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Chemical characterization, safety profile and antileiomyoma effects of *Tetrapleura tetraptera* Taubert (Fabaceae) fruit ethanol extract in Sprague Dawley rats

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Abstract

Background *Tetrapleura tetraptera* Taubert (Fabaceae) fruits are employed by herbal practitioners in the management of uterine leiomyoma, but its usage in this regard and level of safety in chronic administration has not been sufficiently established. This study evaluated the toxicity effects of *T. tetraptera* ethanol fruit extract and explored its antileiomyoma effect in female Sprague Dawley (SD) rats.

Methods Sub-chronic toxicity test of the extract was done, with biochemical and hematological changes as well as histopathology of organs assessed. Leiomyoma formation was induced in SD rats with monosodium glutamate (MSG) and the extract given at 100, 200 and 400 mg/kg doses, following both the preventive and curative methods. Total serum cholesterol, protein and estradiol were determined, as well as histopathology assessment of the uterus. Phytochemical profiling of the extract was evaluated by analytical high-performance liquid chromatography (HPLC).

Results No significant alterations were seen in the biochemical and hematological indices in the toxicity test. The vital organs showed no changes at 200 mg/kg, but at 800 mg/kg it appeared to induce multiplication of glandular epithelium and stromal fibrosis in the uterus, and induced perivascular inflammation around the vessels of the heart. Total serum cholesterol and estradiol were significantly elevated ($P \leq 0.05$) on treating normal female rats with 800 mg/kg MSG. Preventive and curative treatment of MSG-treated animals with the extract significantly decreased the elevated serum cholesterol ($P \leq 0.01$) and estradiol ($P \leq 0.05$). Histological studies of the uterus showed an amelioration of the proliferating fibroid cells with administration of the extract, which was more evident in the curative treatment. Result of HPLC analysis of the extract revealed rich composition in bioactive compounds such as umbelliferone, ferulic acid, aridanin, echinocystic acid, naringenin and hentriacontane.

Conclusion The ethanol fruit extract of *T. tetraptera* is relatively safe in Sprague Dawley rats in low doses and has antifibroid potential as seen in its significant reduction in the elevated total cholesterol and estradiol content as well as its ability to decrease uterine leiomyoma proliferation, which may be due to its array of phytochemical constituents.

Keywords *Tetrapleura tetraptera*, Toxicity, Hematological, Biochemical, Antileiomyoma, Cholesterol, Estradiol, Protein, Histopathology, HPLC

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Background

The most prevalent tumors of the female reproductive system are uterine leiomyomas, often known as uterine fibroid or uterine myomas. It poses a serious risk

to women's reproductive health everywhere [1]. Over 70% of women have it, and about 30% of them develop symptoms [2]. In women who are of reproductive age, it is the most common reason for hysterectomies [3]. It is a benign monoclonal tumor of the uterine smooth muscle cells. It grows in the uterine cavity or on the walls of the uterus in different places. Thus, it is referred to as intramural, submucosal or sub-serosal fibroids. It can have any shape and size, from a pea size to a water melon size [1].

While the exact cause of uterine leiomyoma formation is still unknown, epidemiological studies have provided substantial evidence that estrogen and progesterone stimulate the growth of the tumor, possibly as a result of continuous menstrual cycles, since fibroids rarely develop prior to menarche and disappear following menopause [2]. Women who identify as African-American and who are fat are significantly more at risk. Aside from age and family history, other risk factors for uterine leiomyoma include nutrition, lack of exercise, some chemicals like monosodium glutamate (MSG) and prescription medications that may raise cholesterol, estrogen and total protein levels [4].

Since most women with uterine fibroids have no symptoms, they receive less clinical attention and the tumors are frequently left undetected. Women with symptoms usually report abnormally heavy bleeding from the uterus (which can cause anemia) or painful periods; pelvic fullness; lower abdominal enlargement; frequent urination; pain during sexual activity; lower back pain; complications during pregnancy and labor; and reproductive issues like infertility [5]. The size and location of the fibroids typically determine how severe these symptoms are.

There are extremely few alternatives for treatment and prevention; doctors frequently recommend myomectomy and hysterectomy. Medication treatments, such as hormone replacement therapy, are only beneficial for six to twelve months because prolonged use of the drug might have serious negative effects [6, 7]. Although uterine artery embolization and high-intensity focused ultrasound ablation are viable options, patients may be discouraged from pursuing such treatment due to the associated risks and expenses [8]. Not only are orthodox drugs expensive, but they often only provide brief relief, which makes alternative treatment choices necessary.

The drawbacks of conventional medications, in addition to the accessibility, affordability and cultural significance of herbal remedies, which are favored over the expensive medical services provided by conventional medicine, have sparked a resurgence of interest in these remedies [9]. In one study, various biochemical parameters changed by administration of MSG were recovered by the ethanol extract of *Diodia sarmentosa* leaves. The treated groups' protection against leiomyoma development was also demonstrated by histopathological assessment [7]. Femitol, a herbal mixture manufactured in Ghana, was found to be effective as an antifibroid agent as it greatly reduced MSG-induced uterine hyperplasia and decreased high levels of cholesterol and estrogen, as well as uterus size and weight [10, 10]. Additionally, morphological examination demonstrated that the entire plant of *Labisia pumila* caused apoptosis against SK-UT-1 (uterine leiomyoma cells) in a dose-dependent manner and inhibited the formation of uterine fibroid tumors [11].

Tetrapleura tetraptera Taubert (Fabaceae) is used to treat uterine leiomyoma, according to anecdotal evidence [12]; however, this is not supported by scientific research. The plant is used to cure febrile illnesses and infections, stop postpartum contractions, lower cholesterol, encourage the production of breast milk and speed up the healing of reproductive wounds [13]. Despite the various evidence for its efficacy, there is insufficient scientific evidence to support its safety with regards to its use in herbal medicines. Since the fruit of this plant is used in preparation of herbal remedies for management of leiomyoma which is a chronic illness, it is therefore necessary to evaluate the level of safety of this plant following prolonged repetitive administration.

The objectives of this study, therefore, were to establish the safety of the fruit extract and analyze its protective effect on biochemical indicators such as total cholesterol, protein and estradiol, in order to determine its antiuterine fibroid effect on MSG-induced uterine leiomyoma in Sprague Dawley rats, which will be further confirmed by histopathological assessment of the rat's uterus. Furthermore, identification of the major constituents of the extract was carried out with analytical HPLC.

Methods

Chemicals and reagents

All reagents used in this study were of analytical quality and acquired from reputable local vendors.

Plant collection and preparation

The fruit of *T. tetraptera* was harvested in the savanna area of Odofin Agbegi village, Ikire, Osun state, Nigeria, and authenticated at the Forest Research Institute of Nigeria (FRIN) with the herbarium number FHI 113604 attached. Using a Soxhlet device, the dried and crushed fruit was extracted with ethanol. A rotary evaporator was used to reduce the extract to dryness.

Animal handling conditions

Non-pregnant Sprague Dawley (SD) rats weighing between 125 and 160 g were obtained and placed in groups of five (5) in plastic cages with a wire screen top for adequate ventilation and wood shavings as the bedding material. The animals were supplied clean water and fed with commercial rat pellet (Chikun feed) manufactured by Olam Agricultural enterprise ad libitum. The processes applied in animal handling were in accordance with the National Institute of Health Guidelines for the Care and use of Laboratory Animals. All procedures for using experimental animals were approved by the Research Ethics Committee of the Faculty of Pharmacy, University of Benin (EC/FP/023/04).

Dosing of experimental animals

Doses of *T. tetraptera* extract used in this study were selected based on the LD_{50} of the plants fruit which is greater than 5000 mg/kg [14]. The SD rats were given doses via gavage. Throughout the experiment, animals were dosed once a day, with each dose volume determined by the animal's weekly recorded body weight. The oral route of administration was chosen because it is the commonly utilized route by humans.

Sub-chronic toxicity test

SD rats were divided into four groups comprising of 6 animals each. Group A received 0.5 mL distilled water (control). Groups B, C and D were orally administered different doses of the extract (200, 400 and 800 mg/kg BW), respectively, daily for 28 days. Body weights of the rats were taken on day 0, 7, 14, 21 and 28. Every day, the animals were closely monitored for any changes in their clinical symptoms. Tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were the main areas of focus [15]. On the last day of gavage, the rats were fasted for a period of 12 h before being killed using a chloroform chamber. The blood was drawn by cardiac puncture into two distinct kinds of bottles: Ethylenediaminetetraacetic acid (EDTA) bottles were used to collect the blood's hematological parameters, while plain bottles were used to acquire serum for the analysis of biochemical parameters. Organs for histological analysis were obtained, including the kidney, liver, heart and uterus.

