Journal of Research in Applied and Basic Medical Sciences 2024; 10(4): 361-379

Original Article

RABMS Journal of Research in Applied and Basic Medical Sciences

Methanol extract of *Laportea aestuans* reverses uterine hyperplasia in rats

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Abstract

***Background & Aims*:** *Laportea aestuans* is a medicinal plant used in ethnomedicine as an abortifacient, anthelminthic, anti-fibroid, antipyretic, and anti-microbial agent. Uterine leiomyoma, a menace of reproductive age in women, is characterized by the proliferation of smooth muscle cells (hyperplasia) in the uterus. This study aims to investigate the preventive and curative anti-leiomyoma activity of *Laportea aestuans* using monosodium glutamate-induced uterine hyperplasia models in female Sprague-Dawley rats.

***Materials & Methods*:** The methanol whole plant extract of *Laportea aestuans* (*MELA*) was prepared by extracting the pulverized, air-dried whole plant with 2.5 L of methanol in a Soxhlet apparatus, followed by filtration and oven-drying. The acute toxicity of *MELA* was then assessed using Lorke's method. Subsequently, three doses of *MELA* were selected for this study.

Uterine hyperplasia was induced with 200 mg/kg *p.o*. monosodium glutamate (MSG) for 30 days. *MELA* was either co-administered with the inducing agent (preventive study) or administered post-induction for another 30 days (curative study). The anti-leiomyoma activity of *MELA* was then assessed through haematological, biochemical, and histopathological findings.

***Results*:** Increased serum oestrogen, progesterone, and total cholesterol levels were observed in the untreated fibroid animal groups, but these were significantly attenuated ($p < 0.05$) in animals treated with different doses of *MELA.* This correlated with the histopathological findings, as *MELA* reversed uterine hyperplasia with its therapeutic potential noted from 500 mg/kg.

***Conclusion*:** These findings suggest that *MELA* contains bioactive agents that can reverse MSG-induced uterine hyperplasia. It may therefore be useful in reducing the proliferation of fibroblast cells and managing other symptoms associated with uterine leiomyoma upon successful clinical trials.

***Keywords*:** Fibroid, *Laportea aestuans*, Monosodium glutamate, MSG-induced uterine hyperplasia, Oestrogen, progesterone, Uterine hyperplasia, Uterine leiomyoma, Sex hormones

Received 01 May 2024; accepted for publication 17 September 2024

Introduction

Uterine leiomyoma (UL), also referred to as uterine leiomyoma, is a monoclonal tumor of the uterus's myometrium, or smooth muscle tissue layer. About 25% of women with uterine leiomyoma have clinically significant symptoms that cause substantial quality-of-life problems, even though uterine leiomyomas are thought to be benign (1). Although UL is common, there is currently no long-term, affordable medical treatment for UL that preserves fertility. The dearth of medical treatments available for the treatment of UL is partly due to the incomplete understanding of the etiology of the condition. Surgery is therefore the cornerstone of UL treatment (2). Collagen, fibronectin, and proteoglycans are found in considerable quantities in the extracellular matrix (ECM) of uterine leiomyomas (3-5). Leiomyomas pose a significant threat to public health since, in 30% of cases, irregular uterine bleeding (heavy monthly flow that induces anemia) and pelvic pressure (urinary symptoms, constipation, and tenesmus) cause morbidity (6, 7) associated with leiomyomas. Infertility, abortions, and other obstetric difficulties can also be brought on by leiomyomas (8, 9).

According to Stewart et al. (2013), race is a significant risk factor for developing leiomyomas (10). African American women had a 60% incidence of uterine leiomyomas by the age of 35, rising to > 80% at the age of 50, according to a study, while Caucasian women had a 40% incidence by the age of 35, rising to 70% by the age of 50 (11). Therefore, it is an endemic problem that plagues Sub-Saharan Africa (12).

The primary therapy for leiomyomas is surgery, which is typically expensive, intrusive, and sometimes socially unacceptable. According to a recent retrospective analysis, there were intra-operative complications in 25% of patients and postoperative complications in 29.3% of patients who underwent abdominal myomectomy at University College Hospital in Ibadan, Nigeria, between July 2016 and June 2019 (13). Cardozo et al. (2012) also reported that the US healthcare system's annual cost of treating UL is projected to be $34.4 billion (14). High-intensity focused ultrasound ablation, a non-invasive surgical technique for the excision of uterine leiomyomas, can cost anywhere from NGN 2.5 million to NGN 5.5 million in Lagos, Nigeria (15).

The pathogenesis of fibroids is yet to be fully understood, and this has stalled the development of safe and effective therapies. However, numerous studies have established that leiomyomas are hormone-responsive and express oestrogen (ER) and progesterone (PR) receptors. Laboratory studies with primary cultures of leiomyoma-derived cells indicated that they are responsive to steroid hormones (16, 17). Good evidence also exists that a hyperoestrogenic milieu within the tumors themselves may contribute to tumor growth (18). In addition, an elevated transcriptional response to ER in leiomyomas suggests that these tumors may have an increased responsiveness or are hypersensitive to ER stimulation. Thus, a therapy based on ER inactivation may be successful in abrogating the growth machinery of leiomyomas and achieving tumor reduction.

Herbal formulations have become increasingly popular because there is currently no effective conventional treatment for fibroids other than surgical treatments. *Laportea aestuans* (L.) Chew is one of these common medicinal plants used to treat leiomyomas (19). The approved name for the genus Laportea is *Laportea aestuans* (L.) Chew (20).

The West Indian woodnettle species *Laportea aestuans* (Figure 1) is a member of the Urticaceae family. The plant, which grows in fallows and new cultivations, is a weed. This 1.5-meter-long herbaceous plant is common in the tropical regions of Asia and Africa, where it frequently climbs walls. It goes by several different names in Nigeria, including *ile-nkita* in Igbo, *bulsum fage* in Hausa, *fiyafiya*, *Ofiya*, *Ipe erin*, and *ofuefue* in Yoruba. According to Cheryl (21) and Etukudo (22), the herb has been used with success to treat gynecological disorders, including hastening labor, delivering the placenta during childbirth, and acting as an abortifacient. The cooked leaves of *L. aestuans* are consumed in Gabon as a stomachache cure (23).

Recently, Oloyede et al. (2013) reported its phytochemical constituents and further investigated its toxicity, antimicrobial, and antioxidant effects (24). Adetunji et al. (2021) documented the reno-hepatoprotective and anti-diabetic properties of the methanolic leaf extract of *Laportea aestuans* (25). The extract of the plant has also been reported to contain bioactive compounds that reduce pro-inflammatory cytokines (26).

The littoral and southwestern parts of Cameroon employ a decoction of *L. aestuans* stems, roots, and leaves to treat leiomyoma, anemia, and calcium loss (19). However, there is no scientific evidence supporting the use of *L. aestuans* for this purpose. This study examined the scientific rationale behind the use of *Laportea aestuans* as anti-leiomyoma medicine. It may be of potential benefit in the ongoing search for anti-leiomyoma drugs and minimize the need for surgical interventions in treating the disease. Through histological, hematological, and biochemical results, this study validates the safety and effectiveness of *Laportea aestuans* for the treatment and prevention of leiomyoma.

Fig. 1. *Laportea aestuans* growing in a farm garden in Surulere, Lagos, Nigeria (Photo Credit: Qudus Ojomo).

Materials & Methods

The source of monosodium glutamate was Sigma Aldrich Chemical Co. (St Louis, USA). Bioassay Technology Laboratories, located in Shanghai, China, provided the ELISA kits (catalog numbers in parentheses) for progesterone (CSB-E07282r), oestrogen (CSB-E07293h), triglycerides (CSB-E07287r), cholesterol (CSB-E07152k), and total protein (CSB-E07282g).

Collection and Identification of the Plant:

Laportea aestuans (L.) Chew was found in large numbers at a farm garden within the Nigerian Railway staff quarters, Surulere, Lagos. The plants were uprooted whole. Thereafter, they were authenticated and identified by Dr. Nodza George Isaac at the Department of Botany, University of Lagos, on December 15, 2021. The plant was assigned the herbarium voucher specimen number LUH-9032.

The amount of plant material collected weighed up to 2 kg. It was then prepared for extraction as described in the plant extract preparation section.

Preparation of Monosodium Glutamate:

A stock solution of monosodium glutamate (Sigma Aldrich Chemical Co., St. Louis, USA) was prepared according to methods described by Oyebode et al. (2020) by dissolving 200 mg of the salt in 200 ml of

distilled water. The stock MSG was kept at room temperature and freshly prepared as needed (23).

Experimental Animals:

A total of 62 adult female (16-18 weeks old) Sprague-Dawley rats with weights between 150-200 g were obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos. The animals were acclimatized for 14 days in the animal house and housed in plastic cages at room temperature. They were kept in ventilated cages with a 12-hour light/dark cycle and provided with rat chow (Flour Mills, Nigeria) and pure tap water *ad libitum*. Animals of this age were selected because uterine leiomyoma is observed in adult women of reproductive age.

