Haematological and biochemical changes after exposure to *Phoebe wightii* Meisn methanol extract in rats.

***Devika M & **Mary kensa V.**

*Research Scholar (Full time) Reg: No: 18213152142037, Email Id: deviashi.mv@gmail.com, Abishehapatti, M.S. University, Tirunelveli.

**PG Research Centre of Botany, S.T. Hindu College, Nagercoil.
E.mail.Id: surejkensa@gmail.com, Abishekapatti, M.S. University, Tirunelveli.

Abstract

Acute and subchronic toxicities of the methanolic extract from the *Phoebe wightii*were studied in male rats. Oral administration of the extract at a single dose of 250 and 500 mg/kg body weight did not produce signs of toxicity, behavioral changes, mortality or differences on gross appearance of internal organs. The subchronic toxicity was determined by oral feeding the test substance at the doses of 250 and 500 mg/kg body weight for 28days. The examinations of signs, animal behavior and health monitoring showed no signs of abnormalities in the test groups as compared to the controls. The test and control groups were analyzed by measuring their final body and organ weights, taking necropsy, and examining hematological parameters, blood clinical chemistry and histopathology features. The results suggest that *Phoebe wightii*administered orally did not cause acute or subchronic toxicities to male rats.

Introduction

Toxicological study is the key for survival of herbalformulation across the world (Wal*et al*., 2011; Debbie *et al*.,2012). *Phoebe wightii*is a tree commonly found in wasteland of garden and plains. It is amonotypic to genus, native to Mexico. It belongs to the family Lauraceae. It is a tree and it contains biologically active compounds.Thepresent study was aimed to assess the adverse effects relatedto different doses in order to find the acceptably safe level ofthe methanol extract from *Phoebe wightii*in rats by determiningboth oral acute and subchronic toxicities.

Materials and Methods

Collection of plant sample

*P. wightii*was collected from Kotagiri, Coimbatore of Tamil Nadu, and India andauthenticated by Botanist Dr. R. Murugan, BSI, Southern circle, Kovai, India. A voucherspecimen was deposited in the herbarium of the Botanical Survey of India Coimbatore;Herbarium code No. BSI/SRC/19/710-20/Tech.

Preparation of plant extract

Powder of *Phoebe wightii*500 grams werewrapped in a calico bag and put into a stainless steel boiler.Ten liters of water were added and boiled for 3-4 hours, thenfiltered when it had cooled down. The residue from the filtrationwas boiled and filtered again with the same procedure.The filtrates were collected and evaporated in a rotaryevaporator until concentrated.

Experimental animals

Wistar albino rats weighing 175-225g of either sex maintained under standard husbandry conditions (temp 23±2^{o}C, relative humidity 55±10% and 12 hours light dark cycle) were used for the screening. Animals were fed with standard laboratory food and ad libitum during the study period. The experiments were performed after the experimental protocols approved by the institutional animal ethics committee, India 2009.Acute and subchronic toxicities was assessed using andthe ethical clearance was obtained before the experiment from the Institutional Animal Ethics Committee (Registration number **NCP/IAEC/2019-20/18**). The male Wistar rats, weighing between 100-150g, were procured from National Toxicology Centre, Pune and housed in Animal House of NandhaCollege of Pharmacy, Erode. Rats were kept in the polypropylene cages and fed on a standard laboratory diet with water *ad libitum.* The animals were exposed to alternate cycle of 12 h of darkness and light each. Rats were divided into 6 groups of 2 animals each.

Acute toxicity

According to the World Health Organization (WHO)guideline (WHO, 2000) and the Organization of EconomicCo-operation and Development (OECD) guideline for testingof chemicals, TG420 (OECD, 2001), 10 rats wererandomly divided into two groups of 5 animals per sex. Theextract at a single dose of 5,000 mg/kg body weight wasgiven orally to the treated group (the extract at concentration2,500 mg/ml in distilled water), while the control groupreceived water vehicle. Body weight, signs of toxicity andmortality were observed after the

administration at the first,second, fourth and sixth hour and once daily for 28days. Onthe 15th day, all rats were fasted for 16-18 hours, and thensacrificed for necropsy examination. The internal organs wereexcised and weighed. The gross pathological observationsof the tissues were performed by histopathological examination.