Biochemical analysis

Samples of blood were taken into plain tubes and left to stand at room temperature for 45 min before being centrifuged for 10 min at 3400 rpm. The collected serum stored at − 25 °C was utilized to assess the lipids, renal and liver function tests. These parameters were assessed using an automated chemistry analyzer (Selectra Pro S, Germany). Electrolyte assay was by ion-selective electrode (SFRI 4000, France). The parameters assayed included creatinine (Cr), urea (Ur), uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), serum proteins (Tp), total bilirubin (Tb), triglycerides (TG), total cholesterol (T-CH), low-density lipoproteins (LDL), high-density lipoprotein (HDL) and serum electrolytes (Na^+,K^+,Cl^-, HCO_3^-).

Hematological analysis

Additionally, hematological parameters were evaluated by a blood count utilizing an automated hematology analyzer on blood collected into EDTA bottles (Dymind 2000, China). The parameters analyzed include white blood cell (WBC), red blood cells (RBC), red blood cell distribution width (CV), red blood cell distribution width (SD), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), granulocytes (GRAN), platelets count (PCT) and hematocrit (HCT).

Histopathology analysis of organs

For histological analysis, the liver, heart, kidney and uterus from the killed animals were fixed in 10% neutral buffered formalin solution. These tissues were subsequently dehydrated in ascending grades of alcohol (70%, 90%, 96% and 100%), cleared in xylene, impregnated with molten paraffin wax and sectioned to slides. These sections (4–5 μm thick) were stained with hematoxylin after dewaxing with xylene and hydrating in descending grades of alcohol (100%, 96%, 70%) and water. Differentiation was done in 1% acid alcohol and the sections counter stained with eosin. Dehydration in ascending grades of alcohol was carried out again, and the sections were cleared in xylene and mounted with dibutylphthalate polystyrene using cover slips prior to microscopic examination [16].

Antileiomyoma effect of *Tetrapleura tetraptera* fruit (TTF)

Experimental procedures

Preventive study

This was carried out following the method described in the literature [7]. Female SD rats were placed in five groups of five rats each. Dosing was done once a day through the oral route. Group A (Control) was the no treatment group, given only food and water. Group B was treated with 800 mg/kg monosodium glutamate (MSG) (Lobachem, India) only. Groups C, D and E were treated with 800 mg/kg MSG and *T. tetraptera* fruit extract

(100, 200 and 400 mg/kg) concurrently. All treatments were given concurrently for a 30-day period. On the 31st day, the animals were killed by anesthetizing them in a chloroform saturated chamber and their blood collected through cardiac puncture and transferred into plain bottles which were used to determine total serum cholesterol, protein and estradiol content. The uteri were surgically removed and transferred into sterile tissue bottles containing 10% neutral buffer formalin for histopathology assessment.

Curative study

Following the method described in the literature [4], female SD rats were treated with 800 mg/kg MSG for 30 days to induce the development of uterine leiomyoma. The rats were housed in five groups of five rats each. Treatment with extracts commenced on the 31st day. Group A was the control group, given no MSG but only food and water for the duration of the experiment. Group B, after being administered 800 mg/kg MSG for 30 days, was given food and water only from the 31st to 60th day. Groups C-E were treated with 100, 200 and 400 mg/kg *T. tetraptera* fruit extract, respectively, from the 31st to 60th day, after the 30-day MSG administration. The animals were killed by anesthetizing them in a chloroform saturated chamber and their blood collected through cardiac puncture into plain bottles to determine the total serum cholesterol, protein and estradiol content. The uteri were surgically removed and transferred into sterile bottles containing 10% neutral buffer formalin for histopathology assessment.

Biochemical assays

Determination of total cholesterol content

A day after the experimental period (30 days for preventive treatment and 60 days for curative treatment), the animals' blood was collected and serum separated by centrifugation at 3000 rpm for 10 min, which was used for the experiment.

Total cholesterol content was determined using the semi-automated chemistry analyzer (Mindray BA-88A Reagent system) and the AGAPPE test kit. The total cholesterol kit was programmed on the semi-automated biochemistry analyzer using the information on the instruction manual. In a microtube marked "blank," 1000 µL of cholesterol biuret reagent was added. 10 µL of standard cholesterol and 1000 µL of the reagent were added to and properly mixed in a tube marked "standard." 10 µL of sample A_1 and 1000 µL of cholesterol reagent were added to and properly mixed in a tube with the label "A_1." The same procedure was carried out for the remaining samples (A_1–E_5) in their respective tubes. This was done for samples obtained from both the preventive and curative experiments. All tubes were incubated for 10 min at 37 °C. After incubation, the content of the blank tube was aspirated into the flow cell of the analyzer to measure the absorbance, after which the content of the standard tube was also aspirated into the flow cell to measure the absorbance. Reaction mixture for each sample was then aspirated into the flow cell to measure the absorbance. The values of absorbance for each tube were recorded accordingly [10].

Determination of total protein content

The information in the instruction manual was used to program the kit on the semi-automated biochemistry analyzer. In a microtube marked "blank," 1000 µL of total protein biuret reagent was added. 20 µL of standard total protein and 1000 µL of the reagent were added to and properly mixed in a tube marked "standard." 20 µL of sample A_1 and 1000 µL of total protein reagent were added to and properly mixed in a tube with the label "A_1." The remaining samples (A_1–E_5), from both the preventive and curative experiments in their respective tubes underwent the same procedure. All tubes were incubated for 10 min at 37 °C. After incubation, the content of the blank tube was aspirated into the flow cell of the analyzer to measure the absorbance, after which the content of the standard tube was also aspirated into the flow cell to measure the absorbance. Reaction mixture for each sample was then aspirated into the flow well to measure the absorbance. The value of absorbance for each tube was recorded accordingly [10].

Determination of estradiol content

Estradiol was assayed using the microplate reader (mindray MR-96A), microplate washer (Mindray MW-12A) and E2 AccuBind ELISA Kit. 25 µl of the serum reference and 25 µl of each sample (A_1–E_5) were pipetted into the assigned wells, after which 50 µl of estradiol biotin reagent was added to all wells. Plates were swirled gently for about 30 s and then incubated for 30 min followed by the addition of 50 µL of estradiol enzyme reagent to all the wells and incubation for 90 min at room temperature. The contents of the microplate were discarded by decantation and the plates dried with absorbent paper afterward. 350 µL of the wash buffer was added and the content decanted thrice. 100 µL of substrate was added and incubated for 20 min, after which 50 µL of the stop solution was added to each well and gently mixed for 20 min. The absorbance was read at 450 nm within 15 min of adding the stop solution. The concentration of estradiol in samples was extrapolated from a dose response curve [14].

Histopathology analysis of the uterus

This was carried out following the method earlier stated [16].

HPLC procedure

The HPLC procedure was carried out at Bato Chemical Laboratory in Lagos, Nigeria. HPLC make was Shimadzu (Nexera mx). Column used was ubondapak, a C18 reverse-phase chromatographic column, with dimensions of length: 100 mm, internal diameter: 4.6 mm and thickness: 7 µm. Mobile phase employed was acetonitrile/water, 70:30. The HPLC system was attached to a UV–Vis diode array detector set at an analytical wavelength of 254 nm, within the UV–visible region. Pump pressure employed was 15 mpa. Solutions of standards were first injected into the HPLC system to generate a chromatogram with a given peak and peak profile. These were used to create a window in preparation for the test sample analysis. The analysis adopted a 2 ml/min constant flow rate as 5 ul of extract was injected into the HPLC system also to obtain a corresponding peak area and peak profile in a chromatogram [18].

Data analysis

Bar graphs were obtained by the software GraphPad Prism for Windows, version 6.01, and data were subjected to one-way analysis of variance (ANOVA) with Tukey–Kramer multiple comparison test. $P \leq 0.05$ was considered statistically significant in all analysis.

Results

Sub-chronic toxicity results

Effect of T. tetraptera fruit on food consumption and body weight

In test animals, repeated dosing over a 28-day period did not result in mortality or systemic toxicity indicators. Normal eating habits were observed and a gradual increase in weight was seen in all the test groups, although the control group showed marked weight gain ($P < 0.05$) compared to the groups administered the extract (Table 1).

