(gm)	Body weight Gain (%) 0-14
2000	2FNM	140	160	14.3	200	42.8
2000	2FH	120	160	33.3	160	33.3
2000	2FB	120	150	25	140	16.7
2000	2FT	140	180	28.6	180	28.6
2000	2FFL	130	180	38.5	170	30.8

Animal ID - Animal Identification number.

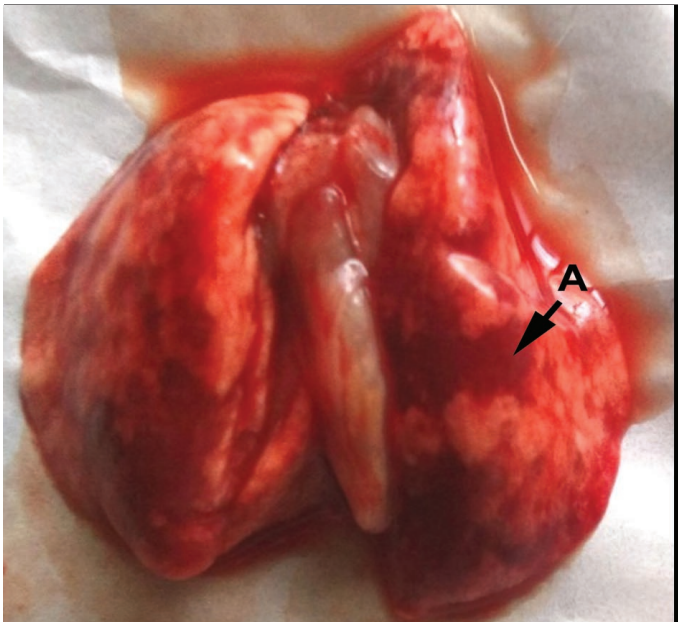

Fig. 2. Lung (Gross) a. multifocal areas of congestion.

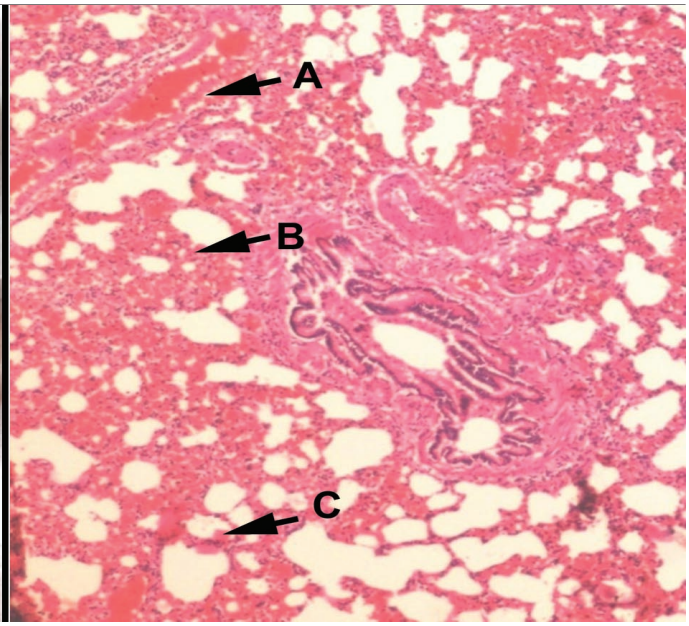

Fig. 3. Lung (tissue section) 10X a.congestion, b. mononuclear infiltrates, c. alveolar wall thickening.