Oestrus Cycle of the Animals::

All rats used in this study were assessed for regular oestrus cycle length for 5 days. The oestrus cycles of the animals were monitored by observing the vaginal smear in the morning between 8:00 am and 9:00 am, following to the procedure described by McLean et al. in 2012 (27).

Phytochemical Screening of the Plant Extract:

The qualitative chemical analysis of *MELA* was conducted to test for the presence of anthraquinones, tannins, saponins, steroids, cardiac glycosides, flavonoids, terpenoids, and alkaloids using methods adopted in similar studies (28, 29).

Preparation of the Plant Extract:

The whole plant (containing stems, leaves, and roots) of *Laportea aestuans* was sorted to remove dirt, weighed, and air-dried for 2 weeks until the weight remained constant for 3 consecutive days. It was then pulverized. The pulverized plant material, weighing about 1 kg, was extracted with 2.5 L of methanol in a Soxhlet apparatus at the Department of Pharmacognosy, University of Lagos, yielding 91% of the plant extract. The filtered extract was then concentrated using a rotary evaporator (EYELA, CA-1111, Rikakikai company Limited, Tokyo, Japan) for about 4 days, and the semi-solid form (20 g) of the extract was oven-dried at 50 ^{o}C. It was stored in an airtight sample bottle and placed in a refrigerator until further use.

The whole plant was used because this conforms to the traditional use of the plant for managing uterine leiomyoma (19). Methanol was chosen for extraction as it ensures an efficient extraction process without destroying the active ingredients in the plant material (23, 26).

Acute Toxicity Study:

The median lethal dose (LD_{50}) was determined using Lorke's method (1983), involving the use of 12 rats in two phases. All doses were administered via the oral route (30).

Dosing of Experimental Animals:

Methanol whole plant extract of *Laportea aestuans* (*MELA*) doses selected for this study were 250, 500, and 850 mg/kg body weight of the experimental animals. This dose selection was based on the acute toxicity study results from this study and previous similar studies (23, 26, 45). The safest oral dose of the extract at 5000 mg/kg was divided by a factor of six (6) to obtain the maximum dose used in this study. The doses were administered orally once daily throughout the experimental period. The oral route was chosen because it is the intended route for human exposure.

Experimental design

Preventive Study:

Twenty-five (25) female Sprague-Dawley rats were divided into five groups (n = 5) as shown in Table 1. Group A received distilled water (control), Group B received 200 mg/kg/day of monosodium glutamate (MSG), Group C received 250 mg/kg *MELA* and 200 mg/kg/day of MSG, Group D received 500 mg/kg *MELA* and 200 mg/kg/day of MSG, and Group E received 850 mg/kg of *MELA* and 200 mg/kg/day of MSG. All treatments were administered daily and orally for 30 days. Twenty-four hours after the final

treatment, the animals were sacrificed humanely via cervical dislocation. Blood was collected via oculopuncture into ethylenediaminetetraacetic acid (EDTA) sample bottles. The reproductive system was harvested and weighed, then preserved in 10% formal saline for histopathology in specimen bottles.

Curative study:

Twenty-five (25) female Sprague-Dawley rats were divided into five groups (n = 5) as shown in Table 1. Group A received distilled water (control) throughout the study, while Groups B to E were pre-treated with 200 mg/kg MSG for 30 days to induce of uterine hyperplasia. Thereafter, Group B did not receive any treatment for another 30 days apart from water and feed. Group C received 250 mg/kg *MELA*, Group D received 500 mg/kg *MELA*, and Group E received 850 mg/kg *MELA* for the next 30 days. Twenty-four hours after the final treatment, the animals were sacrificed humanely via cervical dislocation. Blood was collected via oculopuncture into ethylenediaminetetraacetic acid (EDTA) sample bottles. The reproductive system was harvested and weighed, then preserved in 10% formal saline for histopathology in specimen bottles.

Table 1. Grouping of animals for preventive and curative study

Groups	Preventive	Curative
A	Distilled water (control)	Distilled water (control)
B	200 mg/kg MSG only	200 mg/kg MSG only
C	200 mg/kg MSG + 250 mg/kg *MELA*	200 mg/kg MSG pre-treated, then 250 mg/kg *MELA*
D	200 mg/kg MSG + 500 mg/kg *MELA*	200 mg/kg MSG pre-treated, then 500 mg/kg *MELA*
E	200 mg/kg MSG + 850 mg/kg *MELA*	200 mg/kg MSG pre-treated, then 850 mg/kg *MELA*

Preparation of serum:

Blood (5 ml) was collected via oculopuncture into EDTA-sterilized sample bottles. Serum was prepared by centrifugation (3000 rpm, 20 min) and subjected to analysis. Total estradiol (oestrogen), progesterone, alanine transaminase, aspartate aminotransferase, and total cholesterol were determined according to the manufacturer's instructions (Bioassay Technology Laboratories).

Determination of Serum Estradiol (oestrogen) and Progesterone Levels:

For estradiol and progesterone analysis, with a sensitivity of 0.2 ng/ml and detection range between 0.15 ng/ml – 70 ng/ml, all reagents and rats' sera were brought to room temperature (27 ºC) before starting the procedure. The procedure then followed the ELISA (Enzyme-Linked Immunosorbent Assay) method as outlined by the kit manufacturer (Bioassay Technology Laboratories).

Finally, the absorbance in each well was read at 450 nm (using a reference wavelength of 620-630 nm), and the results were read within 30 minutes of adding the stop solution. A dose-response curve was used to ascertain the estradiol of unknown specimens.

Determination of total cholesterol:

Total cholesterol was determined based on an enzymatic method described by Allain et al., 1974, using an ELISA kit with a sensitivity of 0.2 ng/ml and detection range between 0.15 ng/ml – 70 ng/ml (31).

Total Cholesterol = Absorbance (assay) / Absorbance (standard) X standard concentration

ALT and AST Determination:

The serum concentration of two liver enzymes was determined in the experimental animals. Alanine transaminase (ALT) and Aspartate aminotransferase (AST) analysis were performed with ELISA kits following the procedures outlined by the manufacturer (Bioassay Technology Laboratories, sensitivity: 0.2 ng/ml and detection range: 0.15 ng/ml – 70 ng/ml).

The absorbances were then read at 546 nm using a microplate spectrophotometer. A calibration curve of the standard absorbance values against corresponding concentrations was plotted to obtain the enzyme

concentration from the experimental absorbance values.

Assessment of Haematological Parameters:

Blood samples (1 ml) from experimental animals, collected into EDTA bottles, were analysed using the Swelab ® automated hematology cell counter ($AC970^{EO+}$). The bottles containing the blood samples were first made homogenous using a roller mixer machine (BST/205RM).

The following parameters were determined using the hematology cell counter: white blood cell (WBC), lymphocytes (LYM), packed cell volume (PCV), lymphocyte percentage (LYMPH%), monocyte percentage (MON%), neutrophil percentage (NEU %), red blood cell (RBC), haemoglobin concentration (HGB), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), and platelet cells (PLT).

Tissue preparation for histopathological analysis:

The histopathology of the uteri of the experimental animals was determined following the methods described previously by Khaimaisi et al. 1997 (32).

Statistical Analysis:

Data were analyzed using GraphPad Prism version 6.0 for Windows (GraphPad Software, San Diego, California, USA) and presented as mean ± standard error of mean (SEM). Statistical significance was tested using two-way ANOVA followed by Dunnett's multiple comparison test. The level of significance was set at $p < 0.05$.

Ethical Considerations:

All experimental procedures for this study were carried out in accordance with the ethical guidelines of the College of Medicine, University of Lagos. The animals were handled with care to minimize distress and pain. Before the commencement of this project study, approval was obtained from the Animal Care and Use Research Ethics Committee of the College of Medicine, University of Lagos. This study was approved with the ethical approval number CMUL/ACUREC/07/22/1083.

Results

Oestrous cycle of the animals:

All animals used in this study showed a regular oestrous cycle during the 5-day observation period.

Acute toxicity study:

In Phase I of the LD_{50} determination for the methanol whole plant extract of *Laportea aestuans* (*MELA*), none of the animals died in the three test groups within a 24-hour period, leading to Phase II. In Phase II, none of the animals died in the three test groups within 24 hours either. This shows that the LD_{50} is greater than 5000 mg/kg.

Also, the animals were observed for any signs of delayed toxicity throughout the experimental period. None of the animals exhibited any clinical signs of toxicity, such as frequent stooling and urination, abnormal movements, salivation, weight loss, and irregular breathing when compared to the control group. Examination of the internal organs of the animals upon the completion of the dosing period revealed no visible abnormal changes to the kidney, liver, brain, spleen, and heart in the treatment groups in comparison with the control.

Physicochemical properties of the extract:

The crude extract of the methanol whole plant extract of *Laportea aestuans* (*MELA*) is dark greenish-brown, coarse in nature, with a pungent smell. It is hygroscopic and readily soluble in water.