Effect of T. tetraptera fruit on serum biochemical parameters

Effect on lipid profile tests All the lipid profile parameters obtained from the serum of the control and test groups at different doses were not significantly different ($p > 0.05$), though a slight reduction in total cholesterol was observed at 400 mg/kg compared to the control (Table 2).

Table 2 Lipid profile parameters after 28 days of administration

	Doses (mg/kg)			
Parameters	**Control**	**200**	**400**	**800**
T-CH (mg/dL)	71.80 ± 2.80^{a}	87.60 ± 8.16^{ab}	66.60 ± 2.84^{ac}	71.60 ± 2.54a
TG (mg/dL)	96.20 ± 13.60^{a}	120.6 ± 27.83^{a}	82.00 ± 12.63^{a}	97.20 ± 7.59^{a}
HDL (mg/dL)	34.80 ± 2.27^{a}	38.40 ± 1.63^{a}	33.40 ± 3.17^{a}	34.60 ± 2.50^{a}
LDL (mg/dL)	21.80 ± 1.63a	24.80 ± 2.75^{a}	16.80 ± 1.20^{a}	18.40 ± 3.14^{a}

Data are expressed as mean ± SEM, $n = 6$. Values in the test groups carrying the same letters as the control group are not significantly different according to Tukey–Kramer multiple comparison test $p > 0.05$.

T-CH total cholesterol, *TG* triglyceride, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein

Effect on kidney function test parameters For the kidney function tests, extract administration at all doses did not significantly alter the concentration of sodium, potassium, chloride, urea and creatinine ($P > 0.05$) (Table 3).

Table 3 Kidney function test parameters after 28 days of administration

	Doses (mg/kg)			
Parameters	**Control**	**200**	**400**	**800**
Na^{+} (mmol/L)	139.4 ± 0.68	138.8 ± 0.80	139.4 ± 0.81	140 ± 1.14
K^{+} (mmol/L)	5.06 ± 0.12	5.10 ± 0.21	5.22 ± 0.21	5.26 ± 0.24
HCO_3^{-} (mmol/L)	21.40 ± 0.81	19.60 ± 0.93	20.00 ± 0.77	20.20 ± 0.80
Cl^{-} (mg/dL)	102.80 ± 0.8	102.00 ± 0.95	100.60 ± 1.10	101.80 ± 0.80
Urea (mg/dL)	43.00 ± 1.92	39.80 ± 3.20	38.40 ± 2.58	43.40 ± 2.09
Creatinine (mg/dL)	0.84 ± 0.09	0.84 ± 0.08	0.80 ± 0.06	0.86 ± 0.08

Data are expressed as mean ± SEM, $n = 6$. There was no significant difference between the groups according to Tukey–Kramer multiple comparison test $P > 0.05$

Table 1 Change in mean body weights of the rats at different days of the experiment

Dose (mg/kg)	Change in mean body weights (g) at different days				
	0	**7**	**14**	**21**	**28**
Control	125.86 ± 14.92	9.87 ± 5.56	9.30 ± 2.14	8.30 ± 1.14	9.96 ± 3.34
200	138.30 ± 10.89	7.90 ± 4.45^{a}	8.29 ± 2.43	7.30 ± 2.14	7.22 ± 1.43^{b}
400	143.74 ± 10.18	6.30 ± 3.14^{b}	6.86 ± 4.71^{a}	7.90 ± 3.43	6.87 ± 4.52^{b}
800	133.22 ± 9.24	5.20 ± 4.90^{b}	6.50 ± 2.77^{a}	5.10 ± 5.14^{b}	5.24 ± 4.78^{b}

Data are expressed as mean ± SEM, $n = 6$, a = $p < 0.05$, $b = p < 0.01$ compared to control

Table 4 Liver function test parameters after 28 days of extract administration

Parameters	Doses (mg/kg)			
	Control	200	400	800
ALP (µ/L)	210.20 ± 32.91	251.20 ± 8.79	242.80 ± 28.9	249.80 ± 16.73
ALT (µ/L)	128.20 ± 12.29	112.60 ± 14.87	97.20 ± 15.19	118 ± 14.16
AST (µ/L)	195.20 ± 16.93	213.20 ± 28.52	143.6 ± 14.6	173.2 ± 17.48
Tb (mg/dL)	0.22 ± 0.02	0.22 ± 0.02	0.24 ± 0.02	0.2 ± 0.00
Cb (mg/dL)	0.10 ± 0.00	0.12 ± 0.02	0.1 ± 0.00	0.1 ± 0.00
Tp (g/dL)	6.28 ± 0.21	6.72 ± 0.22	6.22 ± 0.18	6.7 ± 0.27
ALB (g/dL)	3.26 ± 0.05	3.42 ± 0.17	3.20 ± 0.03	3.20 ± 0.06
GLo (g/dL)	3.02 ± 0.19	3.30 ± 0.17	3.02 ± 0.17	2.98 ± 0.22

Data are expressed as mean ± SEM, $n = 6$. There was no significant difference between the groups according to Tukey–Kramer multiple comparison test $P > 0.05$, alkaline phosphatase (ALP), alanine aminotransferase (ALT) Aspartate aminotransferase (AST), total bilirubin (Tb), conjugated bilirubin (Cb), total protein (Tp), albumin (ALB), globulin (GLo)

Effect on liver function test parameters The liver function test parameters were also not significantly altered by administration of the extract (Table 4) although there was a slight elevation in alkaline phosphatase content.

Effect of T. tetraptera fruit on hematological parameters The results of the hematological parameters (Table 5) revealed a decrease ($p < 0.05$) in white blood cell count at 200 and 400 mg/kg BW. The extract did not have a significant effect ($p > 0.05$) on all other hematological parameters tested as they were all within normal range (Table 5).

Histological evaluation of the rat organs

The rat heart muscles were made up of cardiomyocytes arranged in bundles. At 200 mg/mL of the extract, the architecture of the heart was normal (Panel A). 400 mg showed the presence of polymorphs. At 800 mg, there was induction of inflammation around the blood vessels of the heart (polymorphs and fibroblasts) and the blood vessels looked ulcerated.

The liver at 200 mg had increased blood flow in the veins and normal hepatocytes (Panel B). 400 mg revealed the presence of activated Kupffer cells in the sinusoids which is evidence of a boost in the immune system; this can be seen as an extra quality of the extract. At 800 mg, polymorphs around the portal triad were observed along with Kupffer cells. The kidney is made up of the renal corpuscle; there was increased blood flow (active congestion) in the interstitial space and normal architecture was observed even at 800 mg/kg, as no necrosis of tubular epithelium was seen. The uterus at 200 mg showed normal structures (Panel D), but stromal fibrosis and glandular epitheliosis were observed at 800 mg (Fig. 1).

Table 5 Results of hematological assay after 28 days administration

Dose (mg/kg)	0	200	400	800
WBC (10^3/µL)	8.16 ± 0.58^{a}	5.84 ± 0.52^{bc}	5.84 ± 0.35^{bc}	6.42 ± 0.54^{ac}
LYM (%)	83.58 ± 1.94^{a}	79.68 ± 3.85^{a}	76.70 ± 2.12^{a}	79.40 ± 2.04^{a}
MID (%)	12.18 ± 1.25^{a}	13.90 ± 2.26^{a}	15.62 ± 1.52^{a}	13.02 ± 1.35^{a}
GRAN (%)	4.24 ± 0.75^{a}	6.42 ± 1.60^{a}	$7.80 \pm 0.90b^{a}$	7.60 ± 1.00^{a}
RBC (10^6/µL)	5.67 ± 0.30^{a}	5.94 ± 0.28^{a}	5.90 ± 0.05^{a}	6.21 ± 0.19^{a}
HGB (g/dL)	11.06 ± 0.64^{a}	11.74 ± 0.72^{a}	11.60 ± 0.19^{a}	12.20 ± 0.45^{a}
HCT (%)	31.54 ± 1.82^{a}	33.66 ± 1.82^{a}	33.58 ± 0.74^{a}	33.96 ± 1.30^{a}
MCV (fL)	55.74 ± 1.90^{a}	56.82 ± 2.06^{a}	57.02 ± 1.40^{a}	54.74 ± 1.22^{a}
MCH (pg)	19.46 ± 0.29^{a}	19.68 ± 0.61^{a}	19.62 ± 0.22^{a}	19.56 ± 0.24^{a}
MCHC (g/dL)	35.08 ± 0.84^{a}	34.80 ± 0.58^{a}	34.56 ± 0.84^{a}	35.88 ± 0.41^{a}
RDW-SD (fL)	30.74 ± 2.76^{a}	32.48 ± 2.07^{a}	31.60 ± 2.08^{a}	31.73 ± 1.77^{a}
RDW-CV (%)	14.96 ± 0.94^{a}	15.52 ± 0.57^{a}	15.10 ± 0.74^{a}	15.44 ± 0.56^{a}
PLT (10^3/µL)	624.20 ± 76.12^{a}	588.80 ± 19.99^{a}	632.80 ± 70.34^{a}	652.80 ± 39.78^{a}
MPV (fL)	7.64 ± 0.07^{a}	7.26 ± 0.23^{a}	7.34 ± 0.20^{a}	7.30 ± 0.08^{a}
PDW (fL)	9.76 ± 0.41^{a}	9.26 ± 0.61^{a}	9.18 ± 0.33^{a}	9.76 ± 0.12^{a}
PCT (%)	0.47 ± 0.06^{a}	0.43 ± 0.02^{a}	0.47 ± 0.07^{a}	0.47 ± 0.03^{a}
P-LCR (%)	9.10 ± 1.43^{a}	6.54 ± 1.76^{a}	7.92 ± 1.44^{a}	5.62 ± 0.77^{a}

