Research Article

Effect of Alcoholic Pod Extract of *Plumeria Rubra* on Biochemical and Haematological Parameters of Female Albino Rats

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ABSTRACT

The alcoholic extract of the pod of *Plumeria rubra* was assessed for haematological and biochemical effects in albino rats. Extraction of dried pods was carried out with ethanol in soxhlet apparatus. After the oral acute toxicity study, plant extract were administered at three dose levels 100, 200 and 400 mg/kg body weight for 21 days. The extract did not significantly alter the levels of Hb and MCHC while RBC and its other indices were significantly increased at certain doses. Similarly the level of WBC and its differentials remained unaltered throughout the experimental period. In biochemical studies, the extract had no adverse effect on the activity of the liver, as shown by ALP, SGPT and SGOT liver enzyme assay. The pod extract of *Plumeria rubra* showed no change in the serum total cholesterol content of rats. The hormonal assay shows that, there was reduction in the level of FSH and LH hormone, while level of estrogen increased but there was slight decrease in the level of progesterone hormone. The extract shows non-significant change in the body weight but there was significant decrease in weight of ovary and increase in the uterine weight in treated rats. The phytochemical screening of *Plumeria rubra* revealed the presence of alkaloids, flavonoids, steroids, tannins and saponines whereas anthraquinone were not detected. All findings suggest that the *Plumeria rubra* does not cause any toxic effect in rats, and the extract possesses estrogenic and contraceptive activity.

Keywords: Haematology, *Plumeria rubra,* Pods, Female albino rats, Estrogenic activity.

INTRODUCTION

Medicinal plants contain substances that could be used for therapeutic purposes or precursors for the synthesis of useful drugs[1]. Medicinal plants, since time immemorial have been used in virtually all cultures as a source of medicine. Over 5000 plants are known to be used for medicinal purposes in Africa, but only a few have been described or studied[2]. Assessment of haematological and biochemical profile becomes a pre-requisite to understand the normal functioning of the system and to further confirm the toxic nature of the administered plant extract or any drug. Alterations in blood parameters may be due to changes in cellular integrity, membrane permeability of cells or even due to exposure to toxic chemicals[3].

Plumeria rubra L. (Hindi: Lal champa; English: True Frangipani) is a laticiferous tree and shrub, belonging to the Apocynaceae family. The decoction of bark and roots of *Plumeria rubra* plant is traditionally used to treat asthma, ease constipation, promote menstruation, reduce fever and the latex is used to soothe irritation[4]. In India, however, its fruit is used as an abortifacient[5]. The decoction of the flowers of *Plumeria rubra* is reported to be used for control of diabetes mellitus in Mexico[6]. The leaves of *Plumeria rubra* are used in ulcers, leprosy, inflammations, rheumatism, bronchitis, cholera, cold and cough and as rubefacient, antibacterial, antipyretic, antifungal, stimulant etc[7].

The selection of the plant *Plumeria rubra* Linn. was made on the basis of its easy of availability, therapeutic value and degree of research work which is not done[8]. Therefore, the present work was undertaken to validate scientifically the Therapeutic role of *Plumeria rubra* pods on biochemical and haematological parameters on female albino rats.

MATERIALS AND METHODS

Collection of plant material

The plant *Plumeria rubra* was collected during the flowering period of August to October from Melghat region (20° 51′ to 21° 46′ N and to 76° 38′ to 77° 33′ E) of Amravati district of Maharashtra state of India, It was identified and authenticated by experts from Botanical Survey of India, Pune (Accession No. DD- 1).

Preparation of the extract

The pods of *Plumeria rubra* were collected, shade dried, powdered and subjected to soxhlet extraction with ethanol for 48 hrs and filtered. The extract was evaporated to near dryness on a water bath, weighed and kept at 4°C in refrigerator until used for experimental evaluation.

Phytochemical screening

The presence of various plant constituents in the plant extract was determined by preliminary phytochemical screening as per Thimmaiah[9].

Procurement and rearing of experimental animal

Healthy Wistar strain female albino rats were procured from Sudhakarrao Naik Institute of Pharmacy, Pusad (Maharashtra). The rats were housed in polypropylene cages and maintained under environmentally controlled room provided with a 12:12 hrs light and dark cycle approximately at 25 ± 2°C. They were fed on pellets (Trimurti Lab Feeds, Nagpur) and tap water *ad libitum.* The rats were allowed to acclimatize to laboratory environment for 15 days before experimentation.

All experimental protocols were subjected to the scrutinization and approval of Institutional Animal Ethics Committee [registration number 1060/ac/07/CPCSEA (IAEC/7/2009)].

Acute toxicity study

Healthy female albino rats were starved for 3- 4 h and subjected to acute toxicity studies as per Organization of Economic Co-operation and Development (OECD) guidelines No: 423 and a highest dose was selected for treatment. The rats were observed continuously for 2 hrs for behavioural, neurological and autonomic profile and next 24 and 72 hrs for any lethality or death[10].

