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Evaluation of the acute and sub acute toxicity of the methanolic leaf extract of Plectranthus amboinicus (Lour) Spreng in Balb C mice

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ABSTRACT

The present investigation was carried out to evaluate the safety of methanol extract from leaves of Plectranthus amboinicus (Lour) Spreng by determining its potential toxicity after acute and sub acute administration in mice. For the acute study, PAS was administrated to mice in single doses given by oral route. General behavior adverse effects and mortality were determined up to 7 days. In the Sub acute study, the extract was administered orally at doses of 200 and 400 mg/kg for 28 days to mice. Body weight, biochemical and hematological parameters were determined at the end of 28 days of daily administration. The studies on sub acute toxicity reveals that no mortalities or evidence of adverse effects have been observed in Balb C mice following acute oral administration at the highest dose of 2000mg/kg crude extracts of PAS. In sub acute toxicity study daily oral administration of methanol extract 200 and 400 mg/kg body wt of PAS for up to 28 days did not result in death or significant changes in body weight, hematological and biochemical parameters. Studies on histopathological examination of vital organs showed normal architecture suggesting no morphological disturbances.PAS extract found to be less toxic at the doses examined.

Key words: Plectranthus amboinicus, Acute oral toxicity, Sub acute toxicity, His to pathological studies.

INTRODUCTION

Nature has best owned upon us a very prosperous botanical prosperity and a large number of diverse types of plants cultivate wild in different parts of our country. In India, the use of different parts of medicinal plants to alleviate specific ailments was in practice form ancient times[1]. Plectranthus amboinicus (Lour) Spreng (synonym: Coleus amboinicus, Coleus aromaticus family Lamiaceae) is known as Country borage in English, Pathurchur in Hindi [2&3], it is a large succulent aromatic perennial herb, much branched, Fleshy highly aromatic pub scent herb with distinctive smelling leaves. The plant is distributed through out India,

cultivated in the gardens. It is a folkloric medicinal plant used to treat malarial fever, hepatopathy, renal and vesical calculi, cough, chronic asthma, hiccough, bronchitis, colic convulsions and epilepsy [4,5&6]. The phytochemical studies reveal the presence of various Flavonoids like quercetin, apigenin, luteolin, salvigenin, genkwanin and volatile oil in the leaves [7]. The Pharmacological properties have been reported including Urolithiasis [8], Fungitoxic [9&10], antibacterial [11], antimalarial [12&13], anti-inflammatory [14&15]. Because of their wider pharmacological activities PAS has to be identified as a traditional medicine. Herbal medicines have received greater attention as an alternative to clinical therapy and the demand for these remedies has currently increased. Experimental screening method is important in order to ascertain the safety and efficacy of traditional and herbal products and also to establish the active component of the herbal products [15]. Despite the wide use of the leaves of PAS very few investigations have been published in the literature about its toxicological profile. Therefore, the purpose of this study was designed to determine the acute and sub acute oral toxicity of the leaf extract of PAS.

MATERIALS AND METHODS

Plant Material

The leaves of **Plectranthus amboinicus** (Lour) Spreng (Figure1) were collected from the fields of Pathanamthitta, Kerala. It was authenticated by Dr. A.K.Pradeep, Reader, Calicut University Herbarium, Dept of Botany, University of Calicut, Kerala.

Figure 1 Color photograph of the plant & leave

Table1 Phytochemical screening of Methanol extract of PAS

Phytoconstituents	Extract
Carbohydrates	+
Glycosides	+
Alkaloid	+
Sterols	+
Triterpenoids	+
Proteins & Amino acids	-_
Tannins	+
Flavonoids	+
Fixed oils	+

+ = *present* - = *absent*

Experimental animals

Healthy male and female Balb C mice weighing 20-35 gm were acclimatized for 14 days. The animals were housed under standard conditions and room temperature (25±2^{o}C). During the

acclimatization period of 14 days, animals were observed for general condition every day and weighed on the next day of arrival and on the last day of acclimatization. The experimental protocol (Protocol No: HNCP/PH/21/OS) was approved by the Institutional Animal Ethical Committee of Committee of HSNCB's Facility for Animal Breeding and Experimentation (Reg No.879 /ac /05/ CPCSEA).

