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Chemical composition and Toxicological evaluation of the aqueous leaf extracts of Plectranthus amboinicus Lour. Spreng

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ABSTRACT:
Ethnopharmacological relevance: *Plectranthus amboinicus* is used in traditional medicine to treat several diseases and ailments including opportunistic infections associated with HIV/AIDS. However, there is little insight as to the possible toxic effects of this herbal formulation on major organ systems of the body. This study was designed to assess the acute and sub acute toxic effects of the aqueous leaf extract of *P. amboinicus* on the histology of tissues, biochemical (liver and kidney functions) as well as hematological indices in albino swiss mice and wistar rats respectively.

Materials and methods: In this study, phytochemical screening of the aqueous extracts of *P. amboinicus* was carried out using standard qualitative and quantitative (SPME and GCMS) analytical methods. The extracts were tested for acute toxicity in mice and for sub acute toxicity in Wistar rats according to the OECD guidelines No 425. In acute toxicity test, the dose of 5000 mg/kg was used as the limit dose test. The sub acute toxicity test was evaluated through biochemical, hematological, histopathological and body weight of rats using daily doses of 2500 mg/kg, 1250 mg/kg and 625 mg/kg of the aqueous extract for 28 days. Any signs of toxicity were recorded. Histopathological examination was done on the liver, kidneys, lungs and intestines. Body weight changes were measured weekly for 28 days of daily single dose of extract administration.

Results: Phytochemical screening of the aqueous extracts revealed the presence of tannins, saponins, flavonoids, steroid glycosides and polyuronides. GCMS analysis revealed the presence of 11 chemical compounds constituting 97.6% of the total extract composition. The principle constituents include linalool (50.3 %), nerol acetate (11.6%), geranyl acetate (11.7 %) and carvacrol (14.3%). In acute toxicity, the extracts did not cause any mortality or evidence of adverse effects following oral administration of the highest dose of 10,000 mg/kg of crude extract in both mice. In sub acute study, normal body weight gains were observed during the study period compared to the control group. The kidney function parameters (Urea, creatinine, uric acid) were significantly increased following administration of extract. Histopathological examination of extract treated groups showed necrosis of hepatic cells with generalized congestion in the renal cortex, pneumonitis and sloughing of intestinal villi.

Conclusion: The aqueous extract is rich in phytochemical compounds that are of therapeutic value. No mortalities were observed during acute and sub acute toxicity study period. These finding indicate that the extract caused treatment- related toxicological abnormalities which increased with dosage. The aqueous extract of *P. amboinicus* is safe to use as indicated by the high LD_{50} value, but should be used with caution at high doses.

KEYWORDS: *Acute toxicity; Sub acute toxicity; Biochemical; Histopathology; GC-MS; Phytochemical*

I. INTRODUCTION

Medicinal plants are the source of treatment for many diseases and ailments throughout the developing world (Rao et al., 2004) because they contain various bioactive principles which have the potential to cause beneficial and/or detrimental effects (Adewunmi and Ojewole, 2004). Traditionally, people think that medicinal herbs being natural are safe and free from undesirable effects, failing to recognize that herbs are composed of bioactive chemicals some of which may be toxic. Although there is increased acceptance and consumption of herbal remedies worldwide, care must be taken not to consume harmful plants or high doses of plant extracts that could have deleterious effects on vital body organs either in short term or long term. Concerns by medical personnel indicate that herbal medicines may be harmful to vital organs such as liver and kidneys (Ogbe et al., 2012). Toxic effects due to herbal medicine may manifest in a number of organs such as kidney, liver, stomach, nervous system and blood. The liver is a vital organ for maintaining of metabolic functions and detoxification

from exogenous and endogenous substances like xenobiotics, drugs and viral infections. When the liver is exposed to such substances, its protective mechanisms are overpowered due to cellular necrosis and increase in serum levels of biochemical parameters like Alanine aminotransferase (ALP) and Aspartate aminotransferase (AST). Determination of efficacy and safety of herbal remedies is necessary as many people use them for self medication. For majority of herbal products in use, very little is known about their active and /or toxic constituents. Few studies regarding the toxicity of the aqueous extract of *P. amboinicus* are available. Therefore, the present study was undertaken to assess the chemical composition and toxicity profile of the aqueous leaf extract of *P. amboinicus.* Evaluating the prolonged effects of medicinal plant extracts used in humans is useful in assessing the potential toxic effects. This increases the confidence in their safety to humans, particularly for use in the development of pharmaceuticals.

