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Effect of Ethanolic Stem Bark Extract of *Blighia unijugata* (Sapindaceae) on Monosodium Glutamate-Induced Uterine Leiomyoma in Sprague-Dawley Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors GAK and KA designed, wrote the protocol, supervised the study, performed the statistical analysis, and wrote the first draft of the manuscript. Author JOK managed the TLC and HPLC analyses of the study. Authors HKF and EE managed data collection, the literature searches, and laboratory work. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To establish the presence of monosodium glutamate (MSG) as an ingredient of artificial food seasonings on the Ghanaian market, and to evaluate the anti-fibroid property of an ethanolic stem bark extract of *Blighia unijugata* on MSG-induced uterine leiomyoma in Sprague-Dawley rats and its safety for use.

Study Design: Survey and Experimental.

Place and Duration of Study: Department of Pharmacology, CHS; September 2012 and

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Methodology: A survey was conducted to ascertain MSG as an ingredient of Food and Drugs Board approved artificial food seasonings on the Ghanaian Market. Phytochemical screening was performed on an ethanolic, aqueous, and petroleum ether extract of *B. unijugata*. Thin layer and high performance liquid chromatographic analysis were performed on the ethanolic extract of *B. unijugata* (EBU), selected after phytochemical screening, to obtain fingerprint chromatograms for identification. Preventive and curative studies (measuring total plasma cholesterol and plasma estradiol and uterus weight) using 50 and 100 mg kg⁻¹ EBU, *per os*, on 600 and 800 mg kg⁻¹ MSG-induced uterine leiomyoma in Sprague-Dawley rats was conducted. Acute and Delayed toxicity on EBU was tested.

Results: Of 21 FDB approved artificial food seasonings, 85.7% had MSG as an ingredient. MSG administration to rats elevated significantly ($P \leq .001$) cholesterol, estradiol and uterus weight and size (indicating hyperplasia). Curative treatment reduced significantly ($P \leq .01-.001$) the elevated plasma cholesterol and estradiol than preventive treatment. Both treatments however significantly decreased ($P \leq .01-.001$) elevated uterus weight. The lethal dose was less than 1000 mg kg⁻¹ *p.o.*

Conclusion: MSG is found in almost all artificial food seasoning on the Ghanaian market which could be a risk factor to the development of uterine leiomyoma. The ethanolic extract of *Blighia unijugata* reversed hyperplasia induced in the uterus by MSG, making it useful as an anti-fibroid drug.

Keywords: Uterine fibroid; artificial food seasoning; monosodium glutamate; total plasma cholesterol; plasma estradiol.

1. INTRODUCTION

Uterine leiomyoma commonly termed uterine fibroid constitutes a significant reproductive threat in women worldwide. It is the most common uterine tumour described in humans [1, 2]. It is a benign smooth muscle neoplasm, which is frequently found in approximately 50% of fertile women. It is the leading indication for hysterectomies in women of reproductive age [3]. It is a benign monoclonal tumor of the smooth muscle cells found in the uterus. It grows in various locations on and within the uterine walls or in the uterine cavity. It is therefore described as subserosal, submucosal or intramural fibroids. It can be of any size and shape ranging from the size of a pea to an average-sized water melon [4].

A study conducted by Ofori et al. [5] at the Department of Obstetrics and Gynaecology, Korle-Bu Teaching Hospital, Accra, Ghana revealed that out of 584 trans-abdominal pelvic ultrasound scanned images of women analyzed, 143 representing 24.5% were confirmed to have fibroid. The highest prevalence (53.8%) of the fibroid case was found among women aged 30-39 years and the lowest were recorded among women aged 49 years and above. This situation has aroused considerable medical interest and has been considered as a public health problem.

Although pathogenesis of uterine leiomyoma formation remains poorly understood, considerable evidence from epidemiological studies suggest that estrogen and progesterone proliferate the tumour growth, due in part to continuous menstrual cycling, as the fibroids rarely appear before menarche and regress after menopause [6]. Molecular studies have

identified differences in hormone receptor expression and further support a hormone mediated mechanism.

Risk factors for uterine leiomyoma include age, familial history, ethnic origin (African-American women are more likely to develop uterine fibroids than Caucasian women) [7], obesity, diet, lack of exercise, some chemicals (e.g. Monosodium glutamate), some prescription drugs that may lead to an increase in the levels of total protein, cholesterol and estradiol [4]. Monosodium Glutamate (MSG) is a salt of glutamate, synthesized from L-glutamic acids and used as a flavour enhancer in foods [8-10]. Various processed and prepared foods such as traditional seasonings sauce and certain restaurant foods contain significant levels of free glutamate (as MSG), both from natural sources and from added monosodium glutamate [9,10]. Arising from high cholesterol levels caused by Monosodium Glutamate (MSG), there are endocrinological disorder [10].

The majority of women with uterine fibroids are asymptomatic, consequently they get less clinical attention and fibroid tumors often remain undiagnosed. Symptomatic women typically complain about abnormal uterine heavy bleeding (which may lead to anemia) or painful periods, feeling of fullness in the pelvic area, enlargement of the lower abdomen, frequent urination, pain during sexual intercourse, lower back pain, complications during pregnancy and labour, reproductive problems such as infertility. The severity of these symptoms depends usually on the location and size of the fibroids [11, 12].