Acute toxicity study

The toxicity study as carried out using female and male Balb C mice (20-35 g).The acute toxicity studies were conducted as per the OECD guidelines 420(OECD 2000) where the limit test dose of 2000 mg/kg was used. The animals were divided into one control group and one treated group, each group consisting of ten animals (5 males and 5 females). Observations were made at 2,4,8 hrs for seven days for bodyweight, treatment related changes like respiration rate and heart rate and behavioral signs like apathy, reduced locomotor behavior.

Sub acute-Toxicity Study

Healthy adult Female Balb C mice weighing 20-30 gm were divided in to 3 groups of 6 animals each and were housed under standard conditions and room temperature ($25\pm2^{o}C$). The control animals(Group-I) received 0.5ml of vehicle alone and the other two groups(Group-II &III) have received PAS extract for 28 days at doses of 200,400 mg/Kg body wt respectively.

Observations

Toxic manifestations and mortality were monitored daily and body wt changes were recorded every 7 days till the end of the study.

Hematological, biochemical and Tissue analysis

At 28^{th}day animals were fasted for 12 hrs, they anaesthetized with ether and blood was collected from orbital sinus in heparinized tube for the analysis of hematological parameters using Mythic18 , which included Hemoglobin, Red blood cell count, white blood cell count, platelet, reticulocyte, neutrophils, Eosinophils, lymphocytes, monocytes, packed cell volume, mean corpuscular volumn, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin and was centrifuged at 4000 rpm at 4^{o} C for 10 minutes to obtain the serum for biochemical estimations. Both the plasma and serum were stored at -20^{o} C until analyzed for biochemical parameters. The serum was assayed for bilirubin, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, serum alkaline phosphatase, serum proteins, serum total albumin, serum total globulin, serum cholesterol, serum triglycerides, creatinine, blood urea nitrogen, calcium, phosphorus and electrolytes like sodium, potassium and chloride using autoanalyzer. Immediately after collecting the blood samples, animals were then sacrificed by ether anesthesia. The organs such as liver, kidney, lung, spleen, brain, adrenals, gonads, stomach, intestine and heart were removed and weighed immediately and washed and transferred to an ice cold saline solution. The portions of these organs were fixed in 10% formalin for histopathological examinations. Samples were processed using an auto-technicon apparatus through increasing concentrations of ethanol and infiltrated in paraffin. It was followed by microtome and the slides were stained with Hematoxyllin- eosin.

Statistical analysis

All the results are expressed as mean value ± Standard deviation (S.D). Within group comparisons were performed by the analysis of variance using ANOVA test. The significant difference between the groups are considered at $P<0.05$ level [18, 19, 20, 21 &22].

RESULTS

Acute Toxicity Study

The acute toxicity study was conducted as per the OECD guidelines 420, where the limit test dose of 2000mg/Kg was used. The observations are presented in Table 2 .No test substance related mortality was observed at 2000mg/Kg and throughout the observation period there were no significant changes in the body weight and treatment related change like respiration rate and heart rate. Persistent treatment related changes were observed in behavioral signs viz apathy, reduced locomotor behavior but regained after 24 hrs. Consequently, 2000 mg/Kg of plant extract found safe with less toxic effect.