Experimental design

Twenty-eight female albino Wistar rats were used for the study. The rats were divided into four groups of six rats each. Group 1 served as the control and received distilled water. Groups 2, 3 and 4 received 50, 100 and 200 mg/kg body weight of ethanolic pod extract of *Plumeria rubra* for 21 days via gastric intubation. Animals were weighed after every 24 hrs interval during the period of extract administration. After 21 days, the rats were subsequently anaesthetized with anesthetic ether and blood sample were collected by cardiac puncture, into EDTA/ lithium heparin bottles. The heparinized samples were centrifuged at 3,000 rpm for 10 min to obtain the plasma and stored at - 20°C until ready for analysis; while the whole blood samples were maintained at 4°C for further analysis however, the plasma glucose level was determined immediately.

Effect on haematological parameters

The haematological parameters i.e. red blood cell counts (RBC), haemoglobin (Hb) concentration were determined according to the method described by Dacie and Lewis[11]. The packed cell volume (PVC), 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[11]. Platelet (PLT) count and WBC differentials (neutrophils, monocytes, lymophocytes, basophils and eosiniphils) were analyzed according to the standard technique described by Baker, *et al*[12, 13].

Effect on biochemical assays

All biochemical parameters were determined by standard procedure in an auto analyzer. The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated using Reitman and Frankel method[14]; alkaline phosphatase (ALP) estimation was carried out using the phenolphthalein monophosphate method[15]; total bilirubin was estimated using Doumas, *et al* method[16]; total protein was determined using biuret method[17]; glucose level was obtained using the enzymatic GOD-PAP method[18]; creatinine was determined by the method described by Larsen[19], and albumin was estimated by bromo cresol green (BCG) method[20]. The cholesterol and its differentials were estimated by using method of Tietz[21].

Effect on hormonal assay

The sera was analyzed for FSH, LH, Estradiol and Progesterone level by Chemiluminescence immunoassay (CLIA) method with semi automated Chemiluminescence analyzer and autoplex- A processor for CLIA[22, 23].

Effect on body weight and reproductive organ weight

After 21 days of treatment, all the control and experimental groups of female rats were evaluated for any changes in their body weight as well as for their reproductive organ weight by the method of Amini and Kamkar[24].

Statistical analysis: The data are expressed as mean± SE. Statistical analysis was done by using Student's t-test[25].

RESULTS AND 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[26]. The preliminary phytochemical screening of the pod extract of *Plumeria rubra* revealed the presence of alkaloids, flavonoids, steroids, tannins and saponines whereas anthraquinone were not detected. Similar finding was reported by Uboh, *et al*[27] in aqueous extract of *Psidium guajava* leaves in rats.

In the present work clinical toxicity symptoms such as respiratory distress, salivation, weight loss and change in appearance of hair as well as maternal mortality were not observed at any period of the experiment. Similarly no mortality and changes in the behavioural, neurological and autonomic profile were observed in treated groups of the rats up to highest dose of 2000 mg/kg body weight. Hence one-tenth of this dose was used for haematological and biochemical estimations. The acute toxicity study has revealed that the oral administration of the *Plumeria rubra* pod extract has non- toxic effect in Wistar rats. Similar finding was reported in toxicological effect of the aqueous stem bark extract of *Strychnos henningsii* Gilg in Wistar rats[28]. The obtained dose as shown in this study may not predict the human lethal dose of a drug or acute poisoning overdose[29]. However, it may be used to provide a guideline for selecting doses for the acute or sub-acute doses of more clinical relevance.

Table 1: Effect of the ethanolic pod extract of *Plumeria rubra* on haematological parameters in female albino rats

Parameters		Control (Vehicle)	Ethanolic pod extract of *Plumeria rubra* (mg/kg body weight)		
			50	100	200
Red blood cells	RBC (ml/cmm)	6.87±0.29	7.10±0.10*	7.48±0.13*	7.88±0.32**
	Hb (gm/dl)	13.35±0.55	13.57±0.70**	13.50±0.16*	13.32±0.18***
	Hematocrit (%)	53.12±0.48	52.27±0.51*	57.16±0.56 ns	59.28±0.22 ns
	MCV (Cuμ)	51.35±0.30	52.22±0.60***	53.95±0.69***	56.34±1.35***
	MCH (Pg)	17.42±0.98	18.32±0.84**	18.96±0.76**	19.23±0.73***
	MCHC (gm/dl)	34.27±1.21	35.07±1.56**	35.15±0.33***	36.11±0.40**
	PCV (%)	31.48±0.26	39.06±2.02**	38.41±0.85**	38.74±0.52 ns
White blood cells and its differentials	WBC (ml/cmm)	4032±0.64	4100±2.48*	3978±0.19***	4356±0.80**
	Neutrophils (%)	26±0.86	35±1.64 ns	38±0.10***	42±0.48**
	Monocytes (%)	02±0.70	03±0.16***	02±0.29*	03±0.50 ns
	Lymphocytes (%)	55±0.33	57±0.70***	62±0.90**	68±0.90***
	Eosinophils (%)	03±0.16	02±0.38***	01±0.11 ns	02±0.63**
	Basophils (%)	02±0.11	01±0.26*	02±0.12**	01±0.18**
	Platelets (lac/cmm)	7.42±0.08	6.42±0.50***	9.28±0.09***	9.62±0.28*

