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Full Length Research Paper

Phytochemical and preliminary toxicological studies of the aqueous leave extract of *Leucas martinicensis* in wistar rats

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***Leucas martinicensis* (Jacq.) R. Br. Family Lamiaceae and commonly called Whitewort grows widely in the Northern part of Nigeria. It has been traditionally used to treat inflammatory conditions, rashes, diarrhoea, epilepsy and convulsions. The present study was to investigate the safety and phytochemical components of the aqueous leave extract of *Leucas martinicensis*. The quantitative and qualitative phytochemical studies were conducted on the leaf extract. Acute toxicity was studied using the up and down procedure at an oral Limit dose of 3000 mg/kg body weight. Female rats in sequence were used to test for acute toxic effect. Repeat dose toxicity study was carried out at an oral dose of 750 mg/kg daily for 28days. Phytochemical studies revealed the presence of saponins (3.15%), glycosides (1.35%), alkaloids (3.95%) and flavonoids (3.56%). The acute toxicity result showed that the extract has LD_{50} above 3000mg/kg and repeat dose toxicity studies of the extract revealed elevated aspartate transaminase enzyme and haemorrhage around the cardiac myocytes. Though the extract is relatively safe, its prolong use may carry risk of cardiac toxicities.**

Key words: Toxicity, *Leucas martinicensis*, Wistar rats, Phytochemical studies.

INTRODUCTION

Leucas martinicensis (Jacq.) R. Br. Family Lamiaceae and commonly called Whitewort or mosquito plant (Hyde and Wursten, 2009), is an erect strong aromatic annual herb growing up to 1.5m high. It is widely distributed in the tropical parts of Africa, Arabia, Asia and America (Muhammad et al., 2012). In Nigeria, it is commonly found in the Northern part of Nigeria where it is known as Bunsurun fadama (Hausa).

From Ethnobotanical survey, the decoction of leaves and aerial parts are used against kidney disorders, rheumatism and inflammations (Agra et al., 2007). Hot water decoction of *L. martinicensis* has been used to relieve cough (Minja, 1999). It has also been used to prevent and to cure diarrhoea. For the treatment of fevers, the fresh leaves are rubbed on or the whole plant made into an infusion and used as a wash or steam fumigation (Fowler, 2006). In West Africa, the plant ash is used to repel mosquitoes (Muhammad et al., 2012). In Nigeria, the decoction of the leaves is used for the treatment of skin rashes, epilepsy and convulsions.

Previous scientific research has validated the use of *Leucas species* for the treatment of various ailments like cholera (Reddy et al., 1993), malaria (Valsaraj et al., 1997), syphilis (Rahman et al., 2007), diarrhoea (Singh et al., 1978) and dysentery (Sharma et al., 1992). The leaves are also used orally for pain during pregnancy (Qamaruddin et al., 2002). The infusion is used ophthalmically for conjunctivitis (Qamaruddin et al., 2002) and for corneal disease (Dhar et al., 1968). The essential oils obtained from the leaves and flowers of *L. martinicensis* were found to contain 1-hepten-3-ol and germacrene. The volatile oil obtained from *L. martinicensis* was reported to be suitable for use in pharmaceuticals, cosmetics and food products, e.g.,

Table 1. Phytochemical Constituents of aqueous leave extract of *Leucas martinicensis.*

S/N	Constituent	Qualitative analysis	Quantitative analysis
1	Alkaloids	+++	3.95%
2	Tannins	-	
3	Saponins	++	3.15%
4	Flavonoids	++	3.56%
5	Glycoside	++	1.35%
6	Saponin glycosides	-	
7	Steroids	-	
8	Volatile oil	-	
9	Anthraquinones	-	
10	Cardiac glycosides	-	

Moderate (++); Large (+++); Absent (-)

lotions, creams, soaps, shampoos, rinses, gargles and candies. Its antibacterial and antifungal properties have also been reported (Estifanos, 2003).

This present study was to investigate the phytochemical components of the aqueous leave extract of *Leucas martinicensis* and to conduct a preliminary study on its safety profile.

MATERIALS AND METHODS

Fresh leaves of *Leucas martinicensis* were collected at Kara local market of Sokoto in the Month of March, 2009. The plant was identified and authenticated in the Taxonomy unit of the Department of Botany, Usmanu Danfodiyo University, Sokoto (UDUS) where voucher specimen was deposited with voucher number 020.

Preparation of plant material and extraction

The leaves were washed, air dried for about five days to constant weight. The dried leaves were pulverized mechanically into a dried powder. 380 g of the powdered herbs was soaked in 100ml of distilled water. The mixture was stirred continuously for 10 minutes at first instance. It was left overnight and subsequently stirred for 10 minutes before filtering separately first with clean white muslin cloths and later with sterile cotton wool and Whatman filter paper size 15cm. The filtrate was evaporated in oven at a temperature of 50^{o}C. It was weighed after drying and the percentage yield was calculated to be $4.27\%^{w}/_{w}$.