Table 2 Observations of Acute toxicity study (Treatment related changes)

Animal no	Dose mg/ Kg	Body wt.(gm)	Apathy	Ataxia	Circling	Compulsive behavior	Excitability	Locomotor behaviour	Moribund	Drinking	Edema	Paralysis	Reflexes	Heart rate	Respiratory rate	Pruritis	Eyelid closure	Diarrhea	Depression	Body wt . changes	Hunched/stiff posture
A1	200	31	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
A2	200	30	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
A3	200	35	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
A4	200	30	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
A5	200	32	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
A6	200	30	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
A7	200	30	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
A8	200	30	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
A9	200	30	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
A1	200	30	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
C1	C	30	N	N	N	N	N	N	N	N	-	-	N	N	N	N	N	-	-	N	N
C2	C	30	N	N	N	N	N	N	N	N	-	-	N	N	N	N	N	-	-	N	N
C3	C	35	N	N	N	N	N	N	N	N	-	-	N	N	N	N	N	-	-	N	N
C4	C	30	N	N	N	N	N	N	N	N	-	-	N	N	N	N	N	-	-	N	N
C5	C	30	N	N	N	N	N	N	N	N	-	-	N	N	N	N	N	-	-	N	N
C6	C	30	N	N	N	N	N	N	N	N	-	-	N	N	N	N	N	-	-	N	N
C7	C	35	N	N	N	N	N	N	N	N	-	-	N	N	N	N	N	-	-	N	N
C8	C	30	N	N	N	N	N	N	N	N	-	-	N	N	N	N	N	-	-	N	N
C9	C	30	N	N	N	N	N	N	N	N	-	-	N	N	N	N	N	-	-	N	N
C1	C	30	N	N	N	N	N	N	N	N	-	-	N	N	N	N	N	-	-	N	N

+ Significant changes - not observed/no change noticed C- Control N- normal

Sub acute toxicity study

The methanol extract of PAS at dose of 200,400 mg/kg orally for every 24 hr for 28 days did not produce any mortality in tested animals. No sign of observable toxicity was detected during the experimental period. Changes in body weight of the control and PAS treated mice are presented in Table 3. The increase in weight was not significantly different from that of the control. The progressive increase in body weight at dose of 200,400 mg/kg of mice during 28 days of administration of methanol extract of PAS may indicate the improvement in the nutritional state of the animal.

Table 3. Effect of Oral administration of methanol extract of PAS on body weight (g) and organs weight (g) of mice. Values are expressed as mean ± S.D of 6 mice in each group

	Group-I	Group-II	Group-III
Liver	1.45 ± 0.11	1.52±0.13	1.54±0.07
Heart	0.11 ± 0.01	0.12±0.01	0.13±.03
Lung	0.39 ± 0.02	0.40±0.03	0.40±0.03
Spleen	0.14 ± 0.03	0.16±0.02	0.15±0.03
Brain	0.70±0.04	0.74±0.06	0.75±0.032
Kidney	0.34±0.03	0.35±0.05	0.36±0.02
Adrenals	0.020±0.006	0.019±0.006	0.024±0.008
Ovary	0.191±0.006	0.176±0.023	0.186±0.009
Stomach	0.31±0.020	0.32±0.035	0.35±0.035
Intestine	1.49±0.090	1.56±0.21	1.58±0.11
Body weight	21.98±0.52	22.71±1.34	23.05±1.34

Hematological and biochemical parameters

The effect of PAS extract on hematological parameters of the experimental and control mice is presented in table 4. All the tested hematological parameters such as hemoglobin, R.B.C, Platelet count, Reticulocyte count, Mean corpuscular volume, mean corpuscular hemoglobin concentration, Percent of Neutrophils, Lymphocytes and Monocytes, Packed cell volume and mean corpuscular hemoglobin remained within physiological range throughout the treatment period (28 days).

