Pak. J. Sci. Ind. Res. 2006 **49**(6) 410-413

Acute Toxicity Studies of *Bombax cieba* Flowers In Mice and Rats

Zakir-ur-Rehman, Atiq-ur-Rehman* and Shahnaz Ahmad
Pharmaceutical Research Centre, PCSIR Laboratories Complex Karachi,
Karachi-75280, Pakistan

(received November 24, 2005; revised September 29, 2006; accepted October 10, 2006)

Abstract. Aqueous extract of *Bombax cieba* (red silk cotton tree) flowers exhibited a marked action on central nervous system. The signs and symptoms observed in non-lethal doses through oral and intravenous routes in rats and mice were found to be solely functional and short-lived, while lethal doses imparted pharmacological and toxicological action by affecting physiological mechanism of the body. Furthermore, the magnitude and intensity of the toxic symptoms exhibited were found to be highly dose dependent. The mortalities that occurred may be due to the direct action on central nervous system. The LD_{50} as calculated for oral route in rats was 6768.730 mg/kg and for intravenous route in rats and mice were 889.496 mg/kg and 467.84 mg/kg, respectively.

Keywords: *Bombax cieba* flowers, red silk cotton tree, toxicity study, short term toxicity

Introduction

Bombax cieba Linn. (family: Bombaceae), syn. *Salmalia malabarica* Schout, *Bombax malabaricum* DC, is one of the trees of infernal regions. It is commonly known as sainbhal, nirma and red silk cotton tree (Anon., 2002; Krishnamurti and Chadha, 1972; Nadkarni, 1954; Dymock *et al.,* 1890). The plant is found abundantly in Pakistan, India (Eastern Himalayas, Assam, West Bengal), Burma and Sri Lanka (Krishnamurti and Chadha, 1972; Nadkarni, 1954; Dymock *et al.,* 1890). The plant is not only valued for its economic importance (Seth, 2004; Chawla and Sharma, 1972; Sharma and Thakur, 1992), but also for its medicinal importance. Almost all parts of the plant are used for different medicinal purposes, locally as well as systemically (Krishnamurti and Chadha, 1972; Nadkarni, 1954). *B. cieba* is used as a remedy for diarrhoea, dysentery, menorrhagia, leucorrhoea, haemorrhoids, piles, conjunctivitis, skin eruption, boils, sore throat, itch, inflammation, acne, and pimples with great success (Young *et al.,* 2003; Krishnamurti and Chadha, 1972; Yang *et al.,* 1970; Nadkarni, 1954). The plant, specially flowers, also possesses astringent, demulcent, stimulant and aphrodisiac properties. It also acts as a tonic, improves general debility as rejuvenative. Studies have also revealed its antiangiogenic and cytotoxic (Young *et al.*, 2003), musculotropic (Misra *et al.,* 1968), hypotensive (Rubeena *et al.,* 2003; Rubeena and Mohammed, 1999), and diuretic and antifungal activities (Puckhabar and Stipanovic, 2001).

Ample data regarding the phytochemical analysis of the plant is available, which reveals the presence of alkaloids, glycosides, flavonoids, terpenes, sesquiterpenes and tannins (Sankaram *et al.,* 1981; Rizvi and Saxena, 1974; Yang *et al.,* 1970); phenolic compounds, lupeol and anthocyanins (Seshadri *et al.,* 1973; Niranjan and Gupta, 1973); and polysaccharides, sugars, proteins, essential oils, amino acids, colouring matter and trace metals (Bushra and Mohammed, 1987; Haq and Gomes, 1973; Agarwal *et al.,* 1972; Duong *et al.,* 1969). However, very little has been reported on the toxicity of *Bombax cieba*. The use and practical implications of the plant, as a source of drug, requires convincing proofs for the absence of toxic and deleterious affects. Therefore, the present work is aimed to quantify the risk of untowards signs and symptoms according to the dose and route of application.

Materials and Methods

Plant material. Fresh flowers of *Bombax cieba* were collected from the surroundings of the University of Karachi (Karachi, Pakistan) during the month of March 2005. Flowers were authenticated at the Department of Botany, University of Karachi. A voucher specimen was deposited in the same department for further reference.