Subchronic toxicity

According to WHO guideline (WHO, 2000) and theOECD TG408 (OECD, 1981), rats were divided into 6groups of 18 animals. The extract at concentration 250 and 500 mg/ml in distilled waterwas given orally to each group of rats daily for 28 days,while the control group received water vehicle. In order toassess reversibility effect, the extract at the dose of 250mg/kg was given once daily to the fifth group of rats for 28days, and kept for another 28 days post treatment. Toxicmanifestations such as signs of toxicity, mortality and thebody weight changes were monitored daily.Heparinized blood samples were taken fordetermining complete blood count, red blood cell count,platelet count and red cell indices. The serum from non-heparinizedblood was carefully collected for blood chemistryand enzyme analysis.All rats were sacrificed after the blood collection.The internal organs and some tissues were weighed to determinerelative organs weights and observed for gross lesions.All tissues were preserved in 10% buffered formaldehyde solution for histopathological examination.

Biochemical parameters.Biochemical parameters wereevaluated using a Semi-Automatic Biochemical Analyzer,model EMP-168 (Ivdiagnostik, Emperor Medical, Shenzhen,China), according to the manufacturer's specifications. Thelevels of aspartate aminotransferase (AST), alanine aminotransferase(ALT), alkaline phosphatase, total protein,bilirubin, cholesterol, triglycerides, high-density lipoprotein(HDL), low-density lipoprotein (LDL), total albumin,glucose, urea, and creatinine were determined.

Hematological assay.Hematological assays wereperformed using an Automatic Hematology Analyzer KT-6400 (Genius, Med Equipment, Guangzhou, China). Atthe end of the experiment hematocrit, hemoglobin concentration,erythrocyte count, total and differential leukocytecounts, and platelet count were evaluated.

Statistical analysis

Results were expressed as mean + standard error ofmean (S.E.M.). Statistical significance was determined byone-way analysis of variance (ANOVA) and post hoc leastsignificantdifference (LSD) test. The data obtained fromacute toxicity studies were analyzed using Student's paired*t*-test. P values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Overall, the study of acute toxicity revealed no adverse change in the behavior of female rat at 5000 mg/kg as compared to the control and no mortality was registered. On the other hand, there was no significant change in the body weight, as a toxicity indicator and the macrocospicanatomopathological studies did not show any alteration in the analyzed organs.Therefore, the LD50 of the extract is over 500 mg/kg. In the sub-chronic toxicity study, the extract at all doses caused no significant changes in the body weight of the rats and significant decrease in food intake comparatively to the control on days 6 and 28 of treatment at the higher dose 500 mg/kg

The effect of a oral administration of 250 mg/kg and 500 mg/kg body weight of *P. wightii* on haematological parameters of female rat are shown in Table: **1.** The analysed parameters did not record any statistically significant ($P > 0.05$) changes as compared to respective control group increase in haemoglobin , RBC, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), neutrophils, Eosinophils, were observed which are mainly non significant and dose dependant decrease in WBC, platelets, lymphocytes and monocytes at high dose when compared with the control. All the parameters showed non-significant and dose dependant changes when compared with the control.

Table-2portrays the biochemical parameters of female rat after treatment with repeated oral doses of *P. wightii* for 28 days. The results in female rat showed a dose dependant increase in total protein, albumin, SGPT, SGOT, ALP, TG, Chloesterol, HDL, LDL, VLDL which was significance in high dose glucose, urea, sodium, are decreased significantly in a dose dependant manner when compared to control. There was a minor fluctuations was noted in sodium albumin, cholesterol, LDL, are mostly non significant when compared with the control.