A review of the traditional uses of *Plectranthus amboinicus*

Plectranthus amboinicus (Lour.) Spreng is a tender fleshly perennial plant in the family Lamiaceae and native to southern and eastern Africa. The genus *Plectranthus* comprises of about 350 species distributed in Sub Saharan Africa, Australia, Asia, India, Madagascar and Pacific Islands (Alasbahi and Melzig, 2010). *P. amboinicus* is used in the treatment of diseases such as digestive, genitourinary and respiratory disorders, skin infections and pain (Lukhoba et al., 2006). In India, it is used to treat malaria, cough, chronic asthma, bronchitis, colic, epilepsy and hiccups (Nadkarni, 1996). The pharmacological properties recorded include antibacterial, antifungal, antioxidant, analgesic, anti-inflammatory, fungitoxic, and antimalarial activities (Lukhoba et al., 2006). In Uganda, the plant is used in traditional medicine to treat ailments such as, cough, diarrhoea, immunity boosting and fungal infections of the skin (Asiimwe et al., 2013). Basing on the traditional uses of the plant mentioned above, the plant is mainly consumed orally; hence the need for toxicity studies on the possible deleterious effects of *P. amboinicus* on internal body organs after oral administration of the crude aqueous extract of the leaves.

II. MATERIALS AND METHODS

Plant material collection and preparation

The fresh leaves of *P. amboinicus* were collected from Mbarara district, western Uganda in May 2011. The plant was taxonomically identified and authenticated by Protase Rwaburindore at the Department of Biological Sciences, Makerere University, Uganda, under voucher specimen number AS053 for reference. The leaves were air dried under shade for two weeks and ground into powder using a mortar and pestle. The dry powder (100 g) was soaked in 1 litre of warm distilled water for 72 hours at room temperature with vigorous shaking. The mixture was filtered with cotton wool and later with whatman filter paper no: 1. The filtrate was then dried at a temperature of 40^0C for 72 hours to produce a gel-like extract which was stored at 4^0C in a refrigerator for further use. The percentage yield was calculated using the formula: Percentage yield= W_2/W_1 *100, whereby W_1 is the weight of the powdered sample before extraction, while W_2 = weight of semi-solid aqueous extracts. Hence, the percent yield of *P. amboinicus* was 47/200*100= 23.5 % w/w.

Qualitative Phytochemical analysis

Chemical tests were carried out on aqueous extracts of dry leaves using standard procedures to identify bioactive molecules as described by (Harborne, 1998; Trease and Evans., 2002).

Isolation of volatile chemical compounds

The plant leaves were boiled for 30 minutes and the water cooled. The plant volatiles were collected from the warm aqueous extract by head space solid phase microextraction (HS-SPME) using a Polydimethylsiloxae/divinylbenzene 65µm- fiber (PDMS/DBV) for a sample/headspace equilibration time of 1 hour. The extracted materials were subsequently desorbed and analyzed in the GC-MS apparatus.

Gas Chromatography Mass Spectrometry

The volatile compounds extracted by SPME were analyzed by a GC-MS electron impact ionization (EI) method on a Varian GC equipped with an a DB-5 wax (J&W Scientific; column 30 m long × 0.25 mm i.d; 0.25 µm film thickness). Oven temperature was programmed from 40^0C (1 min) with an increase of 3^0C/min to 235^0 for 9 min. Injections were performed with injector temperature of 230 ^{0}C, ion source temperature 200^0C. Volume injected was 1µl of the oil. Helium was used as a carrier gas at a flow rate of 1ml/min. Mass spectra were taken at 70eV, a scan interval of 1sec and a scan range of 30-400m/z, full scan mode that revealed the total ion current (TIC) chromatograms. A Finnigan SSQ 7000 MS was used to identify and quantify the individual compounds. The bioactive compounds were identified by calculating retention indices using a homologous series of n-alkanes (C_5-C_{26}) and comparing retention indices (RIs) with literature values measured on columns with identical polarities (Adams, 2007). The components were also identified by matching their Mass spectra

with those in the available reference computer libraries of NIST (National Institute of Standards and Technology).

