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Pharmacognostical standardization and toxicity profile of the methanolic leaf extract of *Plectranthus amboinicus (Lour) Spreng*

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ABSTRACT

The present investigation was intended to evaluate the toxicity of the methanolic leaf extract of a traditionally used plant *Plectranthus amboinicus* (Lour) Spreng. Plant material was analysed for various pharmacognostical parameters as per WHO guidelines procedure i.e., foreign matter, microscopical sections, loss on drying, water and alcoholic extractive values, Total ash, acid soluble ash, heavy metals, phytochemical analysis and toxicity studies. Acute & Sub acute toxicity of the methanolic extract was evaluated in albino mice (Female) after ingestions of the extract during one day (Acute model) and during 28days (sub acute model).The studies on sub acute toxicity reveals that no mortalities or evidence of adverse effects have been observed in Albino mice following acute oral administration at the highest dose of 2000mg/kg crude extracts of *Plectranthus amboinicus* (Lour) Spreng. Similarly, in sub-acute toxicity study methanolic extract 200,400 mg/kg body wt of Plectranthus amboinicus did not cause any changes in hematological and biochemical parameters. Studies on histopathological examination of vital organs showed normal architecture suggesting no morphological disturbances. *Plectranthus amboinicus* (Lour) Spreng can be considered as safe as it did not cause either any lethality or adverse changes in the general behavior in mice.

Key words: *Plectranthus amboinicus, Heavy metals, acute oral toxicity, sub acute toxicity, histo pathological studies*

INTRODUCTION

Plectranthus amboinicus (Lour) Spreng (synonym: Coleus amboinicus, Coleus aromaticus family Lamiaceae) is known as Country borage in English, Pathurchur in Hindi (Warrier 1994, Nirmala Devi and Viswanathan 2008), it is a large succulent aromatic perennial herb, much branched ,Fleshy highly aromatic pubscent 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 (Kirtikar & Basu 2005, Nadkarni 1996,Chopra etal 1956). The phytochemical studies reveals the presence of various flavonoids like quercetin, apigenin, luteolin, salvigenin, genkwanin and volatile oil in the leaves(Deena et al 2002).The Pharmacological properties have been reported including Urolithiasis (Patel etal 2010), Fungitoxic (Murthy & Ramalashkmi, 2009), antibacterial (Vijaya kumar 2008), antimalarial (Periyanayagam etal 2008 and Kaou etal 2008), anti-inflammatory(Minker etal 2007& Jia etal 2010). Because of their wider pharmacological activities PA 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 (Mythilpriya etal 2007). The present work was undertaken to study the acute and sub acute toxicity of the leaf extract of *Plectranthus amboinicus* (L) Spreng.

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 Colour photograph of the plant & leave

Preparation of Extract

The leaves of ***Plectranthus amboinicus*** (Lour) Spreng were shade dried at room temperature. Then the shade dried leaves were powdered to get a coarse powder. 60g of coarse powder was defatted with petroleum ether and extracted exhaustively with 95% methanol at Temperature 60^{o}C, in a soxhlet extractor. The extract was concentrated in a rotary flash evaporator. The residue was dried in a desiccator over sodium sulfite. This procedure was repeated for 5-6 times to receive sufficient quantity of methanolic extract.

Phyto chemical Investigation

Methanolic extract of *Plectranthus amboinicus* (Lour) Spreng leaves were subjected to further preliminary, qualitative, phytochemical investigation (Table 1) (Kokate 1999 & Khandelwal 2007).

Table1 Phytochemicalscreening of Methanolic extract of *Plectranthus amboinicus* (lour) spreng

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

+ = present - = absent

Quality control methods for medicinal plant materials

The pharmacognostical standardization i.e, quality control methods comprises the various analytical and phytochemical procedures. They are macroscopic and microscopic examination, determination of ash values, microbial content, phytochemical analysis and heavy metal analysis . The study were conducted as per WHO guidelines (Table 2) (WHO-Geneva and Rajesh et al 2010).

Table 2 Standardization of Plectranthus amboinicus (Lour) spreng.