Table 4. Hematological parameters after 28 days oral treatment with methanol extract of PAS. Values are expressed as mean ± S.D. P <0.05 was considered significant .The * symbol represent the statistical significance at P <0.05

Parameters	Group-I	Group-II	Group-III
Hemoglobin G%	15.48±0.54	15.45±0.68	15.93±0.52
RBC X 10^6/cmm	8.46±0.40	8.48±0.37	8.77±0.24
WBC X 10^3/ cmm	4.07±0.51	5.32±0.48*	3.98±0.82*
PLT lakhs/cmm	5.72±0.71	6.3±0.46	6.35±0.37
Reticulocyte%	0.97±0.14	1.02±0.19	1±0.19
Neutrophil %	20.5±3.95	21.67±2.85	24±7.57
Lymphocyte %	78.17±4.09	77.17±5.63	74.83±7.54
Monocyte %	1.33±0.47	1.17±0.37	1.17±0.37
PCV%	45.82±1.13	45.33±2.17	47.15±1.42
MCV FI	54.24±1.66	54.49±2.14	53.77±1.20
MCH pg	18.28±0.50	18.2±0.82	18.1±0.49
MCHC gm/dl	33.77±0.37	34.05±0.59	33.75±0.39

The data for the biochemical parameters in the treated and control mice are presented in Table 5. Sub acute oral administration of PAS extract (daily for 28 days) did not cause any significant changes in some biochemical parameters including serum bilirubin, Serum total proteins, serum total albumin, serum total globulin, serum cholesterol, serum triglyceride, sodium, potassium, calcium and phosphorus and the activity of the marker enzymes of the liver (Serum glutamic oxaloacetic Transaminase, Serum Glutamic pyruvic Transaminase, Serum alkaline phosphatase).

Table 5. Effect of treatment with PAS extract on biochemical parameters Values are expressed as mean ± S.D. The * symbol represent the statistical significance at P <0.05

Parameters		Group-I	Group-II	Group-III
SGOT IU/L		123.17±22.62	115.83±22.50	113.5±32.67
SGPT IU/L		80.83±11.56	77.66±13.63	90.5±13.91
ALP IU/L		581.66±86.20	539.83±47.35	500.83±128.40
BILI mg/dl		0.43±0.094	0.42±0.11	0.5±0.08
PRO g/dl		5.1±0.49	5.12±0.44	4.97±0.45
ALB g/dl		2.33±0.12	2.4±0.13	2.33±0.14
GLB g/dl		2.75±0.22	2.61±0.22	2.87±0.23
Cholesterol mg/dl		82.83±4.16	82.67±5.99	82.67±4.30
TG mg/dl		90.67±3.59	94.17±4.41	93.83±8.74
Electrolytes	Na mEq/L	150.48±7.10	158.68±2.11	150.12±10.37
	K mEq/L	6.8±1.01	6.78±0.58	5.96±0.73
	Cl mEq/L	116.82±6.96	130.45±6.94*	115.47±8.08*
	Ca mg/dl	8.5±0.32	8.53±0.38*	9.88±0.82*
	P mg/dl	7.03±0.47	6.77±0.60	7.18±0.30
BUN mg/dl		18.35±8.50	9.15±0.51*	11.3±2.91*
Creatinine mg/dl		0.33±0.05	0.25±0.08	0.32±0.11