Data are expressed as mean ± SEM, $n = 6$. Values in the test groups carrying the same letter as the control group are not significantly different according to Tukey–Kramer multiple comparison test ($p > 0.05$), white blood cell (WBC), lymphocytes (LYM), mid-range absolute count (MID), granulocytes (GRAN), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width SD (RDW-SD), red blood cell distribution width CV (RDW-CV), platelet (PLT), mean platelet volume (MPV), platelet distribution width (PDW), platelets count (PCT), platelet larger cell ratio (P-LCR)

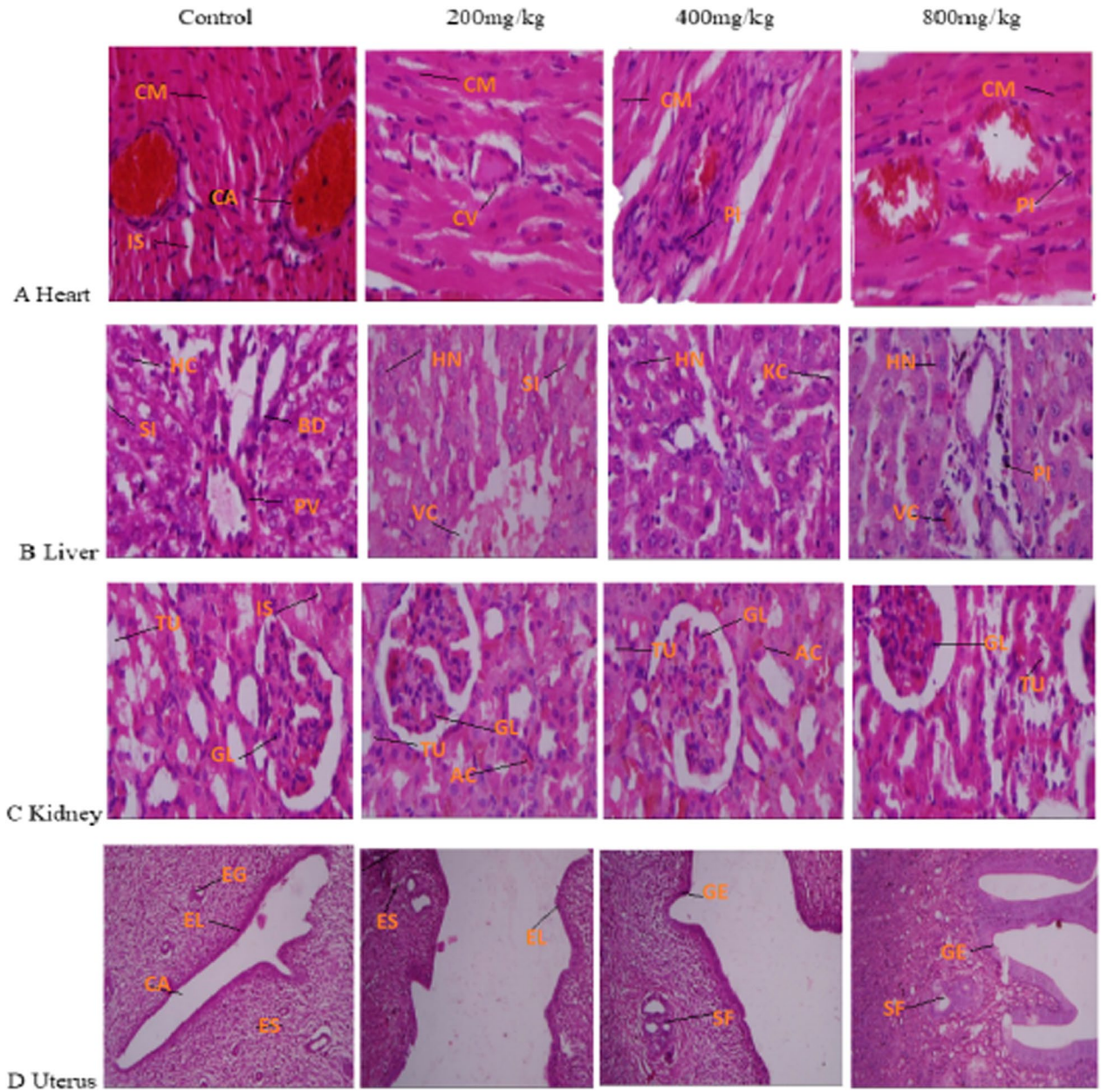

Fig. 1 H&E×400 SD Rat organ sections: Panel **a** shows normal heart architecture, bundles of cardiomyocytes (CM), coronary artery (CA), interstitial space (IS), at 400 and 800 mg/kg perivascular mobilization of inflammatory cells (PI) are seen. Panel **b** shows normal liver architecture, hepatocytes (HC), sinusoids (SI), portal vein (PV), bile duct (BD). 800 mg/kg shows normal hepatocytes with conspicuous nucleoli (HN), periportal infiltrates of inflammatory cells (PI), vascular congestion (VC). Panel **C** shows normal kidney architecture: tubules (TU), interstitial space (IS), glomeruli (GL). Panel D shows normal uterus architecture: cavity (CA), endometrial lining (EL), stroma (ES), glands (EG), 800 mg/kg shows glandular epitheliosis (GE), stromal fibrosis (SF)

Antileiomyoma evaluation results

Total serum cholesterol

There was significant elevation [64.37% ($P \leq 0.05$)] in serum cholesterol in the group administered MSG only for the preventive treatment. When the extract was administered concurrently with MSG, this action was observed to a lesser degree, with 100 and 200 mg/kg having only 20.77 and 6.56% increase in serum cholesterol compared to the control ($P \geq 0.05$) (Fig. 2). After MSG induction of uterine leiomyoma where serum cholesterol was elevated (45.85%), the curative treatment with different doses of the extract decreased this parameter to

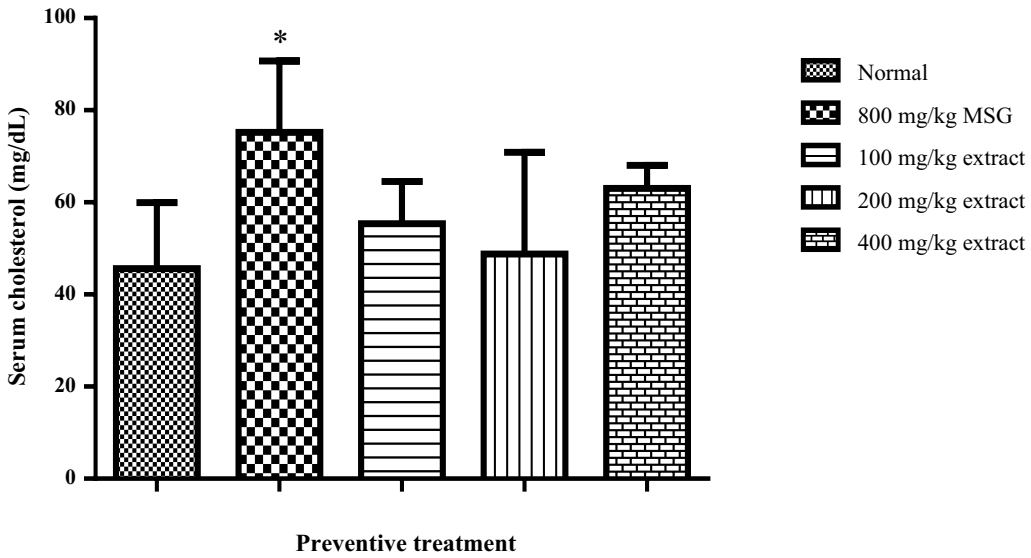

Fig. 2 Preventive effect of the ethanol extract of *T. tetraptera* fruit on total serum cholesterol in female SD rats administered 800 mg/kg MSG. Compared to normal group $^{*}P < 0.05$

normal especially at 200 and 400 mg/kg doses ($P \geq 0.05$) which was vastly reduced compared to the MSG group ($P \leq 0.01$) (Fig. 3).