Standardization procedures	*Plectranthus amboinicus*
Foreign matter	15 % w/w
Loss on drying	10.2 % w/w
Microbial content	Nil
Total ash	33.33 % w/w
Acid insoluble ash	19 % w/w
water extractive value	11.6 % w/w
Alcohol extractive value	11.6 % w/w
Arsenic	Not detected
Lead	Not detected
Mercury	0.29 ppm
Cadmium	Not detected

Microscopy of leaf shows oil glands, trichome, stomata, upper and lower epidermis in Figure 2

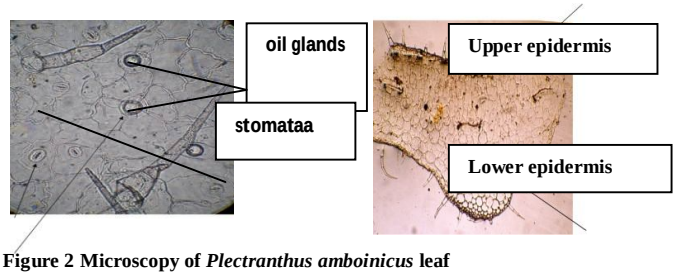


Figure 2 Microscopy of *Plectranthus amboinicus* leaf

Experimental animals

Healthy male and female albino 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 animals were fed with balanced pellet and water *adlibitum*. 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 albino 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 albino mice weighing 20-30 gm were divided in to 3 groups of 6 animals each and were housed

Table 3 Observations of Acute toxicity study (Treatment related changes)

Animal no	Dose mg/ Kg	Body weight(gm)	Apathy	Ataxia	Circling	Compulsive behaviour	Excitability	Locomotor behaviour	Moribund	Drinking	Odema	Paralysis	Reflexes	Heart rate	Respiratory rate	Pruritis	Eyelid closure	Diarrhea	Depression	Body weight changes	Hunched/stiff posture
A1	2000	31	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
A2	2000	30	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
A3	2000	35	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
A4	2000	30	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
A5	2000	32	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
A6	2000	30	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
A7	2000	30	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
A8	2000	30	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
A9	2000	30	+	-	-	-	-	+	-	-	-	-	-	N	N	-	N	-	-	-	-
A10	2000	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
C10	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

under standard conditions and room temperature (25±2^{o}C). The control animals(Group-I) received 0.5ml of vehicle alone and the other two groups(Group-II &III) have received PA extract for 28 days at doses of 200,400 mg/Kg body wt respectively. Toxic manifestations and mortality were monitored daily and body wt changes were recorded every 7 days till the end of the study.

Clinical Test Parameters

At 28thday 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 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.

Animals were then sacrificed by ether anesthesia. The liver, kidney, lung, spleen, Adrenal, Ovary and heart were dissected out, washed and transferred to an ice cold saline solution. The organs were weighed and portions of these organs were fixed in 10% formalin for histopathological examinations.

The hematological parameters like Haemoglobin, Red blood cell (RBC), white blood cell(WBC) and packed cell volume (PCV) were determined by Mythic18.The biochemical parameters SGOT, SGPT, ALP, Bilirubin, total proteins, serum albumin, serum globulin, Total cholesterol and triglyceride, electrolytes, Calcium, Phosphorus, creatinine and blood urea nitrogen were determined by autoanalyzer.

Histopathological study

Histopathological investigation of the organs was done. The organ pieces were fixed in 10% formalin for 24 hrs and washed in running water for 24 hrs. 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

The values are expressed as mean ± Standard deviation (S.D).Results were analyzed statistically using one way Anova. The significant difference between the groups are considered at $P<0.05$ level (Sim 2010, Mounnissamy etal2010, Shylesh 2005, Teo 2002, Godkar & Godkar 2003).

RESULT

Standardization of Plant material

To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. According to WHO the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken. The test for loss on drying determines both water and volatile matter. This is especially for materials that absorb moisture easily or deteriorate quickly in presence of water. The residue remaining after incineration of plant material is the ash content, which represents inorganic salts naturally occurring in crude drug. Another concern related to safety of herbals is presence of contaminants such as heavy metals. The plant material was subjected to heavy metal analysis.

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.

No test substance related mortality was observed at 2000mg/Kg and through out the observation period there were no significant changes in the body weight and treatment related change like respiration rate and heart rate. Table 3 shows the result of acute toxicity study of methanolic extract of Plectranthus amboinicus. Persistent treatment related changes were observed in behavioural signs viz apathy, reduced locomotor behavior but regained after 24 hrs. Consequently, 2000mg/Kg of plant extract found safe with less toxic effect.