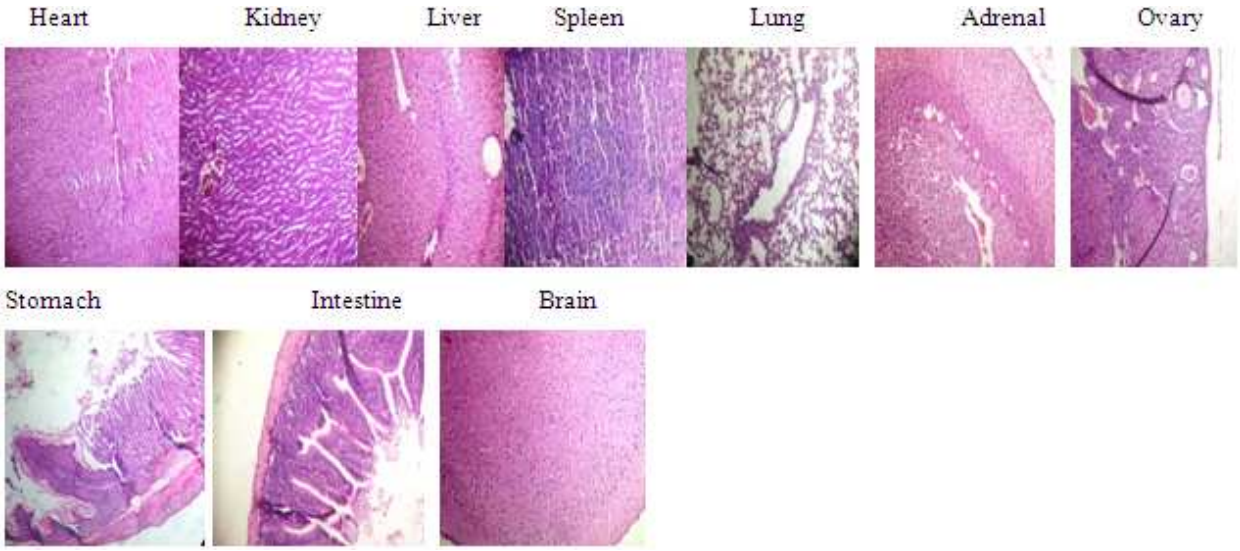
Histopathological study

There were no significant differences between the control and treated groups in the organ weights of animals. Pathological examinations of the tissues indicated that there were no detectable abnormalities. No alterations were seen in the microscopic examination of the internal organs; the cellular appearances were unremarkable in both the groups Histopathological investigations of organs as shown in figure 2.