Preparation of extract. Flowers (without sepals) were washed and dried in air at room temperature. The material was then chopped into small bits and soaked for 72 h in 95% ethyl alcohol (100 g/1 litre), with continuous agitation for 6 h/day. The solvent was decanted and fresh ethyl alcohol was again added to the material. This process was repeated thrice to obtain maximum quantity of the extract. The extracted material was pooled, and the solvent was then removed under reduced pressure at 45 °C ± 5 °C. This afforded a crude ethanolic extract (29.097%). A part (50%) of the alcoholic extract was partitioned with water and petroleum ether

*Author for correspondence

(2 : 1 v/v). The aqueous layer was then separated and concentrated under reduced pressure at room temperature into a semi-solid mass and was marked as the aqueous extract, which was used for further study.

Toxicity studies. *Selection of animals.* Healthy albino mice (Swiss albino strain) and rats (Spraug Dawly strain) of both sexes, reared at the PCSIR animal house, weighing 20-30 g and 130-140 g, respectively, were selected for acute oral and intravenous toxicity studies. Animals were maintained on standard diet and water ad libitum. All animals were kept under optimal experimental conditions for a period of 7 days before the toxicity studies. Cages were marked with their respective doses and the route of administration. Each dose group comprised of nine animals, and each dose was repeated thrice to confirm the results. Control group was run simultaneously, using distilled water. The feeding and injecting volumes were kept constant throughout the study. Care was taken not to injure the animals while feeding. Distilled water was used as a solvent. Feed was withdrawn 24 h before the onset of the studies from the test animals, but not the water.

***Oral route toxicity studies*.** Different concentrations (mg/kg) of the aqueous extract of *Bombax cieba* flowers were given/ administered orally by means of an appropriate gavage in a single dose to the animals of different groups (of only rats). All dose groups were observed for a period of 24 h for gross physical and behavioural changes, and then again for 48 h. The LD_{50} was calculated by the method of Reed and Munch (Turner, 1965) on the basis of 24 h mortality.

Parenteral route toxicity studies. Intravenous toxicity of aqueous extract was done in both mice and rats by injecting the drug through tail vein at different doses to each group. The volume and time taken for injection was kept constant. All animals, (both rats and mice) were observed carefully for gross physical and behavioural changes. Mortality rate was noted, autopsy was done, and macroscopic findings were noted. The LD_{50} was calculated on the basis of 24h mortality by the method of Reed and Munch (Turner 1965).

Results and Discussion

Evaluation of toxicities induced by the aqueous extract of *Bombax cieba* flowers, through oral and intravenous routes, are shown in Figs. 1-3. The nature, severity and depth of all signs and symptoms exhibited by both routes in both animal species used were found to be the same. Marked variations in toxic signs and symptoms and subjective changes in mood and behaviour were found to be proportional to the concentration of the extract used and the route of administration.

Oral toxicity. No mortality was observed upto a dose of 6000 mg/kg, while a dose above that resulted in mortalities. The LD_{50} was found to be 6768.730 mg/kg (Fig. 1). A dose upto 3000 mg/kg caused activeness, alertness, vasodilatation and rapid shallow respiration, whereas doses above that resulted in marked physical and behavioural changes. The common manifestations were vasodilatation, followed by vasoconstriction, piloerection, tachycardia, dried mouth, bulging of eyes, dysponea, discomfort, irritability, restlessness, tremor, frequent urination, retching, gagging, decreased locomotive activity, abnormal gait, convulsion, cardiac decompensation, and cyanosis resulting in generalized weakness, paralysis of hind limbs, loss of concentration, sedation, loss of consciousness, comma and death. Animals also exhibited apathy to external stimuli. In high doses, animals became highly anoxic.

Amelioration of all these signs and symptoms in the surviving animals took 5-45 min in lower doses (3000 mg/ kg), while in higher doses the recovery time was enhanced upto 110 min.

Autopsy findings made on the dead and moribund animals revealed the presence of fluid in abdominal cavity, dilatation of blood vessels, congestion of lungs, haemorrhagic spots on heart, liver and lungs. Haemorrhagic signs and clotting of blood were also seen in brain.

Intravenous toxicity. The aqueous extract of *Bombax cieba* flowers was found to be safe upto a dose of 750 mg/kg in rats (Fig. 2) and 450 mg/kg in mice (Fig. 3), whereas doses above that resulted in mortalities. A dose of 1050 mg/kg and 480 mg/kg in rats and mice, respectively, were found to be lethal and the LD_{50} was 889.496 mg/kg and 467.84 mg/kg, respectively, in rats and mice (Fig. 2 and Fig. 3).