Phytochemical constituents:

Qualitative phytochemical screening of *MELA* confirms the presence of tannins, cardiac glycosides, phlobatannins, flavonoids, terpenoids, and saponins, as shown in Table 2.

Table 2. Results of the phytochemical screening of *MELA*

S/N	Phytochemical constituents	Inferences
1.	Saponins	++
2.	Tannins	+++
3.	Flavonoids	+++
4.	Phlobatannins	+++
5.	Alkaloids	+
6.	Cardiac glycosides	+++
7.	Anthraquinones	-
8.	Terpenoids	++
9.	Phenol	-

Key = + traces, ++ moderately present, +++ abundantly present

Effects of *MELA* on the levels of sex hormones in MSG-treated female rats:

Figures 2a and 2b depict the variation in the levels of serum oestrogen and progesterone following MSG administration and treatment with different doses of the extract, *MELA*. The data reveal that oral administration of 200 mg/kg bw of MSG for 30 days in female Sprague-Dawley rats resulted in an elevation in the levels of serum oestrogen and progesterone when compared with control animals in both the preventive and curative studies.

However, co-administration of MSG with different doses of the extract (250, 500, and 850 mg/kg) resulted in a significant ($p < 0.05$) lowering of the elevated levels of the sex hormones in both the preventive and curative studies when compared with the untreated fibroid groups. In addition, during the preventive study, it can be observed that the greatest activity of *MELA* is at the 500 mg/kg dose, as the extract lowers the oestrogen and progesterone levels better than at other doses. Also, the extract had a comparable lowering effect on the sex hormones during the curative study among the three selected doses.

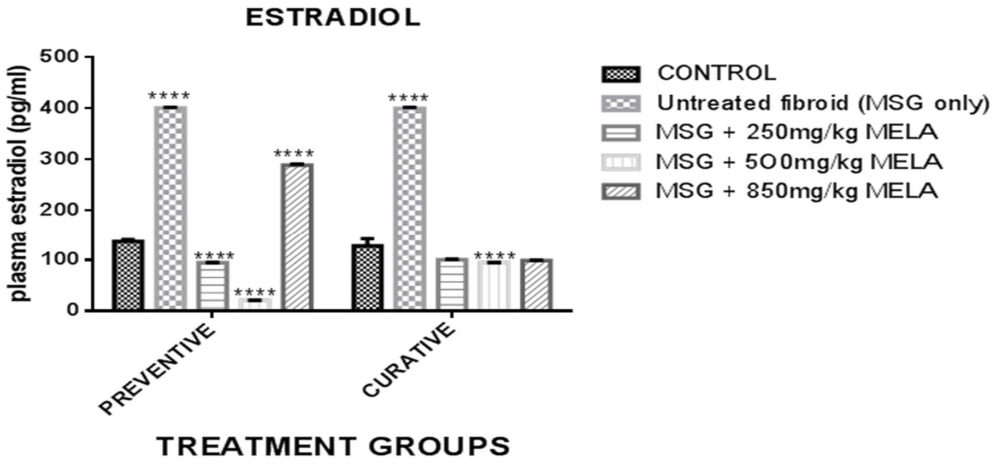

Fig. 2a. Effects of methanol extract of *Laportea aestuans* (*MELA*) on serum oestrogen level in MSG-treated female rats

*Values are Mean ± Standard error of mean (n = 5). Bars with * are significantly (p < 0.05) different from the Control group.*

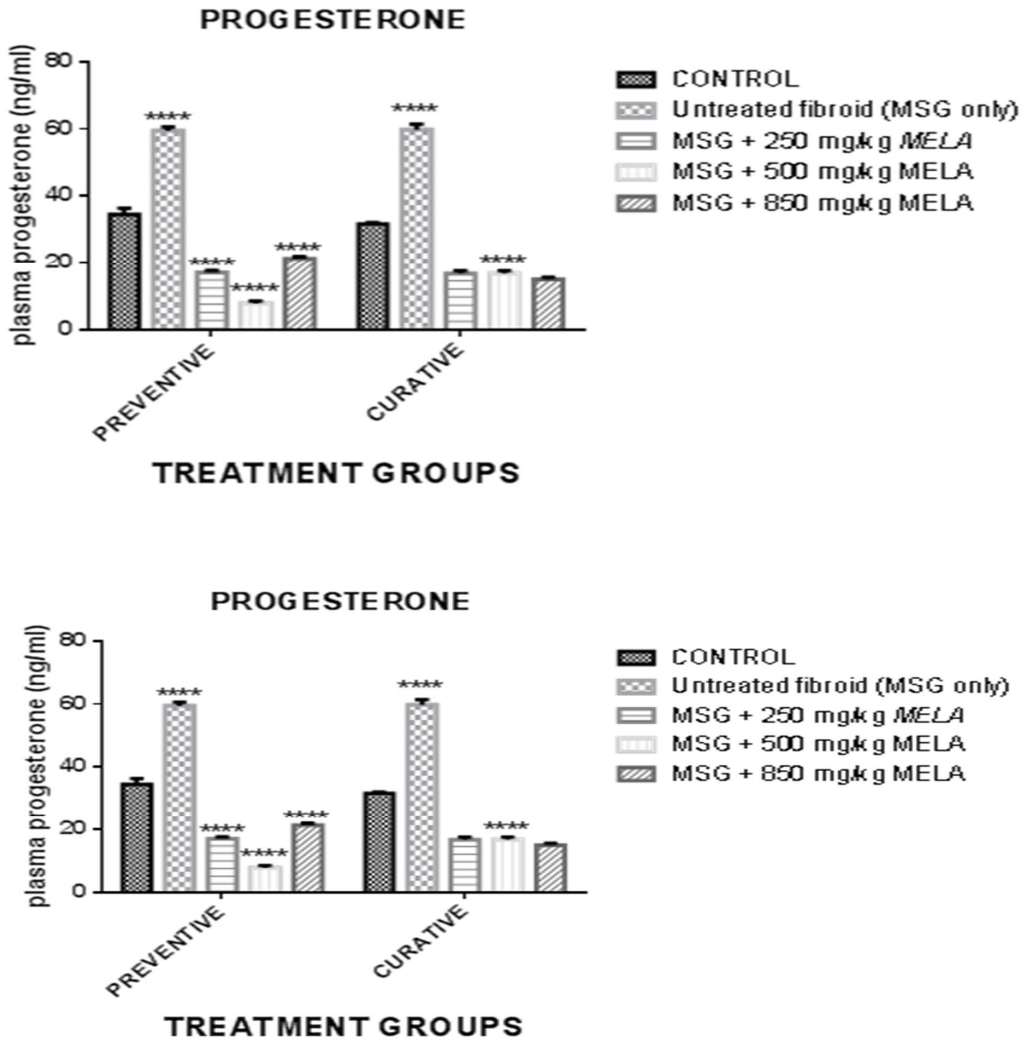

Fig. 2b. Effects of methanol extract of *Laportea aestuans* (*MELA*) on serum progesterone level of MSG-treated female rats.

*Values are Mean ± Standard error of mean (n = 5). Bars with * are significantly (p < 0.05) different from the Control group.*

Effects of the administration of *MELA* on the lipid profile and liver enzymes of MSG-treated female rats:

The effects of *MELA* on serum levels of total cholesterol, aspartate aminotransferase (AST), and alanine transaminase (ALT) are shown in Figures 3a, 3b, and 3c, respectively. The data showed that there were significant ($p < 0.05$) increases in total cholesterol and ALT levels in the untreated fibroid group when compared to the control group after 30 days of oral administration of 200 mg/kg monosodium glutamate. Meanwhile, the AST levels in the untreated fibroid groups are comparable to the control groups in both the preventive and curative studies.

The increase in the serum cholesterol level was attenuated significantly ($p < 0.05$) at the 250 mg/kg and 850 mg/kg doses of *MELA* during the preventive study but remains largely unchanged at 500 mg/kg.

The effect of *MELA* on the serum levels of the two liver enzymes, ALT and AST, revealed contrasting results. The ALT levels, which were significantly elevated in the untreated fibroid groups of both the preventive and curative studies, were significantly reduced ($p < 0.05$) at all doses of the extract in both the preventive and curative study groups. Meanwhile, the AST levels remained unchanged and comparable across all treatment groups except at the 500 mg/kg dose of the extract in the preventive study where the AST level was elevated compared to the other study groups.