Experimental animals

Female wistar rats of 8-10 weeks old (150-180 g) were obtained from Veterinary institute Vom, Jos and kept in the animal house of the Department of Pharmacology UDUS. The animals were kept in well constructed cages for two (2) weeks for acclimatisation before the commencement of the study. Water and standard rat chow were provided *ad libitium* throughout the period of acclimatization and the study. The study was conducted in accordance with the Organization for Economic Development (OECD) guidelines on good laboratory practice (OECD, 2008).

Phytochemical analyses

The quantitative and the qualitative analyses of the plant's constituents were assessed by the methods described by Trease and Evans (1999), El-Olemmy et al.(1994) and Harbone (1993).

Acute toxicity studies

The acute oral toxicity study was done by 'Up-and- Down' method in healthy female wistar rats according to 'OECD' guideline no. 425 (OECD, 2001). A limit dose of 3000 mg/kg was used for the study. Five female rats were randomly selected through a computer generated random numbers and used for the study. An animal was picked at a time, weighed and dosed orally with 3000mg/kg of the extract after overnight fasting. Each animal was observed after dosing for the first 5 minutes for signs of regurgitation and kept in a metallic cage. Each was then observed every 15 minutes in the first 4 hours after dosing, every 30 minutes for 6 hours and daily for 48 hours for the short-term outcome according to the specifications of the OECD. The animals were monitored for a total of 14 days for the long-term possible lethal outcome.

Repeat dose oral toxicity study

The study was conducted according to the methods used

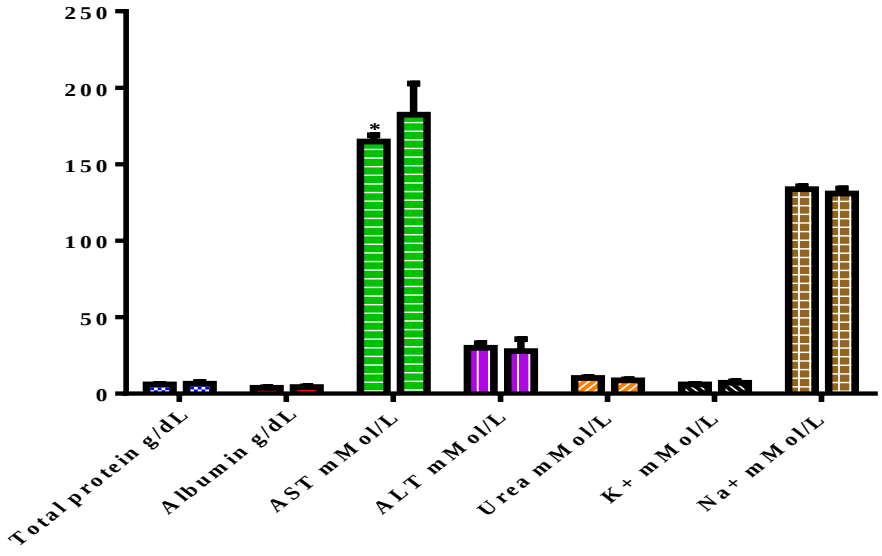

Figure 1. Effect of *Leucas martinicensis* on Biochemical parameters. Data are presented as mean ± SEM. *values are significant at p< 0.05. First bar represents the treated group while the second is the control. AST= Aspartate transaminase; ALT= Alanine transaminase.

by Adeneye et al. (2006) and Ugwah-Oguejiofor et al.(2011). Female wistar rats were divided into two groups, the control (n=5) and the test groups (n=8).

At the beginning of the experiment, the animals were weighed. Daily oral dose of 750 mg/kg body weight of the aqueous leave extract of *Leucas martinicensis* was administered to the test group for 28 days. The control group received 5ml/kg of distilled water for the same period of time. Both the control and the test group were treated under the same experimental conditions.

On the 29th day, rats were weighed and anaesthetised with chloroform. Blood samples were collected by cardiac puncture for biochemical and haematological analyses. The heart, liver, lungs, kidney, spleen and brain were excised, weighed, trimmed and then fixed in Bouin's solution for histological analysis.

