Behavioral assessment of albino rats after oral ingestion of *Catharanthus roseus* aqueous leaf extract

Baby Tabassum[a], Priya Bajaj[a] and Mohammad Hashim[b]
Toxicology Laboratory, Department of Zoology, Govt. Raza P.G. College, Rampur, UP, India

Abstract

India is a country with rich biodiversity & long history of herbal/ tradition medication against an under of ailments. Our study was designed of assess the good / bad effect of one such well known medicinal or ornamental plant *Catharanthus roseus.* The Study was strictly qualitative predicting the effect of *C. roseus* aqueous leaf extract oral ingestion on behavioral, food consumption & water intake of albino rats, and to find a safe dose for further use. For the purpose, twenty five rats were grouped to in five groups- one control and four treated with different doses 100mg/kg, 200mg/kg, 250mg/kg, 500mg/kg of *C. roseus* leaf extract. After detailed examination of 48 hrs it was found to be safe on almost all doses but a dose of 250 mg/kg body weight was safest & suggested to be used for further biochemical/ histological studies.

KEYWORDS:-*Catharanthus roseus*, acute toxicity, Albino Rats, Oral ingestion, behavior

Introduction

India possesses a rich biodiversity of the medicinal plants that are still not explored completely. *Catharanthus roseus* is one of them which is being used as Ayurvedic medicine for treatment of many fatal diseases, all over the world. It is known to possess good antioxidant potential. The decoction of this plant is used to treat diabetes, reduces blood pressure, cancer **(Wiart C *et al.*, 2002)**& Insomnia. Vincristine & Vinblastine which are major known ingredient of the plant, are clinically used as chemotherapeutic agents for acute lyphoblastic, leukaemia, lymphomas, multiple myeloma, Hodgkin`s disease and testicular carcinoma **(James SA *et al.*, 2007).***Catharanthus roseus* is a native to the Indian Ocean, Island of Madagascar, now a popular ornamental plant common in many tropical and subtropical regions worldwide, including the Northern India **(Frode TS., 2008).**The need for the novel pharmaceutical products has attained a great interest in the present research world due to the cost effective and the higher side effects associated with the chemical & herbal allopathic drugs. *Catharanthus roseus* is a potent medicinal plant with many pharmacological actions such as antioxidant, antimicrobial, anthelmintic, anti-feedant, anti-sterility, anti-diarrhoeal, anti-diabetic etc. **(Gajalakshmi S *et al.*, 2013),** that is why it is used to treat many fatal diseases. Alkaloids with various medicinal uses are the major phytochemical constituent of this plant.The objective of this study is to examine toxic abnormal effect of aqueous leaf extract of *Catharanthus roseus*, and to find an adequate dose for further studies. The study was designed to assess the effect on behaviors parameters like - corner sitting, Drowsy, Sleeping & food avoidance etc.

Materials & Methods

Plant Material

The *Catharanthus roseus*plant used in the experiment was grown in the campus of Govt. Raza P.G. College Rampur (U.P) near laboratory & provided standard 12 hrsLight/dark conditions. The leaves were collected with their tender petiole after the complete maturation of plants and subjected to prepare aqueous extract. For the purpose, fresh plant material was washed thoroughly in tap water to remove traces of soil and any other contaminants. Further, known quantities of wet leaves and tender petiole were homogenized with distilled water in a clean and deionized pastel mortar. The homogenized mixture was then centrifuged at 1000g for 15 minutes. Supernatant was decanted and collected to store at 4˚C in prewashed glass bottles for further use**(Kandil*et al.,* 1994).**GC/MS analysis of leaf extract was also performed to find out its major constituents.

Experimental Animals

Twenty five adult male Wister albino rats (*Rattus norvegicus*) of almost same age and weight (150 ± 10 gm), were procured from inbred colony. They were acclimatized at room temperature with 12 hrs dark/light cycle and fed on standard diet and water *ad-libitum.* All experiments were performed as per animal institutional ethical committee (**360/01/CPSEA/2001**).Animals were divided into five groups. **Group I** (control) received distilled water only, whereas the remaining **four groups** orally treated with single dose of 500mg/kg, 250mg/kg, 200 mg/kg and 100mg/kg aqueous leaves extract of *Catharanthus roseus,* respectively for 2 days. All the animals were closely observed after each dosing to examine any change. The changes were captured in high quality camera **(Bakere RI *et al.,* 2011).** Changes in body weight and behaviors, food consumption and water intake were regularly recorded during the experimentation period. The dose schedule is depicted in Table-1.