Preparation of test animals

Disease free mice (n=30) and Wistar rats (n=30) of both sexes were obtained from the College of Veterinary Medicine and Biosafety, Division of Pharmacology & Toxicology, Makerere University, Uganda. The animals were kept in cages for one week to acclimatize and were maintained on standard animal diet and water in excess. However food was withdrawn 18 hours before the start of the experiment according to the method of (Amresh et al., 2008) in acute toxicity.

Method for acute toxicity

Paragraph 22 of the OECD guidelines 425 suggests two types of acute oral toxicity tests, i.e the limit test and the main test. The limit test is used in situations where the experimenter has information indicating that the test material is likely to be non-toxic. The literature survey of this herb indicates that the plant is not toxic (Pillaia et al., 2011). Hence, the limit test was used, where the limit test dose of 5000 mg/kg was used as described by the Organization for Economic Cooperation Development guidelines No. 425 (OECD, 2008a). Acute toxicity test of the plant extracts was carried out using female and male albino mice (1:1, 15 - 25g) accordingly. The aqueous extract was reconstituted by suspending 2 g of crude aqueous extract in 10 ml of distilled water to obtain a stock solution of 200 mg/kg and administered to the study animals. The volume of extract administered was calculated as follows (Gosh, 1984):

$$\text{Volume of extract (ml)} = \frac{\text{weight of mice (kg) x dose rate (mg/kg)}}{\text{Stock solution (mg/ml)}}$$

The aqueous extracts of *P. amboinicus* were administered orally by gavage to 3 groups of mice at different doses to two mice per group. The animals were dosed one at a time, at the test dose of 5000 mg/kg. If the animal did not die, two additional animals were given higher doses of 7500 mg/kg and 10,000 mg/kg body weight respectively. If both animals survived, the LD_{50} was greater than the limit dose and the test was terminated. The control group received 1 ml of distilled water. Animals were observed for general toxicity signs, behavioral changes, and mortality for the first 6 hours. Observations of toxic signs were recorded and the number of survivors was noted after 72 hrs.

Sub-acute toxicity

The sub acute toxicity test was performed following the Repeat-dose oral toxicity protocol described by the OECD guideline 407 for testing of chemicals (OECD, 2008b). Twenty four adult wistar rats were divided into four groups of 6 rats per group. Having determined that the LD_{50} was above the limit test dose of 5000 mg/kg, the extract doses for sub acute test were determined from ½, ¼ and $^1/_8$ of the limit test dose of 5000 mg/kg. Groups I, II and III received 625, 1250 and 2500 mg/ kg body weight respectively, of the aqueous extract once daily for 28 days. Group IV (control) received 1 ml of distilled aqueous. The body weights of individual animals were evaluated weekly and recorded.

Hematological and biochemical parameters

At the end of 28 days of treatment with *P. amboinicus* leaf aqueous extract, the animals were anaesthesized with chloroform in a desiccator and blood was collected via cardiac puncture and placed in non-heparinised and heparinised sample bottles for serum blood chemistry and hematological parameters analyses respectively.

Histopathological examination

After collecting blood, an autopsy was performed for tissue collection from two arts from each treatment groups with the liver, kidney, lung and intestinal tissues fixed in freshly prepared 10% buffered-formalin. After 72hrs the tissue were subjected to automated tissue processing machine with increasing concentrations of ethanol. Thin sections of 5µm were made using microtome and later stained with heamatoxylin and eosin stain ready for microscopical examination.

Statistical analysis of data

Results were presented as mean ± SEM. The data was subjected to one-way analysis of variance (ANOVA) test and differences between the control group and extract treated groups were determined by post hoc Dunnett's multiple comparison tests, using Graph Pad Prism (Version 5) software. Results were considered to be statistically significant at $p \leq 0.05$ with 95% confidence interval.

Ethical clearance

The study was cleared by the higher degree academic committee of Makerere University, Kampala. The experiments were carried out according to international guidelines on care, use and handling of laboratory animals (NIH, 2012).