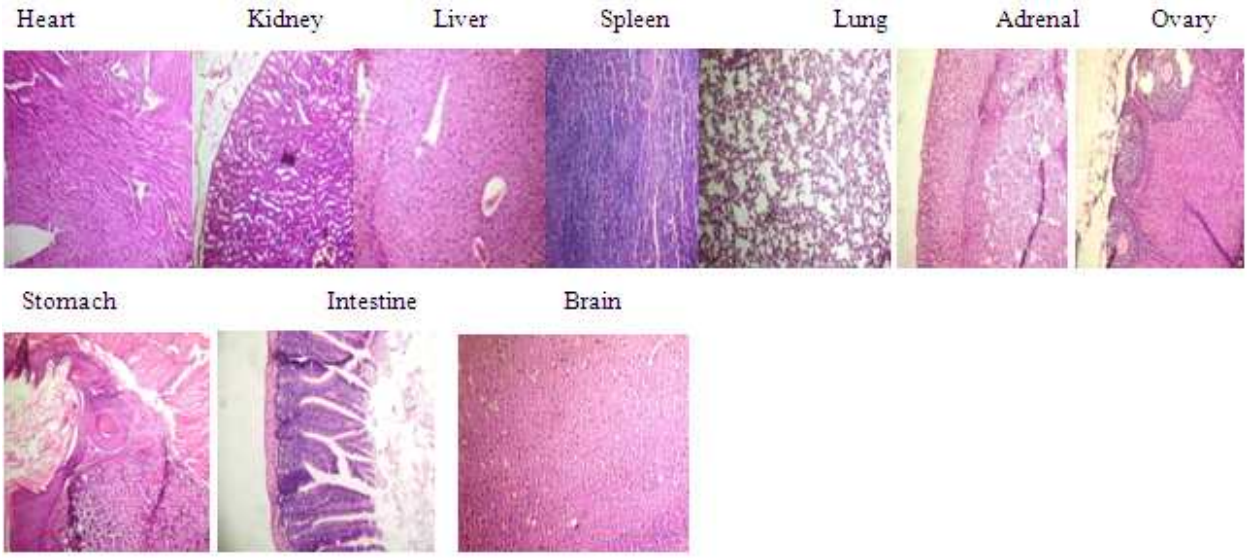
Figure 2

Group-I Control

Group-II Treated with 200 mg/kg of PAS



Group-III Treated with 400 mg/kg of PAS



DISCUSSION

Herbal medicine is still the mainstay of about 75-80% of the world population, mainly in the developing countries for primary health care[23]. Herbal medicines have received greater attention as an alternative to clinical therapy and the demand for these remedies has currently increased. The increase in number of users as oppose to the scarcity of scientific evidences on the safety of the medicinal plants have raisen regarding toxicity and detrimental effects of these remedies. The medicinal plants commonly contain various bioactive principles which have the potential to cause beneficial and/or detrimental effects. Experimental screening method is important in order to ascertain the safety and efficacy of traditional and herbal products and also to establish the active component of the herbal products [15].The results of the acute toxicity reveals that there was no mortality observed up to the maximum dose level of 2000mg/kg b.wt of the extract administered orally, which is the single high dose recommended by OECD guidelines423 for testing acute toxicity. No changes attributable to treatment were found in body weight, respiration rate, heart rate. Treatment related changes observed in behavioral signs viz

apathy, reduced locomotor behavior but regained after 24 hr may be due to the effect of solvent. Thus the present investigation reveals that methanolic extract of PAS does not cause any acute toxicity. Generally the reduction in body weight gain and internal organ weights is a simple and sensitive index of toxicity after exposure to toxic substances. In sub-acute toxicity study mice treated with 200,400 mg/kg doses of methanolic extract of PAS had a progressive increase in body weight. The increase in weight was not significantly different from that of the control. The progressive increase in body weight at dose of 200,400 mg/kg of mice during 28 days of administration of methanolic extract of PAS may indicate the improvement in the nutritional state of the animal. The growth response effect could be as a result of increased food and water intake.

The hematopoietic system is one of the most sensitive targets for toxic chemicals and an important index of physiological and pathological status in human and animal [24].The hematological status after 28 days of oral administration of methanolic extract of PAS was also assessed. The white blood cell was found to be significantly increased ($P<0.05$) in Group –II and decreased in Group-III. With the exception of a transient change in WBC count there were no significant alterations in the hematological parameters. Increase in WBC may indicate the impact of PAS in boosting the immune system of treated groups. However slight changes in WBC did not show any dose responsiveness. All the other hematological parameters in all treated group remained normal without any significant difference.

Transaminases (GOT and GPT) and ALPs are good indices of liver damage. There were no deleterious changes found in the level of transaminases and ALPs in serum of treated groups with control animals. Equally, there also was no marked change in creatinine in these two doses when compared to the control. And creatinine is known as an effective indicator of renal function and any rise in creatinine levels is observed if there is marked damage to functional nephrons. Thus, the results recorded in this study suggest that PAS extract did not affect the renal function. Clearly, this only serves as a preliminary test and that for a better estimation of renal function a creatinine clearance test is required. The liver is the site of cholesterol disposal or degradation and the major site of synthesis. Since, no significant changes were observed in cholesterol levels in this study, it suggests that PAS extract had no effects on the cholesterol metabolism of the mice.

All other biochemical parameters such as total protein, albumin and globulin were remained normal without any significant difference .The levels of electrolytes maintain the body fluid equilibrium. No significant changes were observed in the electrolytes levels, except Calcium, Chloride and blood urea nitrogen. Calcium, Chloride and blood urea nitrogen were significantly changed in treated animals when compared with control group suggesting that PAS extract was relatively low or non-toxic under study conditions.

Furthermore gross examination of internal organs from treated and control animals shoed normal architecture, suggesting no detrimental changes and morphological disturbances caused due to the administration of PAS for 28 days.

CONCLUSION

In conclusion, this study provides the very valuable data on the acute and sub acute toxicity profile of the methanol extract of Plectranthus amboinicus (Lour) Spreng that should be very useful for any future in vivo and clinical study of this plant medicine. PAS extract was found to be less toxic when oral acute and sub acute toxicities in mice were performed. Chronic toxicity,

are necessary to further support the safe use of this herb. These results showed that the use of extract of Plectranthus amboinicus (Lour) Spreng is safe and explained the extensive utilization of the plant in traditional medicine.

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