Results & Discussion

Ingestion of aqueous leaf extract of *Catharanthus roseus* at any dose did not cause mortality. The body weight, food & water consumption showed no significant difference after *Catharanthus roseus* low dose treatment. The results were qualitative and complicated. Thus depicted as a comparative a marker of intensity shown by symbols of plus (+) and Minus(-)in**Table-2**.

The table 2 concludes that **Group I (Control)** did not show any significant change during the entire experiment. In Group-II, administration of 100 mg/kg leaf extract caused same behavioral pattern up to 48 hrs. In Group-III & IV, 200mg/kg & 250 mg/kg leaf extract respectively, increased some behavioral pattern like corner sitting, drowsy, sleep cycle, food consumption & water intake up to 24 hrs but they disappear after 24 hrs. In Group-V 500 mg/kg leaf extract highly increased all behavioral pattern like corner sitting, Drowsy, Itching, sleep cycle, food consumption & water intake except salivation up to 40 hrs but some of them like drowsiness & corner sitting retained even up to 48 hrs. Comparatively loss of body weight ±15 gm has been notice in higher dose consumption of leaf extract after 48 hrs.

Toxicological studies are the platform for hazard identification and safety assessment **(Wallace HM., 2011).** Observations of the experiment suggest that *C. roseus* extract might exert hepato-toxic effect, which increase along with increase in concentration. Similar observation was made by **Pinkerton *et al.* (1988).Kevin LYW *et***

***al.*, 2012** according to the study of suggested that the *C. roseus* extract was highly toxic at doses of 500 mg/kg and 1000 mg/kg and caused the rats to have diarrhoea and significant increase in relative liver weight. Treatment with 500 mg/kg *C. roseus* revealed the targeted toxic effects of this plant extract on rat livers within a short period**.**

Another possible reason for the toxic effect of leaf extract has been given by **Khan 2007** at higher doses may be due to the very slow excretion rate and liver metabolism of *C. roseus* extract which makes the alkaloid to linger in the body in his studies**.** The toxic effect associated with *C. roseus* extract alkaloids as reported by **Rosazza*et al.* (1992** include selective reversible inhibition of monoamine oxidase-B (MAO-B) that is important in the biotransformation of xenobiotic. In addition, alkaloids have been found to decrease red blood cells and the formation of granulocyte and leukocytes giving rise to bone marrow depression and neurotoxicity and further behavior changes**(Barnett *et al.*, 1978; Lobert*et al.*, 1998; Alexandrova*et al.*, 2000; James *et al.*, 2006 & Khan 2007).**

Thus, from the experimental results it could be concluded that a dose of 500mg/kg leaf extract is toxic in albino rats. However, the low doses *i.z.* 100 mg/kg, 200 mg/kg & 250 mg/kg body weight do not produce noticeable behavioral changes. Though, further clinical studies require before implication in human beings. As far as experimental animal albino rat is concerned, on suitability basis, the doses of 200 mg/kg & 250 mg/kg could be used for further experimentation. Whereashigh dose is unsafe may be due to the presence of poisonous, chemical known as vinca alkaloids (Drug Digest: *C. roseus*), *Vincarosea* contain 2 classes of active compounds, the alkaloids and tannins. More than 100 alkaloids have been found in *C. roseus*.

Vinblastine and Vincristine extracted from this plant are reported as treatment of leukemia (Drug Digest: *C. roseus*). Rosenidin is an anthocyanidin pigment found in the flowers of *C. roseus* (**Taki K, *et al.*, 2008**). Because of its detoxification and counteracting poison properties, suitability dose for its beneficial use is required to carry out further experiments.

Acknowledgement

The Authors are thankful to Council of Science & Technology (CST), U.P., India for providing financial assistance to conduct this study.