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

Table 2: Effect of ethanolic pod extract of *Plumeria rubra* on biochemical parameters of female albino rats

Parameters	Control (Vehicle)	Ethanolic pod extract of *Plumeria rubra* (mg/kg body weight)		
		50	100	200
Bilirubin (mg/dl)	0.24±0.06	0.45±0.09*	0.55±0.08 ns	0.39±0.37**
ALP (IU/L)	56.9±0.90	78±1.60**	79±1.48**	110.00±0.74**
Total Protein (g/dl)	5.8±0.11	5.7±0.27 ns	6.9±0.66***	72±0.33***
SGOT (IU/L)	70.20±3.62	73.16±0.36*	73.80±0.68*	73.94±1.34*
SGPT (IU/L)	48.20±0.38	48.33±0.19**	49.56±0.50 ns	49.86±0.48**
Albumin (mg%)	4.0±0.18	3.8±0.33**	4.4±0.33*	4.7±0.55 ns
Glucose (mg/dl)	65±0.87	79±0.46***	87±1.54**	126±2.24*
Creatinine (mg/dl)	0.3±0.16	0.4±0.04***	0.45±0.16**	0.7±0.17**

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

Table 3: Effect of ethanolic pod extract of *Plumeria rubra* on lipids profile of female albino rats

Parameters	Control (Vehicle)	Ethanolic pod extract of *Plumeria rubra* (mg/kg body wt.)		
		50	100	200
Total Cholesterol (mg %)	89±0.46	128±0.68**	132±1.42*	130±2.06***
Triglycerides (mg %)	81±0.16	89±0.58*	94±0.70***	105±0.82***
HDL (mg %) (High density lipoprotein)	21.2±0.33	38±0.29**	39.6±0.56 ns	62±1.44 ns
LDL (mg %) (Low density lipoprotein)	41.8±0.86	44.3±1.23 ns	79.2±0.52***	96±1.32*
VLDL (mg %)	16.2±0.68	21.9±1.35*	26.3±0.40 ns	35±0.40 ns
Cholesterol/ HDL ratio	3.76±0.72	3.91±0.98***	3.65±0.12***	3.84±0.33**
LDL/HDL ratio	1.99±0.18	1.68±0.30*	2.08±0.08*	2.29±0.16*

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

Table 4: Effect of ethanolic pod extract of *Plumeria rubra* on hormonal profile of female albino rats

Parameters	Control (Vehicle)	Ethanolic pod extract of *Plumeria rubra* (mg/kg body weight)		
		50	100	200
FSH (mIU/ml)	0.167±0.01	0.163±0.02**	0.157±0.09***	0.135±0.01**
LH (mIU/ml)	0.13±0.005	0.11±0.04^{ns}	0.08±0.01*	0.07±0.001**
Estrogens (pg/ml)	70.32±0.80	71.16±2.42*	82.56±1.34**	92.80±1.71***
Progesterone (ng/ml)	43.61±0.96	41.00±0.33**	37.67±1.28^{ns}	29.07±1.18***

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

Table 5: Effect of the ethanolic pod extract of *Plumeria rubra* on body weight, reproductive organ-body weight in female albino rats

Treatment groups	Dose (mg/kg body wt.)	Body weight (gm)		Reproductive organ weight (mg)	
		Initial	Final	Ovary	Uterus
Control	Vehicle	154.83±1.80	163.16±1.51*	55±3.66	118.83±3.71
Ethanolic pod extract of *P. rubra*	50	180±2.10	195±2.67**	62±2.61*	178±1.94**
	100	170.50±0.87	186±1.23^{ns}	59±1.60*	145±0.70***
	200	156±1.71	166.33±1.50**	46±1.67***	167.83±2.09*

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

The effect of oral administration of the alcoholic pod extract of *Plumeria rubra* at different doses, investigated on RBCs and its functional indices, in female Wistar rats for 21 days are shown in Table 1. The extract did not significantly alter the levels of Hb and MCHC while those of RBC, hematocrit, MCH, PCV, MCV were significantly increased at certain doses. There were no obvious hemolytic changes in the plasma of the extract treated rats on RBC, Hb, hematocrit and MCHV. These indices suggest that the extract does not posse's toxic substances that can cause an anemic condition in rats. This observation corroborates with the report of Ashafa, *et al*[30] and Oyedemi, *et al*[28]. 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 PVC are associated with the total population of red blood cells. Therefore, the absence of significant effect of the extract on RBC, Hb, PVC, 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 altere[31].