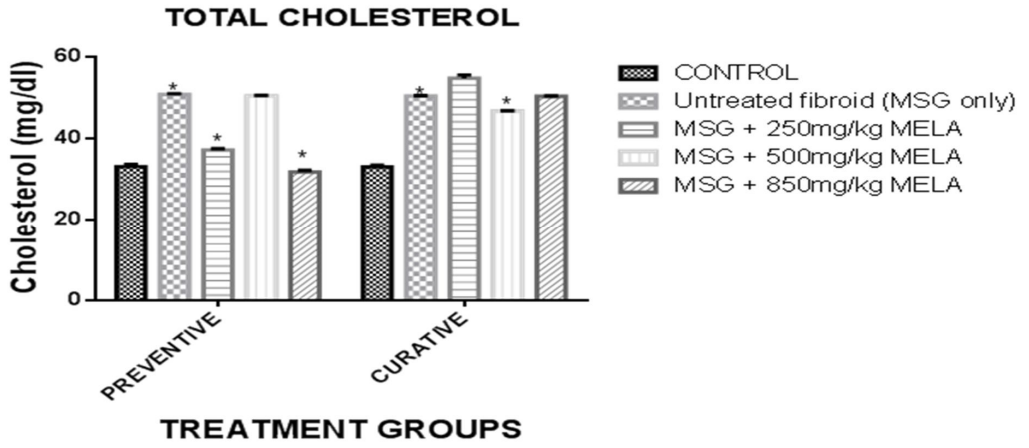

Fig. 3a. Effects of methanol extract of *Laportea aestuans* (*MELA*) on serum cholesterol level of MSG-treated female rats

*Values are Mean ± Standard error of mean (n = 5). Bars with * are significantly ($p < 0.05$) different from Control group*

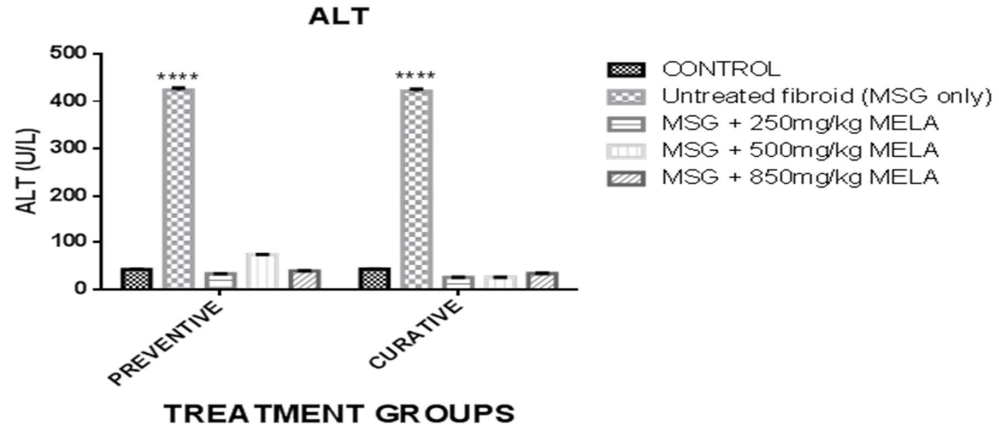

Fig. 3b. Effects of methanol extract of *Laportea aestuans* (*MELA*) on serum alanine transaminase (ALT) level of MSG-treated female rats

*Values are Mean ± Standard error of mean (n = 5). Bars with * are significantly ($p < 0.05$) different from Control group*

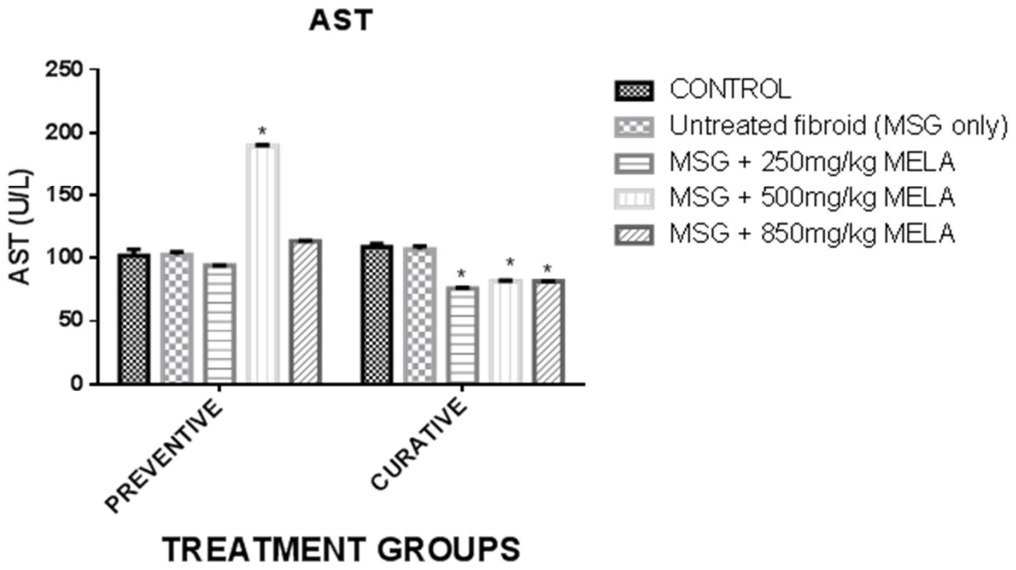

Fig. 3c: Effects of methanol extract of *Laportea aestuans* (*MELA*) on serum aspartate aminotransferase (AST) level of MSG-treated female rats.

*Values are Mean ± Standard error of mean (n = 5). Bars with * are significantly (p < 0.05) different from Control group*

Effect of *MELA* on the haematological parameters of MSG-treated female rats

In the analysis of the haematological parameters, the groups in the preventive study treated with *MELA* had haemoglobin levels of 12.3 g/dL, 14.7 g/dL, and 15 g/dL at 250 mg/kg, 500 mg/kg, and 850 mg/kg, respectively. This is comparable with the haemoglobin level of the control group of 14.4 g/dL. All the groups that were treated with *MELA* had higher haemoglobin levels than the untreated fibroid group (11.2 g/dL) in the preventive study.

Similarly, the groups in the curative study treated with *MELA* had haemoglobin levels of 14.2 g/dL, 14.3 g/dL, and 13.7 g/dL at 250 mg/kg, 500 mg/kg, and 850 mg/kg, respectively. This is comparable with the haemoglobin level of the control group of 15.1 g/dL. All the groups that were treated with *MELA* had higher haemoglobin levels than the untreated fibroid group (9.9 g/dL) in the curative study. Other haematological parameters are outlined below (Table 3 and Table 4).

Table 3. Preventive effects of *MELA* on the haematological parameters of monosodium glutamate-induced uterine hyperplasia in female rats

Parameter	Control	Untreated fibroid (MSG only)	250 mg/kg *MELA* + MSG	500 mg/kg *MELA* + MSG	850 mg/kg *MELA* + MSG
HGB (g/dL)	14.4 ± 0.354	11.2 ± 0.412$^{\#}$	12.3 ± 0.341$^{\#}$	14.7 ± 1.099	15 ± 0.912
WBC X 10^3 /µl	11.3 ± 0.05	3.7 ± 2.010$^{\#}$	6.5 ± 0.851$^{\#*}$	7 ± 1.025$^{\#*}$	6.8 ± 3.451$^{\#*}$
PCV %	50.3 ± 1.019	36.5 ± 0.178$^{\#}$	39.1 ± 2.098$^{\#}$	48.9 ± 0.654*	45.9 ± 1.123*
LYM %	39 ± 3.779	50 ± 3.566	10.2 ± 7.543$^{\#*}$	36.8 ± 0.551*	34.6 ± 0.124*
NEU %	44.2 ± 2.127	40 ± 5.315	80.3 ± 1.524	41 ± 0.458	57.9 ± 6.558

Parameter	Control	Untreated fibroid (MSG only)	250 mg/kg *MELA* + MSG	500 mg/kg *MELA* + MSG	850 mg/kg *MELA* + MSG
MON %	16.8 ± 1.932	10 ± 3.339	9.5 ± 0.763	22.2 ± 1.113	7.5 ± 3.451
PLT cells X 10^3/ µl	466 ± 2.530	203 ± 0.471$^{\#}$	208 ± 2.894$^{\#}$	947 ± 0.741$^{\#*}$	222 ± 5.712$^{\#}$
MCHC (g/dl)	28.6 ± 0.367	32.3 ± 4.225	31.4 ± 7.041	30 ± 0.331	32.6 ± 0.072
MCH (pg)	18.2 ± 3.952	25.5 ± 3.903$^{\#}$	24.7 ± 3.765	17.5 ± 2.760	29.4 ± 3.058
MCV (fl)	63.7 ± 0.541	79.2 ± 0.674	78.7 ± 0.981	58.4 ± 0.472	90.1 ± 0.541
RBC X 10^6 /µl	7.9 ± 0.252	4.82 ± 0.312$^{\#}$	4.97 ± 0.557$^{\#}$	8.38 ± 0.772*	5.1 ± 0.804

Control= Distilled water only without fibroid, Untreated fibroid = Distilled water only with fibroid
*# represents a significant ($p < 0.05$) difference in the specified haematological parameter when compared to control * Represents a significant ($p < 0.05$) difference when compared to the Untreated fibroid group.*

Table 4. Curative effects of *MELA* on haematological parameters in monosodium glutamate-induced uterine hyperplasia in female rats.