REFERNCES

1. Alxeandrova, R; Alxeandrova, I; Velcheva, M. &Varadino, T.(2000) Phytoproducts and cancer. Experimental Pathology and Parasitology. 4: 15-25
2. Bakere RI, Magbaghoeal OA, Akinwande AI, Okunowo OW, Green M.(2011) Antidiarrhoeal activity of aqueous leaf extract of *Momordicacharanthia* in rats. J pharmacognphytother. 3 (1): 1-7.
3. Barnett, C. J; Cullinan, G. J; Gerzon, K; Hoying, R. C; Jones, W. E; Newlon, W. M; Poore, G. A; Robison, R. L; Sweeney, M. J. & Todd, G. C.(1978) Structure-activity relationships of dimeric*Catharnthus* alkaloids. 1-Deacetylvinblastine amide (vindestine) sulfate. Journal of Medicinal Chemistry. 21(1): 88-112.
4. Frode TS, Medeiros YS.(2008) Animal Models to test drugs with potential antidiabetic activity. Journal of Ethanopharamacology. 115:173- 183.

5. Gajalakshmi S, Vijayalakshmi S &Rajeshwari V*.(2013) Apr Pharmacological activities of *Catharanthus roseus*: A Perspective review.Int J pharm Bio Sci. 4(2): (P) 431- 439.
6. James S. A., Bilbis S. L. & Muhammad, B. Y.(2006) Phytochemical studies and Antibacterial activity of aqueous leaf extract of *Cathatanthus roseus*. Standardizer of Nigerian Academic,) Vol. 2 No. 2: 111-119.
7. James SA, Bilbiss L, Muhammad BY.(2007) The effects of *Catharanthus roseus* (L) G Don 1838 Aqueous Leaf extract on some liver enzymes, serum proteins and vital organs. Sci World J. 2 (1): 5- 9.
8. Kandil, O., Radwam, N.M.M .Hassan, A.B.Amber, A.M.M., Ei-banna, H.A.andAmer, W.M.M.(1994) Extract and Fractions of *Thymus capitatus* exhibit antimicrobial activities. Journal of Ethopharmacol. 44(1): 19-24.
9. Kevin LYW, Hussin AH, Zhari I, Chin JH.(2012) Sub- acute oral toxicity study of methanol leaves extract of *Catharanthus roseus* in rats. Jounal of Acute Disease, 38-41.
10. Khan, H. (2007) Vinca alkaloids-Periwinkle Vine. Interscience. http://www3.interscience.wiley.com (retrived 5th March, 2007).
11. Lobert, S; Frankforter, A. &Correla, J. J.(1998) Energetisc of Vinca alkaloid interaction with tubulin isotypes: Implications for drug efficacy and toxicity. Cell motility and the cytoskeleton. 39(2): 107-121.
12. Pinkerton, C. R.; McDermott, B; Philip, J.; Biron, P; Andiet, C; Vandenberg, H. &Brunat-Mentigny, M. (1988)Continuous Vincristine infusion as part of a high dose chemo-radiotherapy regimen: Drug kinetic and toxicity. Cancer Chemotherapy and Phamacology. 22(3): 271-274.
13. Rosazza, J. P. N; Duffel, M. W.; El-Marakby, S. &Ahn, S. H.(1992) Metabolism of the Catharanthus Alkaloids: From Streptomyces Griseus to monoamine oxidase B. Journal of Natural Products. 55(3): 269-284.
14. Taki k, Saito N, Tric Y, Tatsuzawa F, Shigihara A, Honda T. (March 2008). 7-0-Methylated anthocyanistry glycosides from *Catharanthus roseus.*Phytochemistry. 69 (5): 1215-9.
15. Wallace HM. (2011)Risk perception in toxicology – Part II: Toxicology must be the solution not the problem. Toxicol sci. 121(1): 7-10
16. Wiart C. (2002) Medicinal plant of Southeast Asia. Selangor: Prentice Hall; p. 224- 225.

Table: - 1: Dose schedule of *Catharanthus roseus* aqueous leaf extract in Albino rats.

Control		***Catharanthus roseus* aqueous leaf extract treated groups**							
Group I		**Group II**		**Group III**		**Group IV**		**Group V**	
Dose	**B. Weight**	**Dose**	**B. Weight**	**Dose**	**B. Weight**	**Dose**	**B. Weight**	**Dose**	**B. Weight**
Water	150±10	100 gm/kg	150±10	200 gm/kg	150±10	200 gm/kg	150±10	200 gm/kg	150±10

Table: - 2: The Effect of Aqueous Leaves Extract of *Catharanthus roseus* on the behavior in albino rats.