Total protein content

Treatment with MSG alone and concurrent treatments with MSG and the extract at various doses did not have any significant ($P \geq 0.05$) effect on serum protein in relation to the normal rats (Fig. 4). Pre-treatment of normal rats with MSG also did not cause significant elevation of serum protein (Fig. 5).

Total serum estradiol

MSG treatment gave rise to a very significant elevation in estradiol (90.98% $P \leq 0.05$). Concurrent treatments with MSG and extract at various doses produced reduced levels of elevation of 35.35, 52.97 and 8.07% in estradiol content with 100, 200 and 400 mg/kg doses ($P \geq 0.05$)

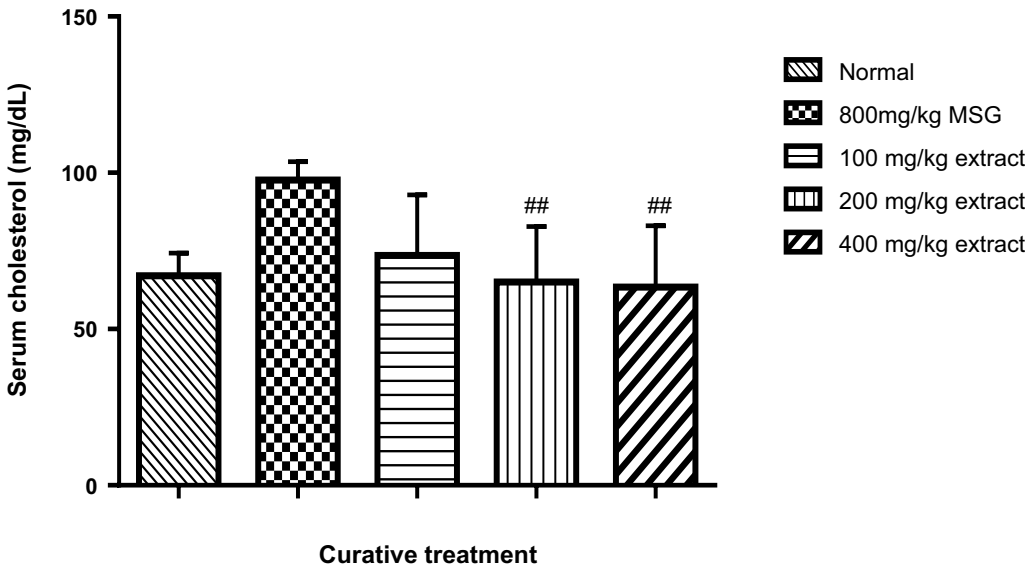

Fig. 3 Curative effect of the ethanol extract of *T. tetraptera* fruit on total serum cholesterol in female SD rats pre-treated with 800 mg/kg MSG. Compared with normal group $^{*}P < 0.05$, compared with MSG group.$^{\#\#}P < 0.01$

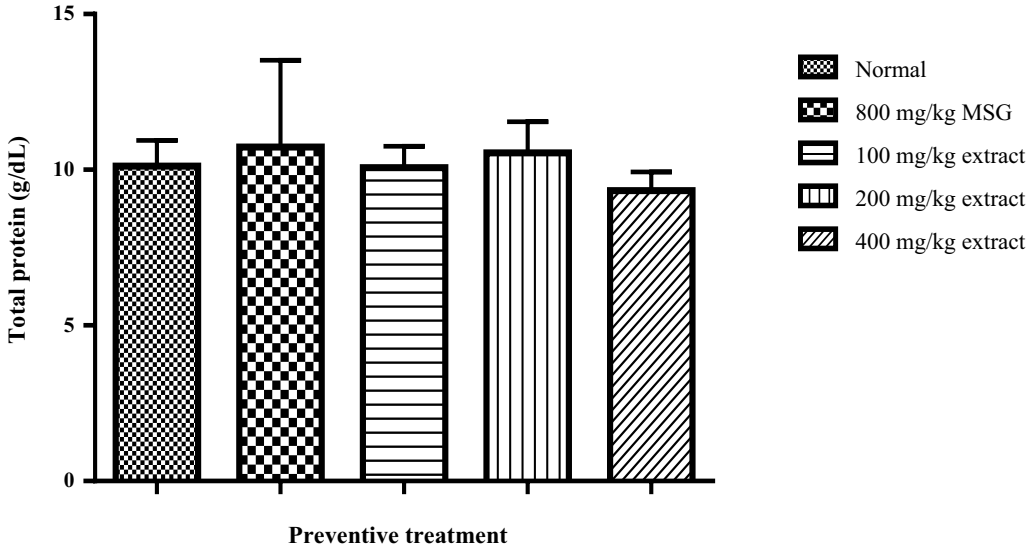

Fig. 4 Preventive effect of the ethanol extract of *T. tetraptera* fruit on total protein in female SD rats administered 800 mg/kg MSG

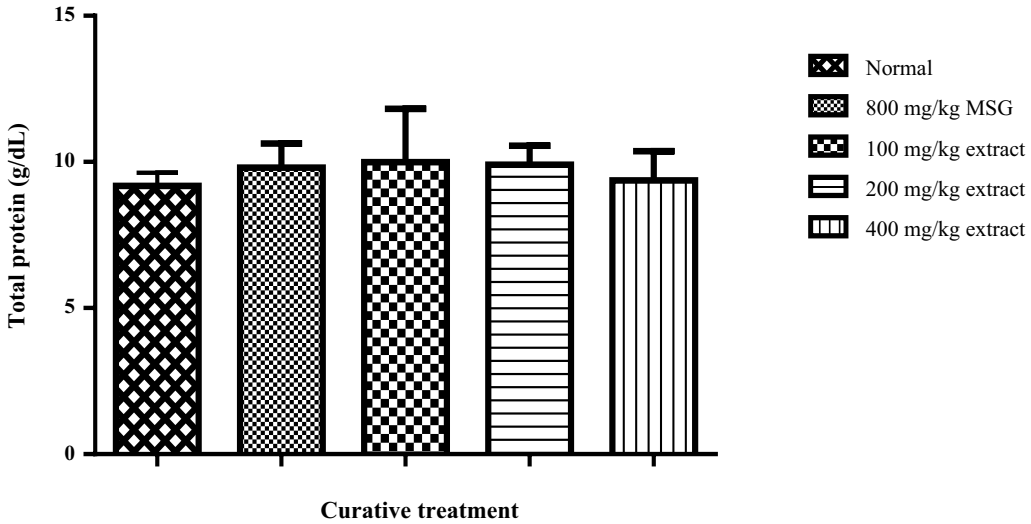

Fig. 5 Curative effect of the ethanol extract of *T. tetraptera* fruit on total protein in female SD rats pre-treated with 800 mg/kg MSG

in relation to the normal group (Fig. 6). Significant difference at $P \leq 0.05$ was observed between the MSG and the 400 mg/kg group. In the curative treatment, serum estradiol in the rats was raised by 90.68% ($P \leq 0.05$) in the MSG group. Pre-treatment of normal rats with MSG and treatment with the extract in graded doses, however, caused significant amelioration of this parameter as elevations of 62.94 and 20.39% were observed with 100 and 200 mg/kg doses (Fig. 7). At 400 mg/kg, there was a significant inhibition of serum estradiol elevation caused by MSG ($P \leq 0.05$). The extract dose-dependently decreased elevated serum estradiol to normal.