Parameter	Control	Untreated fibroid (MSG only)	250 mg/kg *MELA* + MSG	500 mg/kg *MELA* + MSG	850 mg/kg *MELA* + MSG
HGB (g/dl)	15.1 ± 0.340	9.9 ± 0.542$^{\#}$	14.2 ± 0.356$^{\#}$	14.3 ± 1.115	13.7 ± 0.812
WBC X 10^3 /µl	11.6 ± 0.025	3.6 ± 1.010$^{\#}$	7 ± 0.657$^{\#*}$	6.6 ± 1.922$^{\#*}$	7.3 ± 3.531$^{\#*}$
PCV %	50.9 ± 1.056	37 ± 0.219$^{\#}$	48.4 ± 1.874*	48.3 ± 0.654*	45.2 ± 1.624*
LYM %	37 ± 1.653	58 ± 5.830	48.6 ± 0.512	44.1 ± 0.624	38.1 ± 0.165
NEU %	44.8 ± 2.132	40.9 ± 5.225	34.1 ± 1.524	34.9 ± 2.177	40.7 ± 3.100
MON %	17.7 ± 2.002	10.1 ± 3.658	17.3 ± 1.860	21 ± 3.122	21.2 ± 4.458
PLT cells X 10^3/ µl	468 ± 1.530	207 ± 0.370$^{\#}$	539 ± 2.084$^{\#}$	779 ± 1.904$^{\#*}$	997 ± 5.014$^{\#}$
MCHC (g/dl)	27.9 ± 1.007	31.3 ± 4.275	29.3 ± 8.011	30.8 ± 0.931	30.3 ± 0.082
MCH (pg)	18.2 ± 3.952	26.5 ± 3.903$^{\#}$	18 ± 3.765	17.8 ± 2.760	18.3 ± 3.058
MCV (fl)	63.7 ± 0.541	80.1 ± 0.691	61.6 ± 1.001	58 ± 0.472	60.6 ± 0.541
RBC X 10^6 /µl	8.1 ± 0.252	4.61 ± 0.234$^{\#}$	7.86 ± 0.590$^{\#}$	7.99 ± 0.852*	7.46 ± 0.804

*Control = Distilled water only without fibroid, Untreated fibroid = Distilled water only with fibroid, # represents a significant ($p<0.05$) difference in the specified haematological parameter when compared to control. * Represents a significant ($p<0.05$) difference when compared to the Untreated fibroid group.*
***HGB:** Haemoglobin, **WBC:** White Blood Cell, **PCV:** Packed Cell Volume, **LYM:** Lymphocyte, **NEU:** Neutrophils, **MON:** Monocytes, **PLT:** Platelet, **MCHC:** Mean Corpuscular Haemoglobin Concentration, **MCH:** Mean Corpuscular Haemoglobin, **MCV:** Mean Corpuscular Volume, **RBC**: Red Blood Cells*

Histopathological evaluation of the effects of *MELA* on the uteri of MSG-treated female rats:

The wall of the uterus is made up of three (3) layers: the outer layer, the perimetrium, the middle layer, the myometrium, and the innermost layer, the endometrium. The endometrium is further divided into epithelium, muscularis mucosa, and lamina propria. The endometrium encloses the lumen, which is the core of the uterus. Uterine leiomyoma occurs in the myometrium. The myometrium is normally made up of smooth muscles, but upon the development of leiomyoma, the muscles become fibrous. Histological examination of the uteri of the untreated fibroid groups showed the presence of fibers instead of smooth

muscles that are normally found in the myometrium. However, the presence of such myometrium fibers was not observed at the 500 and 850 mg/kg doses of *MELA* (Figure 4 and Figure 5).

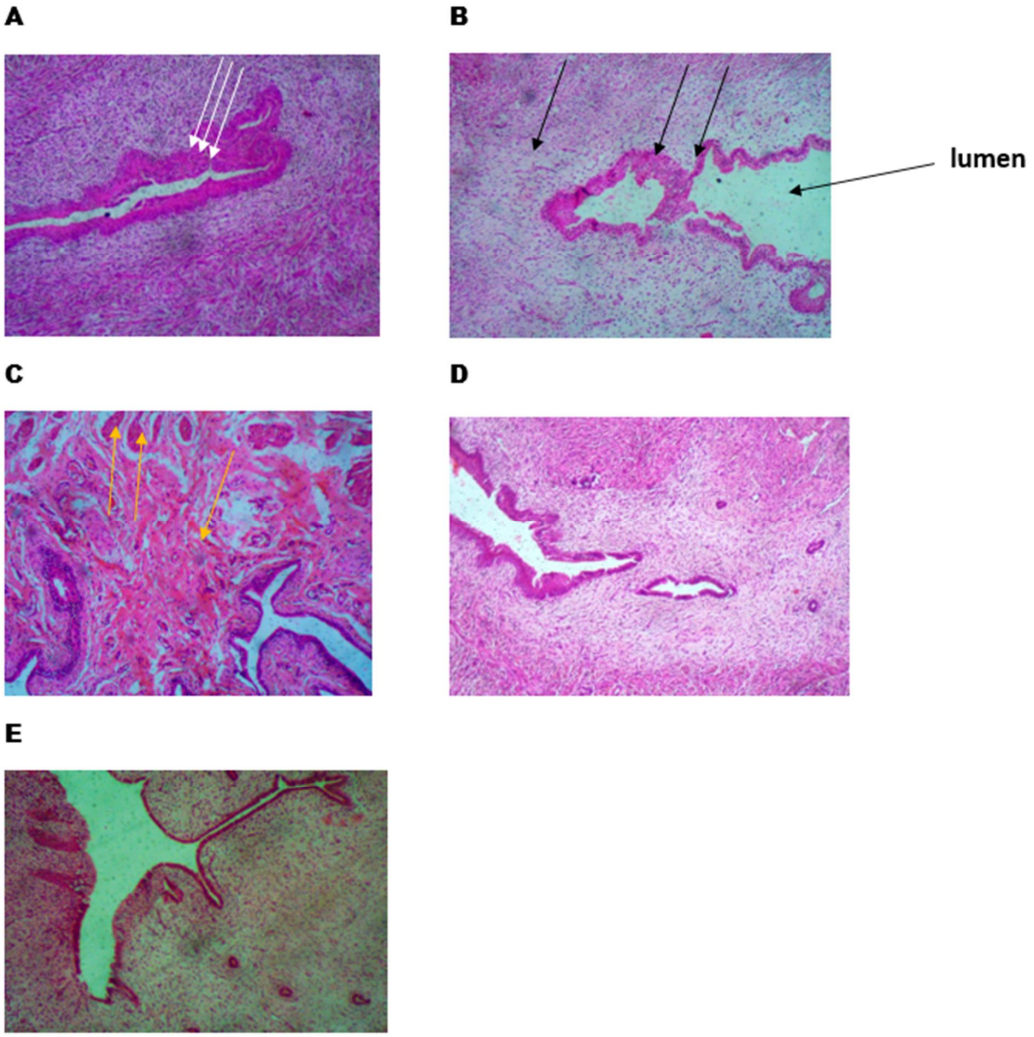

Figure. 4. Photomicrograph of sections of rats' uteri for the preventive study

(A) Photomicrograph of the uterus section of a control rat showing a normal uterus (normal myometrium) indicated by white arrows (H & E staining, x 100) (B) Photomicrograph of the uterus section of a rat in the untreated fibroid group exhibiting uterine atypical hyperplasia indicated by black arrows (H & E staining x 100). (C) Photomicrograph of the uterus section of a rat at 250 mg/kg *MELA* showing uterine atypical hyperplasia with vascular congestion indicated by yellow arrows (H & E staining x 100). (D) Photomicrograph of uterus section of a rat at 500 mg/kg *MELA* showing intact endometrium in a normal uterine architecture (H & E staining x 100). (E) Photomicrograph of the uterus section of a rat at 850 mg/kg *MELA* showing an intact endometrium in a normal uterine architecture (H & E staining x 100).