Groups	**Body Weight**	**Time**	**Symptoms**							
			Mortality	Corner Sitting	Salivation	Drowsy	Itching	Sleeping	Food Consumption	Water consumption
Group I (Control)	No Loss	8 hrs	Nil	-	-	-	-	-	-	-
		16 hrs	Nil	-	-	-	-	-	-	-
		24 hrs	Nil	-	-	-	-	-	-	-
		32hrs	Nil	-	-	-	-	-	-	-
		40 hrs	Nil	-	-	-	-	-	-	-
		48hrs	Nil	-	-	-	-	-	-	-
Group II (100 mg/kg)	No Loss	8 hrs	Nil	-	-	+	-	+	-	-
		16 hrs	Nil	-	-	-	-	+	-	+
		24 hrs	Nil	-	-	-	-	-	-	-
		32hrs	Nil	-	-	-	-	-	-	-
		40 hrs	Nil	-	-	-	-	-	-	-
		48hrs	Nil	-	-	-	-	-	-	-
Group III (200 mg/kg)	±10g	8 hrs	Nil	++	-	++	-	++	++	++
		16 hrs	Nil	+	-	+	-	+	+	+
		24 hrs	Nil	-	-	-	-	-	-	-
		32hrs	Nil	-	-	-	-	-	-	-

		40 hrs	Nil	-	-	-	-	-	-	-
		48hrs	Nil	-	-	-	-	-	-	-
Group IV (250 mg/kg)	±11g	8 hrs	Nil	++	-	++	+	++	++	++
		16 hrs	Nil	+	-	+	-	+	+	+
		24 hrs	Nil	+	-	-	-	-	+	-
		32hrs	Nil	-	-	-	-	-	-	-
		40 hrs	Nil	-	-	-	-	-	-	-
		48hrs	Nil	-	-	-	-	-	-	-
Group V (500mg /kg)	±15g	8 hrs	Nil	+++	-	+++	+	+++	+++	++++
		16 hrs	Nil	++	-	++	++	++	++	++
		24 hrs	Nil	+	-	+	+	+	++	+
		32hrs	Nil	+	-	+	+	+	+	+
		40 hrs	Nil	+	-	+	+	-	+	-
		48hrs	Nil	-	-	+	-	-	-	-

Fig. 1:-In Group-V 500 mg/kg leaf extract effect on sleep cycle drowsy retained even up to 48 hrs after dosing.

Fig. 2:-In Group-V 500 mg/kg leaf extract effect on behavior shows corner sitting retained even up to 40hrs after dosing.

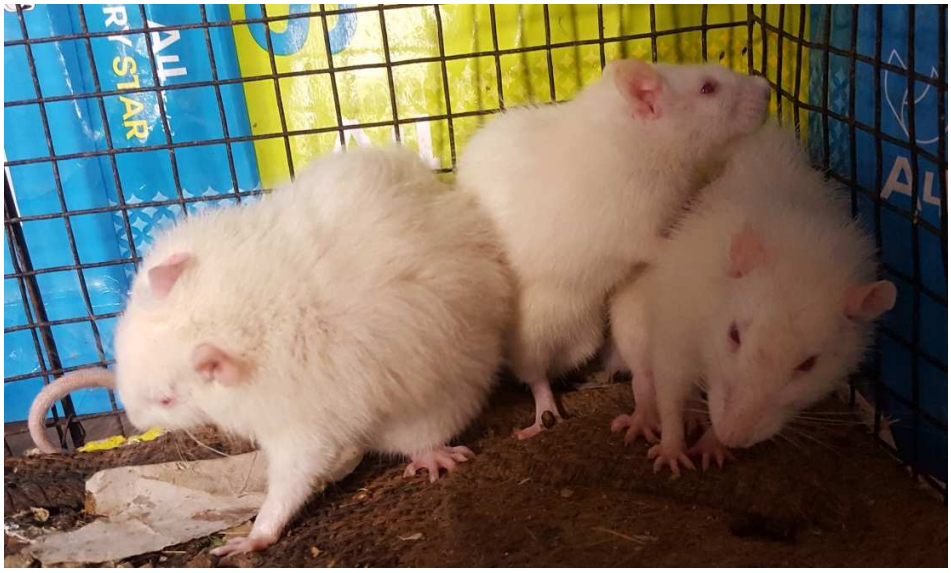

Fig. 3:-In Group-V 500 mg/kg leaf extract effect on behavior showsdrowsy retained even up to 48 hrs after dosing.