Histopathology results of the uterus

Section of the uterus showed normal tissue architecture: endometrial stroma (ES), endometrial lining (EL), endometrial glands (GL) and uterine cavity (UC). When the female rats were treated with 800 mg/kg MSG only, the sections showed thick bundles of smooth muscle fibers which were spindle-shaped (SP), arranged in haphazard fashion and crisscrossing the endometrial glands and stroma which is characteristic of leiomyoma.

Simultaneous administration of graded doses of *T. tetraptera* fruit and MSG showed an amelioration of the proliferating fibroid cells. The activity of the extract

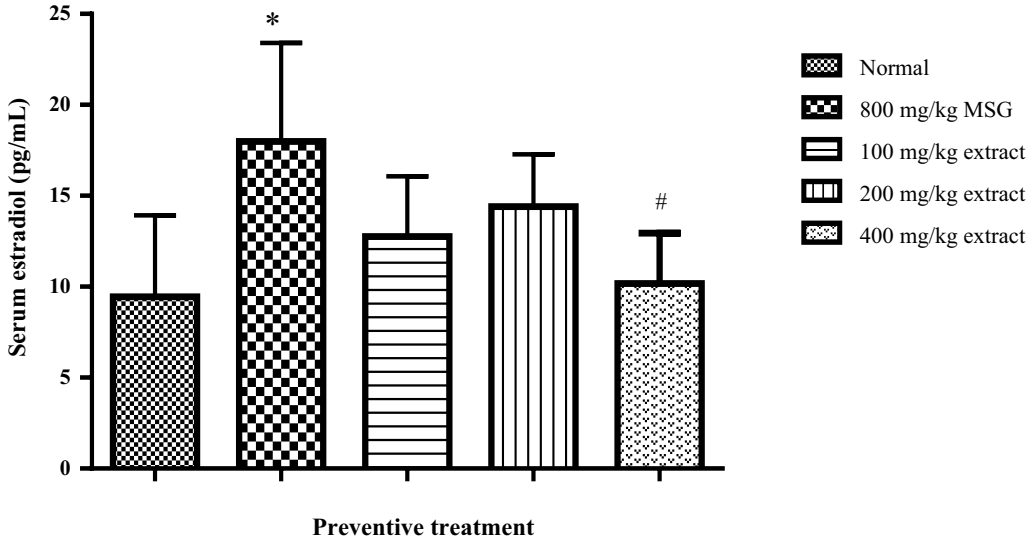

Fig. 6 Preventive effect of the ethanol extract of *T. tetraptera* fruit on total serum estradiol in female SD rats administered 800 mg/kg MSG. Compared with normal group $^{*}P < 0.05$, compared with MSG group.$^{\#}P < 0.05$

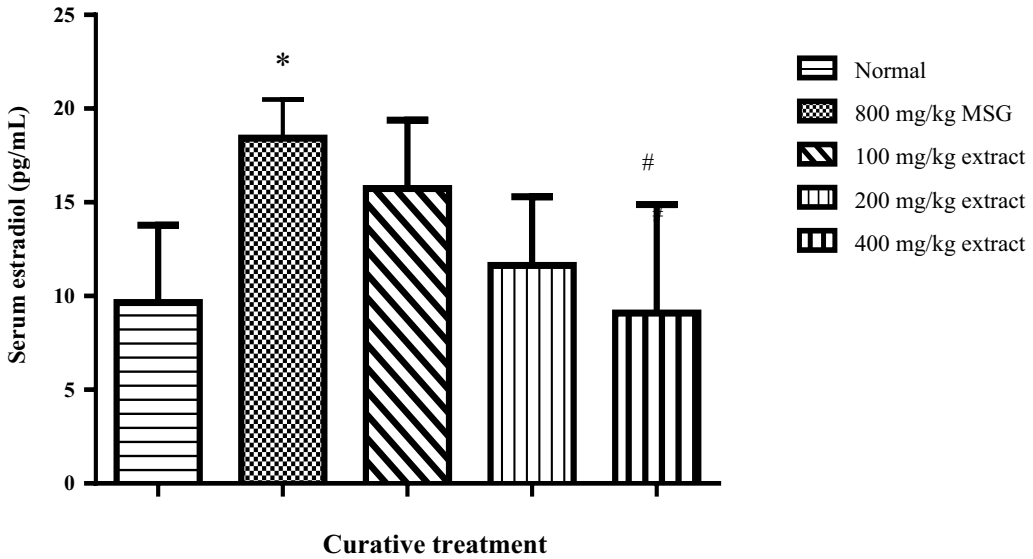

Fig. 7 Curative effect of the ethanol extract of *T. tetraptera* fruit on total estradiol in female SD rats pre-treated with 800 mg/kg MSG. Compared with normal group $^{*}P < 0.05$, compared with MSG group.$^{\#}P < 0.05$

was more evident in the curative experiment as the thick band of smooth muscle fibers indicating the formation of leiomyoma was minimal at 200 and 400 mg/kg and normal tissue architecture was mostly observed (Fig. 8).

HPLC analysis result

HPLC analysis of the ethanol extract of *T. tetraptera* revealed the presence of majorly umbelliferone, ferulic acid, echinocystic acid, piperazine, aridanin and naringenin. Other compounds present but in lesser amounts were octodrine, hentriacontane, butein and isoliquiritigenin (Table 6). HPLC chromatogram showing the various peaks of the identified compounds is presented in Fig. 9.

Discussion

Loss of body weight is typically seen to be a harmful impact of the extract on the animal, caused by less consumption of food and liquids. The extract induced a gradual rise in body weight, indicating the extract's relative