Sub acute toxicity study

The methanolic extract of *Plectranthus amboinicus* (Lour) Spreng 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. The effect of 200,400 mg/kg of methanolic extract of *Plectranthus amboinicus* (Lour) Spreng on body weight and organ weight were shown in Table 4. Table 5& 6 shows the effect of PA on hematological and biochemical parameters. All the tested haematological 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 were within normal. W.B.C count was slightly increased in group-II and decreased in Group-III than the Group-I. Biochemical parameters such as serum bilirubin, Serum glutamic oxaloacetic Transaminase, Serum Glutamic pyruvic Transaminase, Serum alkaline phosphatase, Serum total proteins, serum total albumin, serum total globulin and serum cholesterol and serum triglyceride were within the normal.

Table4. Effect of Oral administration of methanolic extract of *Plectranthus amboinicus* (Lour) Spreng 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 (Control)	Group-II (200 mg/Kg b.wt)	Group-III (400mg/Kg b.wt.)
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
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
Kidney	0.34±0.03	0.35±0.05	0.36±0.02
Body weight	21.98±0.52	22.71±1.34	23.05±1.34

Histopathological study

Histopathological investigations of adrenal, heart, liver, spleen, kidney, Ovary and lung as shown in figure 3, reveals that no abnormalities were detected in histopathology of organs of treated group. All the organs gross histopathology were found to be normal.

Table 5. Hematological parameters after 28 days oral treatment with methanolic extract of *Plectranthus amboinicus* (Lour) Spreng. 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 Control	Group-II (200mg/Kg b.wt)	Group-III (400mg/Kg b.wt.)
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

DISCUSSION

In recent years there has been an emphasis in standardization of medicinal plants of therapeutic potential. Despite the modern techniques, identification and evaluation of plant drugs by pharmacognostical study is still more reliable, accurate and inexpensive. According to WHO the microscopic description, microbial, heavy metal analysis are the first step towards establishing its identity and purity and should be carried out before any tests are undertaken. The all pharmacognostical standardization procedures were shown in Table 2. The studies on 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 behavioural 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 *Plectranthus amboinicus* (Lour) Spreng 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 *Plectranthus amboinicus* (Lour) Spreng 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 *Plectranthus amboinicus* (Lour) Spreng 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.

Table 5. Hematological parameters after 28 days oral treatment with methanolic extract of *Plectranthus amboinicus* (Lour) Spreng. 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 Control	Group-II (200 mg/Kg b.wt)	Group-III (400mg/Kg b.wt.)
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 haematological status after 28 days of oral administration of methanolic extract of *Plectranthus amboinicus* (Lour) Spreng was also assessed. Results of hematological parameters .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 PA in boosting the immune system of treated groups. However slight changes in WBC did not show any dose responsiveness. All the other parameters in all treated group remained normal without any significant difference.

Transaminases (GOT and GPT) and ALPs are good indices of liver and kidney damage respectively. Results of serum biochemistry were showed that there were no deleterious changes found in the level of transaminases and ALPs in serum of treated groups with control animals. All biochemical parameters were remained normal without any significant difference except Calcium, Chloride and blood urea nitrogen. Calcium, Chloride and blood urea nitrogen were significantly changed in treated animals when compared with control group. Further more gross examination of internal organs like Liver, Lung, Heart, spleen, adrenal, Ovary and kidney were also found to be normal. No abnormalities were detected in treated animals when compared to the control.

Table 6. Effect of treatment with *Plectranthus amboinicus* (Lour) Spreng extract on biochemical parameters Values are expressed as mean ± S.D. The * symbol represent the statistical significance at P <0.05