Methods for the bioassays

The hematocrit was determined by the micro-hematrocrit method. The total and differential

leucocytes count and platelets count were made from blood smear stained with Giemsa (Schlam et al., 1975). Beckman model spectrophotometer was used to determine the

Haemoglobin concentration using cyanomethemoglobin method (Drabkin and Austin, 1932). For the biochemical parameters- alkaline phosphatase was estimated by methods of (Sigma Diagnostic, 1987) while those of aspartate aminotransferase (AST) and alanine amino

transferase (ALT) were estimated by colorimetric method (Sigma Diagnostic, 1985). Sodium (Na^+) and potassium (K^+) were estimated by modified diacetylmonoamine method (Marsh, 1965). The total protein was estimated by Biuret method (Treitz, 1970) while the albumin was determined by bromocresol green (Lowry et al., 1957). For the histological analysis the fixed tissues were dehydrated in an ascending series of alcohol, cleared in xylene, and embedded in paraffin wax melting at 60^{o}C. Serial sections (5-µm thick) were mounted on 3-aminopropyl triethsilane – coated slides and dried for 24 hours at 37^{o}C (Baravalle et al., 2006). The sections on the slides were deparaffinised, hydrated, and stained with Mayer's hematoxylin and eosin dyes, dried and mounted.

STATISTICAL ANALYSIS

Data were analyzed by students T-test. Statistical evaluations were performed using GraphPad prism version 6, and a difference was considered statistically significant at P<0.05.

RESULTS

Phytochemical Analyses

The result of the phytochemical analyses showed the presence of alkaloids (3.95%), saponins (3.15%), flavonoids (3.56%) and glycosides (1.35%) (Table1).

Acute Toxicity study

No mortality was recorded on the administration of 3000

mg/kg body weight of the extract. However, there was reduced activity of the rats at the initial stage (10-15 minutes).

Repeat dose oral toxicity study

Effect of *Leucas martinicensis* on the Body weight of the rats

Aqueous extract of *Leucas martinicensis* (750 mg/kg) administered daily to the rats for 28 days showed no significant difference ($p > 0.05$) in weight of the rats when compared to the control group.

Effect of *Leucas martinicensis* on Haematological responses

At the end of 28 days treatment, *L. martinicensis* extract showed no statistically significant differences on the haematological parameters when compared to the control.

Effect of *Leucas martinicensis* on Biochemical parameters

The extract elevated the serum aspartate transaminase (AST) of the rats while the other parameters were not affected when compared to the control (Figure 1).

Effect of *Leucas martinicensis* on Histopathology

The histological views of all the organs appear normal. However, the heart showed cardiac myocytes covered with haemorrhage (figure not shown).

DISCUSSION

This study sought to evaluate the phytochemical studies of the aqueous leave extract of *L. martinicensis* which has been utilised traditionally among the Sokoto indigenes, North West Nigeria for the treatment of convulsion and epilepsy amongst so many other diseases. Previous studies have analysed the non aqueous phytochemical components of this extract. Our result showed that aqueous extract of this plant does not contain cardiac and saponin glycosides, tannins, anthraquinones and volatile oil but are reported present in chloroform and methanolic extracts (Das et al., 2012). This means that the efficacy of this extract for traditional medicine could greatly depend on the mode of extraction.

The acute oral toxicity study of the aqueous extract of *L. martinicensis* showed no mortality at 3000 mg/kg body weight. This implies that the LD_{50} is above 3000 mg/kg. Plants with LD_{50} above 1000 mg/kg by oral route is considered safe or of low toxicity (Clarke and Clarke, 1977). The reduced activity that was noticed in the rats could suggest that the extract may possess sedative activity or a central nervous activity, however, more studies are needed to evaluate this.

In the repeat dose toxicity study, the biochemical studies showed elevated AST which is a liver enzyme. There was no noticeable damage in the liver cells in the histological picture. Therefore, since AST can also be found in other tissues (USDVA, 2007), it is possible that the haemorrhage noticed in the cardiac myocytes could be responsible. Since the cardiac myocytes were found to be covered by haemorrhage, it is possible that the extract may be deleterious to the heart.

CONCLUSION

The aqueous leave extract of *L. martinicensis* contains alkaloids (3.95%), saponins (3.15%), flavonoids (3.56%) and glycosides (1.35%). It has an oral LD_{50} above 3000 mg/kg and appears to be toxic to the heart at a dose of 750 mg/kg.

REFERENCES

Adeneye AA, Ajagbonna OP, Adeleke TI, Bello SO (2006). Preliminary toxicity and phytochemical studies of the stem bark aqueous extract of *Musanga cecropioides* in rats. J. Ethnopharmacol. 105: 374-379.

Agra MF, Freitas PF, Barbosa-filho JM (2007). Synopsis of the plants known as medicinal and poisonous in Northeast of Brazil. Brazilian J. Pharmacognosy 17(1): 114-140.

Baravalle C, Salvetti NR, Mira GA, Pezzone N, Orteaga HH (2006). Microscopic characterization of follicular structures in Leotrozole induced polycystic ovarian Syndrome in the rat. Arch. Med. Res. 37: 830-839.

Clarke EGC, Clarke ML (1977). Veterinary Toxicology. London, Cassel and Collier Macmillan Publishers pp. 268-277.