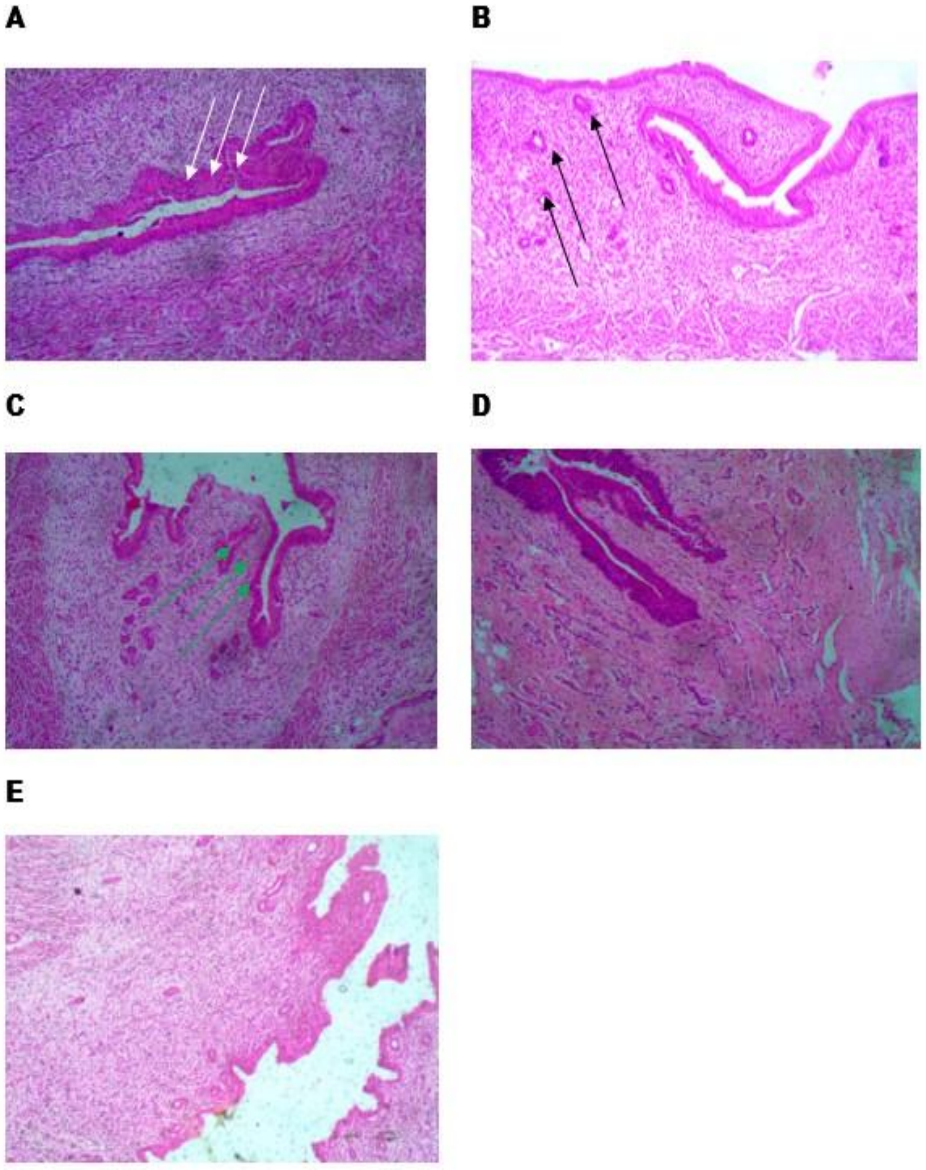

Figure. 5. Photomicrograph of sections of rats' uteri for the curative study

(A) Photomicrograph of the uterus section of a control rat showing a normal uterus (normal myometrium) indicated by white arrows (H & E staining, x 100). (B) Photomicrograph of the uterus section of a rat in the untreated fibroid group exhibiting uterine atypical hyperplasia indicated by black arrows (H & E staining x 100). (C) Photomicrograph of the uterus section of a rat at 250 mg/kg *MELA* showing uterine atypical hyperplasia with vascular congestion and proliferation of endometrial glands, resulting in an increase in gland-to-stroma ratio, indicated by green arrows (H & E staining x 100) (D) Photomicrograph of the uterus section of a rat at 500 mg/kg *MELA* showing an intact myometrium with a normal thickness of the myometrial layer (H & E staining x 100) (E) Photomicrograph of the uterus section of a rat at 850 mg/kg *MELA* showing normal uterine architecture, with an intact myometrium and a patent wide uterine lumen (H & E staining x 100).

Discussion

There is a great interest in exploiting plants for medicinal purposes, and this is true in several parts of the world (33, 34). This is because plants have shown therapeutic potential against several disease conditions, including infectious diseases like malaria (35) and non-communicable diseases such as cancer (36). It has been estimated that more than 25% of prescription pharmaceuticals contain plant-derived ingredients; yet, only a small percentage of plants in the world have been evaluated for potential pharmaceutical use (37, 38). The present study aimed to evaluate the anti-leiomyoma activity of the methanol whole plant extract of *Laportea aestuans* (*MELA*) in female Sprague-Dawley rats (preventive and curative drug candidate). The whole plant (aerial and root parts) was used for this study because of the possible presence of bioactive components in all parts of the plant, which could result in synergistic pharmacological activity (39). Uterine leiomyoma is characterized by increased proliferation of disordered smooth muscle cells (hyperplasia), altered extracellular matrix (ECM) deposition, and enhanced responsiveness to sex steroid hormones (40). One of the plants that has been employed traditionally to manage leiomyoma is *Laportea aestuans* (19).

Preliminary phytochemical screening of *MELA* detected the presence of tannins, cardiac glycosides, phlobatannins, flavonoids, and saponins, which conforms with earlier reports (41, 42, 43). The presence of bioactive components in plants is the basis for their therapeutic effect making *Laportea aestuans* worthy of pharmacological investigations. The abundance of flavonoids in *MELA* explains its possible mechanism of action as an anti-leiomyoma agent. Flavonoids slow down cell proliferation as a consequence of their binding to the oestrogen receptor (37). The attenuation of serum oestrogen levels in the MSG-induced uterine hyperplastic rats could thus be associated with the abundance of flavonoids in *MELA.*

In the acute toxicity test of *MELA,* the oral dosage of 5000 mg/kg was shown to be relatively safe, as there was no death recorded in the test groups within 24 hours post-administration (30). Thus, the doses for this study were safely selected as 250, 500, and 850 mg/kg.

Previous studies have shown that monosodium glutamate is a candidate agent for the experimental induction of uterine hyperplasia in animal models (23, 26). Although several dosing paradigms for such induction have been reported, this study employed an oral dose of 200 mg/kg/day of monosodium glutamate (MSG) for 4 weeks. The results of the haematological, biochemical, and histopathological studies revealed that the dosing paradigm employed is a viable model for experimental fibroid study.

Several risk factors have been linked to the development of uterine leiomyoma (UL), but the most established of these risk factors is the role of sex steroid hormones and, recently, mutations to the MED12 gene in the pathogenesis of UL (44). The role of ovarian steroid hormones in the pathogenesis of uterine leiomyoma is supported by epidemiological, clinical, and experimental evidence (9). Estradiol and progesterone induce mature leiomyoma cells to release mitogenic stimuli to adjacent immature cells, thereby providing uterine leiomyoma with undifferentiated cells likely to support tumor growth (44). In the analysis of serum oestrogen and progesterone levels of the MSG-treated rats, an elevation of the sex hormones was observed in the untreated fibroid groups in both the preventive and curative studies (Figures 1a and 1b).

However, co-administration of MSG with different doses of the extract (250, 500, and 850 mg/kg) results in a significant ($p < 0.05$) lowering of the elevated levels of the hormones in both the preventive and curative studies (Figures 1a and 1b). In addition, during the preventive study, the greatest activity of *MELA* was recorded at the 500 mg/kg dose, as the extract lowered the oestrogen and progesterone levels better than at other doses. However, the extract had a similar and comparable lowering effect on the sex hormones during the curative study among the three selected doses. A study reported that *MELA* has its greatest gastroprotective potency at a dose of 400 mg/kg, which could be the reason why *MELA* also demonstrated its

greatest anti-leiomyoma activity at a similar dose of 500 mg/kg (45).

It has been established that cholesterol is a precursor to the biosynthesis of hormones in the human system (43); thus, elevated levels of total cholesterol in the untreated fibroid groups of the MSG-treated animals were observed in this study (Figure 2a). This correlates with the increased concentrations of oestrogen and progesterone levels in the untreated fibroid groups discussed earlier. However, this increase in serum cholesterol levels was ameliorated significantly ($p < 0.05$) only at 250 mg/kg and 850 mg/kg of the extract in the preventive study. The cholesterol levels remained elevated at other doses of the extract in the preventive and curative studies. The variations in the potency of *MELA* as a pharmacological agent acting as an anti-leiomyoma agent at a dose of 500 mg/kg but not at 250 mg/kg, and as an anti-cholesterolemic agent at other doses, call for further studies to understand these variations and improve the safety profile of the extract as a drug candidate.

In the histopathological examination of the uteri of MSG-treated rats, the uteri of the control animals showed normal connective tissues within the endometrium layer, with smooth muscles within the myometrium appearing normal. Meanwhile, uterine hyperplasia with reduced endometrial lumen was observed in the myometrium of both the untreated fibroid groups and the 250 mg/kg groups for both the preventive and curative studies (Figures 3 and 4). This suggests that 250 mg/kg *MELA* is not potent enough to inhibit the growth of uterine fibroids despite its ameliorating effect on all other measured parameters. In the groups that received 500 mg/kg and 850 mg/kg *MELA*, the histology section shows intact endometrium in a normal uterine architecture, presented with intact myometrium and a patent, wide uterine lumen. This result is consistent in both the preventive and curative studies of MSG-treated animals. Hence, the therapeutic effect of *MELA* as an anti-leiomyoma agent can be inferred from doses of 500 mg/kg and above.