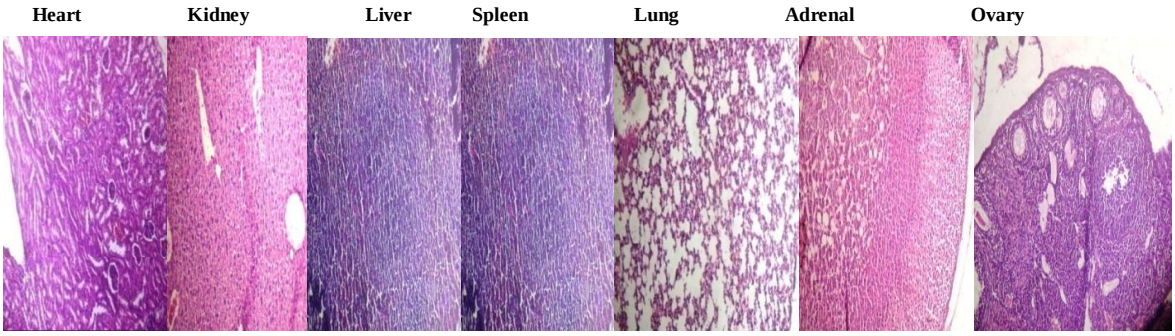
Parameters		Group-I Control	Group-II (200 mg/Kg b.wt)	Group-III (400mg/Kg b.wt.)
SGOT IU/L		123.17±22.62	115.83±22.50	113.5±32.67
SGPT IU/L		80.83±11.56	77.66±13.63	90.5±13.91
ALP IU/L		581.66±86.20	539.83±47.35	500.83±128.40
BILI mg/dl		0.43±0.094	0.42±0.11	0.5±0.08
PRO g/dl		5.1±0.49	5.12±0.44	4.97±0.45
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

CONCLUSION

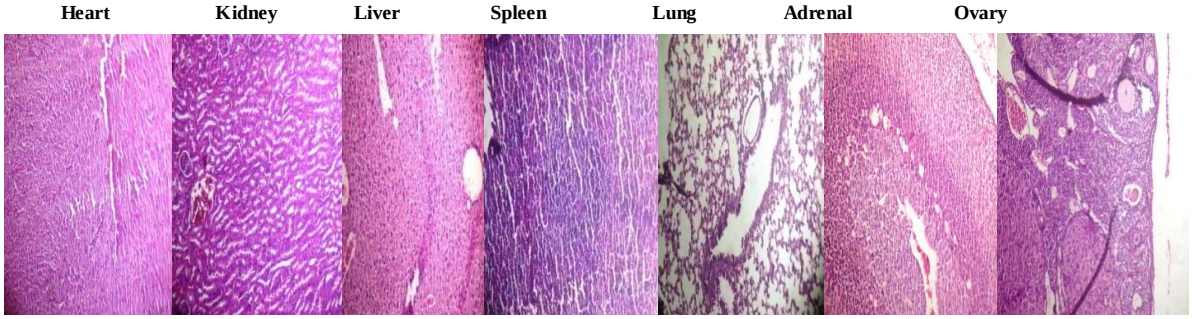
The present investigation of *Plectranthus amboinicus* (Lour) Spreng can be concluded that pharmacognostical study and yielded a set of qualitative and quantitative parameters or standards that can serve as an important source of information to ascertain the quality and purity of the plant material. Toxicity studies

Figure 3 Histopathology of various organs

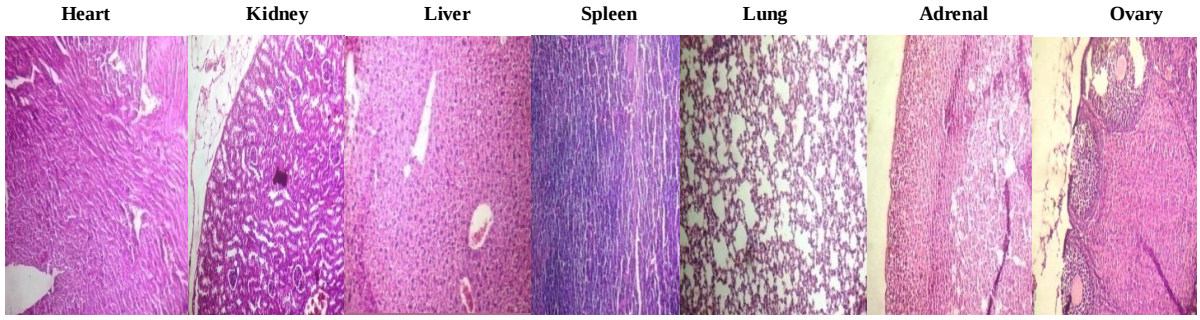
Group-I Control



Group-II Treated with 200 mg/kg of PA



Group-III Treated with 400 mg/kg of PA



presents strong evidence of non toxic effect of methanolic extract of *Plectranthus amboinicus* (Lour) Spreng. 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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