Das SN, Patro VJ, Dinda SC (2012). A review: Ethnobotanical survey of genus *Leucas*. Pharmacognostic rev. 6(12): 100-106.

Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN, Ray C (1968). Screening of Indian plants for biological activity: Part I. Indian J. Exp. Biol. 6: 232-247.

Drabkin DL, Austin JM (1932). Spectrophotometric constants for common haemoglobin derivatives in human, dog, rabbit-blood. J. Biol. Chem. 98: 719-733.

El-Olemmy MM, Muhtadi FA, Affifi AA (1994). Experimental Phytochemical. A laboratory Manual (ed) Department of Pharmacognosy, King Saudi University, Riyadh: King Saudi University Press, p.1415.

Estifanos E (2003). Chemical Studies of *Leucas martinicensis.* M.Sc. Thesis submitted to the Department

of Chemistry, Faculty of Science, Addis Ababa University, Ethiopia, pp. 23-25.
Fowler DG (2006). Traditional Fever remedies: a list of Zambian plants, p. 27.
Harbone JB (1993). Phytochemical methods ed. London, Chapman and Hall, p. 68.
Hyde MA, Wurstem B (2009). Flora of Zimbabwe Information on *Leucas martinicensis* (http://www.zimbabweflora.co.zw/speciesdata /specie.php?speciesid149300. Retrieved 6 May, 2009.
Lowry OH, Rosebrough NJ, Farr L, Randall RJ (1957). Protein measurement with Folin phenol reagent. J. Biol. Chem. 193: 265.
Marsh WH (1965). Automated and manual direct methods for the determination of blood urea. Clin. Chem. 2: 624-625.
Minja MMJ (1999). The Maasai Wonder Plants. Tropical Pesticides Res. Institute, Arusha, Tanzania, p. 3.
Muhammad S, Fatima SA, Yahaya MM (2012). The Phytochemical Components of *Leucas Martinicensis* that Cause Repellence of Adult Mosquito. Int. J. Modern Botany. 2(1): 1-5
Organization for Economic Development (OECD) (2001). Guideline for testing of chemicals. Guideance no 425. Up and Down procedure, Adopted: 17th December 2001.
Organization for Economic Development (OECD) (2008). Principles of Good Laboratory Practice: In hand Book of Good Laboratory Practice (GLP) TDR, PRD/GLP/01.2.
Qamaruddin, Parveen N, Khan NU, Singhal KC (2002). *In vitro* antifilarial potential of the flower and stem extracts of leucas cephalotes on cattle filarial parasite setariacervi. J. Natural Remedies. 2:155-163.
Rahman MS, Sadhu SK, Hasan CM (2007). Preliminary antinociceptive, antioxidant and cytotoxic activities of leucas aspera root. Fitoterapia. 78: 552-555.
Reddy MK, Viswanathan S, Thirugnanasambantham P, Kameswaran L(1993). Effect of *leucas aspera* on snake venom poisoning in mice and its possible mechanism of action. Fitoterapia. 64: 442-446.
Schlam OW, Jain NC, Caroll EJ (1975). Veterinary haematology. 3rd edn. Philadelphia, Lea and Tebiger publishers, pp. 207-209.
Sharma RN, Gupta AS, Patwardhan SA, Hebbalkar DS, Tare V, Bhonde SB (1992). Bioactivity of lamiaceae plants against insects. Indian J. Exp. Biol. 30: 244-246.
Sigma Diagnostic (1985). Transaminase (ALT/GPT) and (AST/GOT) Procedure No. 505.
Sigma Diagnostic (1987). ALP Optimised Alkaline Phosphatase. Procedure No. DG 1245.
Singh N, Nath R, Singh DR, Gupta ML, Kohli RP (1978). An experimental evaluation of protective effects of some indigenous drugs on carbon tetrachloride-induced hepatotoxicity in mice and rats. J. Crude Drug Res. 16: 8-16.
Trease and Evans (1999). Pharmacognosy, edited by Evans W. L. 14th ed. London, W. B. Saunder Company Ltd.,pp. 109-334.
Treitz NW (1970). Fundamentals of Clinical Chemistry with Clinical Correlation. Philadelphia, W.B. Sanders, ,pp. 280-284.
Ugwah-Oguejiofor CJ, Bello SO, Etuk EU, Igbokwe VU, Ugwah OM, Okolo RU (2011). Preliminary toxicity and phytochemical studies of the aqueous extract of *Ficus platyphylla* in female albino rats. Int. Res. J. Pharm. Pharmacol. 1(5): 086-092.
United States Department of Veterans Affairs (2007). Hepatitis C. Understanding lab tests AST (SGOT) Liver Function Test.
Valsaraj R, Pushpangadan P, Smitt UW, Adsersen A, Nyman U (1997). Antimicrobial screening of selected medicinal plants from India. J. Ethnopharmacol. 58: 75-83.