A clinical feature of both benign and malignant uterine leiomyoma is the heavy loss of blood during menstruation (47, 48). Consequently, drug candidates for the management of this disease should possess the ability to preserve haemoglobin levels. Additionally, alterations in haematopoietic and/or biochemical parameters can indicate ~~of~~ toxicity before clinical studies in human subjects (48). In the analysis of the haematological parameters of MSG-induced uterine hyperplasia, the untreated fibroid groups for both the preventive and curative studies had the lowest haemoglobin levels, 11.2 g/dL, and 9.9 g/dL, respectively, (Tables 3 and 4) among the treatment groups. This is significantly below the rat haematological reference range for haemoglobin, 11.5-16.1 g/dL (47). The ability of *MELA* at all tested doses (250, 500, and 850 mg/kg) to restore the depleted haemoglobin levels in both the preventive and curative studies proves that the extract does not induce anaemia and is an ideal drug candidate for uterine fibroids in women with heavy blood loss.

Drug-induced liver injury (DILI) is one reason why potential therapeutic agents have been withdrawn from clinical use. Ulipristal acetate (UPA), a selective progesterone-receptor antagonist, was approved for the treatment of uterine leiomyoma but was subsequently withdrawn due to the occurrence of serious liver injuries in patients (49, 50). Thus, it is important to assess the potential effects of *MELA* on liver enzymes. In this study, the serum levels of aspartate aminotransferase (AST) in the untreated fibroid groups and different doses of *MELA* were comparable to that of the control group, except at the 500 mg/kg dose (Figure 2c) in the preventive study, where the AST level was elevated.

In the analysis of the serum level of alanine transaminase (ALT), an increase in its serum concentration was observed in the untreated fibroid groups of the MSG-treated rats (both preventive and curative studies), but this increase was subsequently attenuated at all treatment doses of *MELA*. This suggests that *MELA* should be further evaluated for its effect on liver injury. Although it shows a promising

hepatoprotective effect when considering the serum level of alanine transaminase (ALT), this effect is not observed at all doses of the extract when considering aspartate aminotransferase (AST). This is an important aspect to consider when *MELA* is evaluated for clinical studies, as most drug candidates fail at the clinical phase due to DILI (51).

Conclusion

The experimental findings in this study reveal that the whole plant extract of *Laportea aestuans* possesses anti-leiomyoma activity. It can arrest the growth of uterine leiomyoma in high-risk women and minimize the need for surgical interventions. The anti-leiomyoma activity of *Laportea aestuans* is possibly mediated through its attenuating effect on the ovarian hormones oestrogen and progesterone. The results obtained in this study justify its traditional use in the management of uterine fibroids. Although the anti-leiomyoma activity of *Laportea aestuans* was best demonstrated at a dose of 500 mg/kg, its effect on liver enzymes at this dose should be monitored to prevent drug-induced liver injury during clinical trials.

Acknowledgments

The authors are grateful for the technical support and assistance rendered by staff of the laboratories of the Department of Physiology, Department of Pharmacognosy, Department of Anatomic and Molecular Pathology, and Department of Pharmacology, Therapeutics, and Toxicology of the College of Medicine, University of Lagos.

Ethical statement

All experimental procedures for this study were carried out in accordance with the ethical guidelines of the Animal Care and Use Research Ethics Committee of the College of Medicine, University of Lagos. Consequently, this study was approved with the ethical approval number CMUL/ACUREC/07/22/1083. These guidelines are similar to international recommendations on animal use in biomedical research.

Data availability

Data is provided within the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding/support

This project was made possible by a partial research grant from the ABISA Research Support Fellowship and a full research grant from the African Centre of Excellence for Drug Research, Medicine Development, and Regulatory Science, University of Lagos, Akoka, Lagos, Nigeria.

CRediT authorship contribution statement

Qudus Ojomo designed the study, performed the experiment, analyzed the data, wrote the paper, reviewed the manuscript, and secured funding. Esther Agbaje contributed to the study concept, analyzed the data, wrote the paper, and made critical revisions to the manuscript for important intellectual content. Joseph Olamijulo contributed to the study concept, reviewed the manuscript, and made critical revisions to the manuscript for important intellectual content. All authors have read and approved the final manuscript.

List of Abbreviations

MELA - Methanol whole plant extract of *Laportea aestuans*

UL - Uterine Leiomyoma

ECM - Extracellular matrix

ER - Oestrogen receptor

PR - Progesterone receptor

MSG - Monosodium glutamate

EDTA - Ethylenediaminetetraacetic acid

References

1. McWilliams MM, Chennathukuzhi VM. Recent advances in uterine fibroid etiology. Semin. Reprod. Med 2017;35(2):181–9. http://dx.doi.org/10.1055/s-0037-1599090

2. Wu HY, Wang KC. Minimally invasive approaches to the surgical management of fibroids. Semin. Reprod. Med 2017;35(6):533–48. http://dx.doi.org/10.1055/s-0037-1606304
3. Parker WH. Etiology, symptomatology, and diagnosis of uterine myomas. Fertil. Steril In 2007;87(4):725–36. http://dx.doi.org/10.1016/j.fertnstert.2007.01.093
4. Sankaran S, Manyonda IT. Medical management of fibroids. Best Pract. Res. Clin. Obstet. Gynaecol 2008;22(4):655–76. http://dx.doi.org/10.1016/j.bpobgyn.2008.03.001.
5. Kim JJ, Sefton EC. The role of progesterone signaling in the pathogenesis of uterine leiomyoma. Mol. Cell. Endocrinol 2012;358(2):223–31. http://dx.doi.org/10.1016/j.mce.2011.05.044.
6. Donnez J, Jadoul P. What are the implications of myomas on fertility? A need for a debate? Hum. Reprod 2002;17(6):1424–30. http://dx.doi.org/10.1093/humrep/17.6.1424
7. Donnez J, Donnez O, Dolmans M-M. With the advent of selective progesterone receptor modulators, what is the place of myoma surgery in current practice? Fertil. Steril 2014;102(3):640–8. http://dx.doi.org/10.1016/j.fertnstert.2014.06.04.
8. Surrey ES, Lietz AK, Schoolcraft WB. Impact of intramural leiomyomata in patients with a normal endometrial cavity on in vitro fertilization-embryo transfer cycle outcome. Fertil. Steril 2001;75(2):405–10. http://dx.doi.org/10.1016/s0015-0282(00)01714-3
9. Hart R, Khalaf Y, Yeong CT, Seed P, Taylor A, Braude P. A prospective controlled study of the effect of intramural uterine fibroids on the outcome of assisted conception. Hum. Reprod 2001;16(11):2411–7. http://dx.doi.org/10.1093/humrep/16.11.2411.
10. Stewart EA, Nicholson WK, Bradley L, Borah BJ. The burden of uterine fibroids for African-American women: results of a national survey. J. Womens. Health (Larchmt) 2013;22(10):807–16. http://dx.doi.org/10.1089/jwh.2013.4334
11. Baird DD, Dunson DB, Hill MC, Cousins D, Schectman JM. High cumulative incidence of uterine leiomyoma in black and white women: ultrasound evidence. Am. J. Obstet. Gynecol 2003;188(1):100–7. http://dx.doi.org/10.1067/mob.2003.99
12. Igboeli P, Walker W, McHugh A, Sultan A, Al-Hendy A. Burden of uterine fibroids: An African perspective, A call for action and opportunity for intervention. Curr. Opin. Gynecol. Obstet 2019;2(1):287–94. http://dx.doi.org/10.18314/cogo.v2i1.1701
13. Agboola AD, Bello OO, Olayemi OO. A clinical audit of the patterns of presentations and complications of abdominal myomectomy at the University College Hospital, Ibadan, Nigeria. J. Obstet. Gynaecol 2021;41(7):1145–50. http://dx.doi.org/10.1080/01443615.2020.1845632
14. Cardozo ER, Clark AD, Banks NK, Henne MB, Stegmann BJ, Segars JH. The estimated annual cost of uterine leiomyomata in the United States. Am. J. Obstet. Gynecol. 2012;206(3):211.e1-9. http://dx.doi.org/10.1016/j.ajog.2011.12.002
15. Nordica Fibroid Care. FAQs. [Cited 2024]. https://fibroidcarecentre.nordicalagos.org/faqs/
16. Brandon DD, Erickson TE, Keenan EJ, Strawn EY, Novy MJ, Burry KA, et al. Estrogen receptor gene expression in human uterine leiomyomata (1995). J Clin Endocrinol Metab 80(6), 1876–1881. https://doi.org/10.1210/jcem.80.6.7775635
17. Wilson PD. Pathogenesis of polycystic kidney disease: altered cellular function. Polycystic Kidney Disease. Oxford University PressNew York, NY; 1996. p. 125–63 .
18. Pollow K, Sinnecker G, Boquoi E, Pollow B. In vitro conversion of estradiol-17beta into estrone in normal human myometrium and leiomyoma. J. Clin. Chem. Clin. Biochem 1978;16(9):493–502. http://dx.doi.org/10.1515/cclm.1978.16.9.493
19. Jiofack TC, Fokunang N, Guedje V, Kemeuze E. Fongnzossie BA. Nkongmeneck PM. Mapongmetsem, N. Tsabang. Ethnobotanical uses of some plants of two ethnoecological regions of Cameroon. Afr. J. Pharm. Pharmacol 2009,3(13). 664-84. http://www.academicjournals.org/ijmms
20. Chew (L). “Laportea aestuans” [Cited 2024]. https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:135476-2