III. RESULTS AND DISCUSSION

Phytochemical screening of the aqueous extracts of the plant revealed the presence of various bioactive compounds (Table 1). Previous studies on *P. amboinicus* indicated the presence of alkaloids, tannins, Saponins, cardiac glycosides, terpenoids and flavonoids (Sathasivam and Elangovan, 2011).

Table 1. Phytochemical profile of the aqueous extract of *P. amboinicus*

Compound	Aqueous Extract
Saponins	++
Polyuronides (pectins, mucilages, gums)	++
Tannins (gallic)	+
Reducing compounds	++
Alkaloids	-
Anthracenosides	-
Flavonosides	+
Anthocyanosides	-
Steroid glycosides	+++

+ trace amount ++ moderately present +++ highly present - absent

In GC-MS analysis of the aqueous extract, 11 bioactive compounds were identified representing a total of 97.6% of the compounds in the extract. The main components of the aqueous extract include linalool (50.3 %), nerol acetate (11.6), geranyl acetate (11.7 %) and carvacrol (14.34) (Table 2). The study of possible effects of these volatile compounds on rats is being reported for the first time. These compounds may exert deleterious effects at high doses. Studies indicate that linalool, (a naturally occurring compound found in oils of herbs, leaves, flowers) increases hepatic microsomal enzyme activity in rats (Parke et al., 1974b) which can result into inducement of drug interactions. However, oral administration of linalool does not affect any of the lung microsomal parameters (Chadha and Madyastha, 1984). Further studies on these compounds are necessary to elucidate the metabolism and mechanism of action of these compounds after oral administration of plant extracts.

Table 2. Percentage composition of the compounds isolated from the aqueous extract of *P. amboinicus*

Constituents	Area %	Retention Index
β - Myrcene	0.8	1158
4 – Carene	1.3	1007
γ-Terpinene	3.2	1262
β – Ocimene	2.0	1290
β- Cymene	2.9	1267
Linalool	50.3	1544
α-Caryophyllene	1.2	1897
Nerol acetate	11.6	1343
Geranyl acetate	11.7	1352
Nerol	2.3	1753
Carvacrol	10.3	1330

Acute toxicity

In the acute toxicity study, a dose of 10,000 mg/Kg produced no mortality after 72 hours of observation. The clinical toxicity signs observed were; ataxia, reduced feed intake, twitching of GIT muscles, waging of tail, hyperurination, drowsiness and urine color change (yellowish-brown urine). The results of the current study showed that the LD_{50} of the crude aqueous extract was found to be greater than 5000mg/kg, which may be accepted as experimentally safe (OECD, 2008a).

Table 3. Variation in weight of rats (g) treated with *P. amboinicus* extracts for four weeks

	Weight (g)				
Dose (mg/kg)	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Control	83.47 ± 3.73	92.62 ± 3.83	106.04 ± 4. 83*	115.42 ± 5.70***	110.34 ± 6.90**
625 mg/kg	88.87 ± 5.00	101.63 ± 3.62	112.77 ± 4.13 *	131.37 ± 6.40 ***	134. 44 ± 6.47***
1250 mg/kg	85.76 ± 8.00	95.73 ±6.90	104.72 ± 5.96	116. 45 ± 6.03*	116.34 ± 5.76*
2500 mg/kg	82. 28 ± 7.66	101.87 ± 7.91	111.69 ± 6.30*	126.30 ± 5.65***	127.91 ± 5.67***

Values are expressed as mean ± SEM of 6 rats treated for 28 days. Values are statistically different from control at p<0.05*, p<0.001 ** and p<0.001*).**

In sub acute toxicity studies, *P. amboinicus* extracts at dose of 2500 mg/kg given orally for 28 days did not produce any mortality in rats. No signs and symptoms of toxicity, changes in behavior or other physiological abnormalities were observed during the experimental period.

Body weight changes are summarized in table 3. Daily administration of the aqueous extract for 28 days showed significant increase in body weight after seven days compared with the control ($p<0.05$). There was no significant difference between the initial weight and weight on the seventh day ($p>0.05$). The increase in body weight is consistent with increase in doses of the extract. This may be an indication that the drug does not affect the feed utilization and normal growth of the rats.