Signs and symptoms observed through this route of extract application were found to be the same as observed for oral route application. The difference was only in the time of onset of toxic signs, symptoms and the duration of action. The important features observed through this route were retching and gagging, followed by marked depression, decreased physical activities, frequent urination, ataxia, paraplegia, cynosis, mental cloudiness, sedation, hypnosis, coma and finally death. The induction time for all these signs and symptoms ranges from 2-10 min, while the amelioration time ranged from 15-95 min in non-lethal doses; and in lethal doses the time was enhanced to 170 min. It was interesting to note that the extract was more toxic towards males as compared to their female counterparts.

The aqueous extract of *B. cieba* exhibited marked action on central nervous system. It increased the pain threshold and decreased the awareness of the animal to external stimuli.

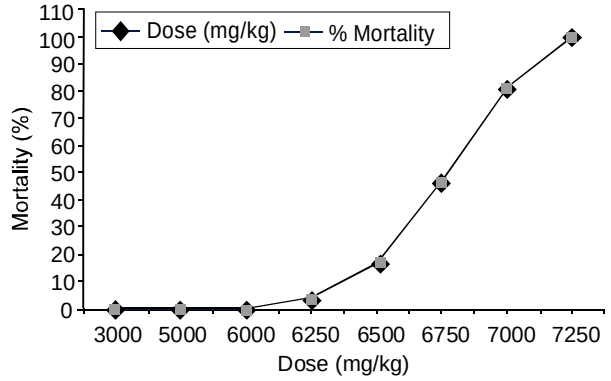

Fig. 1. Oral toxicity of the ethanol extract of *Bombax cieba* flowers in rats.

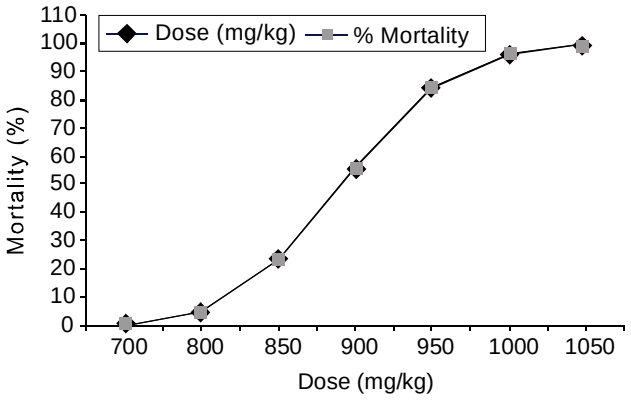

Fig. 2. Intravenous toxicity of the ethanol extract of *Bombax cieba* flowers in rats.

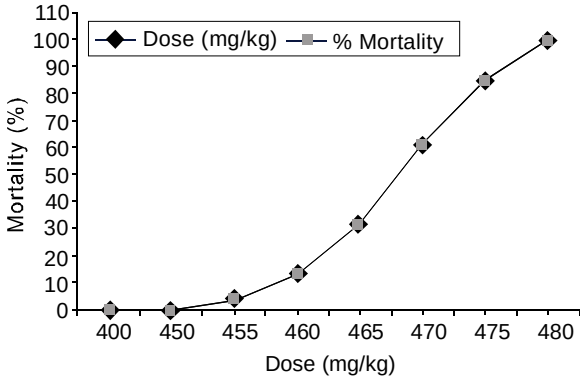

Fig. 3. Intravenous toxicity of the ethanol extract of *Bombax cieba* flowers in mice.

Furthermore, in the doses where the animal survived, the effects were observed on gross motor activity, such as paralysis, impairment of reflexes, sensation, and produced anoxia. These functions were improved as soon as the animal was out of the toxic effects, i.e., improvement of cardiac decompensation. Furthermore, hypoxia leads to decreased heart rate, which resulted in circulatory failure. Hypoxia also affects the central nervous system (CNS), both functionally and morphologically. The brain is usually the first organ to manifest hypoxic damage due to circulatory oxygen insufficiency as a result of accumulation of carbon dioxide. The aqueous extract of *B. cieba* also depressed the excitatory activity of the cerebral cortex and decreased the threshold for the production of seizures, which was evident by retching, gagging and convulsion. It is also evident that hypoxic condition stimulates the rate and depth of respiration. Reduction in oxygen saturation fails to increase the respiratory minutes volume, which results in death of animals.

