



STUDY OF HERBAL EXTRACT ON HAEMATOLOGICAL PARAMETERS IN TREATED RATS

Zoology

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ABSTRACT

The assessment of haematological parameters in rat can be used to determine the extent of deleterious effects of a plant extract on animal blood. Oral administration of alcoholic extract of *Clitoria ternata* (L.) leaves show significant changes in the RBCs, Haemoglobin, platelets and lymphocytes, while other parameters are not altered significantly. This data suggests that the plant extract had no adverse effect but are safe as food and is in use as herbal medicine for other treatments by tribal people.

KEYWORDS

INTRODUCTION :

Herbal medicine system still plays a major role in the delivery of primary health-care in rural and also in urban areas. These medicines have less side effects and man can get it easily from nature (Mishra and Tandon, 2012). Search of an herbal agent that can control and capable of normalizing biochemical and hematological indices in human is occurring from prehistoric times.

The medicinal plants are utilized by traditional medicinal practitioners for treatment of a diverse variety of diseases, against some of which the medicine has no known cure. Over 5000 plants are known to be used for medicinal purposes all over the world, but only a few have been described or studied (Taylor *et al.*, 2001).

Folk medicine is the most widely used form of traditional medicine in our country. These practitioners believe that ailments can occur through accumulation of toxins in blood, which in turn results from undigested food or improper emptying of bowels on a regular basis. This accumulation of toxins in blood and purification of blood through ingestion of medicinal plants is quite an established concept among various traditional medicinal systems and indigenous communities throughout the Indian sub-continent.

The Amravati district of Maharashtra lies in close vicinity of Satpuda Mountains. The Melghat region and Satpuda Mountains of Amravati district are known for their rich flora. The tribals of this region have been using various plants as blood purifier. Unfortunately, the ethanobotanical enumerations for its effect on haematological and biochemical parameters and regulatory properties were not recorded for this region. Therefore, the present study is a small effort to gain insight in the knowledge of traditional medicine of this region and establishing their haematological and biochemical parameters regulatory properties.

MATERIAL AND METHODS:

The plant, *Clitoria ternatea* belonging to the family Fabaceae, Order-Fabales is a perennial twining herb, Flowers - bright blue, violet or sometimes white, widely distributed throughout Indian subcontinent, used as ornamental plant. Various parts of *C. ternatea* have been reported to have tranquilizing property, anti-inflammatory, analgesic activity, antipyretic, and immunomodulatory activities (Mukherjee *et al.*, 2008). The flavonol glycoside present in roots is reported to have antibacterial activity (Yadava *et al.*, 2003). *C. ternatea* has been reported to have anti-inflammatory, hepatoprotective (Solanki and Jain, 2011), antihyperlipidemic (Solanki and Jain, 2010) and immunoinhibitory activities, antimicrobial properties for the control of infectious diseases (Nadkarni, 2000).

Preparation of extract: The leaves of above mentioned plants were collected, shade dried, powdered and subjected to Soxhlet extraction with ethanol. The extract was evaporated to near dryness on a water bath, weighed and kept at 4 °C in refrigerator until use. The presence of various plant constituents in the plant extract was determined by preliminary phytochemical screening as per Thimmaiah (2004).

Procurement and rearing of experimental animal: Healthy Wistar strain female albino rats of about two month old and weighing 150-300 gm allowed to acclimatize to laboratory environment for 15 days before experimentation.

Acute toxicity study: Healthy female albino rats were starved for 3-4 hr and subjected to acute toxicity studies as per OECD guidelines No: 423 (2001). For experimentation they were divided into 2 groups of 6 animals each and kept singly in separate cages during the experiment.

Group 1: served as the control and received distilled water.

Groups 2: received the alcoholic extract of *Clitoria ternatea* leaves orally at the dose of 350mg/kg body weight in 2 ml of distilled water respectively for 21 days via gastric intubation.

After 21 days, the rats were subsequently anaesthetized and blood sample were collected into EDTA/ lithium heparin bottles.

The haematological parameters i.e. red blood cell counts (RBC), haemoglobin (Hb) concentration, The packed cell volume (PCV), white blood cell count (WBC), mean cell volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were determined according to the method of Dacie and Lewis (1994). Platelet (PLT) count and WBC differentials (neutrophils, monocytes, lymphocytes, basophils and eosinophils) were analyzed according to the standard technique described by Beker *et al.*, (1998) and Cheesbrough, (2000).

All the data are expressed as mean $\pm$ S.E. Statistical analysis was done by Student's t-test (Mahajan, 1997).

RESULT

The effects of the doses of the alcoholic leaves extract of *Clitoria ternatea* on haematological parameters of Wistar rats are shown in Table 1. The extract did not significantly alter the levels of Hb and MCHC. The differential white blood cell counts in the treated groups were not significantly different from that of the control group, while those of RBC, hematocrit, MCH, PCV, MCV were significantly increased at certain doses. These indices suggest that the extract does not possess toxic substances that can cause an anaemic condition in rats. The administration of the extract did not alter the level of WBC and its differentials, including basophils, eosinophils and monocytes as well as platelets throughout the experimental period, while that of lymphocytes was only increased significantly at 400 mg/kg body weight.