Assessment of biochemical parameters

The effects of the plant extract on biochemical parameters are summarized in table 4. Biochemical analyses are useful in toxicity studies by providing information about in vivo effects of test substance (Ode et al., 2011). Renal function indices such as urea, creatinine and uric acid can be used to evaluate the functional capacity of the nephrons of study animals (Yakubu et al., 2002) and are considered as good indicators of liver and kidney function. From this study, *P. amboinicus* caused a statistically significant increase in creatinine ($p<0.05$), Uric acid ($p<0.05$) and a decrease in Urease ($p<0.01$) respectively**.** Urea and creatinine are waste products of protein metabolism that need to be excreted by the kidney. Therefore, marked increase in serum urea and creatinine are indications of functional damage to the kidneys. The kidney is the primary organ for clearance and excretion of xenobiotics including drugs and drug products from the body. Damage to the kidney may arise due to the administration of plant extracts, although incidences of toxicity in local settings are hardly reported.

The liver enzymes, Alanine and aspartate aminotransferase (ALT and AST) have been established as markers of hepatocellular injury while alkaline phosphate (ALP) is a marker of cholestasis (Vasudevan and Sreekumari, 2007). These enzymes are also involved in amino acid metabolism and are useful in assessing the functional integrity of the liver. ALT is produced within liver cells, recording increases in conditions where liver cells have been inflamed or undergone cell death. As the cells are damaged, the ALT leaks into the blood stream leading to a rise in the serum levels. It is the most sensitive marker for liver cell damage. In this study, the extract did not exhibit a significant ($p>0.05$) effect on Alanine aminotranferase (ALT), alkaline phosphate (ALP) and aspartate aminotransferase (AST) at all treatment doses relative to the control. The non significant changes in ALT, AST and ALP in rats at all doses suggest that sub acute administration of the extract does not affect hepatocyte function in rats or induce any cytotoxic damage to the liver. Serum levels of AST and ALT become elevated whenever disease processes affect liver cells.

Table 4. Effect of aqueous leaf extract of *P. amboinicus* on liver and kidney function parameters

Parameters	Dose (mg/kg)				
	Control	625	1250	2500	p-value
Creatinine (µmol/L)	40.50±1.586	45.33±2.124	45.33±3.756	50.33±1.647*	p< 0.05
Uric acid (mg/dl)	2.267±0.27	2.333±0.35	3.550±0.38*	3.833±0.38*	p< 0.05
Urease enzyme (mMol/L)	8.267±0.13	6.63±0.16***	5.78±0.21***	5.73±0.04***	p< 0.01
ALT (U/L)	236.7± 22.45	191.4± 10.18	207.32 ± 3.25	256 ± 16.87	p> 0.05
AST (U/L)	229.1 ±22.58	198.6 ± 12.77	191.70 ±10.12	203.60 ±11.31	p> 0.05
ALP (U/L)	299.2 ±44.19	223.8 ± 42.93	219.4 ± 72.23	229.0 ±42.20	p> 0.05

each value represents the mean ± SEM (n=6), values are statistically different from control at p<0.05*, and p<0.001*).**

Assessment of hematological parameters

Hematological analysis of the plant extract is one of the important methods of assessing the toxicity of foreign compounds including plant extracts on blood constituents of an animal (Ashafa et al., 2009a). Assessment of red and white blood cell count, hematocrit and hemoglobin concentration is useful in determining the effect of some chemical substances on hematopoietic system. In this study, *P. amboinicus* caused a significant increase ($p<0.05$) in lymphocytes, platelets, basophils, and neutrophils (Table 5). The significant increase in lymphocytes in the test animals suggests that the extract may have immunostimulating properties. This is due to the presence of Saponins, flavonoids and alkaloids which enhance immune functions by stimulating cell division and transformation in lymphocytes (Bruneton, 1995; Govind et al., 2012; Hoffmann, 2003). This makes the plant important especially in immunocompromised patients with HIV/AIDS to stimulate production of T-lymphocytes thereby boosting the defense system of the patient. This may account for the use of the plant for immunity boosting by traditional medical practitioners in the study area. Herbal extracts have potential application as immunostimulants and could act against a broad spectrum of pathogenic microorganisms (Ogbe et al., 2012). The significant increase in platelets indicates that the plant compounds present in the extract promote blood clotting.