Experimental Procedure and Observations

The study was conducted according to OECD Test Guidelines 425 Acute oral toxicity-Up- and Down-Procedure using the limit dose test at 2000 mg/kg body weight. The ten female rats were deprived of feed for 6h prior and 3h after administration of drug on each occasion. Water was provided ad libitum during the fasting period. The extract was solubilized in one millilitre of distilled water in case of aqueous extract and four drops of gum acacia and made up to 1 ml with distilled water for methanolic extract. The solubilized extracts were administered by oral gavage to rats using an intubation

Table 5. Phytochemical analysis - acute oral toxicity study of aqueous and methanolic extract of whole plant of *Blumea virens.*

Phytochemical	Test	Result (aqueous)	Result (alcoholic)
Saponins	Foam test	—	—
Alkaloids	Dragendroff's test	+	+
	Mayer's test	—	+
	Wagner's test	—	+
	Hager's test	+	+
Tannins	Ferric chloride test	—	+
	Gelatin test	—	—
Flavonoids	Lead Acetate test	—	—
	Ferric chloride test	+	+
Glycosides	Sodium hydroxide test	+	+
Steroids	Salkowski's test	+	+
Diterpene	Diterpene detection test	+	+
Triterpenes	Salkowski's test	—	+

—not detected, + present.

needle of 18gauge size fitted into a syringe. One animal was dosed at a dose rate of 2000mg/kg orally at first. Then four additional animals were dosed sequentially at a dose rate of 2000 mg/kg orally. The animals were observed individually for first 10 min, 30 min, 1h, 2h, 4h and 6h after dosing and thereafter twice daily for mortality and once a day for clinical signs, for 14 days.

The body weight of the animals was recorded and weekly body weight gain was calculated. After the observation period of 14 days, the surviving animals were sacrificed and subjected to complete necropsy.

Phytochemical analysis

The plant extract was subjected to qualitative phytochemical analysis to detect the presence of steroids, alkaloids, saponins, tannins, flavonoids, diterpenes, triterpenes, glycosides and cardiac glycosides (Harborne 1998).

RESULTS AND DISCUSSION

The study was designed to determine the acute oral toxicity profile of aqueous and methanolic extract of *B. virens* in female Sprague-Dawley rats. Rats are one of the recommended rodent species by the regulatory authorities for conducting preclinical toxicity study among rodents. Also, female rats are the most sensitive species for expression of toxic responses. Two animals, one for each extract dosed at the dose level of 2000 mg/kg body weight did not show any mortality or major abnormal clinical signs and the animals survived throughout the study period (Table 1 and Table 2). The treated animals showed overall normal body weight gain during 14 days observation period (Table 3 and Table 4). The gross pathological examination did not reveal any major abnormalities for aqueous extract. Congested lung with mononuclear infiltrates, alveolar wall thickening and focal fibroblast proliferation and multi-focal pale areas in liver with focal enlargement of hepatocytes having an eosinophilic cytoplasm between the normal parenchyma were obtained on gross and histopathology for methanolic extract (Fig. 2,3,4 and 5). Since all the ten animals survived with normal body weight gain and without exhibiting any clinical signs and mortality, it was inferred that the LD_{50} of aqueous and methanolic extract of *B. virens* was more than 2000 mg/Kg. However, the methanolic extract showed some histopathological changes in two vital organs like lungs and liver which indicates that the extract may produce some adverse effects and it may be a probable cause of death in animals (goats) who has consumed the plants for a long period in Kerala.

Phytochemical analysis revealed the presence of steroids, alkaloids, flavonoids, diterpenes and glycosides in case of aqueous extract and tannins and triterpenes were additionally detected for methanolic extract (Table 5). The toxicity, if any, could be attributed to the presence of phytoconstituents of the plant under investigation. Many of the phytoconstituents like steroids, alkaloids, triterpenes and glycosides in plant have been associated with toxicity (Hoffman 2003). This is the first report of the phytochemical analysis and acute oral toxicity study of aqueous and methanolic extract of *B. virens.*

CONCLUSION

Based on the findings of the present study, it can be concluded that the aqueous and methanolic extract of *B. virens* was found to be safe up to 2000 mg/kg body weight after single dose oral administration to female Sprague-Dawley rats and the LD_{50} was more than 2000