Table 1: Effect of the ethanolic leaves extract on haematological parameters in female rats

Parameter		Control	Experimental group <i>Clitoria ternatea</i>
		Vehicle	400 mg/kg b. w.
Red blood cell	RBC	6.87 $\pm$ 0.29	5.68 $\pm$ 0.57*
	Hb (gm/dl)	13.35 $\pm$ 0.35	12.41 $\pm$ 0.13**
	Hematocrite (%)	53.12 $\pm$ 0.14	38.25 $\pm$ 0.38***
	MCV(cuu)	51.35 $\pm$ 0.30	65.4 $\pm$ 0.14**
	MCH (pg)	21.42 $\pm$ 0.98	24.1 $\pm$ 0.15*
	MCHC (gm/dl)	34.27 $\pm$ 1.21	33.15 $\pm$ 0.13***
	PCV (%)	31.48 $\pm$ 0.26	33.45 $\pm$ 0.66**
White blood cell and its differentials	WBC (ml/cmm)	4032 $\pm$ 0.64	6175.06 $\pm$ 18.46***

Neutrophil (%)	16±0.86	15.25±0.38*
Monocyte (%)	02±0.70	2.58±0.43 ^{ns}
Lymphocyte (%)	55±0.33	81±0.53**
Eosinophil (%)	03±0.16	2.05±0.16*
Basophil (%)	02±0.11	0.2±0.66*
Platelets (lac/cmm)	8.42±0.08	11.1±0.11**

Values in means + S.E. (Standard error), n=6, *P<0.05, **P<0.01, ***P<0.001, When compared with control, ns= non-significant

DISCUSSION:

Phytochemicals are known to perform several general and specific functions in plants and may exhibit different biochemical and pharmacological actions in different species of animals when ingested. Their actions range from cell toxicity to cell protective effect (Trease and Evans, 1996) Similar finding was reported by Uboh *et al.*, (2010) in aqueous extract of *Psidium guajava* leaves and Dabhadkar *et al.*, (2013) while working on alcoholic extract of *Plumeria rubra* in rats.

In the present work, The acute toxicity study has revealed that the oral administration of the *Clitoria ternatea* leaves extract has non-toxic effect in Wistar rats. The assessment of haematological parameters in rats can be used to determine the extent of deleterious effect of a plant extract on animal blood (Yakubu *et al.*, 2007). It is inferred from this study that the alcoholic extract of *Clitoria ternatea* have no toxic effect on the haematological parameters which include red blood cell count, haemoglobin concentration, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin and red cell distribution width. This showed that the oxygen carrying capacity of the red blood cells was not compromised thus; the plant may be safe as used in traditional medicine. Some plants have been reported to cause destruction of the red blood cells which leads to anaemia and ultimately cell death (Blood and Radostits, 1989; Adedapo, 2002; Adedapo *et al.*, 2004; 2007a). These indices suggest that the extract does not possess toxic substances that can cause an anemic condition in rats. This observation corroborates with the report of Ashafa, *et al.*, (2009) and Oyedemi, *et al.*, (2010). The non-significant effect of the extract on the RBC levels may be an indication that the balance between the rate of production (erythropoiesis) and destruction of blood corpuscles was not altered. MCHC and MCH relate to individual red blood cells while Hb, RBC and PCV are associated with the total population of red blood cells. Therefore, the absence of significant effect of the extract on RBC, Hb, PCV, MCH and MCHC could mean that neither the incorporation of hemoglobin into red blood cells nor the morphology and osmotic fragility of the red blood cells was altered (Adebayo *et al.*, 2005).

The administration of the extract did not alter the level of WBC and its differentials, including basophils, eosinophils and monocytes as well as platelets, throughout the experimental period, while that of lymphocytes was only increased significantly at 200 mg/kg body weight. Similar finding was observed by Adedapo, *et al.*, (2008), while working on aqueous extract of *Acacia karroo* stem bark in rats and mice. Report about WBC counts and its differentials have pointed out that increased count of lymphocytes is supposed to be helpful in boosting immune system (Adedapo *et al.*, 2007; Mohajeri *et al.*, 2007). The platelets affect the viscosity of blood, which is correlated positively to blood pressure (Adedapo *et al.*, 2007) but, the alcoholic leaves extract of different plants does not produce any observable change in the count of blood platelets.

Mishra and Tandon (2012) studied treatment of mice with crude extract of *Hibiscus rosasinensis* flowers (500 mg/kg BW) and *Bougainvillea spectabilis* leaves (800 mg/kg BW) for a period of 30 days indicates a significant increase in the level of haemoglobin and count of RBC but a significant decline in the level of MCH and MCV in the former case. On the other hand, in *B. spectabilis* treated animals, the level of hemoglobin, RBC count and PCV declined significantly. Alterations in blood parameters may be due to changes in cellular integrity, membrane permeability of cells or even due to exposure to toxic chemicals (Mishra and Tandon, 2012)

CONCLUSION:

This study has shown that administration of *Clitoria ternatea* alcoholic leaves extract appears to be relatively non-toxic to animals. Thus, this study may prove to be an effective and safe alternative remedy in haematological related disorders. Work is needed on the isolation and

characterization of the actual mechanism of action of the crude extract and bioactive agents.

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