There were no significant changes in body weight in female rat treated with the powder sample of *P. wightii* when compared to the control group (Table: **3**). However a significant change occurred in kidney and stomach in the fourth week of 500 mg/kg/body weight dose

group, with the body weight decreasing from 0.34±0.08 and 0.48±0.13 (control group) to 0.41±0.18 and 0.54±0.25 g (P <0.05) respectively. The relative weight of heart, lungs, liver of female rats treated in 28 day period did not show any significant changes when compared with the control group.

Changes in general behaviors, body weight and internalorgan weight are critical for the objective evaluation ofthe effect of a compound on test animals, since such changesare often the first signs of toxicity (Auletta, 1995).All these findings indicate that the extract had no deleterious effect on liver function. However, the liver histology presented slight periportal fibrosis and vascular congestions at all doses but, these histological modifications are of less importance when not linked with modifications in biochemical parameters as in this study; macroscopic and histopathological observations and investigations of additional clinical biochemistry parameters allows the confirmation of hepatotoxicity Creatinine, urea and electrolytes are common parameters often measured to assess the state of the kidneys (Eteng*et al*., 2009).

Administration of methanol extract of *P.wightii* did not lead to a significant effect on creatinine levels when compared to the control. The creatinine is known as an effective indicator of renal function and especially of the glomerular filtration rate. Kidney malfunction causes a rise above the normal threshold of serum creatinine and decreased urinary levels (SchafflerandMenche, 2004). Thus, this results recorded suggest that methanol extract of *P.wightii*did not affect the renal function. Also the K+ and Na+ serum levels were not affected by the administration of different doses of methanol extract of *P.wightii* while they were decreases on urinary K+ levels and on blood urea levels in all treated rats. Moreover, an increase of the urinary urea levels in animals treated with methanol extract of *P.wightii* at the 250 mg/kg doses was noticed and no adverse morphological and histological effects were observed on the kidneys of the experimental rats. These observations proved that extract has no deleterious effect on the kidneys but may improve their function.In this study, there were a decrease in the total cholesterol, HDL and LDL of all treated animals with significance on HDL level at the dose of 250 mg/kg compared to the control. This would be due to the fact that this extract has no effect on lipid metabolism, since the index of arteriosclerosis was not affected. It therefore presents no risk of cardiovascular disease. According to Schaffer and Menche (2004), excess "bad" cholesterol (LDL) and the lack of "good" cholesterol (HDL) are major risk factors for

cardiovascular disease. This hypothesis is supported by the trigycerides level which did not significantly change in the experimental groups as compared to the control. Indeed, higher levels of triglycerides measured in a fasting specimen indicate a lack of clearance or over-production; it could increase the risk of developing cardiovascular diseases(Cole *et al*., 2000). This suggests that methanol extract of *P.wightii* has no effect on the cholesterol metabolism in the rat and consequently its consumption leads to no risk of developing cardiovascular disease.

Table: 1

Effect of *P. wightii* on the relative weight of the organ in rats treated for 28 days.

S.NO	Organ	Control	Dose group (mg/kg/day)	
			250	500
1	Heart	0.34±0.14	0.33±0.07	0.35±0.11
2	Lungs	0.88±0.13	0.90±0.21	0.89±0.26
3	Liver	2.19±0.24	3.21±0.20	3.01±0.22
4	Kidney	0.34±0.08	0.40±0.21	0.41±0.18
5	Stomach	0.48±0.13	0.53±0.12	0.54±0.25

Values are expressed as mean ± SD. No significant difference was observed when compared with controls.

Table: 2

Biochemical parameters of female rats after treatment with repeated oral doses of *P. wightii*for 28 days