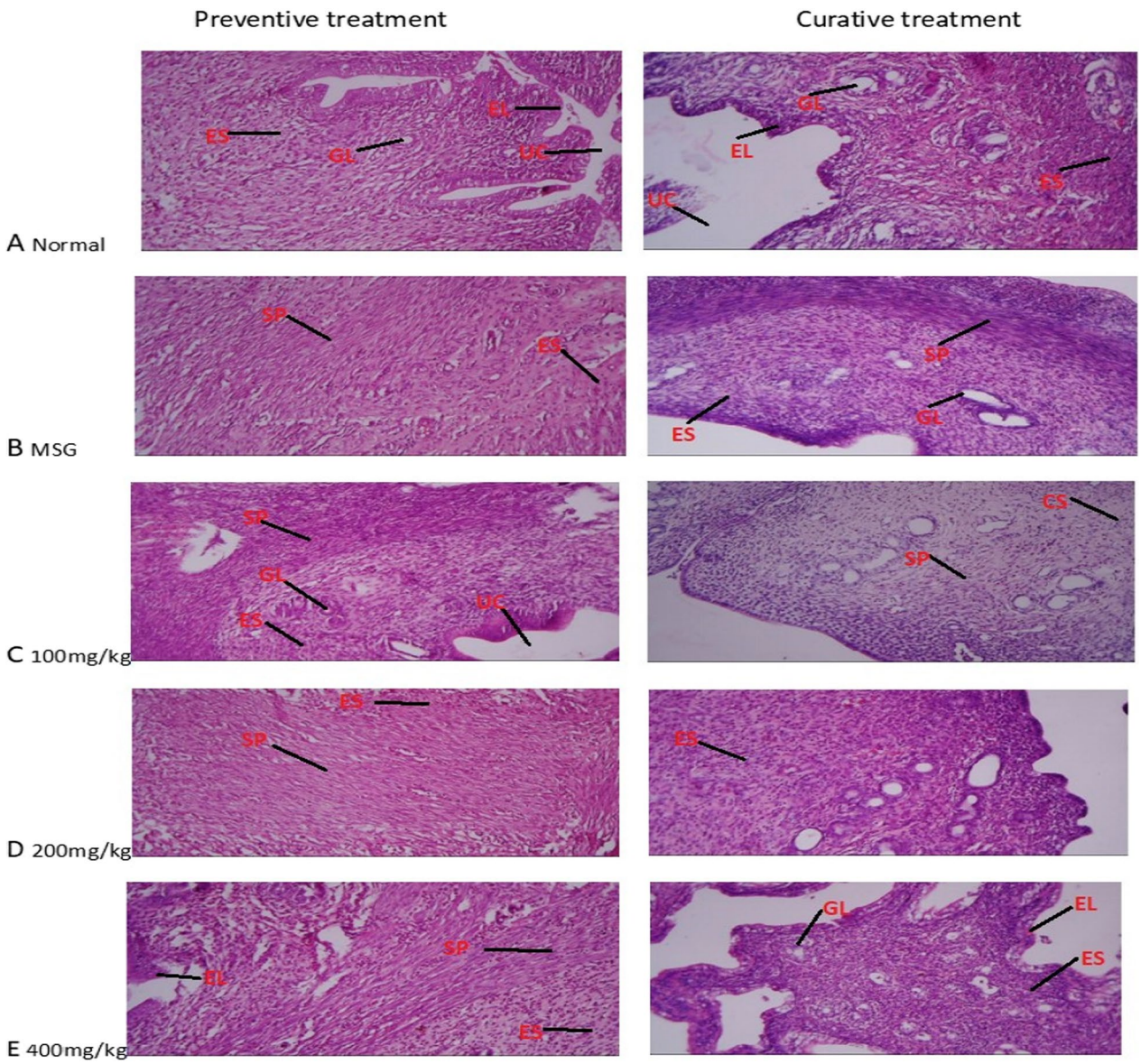

Fig. 8 H&E × 400 SD Rat uteri sections: Panel A shows normal uterus architecture: uterine cavity (UC), endometrial lining (EL), endometrial stroma (ES) , endometrial glands (GL) are seen. Panel B shows rat uterine wall given MSG only: composed of endometrial glands (GL) and stroma (ES) flanked by spindle-shaped proliferating bundles of smooth muscle fibers (SP) characteristic of leiomyoma. Panel C shows 100 mg/kg treated group: Composed of endometrial glands (GL) and stroma (ES) with reduced spindle-shaped proliferating bundles of smooth muscle fibers (SP) in both treatments. Panel D shows 200 mg/kg treated group: composed of stroma (ES) with mild form of proliferating bundles of smooth muscle fibers (SP). Panel E shows 400 mg/kg treated group: preventive group shows mild form of proliferating bundles while curative is mostly normal uterus architecture, uterine cavity (UC), endometrial lining (EL), stroma (ES), glands (GL)

safety for the rats; yet, there was a noteworthy distinction in the average weekly change in weight acquired ($p < 0.05$) when compared to the control group (Table 1). In fact, a number of earlier studies have shown that *T. tetraptera* fruit extract has antiobesity potential [19].

LDL, total cholesterol and triglycerides are three primary components of the lipid profile that are linked to cardiovascular disease. A dysregulated lipid metabolism is indicated by changes in LDL and HDL levels, which may be caused by interference with lipolysis and the release of free fatty acids from peripheral depots [20]. Although the levels of triglycerides, LDL, HDL and total cholesterol were not significantly different ($p > 0.05$) from the control group, other studies have shown that *T. tetraptera* fruit extract has the ability to decrease lipids [21]. It has been reported to elicit reduction in serum

Table 6 HPLC result of *T. tetraptera* fruit ethanol extract

S/N	Component	Retention time (mins)	Area	Height
1	Ferulic acid	1.350	303.6740	23.900
2	Echinocystic acid	1.650	422.7495	47.384
3	Umbelliferone	1.983	8447.0015	222.357
4	Piperazine	3.166	812.8600	25.900
5	Aridanin	4.016	303.6600	12.070
6	Octodrine	4.733	16.8530	0.600
7	Hentriacontane	6.350	115.4450	3.201
8	Naringenin	7.350	359.5600	11.188
9	Butein	8.616	56.3630	1.203
10	Isoliquiritigenin	9.616	41.3410	2.201

triacylglycerols as well as elevation of LDL in male rabbits [22].

The primary function of the kidneys is to remove the harmful waste produced by the normal functioning of the body and transported by the blood. In fact, additional research amply supports the capacity of plant extracts to function as potent free radical scavengers in the kidney, avoiding their harmful effects on lipid peroxidation, which raises biochemical markers like creatinine and urea by rupturing membranes [10]. *T. tetraptera* fruit has been reported to possess alkaloids, tannins, triterpenes, flavonoids, steroid glycosides and coumarins as dominant secondary metabolites [21, 23].

The protective effect of the extract could be explained by the antioxidant potential of some of *T. tetraptera* extract's constituents, such as flavonoids and total phenols, which would inhibit membrane lysis and contribute to the nephroprotective action of the extract [24]. This study's findings support those of another, which established that some methanol plant extracts protect kidney cells by inhibiting xanthine oxidase, an enzyme that causes lipid peroxidation and membrane instability [25].

The liver is essential for the detoxification and excretion of several endogenous and exogenous substances, and any harm or impairment to it can have a wide range of health effects on both humans and animals. Cellular necrosis, elevated tissue lipid peroxidation and glutathione depletion are linked to liver injury. Furthermore, liver illness is associated with increased serum levels of many biochemical indicators, including transaminases, alkaline phosphatase, triglycerides and cholesterol [26]. All of these parameters, at all tested doses, revealed no significant difference ($p > 0.05$) compared to the control. These findings suggest that the extract had no effect on the liver's ability to function normally because any hepatocellular damage would have raised ALT and AST levels in the serum [27]. The extract's phenolic components, which function as antioxidants to inhibit membrane lipid

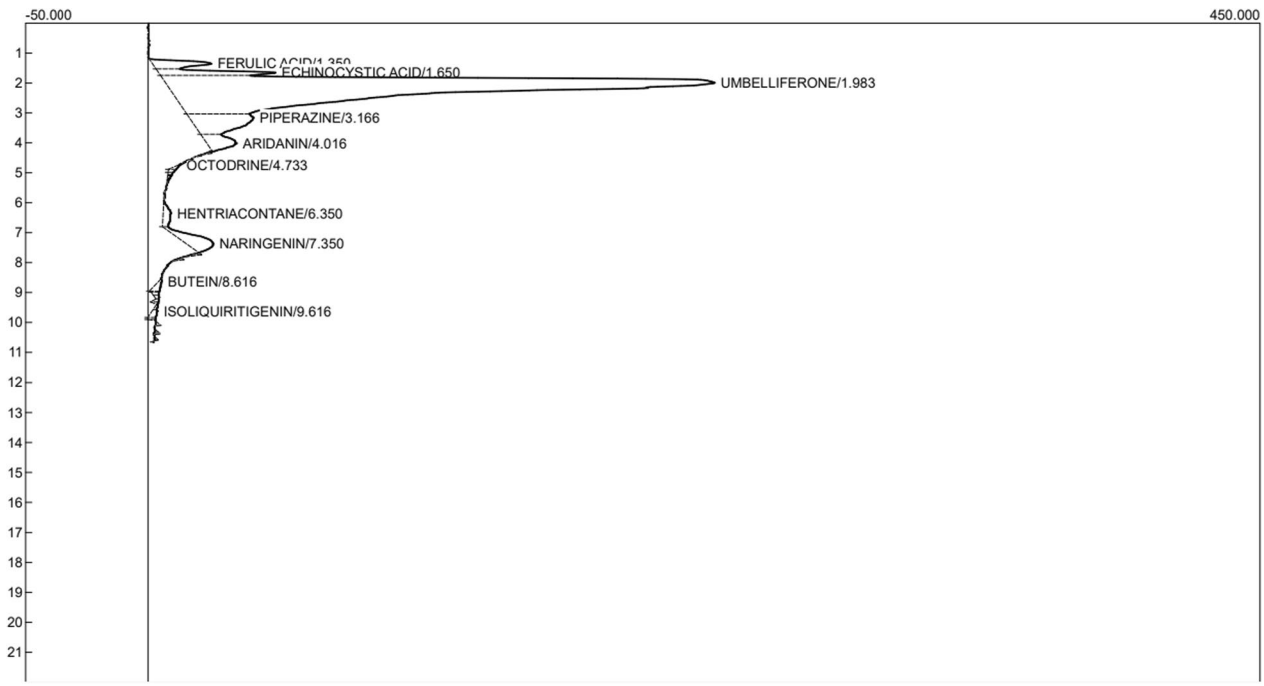

Fig. 9 High-performance liquid chromatography (HPLC) chromatogram of compounds in *T. tetraptera* ethanol fruit extract

peroxidation, may have a hepato-protective effect and could be the reason for this [24].