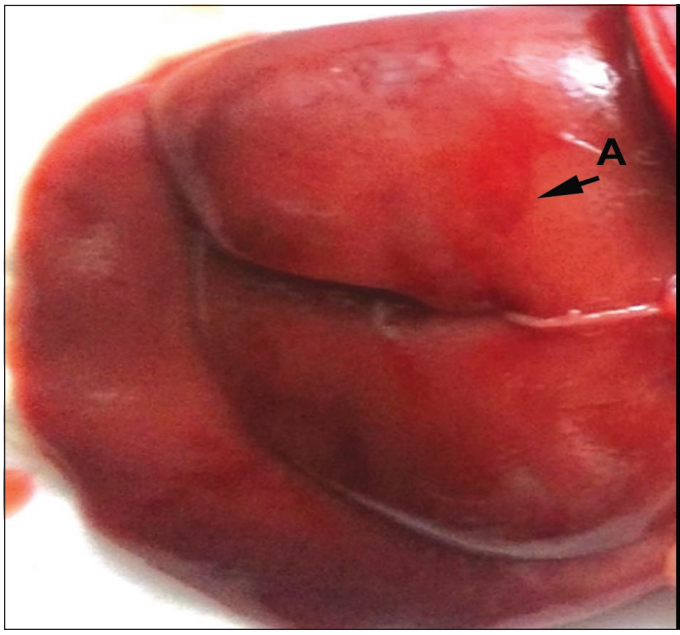

Fig. 4. Liver (Gross), a. Multi-focal pale areas.

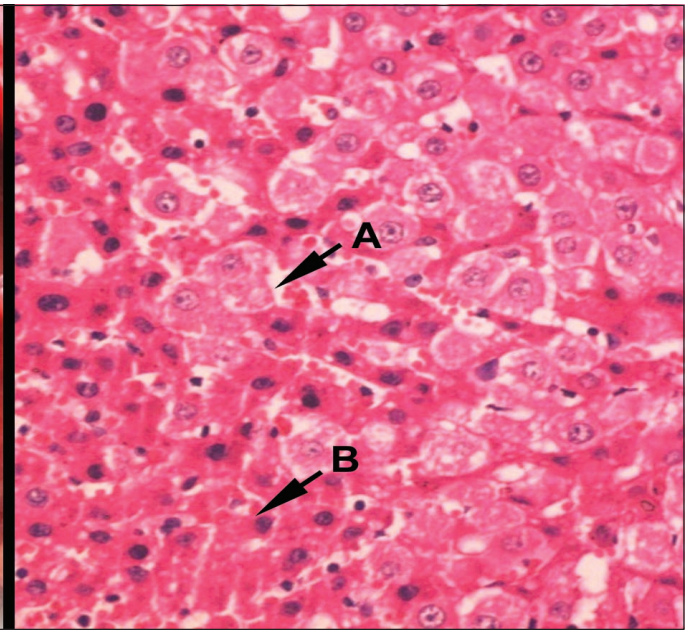

Fig. 5. Liver (tissue section) 40X.
a. enlarged hepatocytes b. normal parenchyma.

mg/Kg. Gross and histopathological findings evinced the hepatotoxicity and pulmonary toxicity of methanolic extract. The aqueous extract of the plant was found to be safer compared to methanolic extract.

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REFERENCES

Handa SS, Khanuja SP, Longo G, Rakesh DD (2008) Extraction Technologies for Medicinal and Aromatic Plants. International Centre for Science and High Technology. 23.

Harborne JB (1998) Phytochemical methods: A guide to modern techniques of plant analysis (3rd edn.) Chapman and Hall, London. 302.

Hoffmann D (2003) Medical Herbalism: The Science and Practice of Herbal Medicine, Inner Traditions/Bear & Co. Publishers. 672.

Nusrat Zahan M, Reza MA, Talukder M, Ali MS, Paul TK, Parvej MS (2015) *Blumea lacera* Plant Poisoning in Cattle; Epidemiology and Management. Turkish J Agri Food Sci Tech 3(8): 635-638.

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