Table 5. **Effect of aqueous extract on hematological parameters of wistar rats**

Parameters	Extract (mg/kg body weight)			
	Control	**625**	**1250**	**2500**
WBC (10^3UL)	10.54 ± 1.40	10.97 ± 1.32	13.72 ± 2.13	13.04 ± 2.45
NEUT (10^3UL)	2.03 ± 0.29	2.22 ± 0,05	1.58 ± 0.01	3.77 ± 0.63**
LYMPH (10^3UL)	5.56 ± 0.04	5.03 ± 0.58	5.11 ± 0.13	9.13 ± 1.20**
MONO (10^3UL)	0.94 ± 0.28	0.80 ± 0.13	1.06 ± 0.20	0.78 ±0.16
EO (10^3UL)	0.36 ± 0.05	0.36 ± 0.06	0.58 ± 0.12	0.46 ± 0.06
BASO (10^3UL)	0.04 ± 0.00	0.04 ± 0.00	0.02 ± 0.00	0.09 ± 0.03*
RBC (10^3UL)	7.40 ± 0.20	7.57 ± 0.25	7.43 ± 0.30	7.78 ± 0.33
HGB (g/dl)	13.43 ± 0.40	13.87 ± 0.42	14.30 ± 0.50	14.40 ± 0.43
HCT (%)	39.73 ±1.53	41.33 ±1.14	41.59 ± 1.21	43.10 ± 1.20
MCV (fL)	53.70 ± 1.30	54.63 ± 0.70	55.82 ± 0.90	53.93 ± 0.91
MCH (pg)	18.15 ± 0.24	18.40 ± 0.17	18.98 ± 0.30	18.40 ± 0.23
MCHC (g/dl)	33.88 ±0.44	35.38 ± 1.75	33.67 ± 0.32	34.10 ± 0.32
RDW-CV (%)	19.32 ± 0.60	16.93 ± 0.69*	17.23 ± 0.51	18.75 ± 0.75
PLT (10^3UL)	537.50 ± 92.52	622.00 ± 88.22	688.20 ± 68.17	829.40 ± 16.44*
MPV (fL)	8.20 ± 0.25	7.72 ± 0.11	7.65 ± 0.12	7.52 ± 0.16*

Mean ± SEM (n=6), * p<0.05, **p<0.01

The extract did not exhibit a significant ($p>0.05$) effect on red blood cells, hematocrit (HCT), hemoglobin (HGB) and mean corpuscular hemoglobin concentration (MCHC) at all treatment doses relative to the control.

Histopathological studies

The tissues of the liver, kidney, lungs and intestines of two randomly selected animals in all the study groups were collected for major Histopathological examination. The results of histopathological studies revealed a dose – related effect in the liver, lungs, kidney and intestines. Although there was no mortality from sub acute toxicity test, there was evidence of tissue necrosis, lung hemorrhages and sloughing of intestinal villi at high doses (Fig1). Hematocrit (HCT) level was increased though not statistically significant ($p>0.05$), suggesting that the extract could stimulate erythrocyte synthesis. Phytochemicals are known to exhibit different biochemical and pharmacological actions ranging from cell toxicity to cell protective effect in different species of animals when ingested. Research shows that xenobiotics like saponins and tannins cause haemolysis, nutrient malabsorption and abnormal haemopoesis which could arise from kidney and liver damage. Some alkaloids have cytotoxic effect on organs by damaging the cells of the liver, lungs, heart and kidney. Hence, the toxic effects of *P. amboinicus* may be due to the combined effect of the chemical constituents present in the extract such as tannins, alkaloids.

The therapeutic importance of the leaves of *P. amboinicus* in folk medicine has been documented in earlier studies (Asiimwe et al., 2013; Khare et al., 2011; Lukhoba et al., 2006). Previous studies on the methanolic extract of *P. amboinicus* found the extract to be less toxic at the tested doses (Pillaia et al., 2011). The current study showed that the aqueous extract of this plant could be potentially toxic when high doses and the duration of use is extended. The traditional use of herbal medicine is an old practice in most regions of the world and has played a central role in drug bioprospecting (Heinrich et al., 1998). The administration of herbal preparation without any standard dosage coupled with inadequate scientific studies of their safety has raised concerns on their toxicity. Toxicity studies in animals help to assess the potential health risk in humans caused by intrinsic adverse effects of chemical compounds present in plant extracts. The adverse effects may manifest in form of alterations in levels of biomolecules such as enzymes and metabolic products, normal functioning and histomorphology of the organs (Ashafa et al., 2009b). The liver, being the primary organ for the detoxification and distribution of drugs, and the kidney, the major excretory organ, could be assessed to establish the safety of a substance (Guptan *et al.,* 1994).