21. Lans C. Ethnomedicines used in Trinidad and Tobago for reproductive problems. J. Ethnobiol. Ethnomed 2007;3(1):13. http://dx.doi.org/10.1186/1746-4269-3-13
22. Etukudo I. Ethnobotany: Conventional and Traditional Uses of Plants. CSRIP 2003;11(3): 191. https://www.scirp.org/reference/referencespapers?referenceid=3042123.
23. Oyebode OT, Obiekwe ME, Olorunsogo OO. Protective effects of alpha stone on monosodium glutamate-induced uterine hyperplasia in female wistar rats. J. Ayurveda Integr. Med 2020;11(3):217–23. http://dx.doi.org/10.1016/j.jaim.2019.05.001
24. Oloyede GK, Oyelola MS. Chrysen-2-ol derivative from West Indian Wood Nettle Laportea aestuans (L.) Chew inhibits oxidation and microbial growth in vitro (2013). EXCLI Journal ;12:894-906. PMID: 27092035; PMCID: PMC4827076
25. Wardani NPA. In Vitro Anti-Inflammatory Activity Test of Laportea Decumana Leaves Methanol Extract [Internet] [Thesis]. Indonesia International Institute for Life Sciences; 2019 [cited 2024 Nov 4]. Available from: http://repository.i3l.ac.id/jspui/handle/123456789/151
26. Adetunji OA, Olugbami JO, Adegoke AM, Gbadegesin MA, Odunola OA. Reno-hepatoprotective and antidiabetic properties of methanol leaf extract of Laportea aestuans in Wistar rats. J. Evid. Based Integr. Med 2021;26:2515690X211017464. http://dx.doi.org/10.1177/2515690X211017464
27. McLean AC, Valenzuela N, Fai S, Bennett SAL. Performing vaginal lavage, crystal violet staining, and vaginal cytological evaluation for mouse estrous cycle staging identification. J. Vis. Exp 2012;(67):e4389. http://dx.doi.org/10.3791/4389
28. Kayode OT, Kayode AA, Mgbojikwe I, Rotimi D. Effect of ketogenic diet on monosodium glutamate-induced uterine fibroids in female Wistar rats. J Babol Univ Med Sci 2021; 23 (1) :1-8. http://jbums.org/article-1-9224-en.html
29. Sofowora, A. Phytochemical Screening of Medicinal Plants and Traditional Medicine in Africa Edition. Spectrum Books Ltd: Nigeria; 1993 150-6.
30. Lorke D. A new approach to practical acute toxicity testing. Arch. Toxicol 1983;54(4):275–87. http://dx.doi.org/10.1007/bf01234480
31. Charles A, Poon LS, Chan CS, Paul C. Enzymatic Determination of Total Serum Cholesterol. Enzymatic Determination of Total Serum Cholesterol. Clin chem 1974;20(4):470-5. https://doi.org/10.1093/clinchem/20.4.470
32. Khamaisi M, Potashnik R, Tirosh A, Demshchak E, Rudich A, Trischler H, et al. Lipoic acid reduces glycemia and increases muscle GLUT4 content in streptozotocin-diabetic rats. Metabolism 1997;46(7):763–8. http://dx.doi.org/10.1016/s0026-0495(97)90120-7
33. Adeniyi SA, Orjiekwe CL, Ehiagbonare JE, Arimah BD. Evaluation of chemical composition of the leaves of Ocimum gratissimum and Vernonia amygdalina. Int. J. Biol. Chem. Sci 2012;6(3). http://dx.doi.org/10.4314/ijbcs.v6i3.34
34. Amusa, N.A, Ashaye O.A, Oladapo M.O. Biodeterioration of the African star apple (*Chrysophyllum albidum*) in storage and the effect on its food value. Afr. J. Biotechnol 2003;2(3):56-59. https://doi.org/10.5897/AJB2003.000-1011
35. Agbaje EO, Ajidahun OA. Analgesic, anti-inflammatory and antipyretic effects of dried root ethanolic extract of *Strophanthus sarmento* susp. Int. J. pharm. pharm. Pharmacol 2020. 1(4), 62-69. http://www.interesjournals.org/IRJPP
36. Naghibi F, Khalaj A, Mosaddegh M, Malekmohamadi M, Hamzeloo-Moghadam M. Cytotoxic activity evaluation of some medicinal plants, selected from Iranian traditional medicine Pharmacopoeia to treat cancer and related disorders. J. Ethnopharmacol 2014;155(1):230–9. http://dx.doi.org/10.1016/j.jep.2014.05.025
37. Miller JS. The global importance of plants as sources of medicines and the future potential of Chinese plants. Drug Discovery and Traditional Chinese Medicine. Boston, MA: Springer US; 2001. 33–42. 2001.https://doi.org/10.1007/978-1-4615-1455-8_4

38. Aatifa R, Khalid M, Altaf A, Aarifa J, Shaziya H. Medicinal plants: Role, distribution and future. J. pharmacogn. Phytochem 2020;9(2):2111-2114. https://www.phytojournal.com/archives/2020.v9.i2.11165/medicinal-plants-role-distribution-and-future
39. Olufunke D. Moronkola, Oladosu I. Adebayo, Ogunwande I. Ajani and Olubomehin Ose Comparisons of the essential oils from the aerial, fruits and roots of Nigerian *Laportea aestuans* (L.) Chew (Urticaceae) Nat. Prod. Commun. 2008;3(11):1921 – 1924. https://doi.org/10.1177/1934578X0800301126
40. Bulun SE. Uterine fibroids. N. Engl. J. Med. 2013. 369(14):1344–55. https://www.nejm.org/doi/10.1056/NEJMra1209993
41. Essiett UA, Edet NI, Bala DN. Phytochemical and physicochemical analysis of the leaves of *Laportea aestuans* (Linn.) Chew and *Laportea ovalifolia* (Schumach.) Chew (male and female). AJPSKY 2011;1(2):35-42. https://api.semanticscholar.org/CorpusID:91048381
42. Oloyede GK. Toxicity, antimicrobial and antioxidant activities of methyl salicylate dominated essential oils of Laportea aestuans (Gaud). Arab. J. Chem 2016;9:S840–5. http://dx.doi.org/10.1016/j.arabjc.2011.09.019
43. S.c O, Department of Biochemistry, Faculty of Biological & Physical Sciences, Abia State University, Uturu, Nigeria, I E. Studies on the in vitro antioxidant activity of Laportea aestuans leaf extract. IOSR-JESTFT 2014;8(1):33–41. Available from: http://dx.doi.org/10.9790/2402-08123341
44. Reis FM, Bloise E, Ortiga-Carvalho TM. Hormones and pathogenesis of uterine fibroids. Best Pract. Res. Clin. Obstet. Gynaecol 2016;34:13–24. http://dx.doi.org/10.1016/j.bpobgyn.2015.11.015
45. Primiano T, Yu R, Kong A-NT. Signal transduction events elicited by natural products that function as cancer chemopreventive agents. Pharm. Biol 2001;39(2):83–107. http://dx.doi.org/10.1076/phbi.39.2.83.6256
46. Mäkinen N, Mehine M, Tolvanen J, Kaasinen E, Li Y, Lehtonen HJ, et al. MED12, the mediator complex subunit 12 gene, is mutated at high frequency in uterine leiomyomas. Science 2011;334(6053):252–5. http://dx.doi.org/10.1126/science.1208930
47. Nelson AL, Ritchie JJ. Severe anemia from heavy menstrual bleeding requires heightened attention. Am. J. Obstet. Gynecol 2015;213(1):97.e1-6. http://dx.doi.org/10.1016/j.ajog.2015.04.023
48. Walker CL. Role of hormonal and reproductive factors in the etiology and treatment of uterine leiomyoma. Recent Prog. Horm. Res 2002;57(1):277–94. http://dx.doi.org/10.1210/rp.57.1.277
49. Walker PA, Ryder S, Lavado A, Dilworth C, Riley RJ. The evolution of strategies to minimize the risk of human drug-induced liver injury (DILI) in drug discovery and development. Archives of toxicology, 94,8 (2020): 2559-85. https://doi.org/10.1007/s00204-020-02763-w
50. European Medicines Agency. PRAC recommends revoking marketing authorisation of ulipristal acetate for uterine fibroids [Internet]. 2020. Available from: https://www.ema.europa.eu/en/documents/referral/ulipristal-acetate-5mg-medicinal-products-article-31-referral-prac-recommends-revoking-marketing-authorisation-ulipristal-acetate-uterine-fibroids_en.pdf
51. Sisay W, Andargie Y, Molla M, Norahun A. Hydromethanolic crude extract of the leaf of Urtica simensis Hochst. ex. A. Rich.(Urticaceae) acquires appreciable antiulcer effect: validation for in vivo antiulcer activity. J Evid Based Complementary Altern Med 2021;2021(1):6591070. https://doi.org/10.1155/2021/6591070