S. NO	Parametes	Control	Dose group mg/kg/day.	
			250	500
1	Glucose	196.5±26.11	190.4±0.80	191±15.03
2	Creatinine	0.48±0.04	0.72±0.1	0.60±0.05
3	Urea	48.8±5.20	49.4±3.78	46.5±4.57
4	Sodium	142.06±4.80	140.05±3.81	141.05±3.71
5	Total protein	5.04±0.07	5.24±0.07	5.48±0.10
6	Albumin	3.56±0.07	3.59±0.07	3.85±0.05
7	Bilirubin	0.16±0.01	0.13±0.01	0.17±0.01
8	SGPT	31.50±1.65	33.40±2.75	40.10±18.01
9	SGOT	102.60±2.19	101.50±7.61	115.30±10.55
10	ALP(Iμ/L)	278±56.86	255±44.2	320±62.11
11	Triglyceride	160.4±18.1	151.6±21.06	170.20±21.17
12	Cholestrol	92.8±1.02	97.8±6.28	98.1±1.29
13	LDL	16.7±3.99	16.7±4.45	17.76±3.98
14	VLDL	13.5±2.11	15.21±2.31	16.91±2.11
15	HDL	44±1.55	46.6±4.85	56.4±1.96

Values are expressed as mean ± SD. No significant difference was observed when compared with controls. ALP- Alkaline phosphate; LDL- Low density lipoprotein; HDL- high density lipoprotein; SGPT-Serum glutamic pyruvic transaminase; SGOT-Serum glutamic oxaloacetic transaminase ; VLDL-Very low density lipoprotein.

Table: 3

Haematological parameters of female rats after treatment with repeated oral dose of *P. wightii*for 28 days

S. NO	Parameters	Dose group (mg/kg/ day)		
		Control	250	500
1.	Haemoglobin (gms/dL)	14.13±0.24	14.71±0.61	14.92±0.68
2.	Red blood corpuscles (10^6/µL	7.13±0.34	7.30±0.51	7.99±0.44
3.	White blood corpuscles (10^3/µL)	8.18±0.97	7.90±1.35	7.17±1.28
4.	Platelets (105/mm3)	7.54±0.41	4.31±0.41	3.05±0.32
5.	Mean corpuscular haemoglobin MCH(fL)	18.08±140	18.51±1.88	20.61±1.31
6.	Mean corpuscular haemoglobin concentration(MCH) (%)	33.72±2.10	32.18±1.70	34.15±1.58
7.	Neutrophils(%)	20.13±4.28	36.93±5.41	50.21±4.45
8.	Lymphocytes(%)	70.12±5.16	48.5±4.30	45.46±4.19
9.	Eosinophils(%)	2.64±0.77	2.65±1.36	4.6±0.78
10.	Monocytes(%)	2.60±0.50	2.75±0.31	2.56±0.48

Value are expressed as mean ± SD difference between the control group were not significant.

CONCLUSION

The present research provided firsthand information on acute and sub chronic toxicity of *P.wightii*.

References

Auletta, C.S. 1995. Acute, Subchronic and Chronic Toxicology.*In* CRC Handbook of Toxicology, M.J. Derelanko,M.A. Hollinger, editors. CRC Press, Inc., U.S.A., pp51-104.

Cole TG, Klotzsch SG, McNamara JR. 2000. Measurement of Triglyceride Concentration. In Handbook of Lipoprotein Testing (2nd edition), Rifai N, Warnick G.R., Dominiczak MH. AACC Press: Washington DC, 207-219.

Debbie S, Ladds G, Duez P, Williamson E, Chan K.Pharmacovigilance of herbal medicine. *Journal of Ethnopharmacology*,2012; 140 (3):513-518.

Eteng MU, Ibekwe HA, Abolaji AO, Okoi AI, Onwuka FC, Osuchukwu NC. 2009. Effect of RauwolfiavomitoriaAfzel (Apocynaceae) extract on serum amino transferase and alkaline phosphatase activities and selected indices of liver and kidney functions. African Journal of Biotechnology 8 (18), 4604-4607.

Schaffler A, Menche N. 2004. Anatomie, Physiologie, Biologie 2e édition. *Malome S. A. éditeur*, 61p.

Wal P, Wal A, Gupta S, Sharma G, Rai, AK. Pharmacovigilance of herbal medicine in India.*Journal of YoungPharmacists*, 2011. 3(3): 256-258.

World Health Organisation, Geneva). A.I.T.B.S. Publishers & Distributors, New Delhi. 2002.