The increase in number of users of herbal medicines as opposed to the scarcity of scientific evidences on the safety of the medicinal plants have risen regarding toxicity and detrimental effects of these remedies. Experimental screening method is important in order to ascertain the safety and efficacy of traditional herbal products and also to establish the active components of the herbal products. It is therefore pertinent to establish the safety of medicinal plant preparations through toxicological assessments.

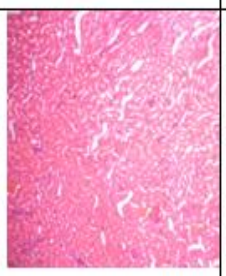
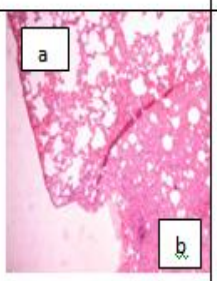
Treatment group	**Organs**				
	Liver	**Kidney (renal cortex)**	**Kidney (Medulla)**	**Lungs**	**Intestine**
Control group	 Normal Liver	 Kidney with normal renal cortex	 No significant lesion in the medulla	 Normal lungs	 Intestine with normal mucosa
Group I (625 mg/kg)	 Focal area of necrosis & perivascular degeneration	 No significant lesion in the renal cortex	 No significant finding	 Part a is normal, part b is pneumonitic	 No significant lesion

Fig 1. Effect of treatment with *P. amboinicus* extract on histopathological parameters; X50; 100µm line bar

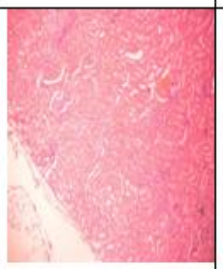
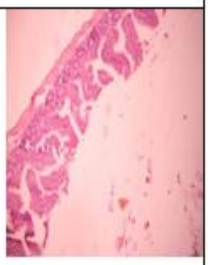
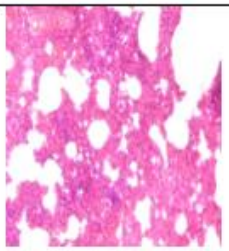
Treatment group	Organs				
	Liver	**Kidney (renal cortex)**	**Kidney (renal Medulla)**	**Lungs**	**Intestine**
Group II 1250 mg/kg	 Perivascular degeneration, congestion and necrosis	 Hyaline degeneration in the renal cortex	 No significant lesion in the medulla	 Lung congestion, oedema and pneumonitis	 Intestine villi tips sloughed off
Group III 2500 mg/kg	 Perivascular hepatic degeneration and necrosis	 Congestion in the renal cortex	 No significant finding in the medulla	 Lung hemorrhages, hemolyzed Red Blood Cells, thickened interalveolar septa (pneumonitis)	 Intestinal ulcers and mucosal erosion

Fig 1 (cont'd) Effect of treatment with *P. amboinicus* extract on histopathological parameters; X50; 100µm line bar

IV. CONCLUSION

It was observed that it would take more than 10,000 mg/kg of extract to kill 50% of the test animals. This gives a wide safety margin for the lethal dose of *P. amboinicus* extract in test animals since the LD_{50} is above the limit dose of 5000 mg/kg according to OECD guidelines. This is the LD_{50} for the aqueous extract which is commonly used in folk medicine. Results of hematology test indicate that the plant extract can be used to boost the immune system, which is an important component in caring for immunocompromised people, particularly HIV/AIDS patients. However, observations indicate that prolonged use of the extract of *P. amboinicus* at high doses could cause injury to the liver, intestine, kidney and lungs. Hence, the extract should be used with caution at high doses. Chronic toxicity tests are recommended to determine the long term effects of the extracts to further support the safe use of this plant.

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VI. CONFLICT OF INTEREST

The authors hereby declare no conflict of interest in this work

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