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 lymophocytes was only increased significantly (P<0.001) at 200 mg/kg body weight (Table 1). Similar finding was observed by Adedapo, *et al* [32], 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[33-34]. The platelets affect the viscosity of blood, which is correlated positively to blood pressure[32] but, the pod extract of *Plumeria rubra* does not produce any observable change in the count of blood platelets. Probably the duration of the treatment may prove non-toxic to animals[32].

Liver enzymes are liberated into blood whenever liver cells are damaged and enzyme activity in the plasma is increased[35]. The pod extract of *Plumeria rubra* at doses of 50, 100 and 200 mg/kg body weight showed varied effect on biochemical parameters. The extract had no adverse effect on the activity of the liver, as shown by ALP, SGPT and SGOT liver enzyme assays. The present data suggest that pod extract of *Plumeria rubra* dose not exert possible hepato toxic effect. There was significant increase in total protein and glucose level in the experimental animal treated with 50, 100 and 200 mg/kg body weight of *Plumeria rubra* pod extract respectively (Table 2). The effect of the *Plumeria rubra* pod extract on the level of albumin and total protein gives useful information on the lack of ability of the extract to inhibit protein biosynthesis[36]. The non-significant effect of the *Plumeria rubra* pod extract on the level creatinine clearly shows the functional status of kidney[37].

Cholesterol derived from the different sources is the precursor for the streoidogenesis of ovarian endocrine tissue[38]. It has been reported that pod extract of *Plumeria rubra* showed no change in the serum total cholesterol content of rats. The significance elevation of low density lipoprotein, high density lipoprotein cholesterol triglycerides and VLDL level as well as in the HDL/total cholesterol and LDL/HDL ratio were observed in treated rats compared with the control rats (Table 3). Similar finding was reported in toxicological effect of the aqueous stem bark extract of *Strychnos henningsii* Gilg in Wistar rats[28]. A non-significant change in the serum cholesterol content of rats treated with aqueous extract of rhizome of *Curcuma longa* indicated no adverse effect on cholesterol metabolism[39].

The most important hormone involved in the regulation of the alterations detected in the feminine genital tract is estrogen and progesterone, which are regulated by pituitary- secreted gonadotropin hormones[40]. FSH indirectly stimulates gametogenesis in both sexes and directly stimulates estrogen synthesis and follicular development. It also maintains the structure of the gonads in conjugation with LH[41]. On the other hand, LH is critical to luteinization of the ovarian follicle and post-ovulatory follicular function[42]. In the present study it was observed that, there was reduction in FSH and LH serum level in treated animal as compared to control. Our report is in line with Gbotolorun, *et al*[43] and Raji, *et al*[44]. The reduced FSH and LH level in serum of treated animals is an indication towards the possibility of the effect of extract on the anterior pituitary or the hypothalamus, since the secretion of FSH and LH is regulated by the gonadotropic releasing factor secreted by the hypothalamus[45]. Reduced levels of FSH may result in the inability of follicular cells to reach maturation, and hence situation may inhibit the synthesis of the LH whose limitation to the normal physiologic process may be exploited in contraception, hence this may be useful as a contraceptive[46]. In the present study it was reported that the level of estrogen gets increased while slight decreased in the progesterone hormone were observed in the extract treated animals (Table 4). These results may suggest that high dose of estrogen disproportionate to progesterone leads to resorption of fetuses. This effect may due to the imbalance in the estrogen- progesterone environment. Similar observation was made by Pradeepa, *et al*[47], while working on antifertility effect of herb of *Indigofera linnaei ali* in female albino rats. .

The doses 50, 100 and 200 mg/kg body weight of *Plumeria rubra* pod extract shows a non- significant change in the body weight of the experimental animals but there was a significant decrease in the weight of ovary and increase in the uterine weight in treated rats compared with control (Table 5). These results suggest that alcoholic pod extract of *Plumeria rubra* possesses estrogenic activity which might be responsible for fetus resorption (abortifacient)[48, 49]. Similar finding was reported by Mutreja, *et al*[50], while working on effect of *Nulumbo Nucifera* seed on the reproductive organs of female rats.

CONCLUSION

This study has shown that administration of *Plumeria rubra* pod extract appears to be relatively non-toxic to animals. The study has provided evidence for the antifertility activity of ethanolic extract of *Plumeria rubra* pods administered orally possessed estrogenic activity, which might contribute to its contraceptive effect.

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