Significant changes in blood indices (white blood cells, red blood cells, platelets and their differentials) suggest that the chemical being administered is either toxic or protective to the hemopoietic tissue. The blood indices are used to monitor the physiological and pathological state of the body. Findings from our study report nonsignificant effects on most of the important blood indices by the ethanol extract of *T. tetraptera* fruit. The major functions of WBCs and its differential are to provide immunity and defend the body against invasion by pathogens or toxins. A significant reduction in WBC count at 200 and 400 mg/kg showed that this defense mechanism was not unusually elicited, which proves further the non-toxic nature of the extract. This is consistent with past studies that found that treating white rabbits with an ethanol extract of this plant's fruits reduced the number of white blood cells [22]. Conversely, the dichloromethane methanol extract of this plant resulted in a rise in WBC, which may be explained by the different chemicals that the extraction solvent produced [28]. The extract's nonsignificant effect on the RBC could mean that there was no change in the balance between blood corpuscle destruction to erythropoiesis and the rate of blood production. HGB, PCT and MCH levels did not significantly decrease ($p > 0.05$) in female rats treated with the various doses; this could indicate that hemoglobin incorporation into red blood cells and red blood cell morphology were unaffected [16]. The dichloromethane methanol extract showed an alteration in this regard.

Toxicity generally happens at the cellular and sub-cellular levels before being seen in tissues. In this study, the low extract dose levels (200 and 400 mg/kg) exhibited little or no significant effect on the histomorphology of the vital organs (Fig. 1). The presence of activated Kupffer cells in the sinusoids at 400 mg/kg is evidence of a boost in the immune system; this can be seen as an extra quality of the extract. Normal architecture observed in the kidney at all doses used shows the level of safety of the extract. This is in tandem with the normal features reportedly observed with the dichloromethane methanol extract of the fruit [28].

The potential benefits of TTF in preventing uterine fibroids were examined in this study. A vital building block for the manufacture of several steroid hormones, which are potent signaling molecules that control a number of bodily processes, is cholesterol [29]. A rise in total serum cholesterol is usually attributed to the activation of the enzyme 3-hydroxyl-3-methoxylglutamyl-CoA reductase (HMGR) which catalyzes the conversion of HMG-CoA to mevalonate; this is the rate-limiting step of cholesterol synthesis [17].

It has been demonstrated that ovarian steroid hormones are important molecular indicators linked to the formation and proliferation of uterine fibroids. The formation and development of uterine fibroids has been attributed primarily to estrogen, but progesterone and its receptors may also have a significant impact [10].

Due to its ability to bind to ERα receptors in the uterus and form a complex that interacts with DNA in the nucleus to activate transcriptional promoter and enhancer regions that govern gene expression, estradiol is unique in its ability to promote the growth of uterine cells. This enables RNA polymerase II binding and the subsequent start of transcription, which results in the production of proteins and higher uterine and ovarian cell proliferation [10].

T. tetraptera fruit extract at graded doses of 100, 200 and 400 mg/kg was evaluated in this study for its ability to inhibit the effect of MSG on these biochemical parameters tested as well as the histology of the uterus because it has been reported to be non-toxic at oral doses as high as 5000 mg/kg [30].

There was a significant ($P < 0.05$) increase in serum cholesterol levels of the MSG group compared with the normal group (Fig. 2). Treatment with the ethanol extract of *T. tetraptera* fruit reduced the elevated cholesterol levels almost to normal ($P > 0.05$) in our study, in both the curative and preventive experiments. The fruit extracts ability to lower cholesterol may be due to a decrease in dephosphorylated HMGR levels as well as an adverse effect on cholesterol production caused by the activation of glucagon and adrenaline [11]. Indeed, the fruit of this plant has been shown in numerous earlier studies to have a lipid-lowering impact [21].

Total protein content in the MSG and treated groups showed no significant difference in relation to the normal. This is similar to results obtained in previous works [7, 17].

Treatment with the ethanol extract of *T. tetraptera* fruit reduced the elevated estradiol levels in a dose-dependent manner (Fig. 6), in both the curative and preventive experiments. Its action on estradiol could possibly be attributed to suppression of the enzyme aromatase responsible for aromatization of androstenedione and testosterone to estrogens in the biosynthesis of estradiol from cholesterol [4]. It might also be due to an inducer of liver microsomal enzyme that increases the metabolism of estradiol, or it might contain phytochemicals that act as gonadotropin-releasing hormone (GnRH) agonists, which when stimulated continuously reduce the expression or downregulates GnRH receptors on the anterior pituitary [31]. Consequently, less estradiol would be produced. The decline in cholesterol production may potentially also be the cause of the estradiol decline.

Additional histology investigations demonstrated *T. tetraptera's* impact on the proliferation of leiomyoma cells in the uterus. A section of the uterus in rats given only food and water revealed normal tissue architecture, but after the female rats were administered 800 mg/kg of MSG, the sections revealed thick bands of spindle-shaped, haphazardly arranged smooth muscle fibers that crisscrossed the endometrial glands and stroma, a characteristic of the formation of leiomyoma.

Gradually increasing dosages of the extract and MSG were administered simultaneously, and the results showed a dose-dependent, stepwise improvement of the proliferating leiomyoma lesion, with the highest dose having the most effective impact. The curative treatment showed a greater reduction in the production of leiomyoma cells than the preventive treatment (Fig. 8).

T. tetraptera fruit has been observed to contain flavonoids, alkaloids, tannins, saponins, steroids, sterols and phenols [23, 28].

Part of the health benefits of saponins include immune system activation and a reduction in cholesterol levels in the body [21]. Additionally, studies have shown that saponin inhibits the enzyme aromatase [14], which is involved in the production of estrogen. Our results are supported by a previous investigation that found a substantial reduction in estrogen induced by a methanol extract of *T. tetraptera* fruit [32].

Phenols' antioxidant properties are also crucial in preventing chronic diseases because they can shield essential molecules like DNA, lipids and proteins from oxidative damage brought on by reactive oxidant species. While dietary antioxidants can have preventive effects, a low-antioxidant diet can raise the incidence of uterine fibroids [33].

HPLC analysis of the extract of *T. tetraptera* revealed the presence of major constituents such as umbelliferone, ferulic acid, echinocystic acid, aridanin and naringenin, with hentriacontane, butein and isoliquiritigenin present in reduced amounts (Table 6).

It has been found that the phenylpropanoid umbelliferone possesses antioxidant qualities and effectively inhibits type 3 17β-hydroxysteroid dehydrogenase, which is the main enzyme responsible for converting 4-androstene-3,17-dione into testosterone [34].

Ferulic acid is also a phenolic substance with a variety of biological activities, especially in oxidative stress and inflammation, and also plays an antifibrosis role [35].

Echinocystic acid has been reported to possess antiviral, anti-inflammatory and antioxidation activities [13] while naringenin, a flavanone, has anti-inflammatory, antioxidant and antiproliferative activities [36], and it also showed lipid-lowering properties. Butein, hentriacontane and isoliquiritigenin have numerous pharmacological properties including anti-inflammatory and antioxidative activities [13].

In conclusion, the decreased level of cholesterol and estradiol by effect of *T tetraptera* fruit extract contributed largely to decreased uterine leiomyoma proliferation. Further studies to determine its exact mechanism as antifibrotic agent against uterine leiomyoma needs to be established.

Conclusion

When administered acutely, *T. tetraptera* ethanol fruit extract is safe. The biochemical and hematological markers examined did not show any discernible alterations; nonetheless, long-term high-dose consumption may have harmful consequences on organs like the heart and uterus. This work aimed to demonstrate the antileiomyoma activity of the ethanol extract of *T tetraptera* fruit on Sprague Dawley rats. The results shown suggest that the extract is effective in reducing the biochemical parameters elevated by MSG and also inhibiting the formation of leiomyoma cells. This validates its usage in ethnomedicine in the management of uterine leiomyoma.

Abbreviations

MSG	Monosodium glutamate
SD rats	Sprague Dawley rats
TTF	*Tetrapleura tetraptera* fruit
HPLC	High-performance liquid chromatography
HMGR	3-Hydroxyl-3-methoxylglutamyl-CoA reductase
GnRH	Gonadotropin-releasing hormone

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Author contributions

ROI, BAA, and AU conceptualized the project and designed the study and methodology, SI, AAU, OVA and JMA performed the study, ROI performed the statistical analysis and data interpretation, ROI wrote the paper, and the final manuscript was proofread and approved by all authors.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Approval for the use of experimental animals was obtained from the Research Ethics Committee of the Faculty of Pharmacy, University of Benin, Nigeria (EC/

FP/023/04). Appropriate permission to research on the study plant was duly solicited from the local legislations.

Competing interests
Authors declare no conflict of interest

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