Conclusion

The aqueous extract of *Bombax cieba* was added to have a great margin of safety and having a definite action on the central nervous system. Higher concentrations of the extract affected physiological activities and mechanism of the body due to the specific toxic action of the aqueous extract. These actions can be avoided by using appropriate and accurate adjustment of dose.

References

Agarwal, G.D., Rizvi, S.A, Gupta, P.C., Tewari, J.D. 1972. Study of polysaccharides from the stamen of *Bombax cieba* flowers. *Planta Med.* **3:** 293-303.

Anon, 2002. *Herbal Health Care.* Himalaya Herbal Monograph. p.107 Himalaya Drug Company, Himalaya.

Bushra, K., Mohammad, A.K. 1987. Amino acid and sugar constituents of *Bombax malabaricum. Pak. J. Sci. Ind. Res.* **30:** 148-149.

Chawla, J.S., Sharma, A.N. 1972. Hardwoods for papers. *Indian Pulp. Pap.* **26:** 116-118.

Duong, T.P., Meier, H., Wiesemauller, W. 1969. Protein and amino acid content in some extracted oil meals of Vietnam Democratic Republic. *Wiss. Z. Univ. Rostock Math Naturwiss Reihe.* **18:** 151-153.

Dymock, W., Warden, C.J.H., David, H. 1890. *A History of the Principal Drugs of Vegetable Origin met within British India.* vol. **1**, p. 215, William Kegan Paul, Trench, Trubner & Co. LD.

Haq, Q.N., Gomes, J. 1973. Water soluble polysaccharides from roots of *Salmalia malabarica*. *Bangladesh J. Sci Ind. Res.* **8:** 16-20.

Krishnamurti, S.A., Chadha, Y.R. 1972. *The Wealth of India*, vol. **9**, pp.177-183. Publication and Information Directorate, CSIR, Hillside Road, New Delhi, India.

Misra, M.B., Misra, S.S., Misra, R.K. 1968. Pharmacology of

Bombax malabaricum DC. *Indian J. Pharm.* **30:** 165-168.

Nadkarni, K.M. 1954. *Indian Materia Medica.* vol. **1**, pp. 207-209, 3rd edition, Popular Book Depot, Bombay 7. Dhootapapeshwar Parkashan Ltd. Panvel, India.

Niranjan, G.S., Gupta, P.C. 1973. Anthocyanins from flowers of *Bombax malabaricum. Planta Med.* **24:** 196-199.

Puckhaber, I.S., Stipanovic, R.D. 2001. Revised structure for a sesquiterpene lactose from *Bombax malabaricum. J. Nat. Prod.* **64:** 260-261.

Rizvi, S.A.I., Saxena, O.C. 1974. New glycosides terpenoids coloring matters, sugars and fatty compounds from flowers of *Salmalia malabarica*. *Arzneim-Forsch.* **24:** 285-287.

Rubeena, S., Syed, I.A., Mohammad, A., Zareen, F. 2003. Hypotensive activity and toxicology of constituents from *Bombax cieba* stem bark. *Biol. Pharm. Bull.* **26:** 41-46.

Rubeena, S., Mohammad, A. 1999. Hypotensive, hypoglycemic and toxicological studies on flavonol C glycoside shamimin from *Bombax cieba*. *Planta Med.* **65:** 331-334.

Sankaram, A.V.B., Reddy, N.S., Shoolery, J.N. 1981. New sesquiterpenoids of *Bombax malabaricum*. *Phytochemistry* **20:** 1877-1881.

Seshadri, V., Batta, A.K., Rangaswami, S. 1973. Phenolic component of *Bombax malabaricum*. *Indian J. Chem.* **11:** 825-827.

Seth, M.K. 2004. Trees and their economic importance. *Botanical Review* **69:** 321-376.

Sharma, K.R., Thakur, N.S. 1992. Evaluation of wooden boxes fabricated from lesser valued farm tree species for packaging and transportation of plum. *J. Food Sci. Technol.* **29:** 233-236.

Turner, R.A. 1965. *Screening Methods in Pharmacology*. pp. 62-63, Academic Press, New York and London, UK.

Yang, T.H., Chen, K.T., Ch'en, C.H., Kao, Yu-Pe. 1970. Constituents of *Bombax malabaricum*. *Taiwan K'O Hsuch.* **24:** 15-18.

Young, J.Y., Nguyen-HaiNam., Young, K., Ki-Hwan, B., Byung, Z.A. 2003. Antiangiogenic activity of lupeol from *Bombax cieba*. *Phytother. Res.* **17:** 341-344.