Treatment options and preventative measures are very limited. Myometry (Abdominal myomectomy, Laparoscopic myomectomy or Hysteroscopic myomectomy) and hysterectomy are often suggested by doctors. Treatment by using medication (Hormone replacement therapy) is only effective for 6-12 months since considerable side effects develop from long term usage [13]. Uterine Artery Embolization as well as High Intensity Focused Ultrasound Ablation can be used [14]. The risks and the cost of these procedures could deter a patient from undergoing such treatment. In addition to cost, adverse effects and temporal symptomatic remedy offered by the orthodox drugs calls for a better treatment option with less risk and adverse effects on the individual coupled with high accessibility and relatively cheaper to afford.

Yarrow (*Achillea millefolium*), Nettles (*Urtica dioica*), Shepherd's Purse (*Capsella bursa pastoris*), Lady's Mantle (*Alchemilla vulgaris*), Cinnamon (*Cinnamomum zeylanicum*) and Bilberry (*Vaccinium myrtillis*) have been recorded to help in reducing prolonged or heavy bleeding associated with uterine fibroids. Anecdotal evidence of *Blighia unijugata* suggests its use in the treatment of uterine fibroid but not based on scientific evidence.

Blighia unijugata (Sapindaceae) is widespread in tropical Africa, extending from Guinea Bissau eastwards to Ethiopia and Kenya, and through DR Congo southwards to Angola, Zimbabwe and Mozambique and South Africa [15]. It is a tree, 3-18 metres high, flowers whitish; very fragrant; calyx-lobes about 1 mm long; petals 1-1.5 mm long; ripe fruits red or pinkish red with three (3) shining black seed, each with a yellow aril [16]. It is a forest tree. The bark pulp is used as an enema or is macerated by draught and taken as febrifuge and purgative. The seeds, because of their oil content, and the jacket because of its potash content, are burned and the ashes used in making soap. It is also recognized for its sedative and analgesic properties in the treatment of rheumatism [17]. The roots are used to treat post-partum bleeding (haemorrhage) and boils, and the seeds to treat vomiting [16].

The aim of this study was to establish the presence of monosodium glutamate (MSG) as an ingredient of artificial food seasonings on the Ghanaian market, to evaluate the anti-fibroid property of an ethanolic stem bark extract of *Blighia unijugata* on MSG-induced uterine leiomyoma in Sprague-Dawley rats and to ascertain its safety for use.

2. MATERIALS AND METHODS

2.1 Presence of MSG in Artificial Food Seasoning in Ghana

A survey to establish the presence of MSG as an ingredient of various FDB registered artificial food seasonings was conducted at the Ayigya market, located at Ayigya, a suburb in the Kumasi metropolis of the Ashanti Region of Ghana, in September, 2012, by inspecting the various artificial food seasonings from vendors at the market place. Twenty one (21) products were sampled.

2.2 Anti-leiomyoma Property of *Blighia unijugata*

2.2.1 Plant collection

The stem bark of *Blighia unijugata* was collected from South Suntreso, a suburb in the Kumasi Metropolis of the Ashanti Region of Ghana, by Mrs Juliana Nkrumah Gyimah (Madam Adutwumwaa), a renowned Herbalist in the Kumasi Metropolis and identified at the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, Ghana. The bark was air dried for use in this study.

2.2.2 Preparation of *Blighia unijugata* stem bark extract

The dried stem bark of *Blighia unijugata* was powdered using a hammer mill (Schutte Buffalo, New York, USA). Seven hundred (700) gram quantities of the powder were extracted with 70% ethanol, distilled water, or petroleum ether using cold maceration and concentrated in a rotary evaporator (Rotavapor R-210, Buchi, Switzerland). The extracts were finally dried in a Gallenkamp hot air oven (Oven 300 plus series, England) at 40°C and labeled EBU, ABU, and PBU respectively for this study. The weight of residue and percentage yield obtained for EBU were 24.39 g and 3.48% respectively ABU and PBU were 8.77 g and 4.39%, and 1.24 g and 0.62% respectively.

2.2.3 Phytochemical screening

EBU, ABU and PBU were subjected to phytochemical screening in accordance with the standard procedure described by Wagner and Bladt [18], Harborne [19], and Kujur et al. [20]. EBU, being the extract with the highest number of phytochemicals, was selected and subsequently used as the extract in this study.

2.2.4 Thin layer chromatography

Aluminium precoated silica gel plates 60 F254 (0.25 mm thick) was cut to an appropriate size so as to fit in a chromatank. EBU (5 mg) was constituted in ethanol (95%) and applied on the TLC plates as spots with the aid of capillary tubes at one end of the plate in a straight line about 2 cm above the edge and 1.5 cm away from the margins. The spots were dried and the plates placed a chromatank saturated with the mobile phase (a mixture of ethyl

acetate, chloroform and glacial acetic acid in the ratio 3:1:1) for development by the one way ascending technique. The zones on TLC plates corresponding to separated compounds were detected under UV light 254 nm and also by spraying with anisaldehyde 0.5% w/v in $\text{CH}_3\text{COOH}/\text{H}_2\text{SO}_4/\text{CH}_3\text{OH}$ (10:5:85) followed by heating at 105°C for 5-10 minutes.

2.2.5 High performance liquid chromatography

Approximately 2 ml of a 0.1% w/v ethanolic (absolute) solution of EBU was transferred into a 1cm square cuvette and placed in a double beam UV machine (T90 + UV/Visible Spectrophotometer, PG Instruments Ltd., UK) to obtain a UV/Visible spectrum. The wavelength of maximum absorption was selected from the spectrum and used as the wavelength for the HPLC determination. The HPLC set up consisted of an LC-10AD Shimadzu pump (Shimadzu Corporation, Kyoto, Japan) connected to a Perkin Elmer 785A UV/Visible detector (Perkin Elmer Inc., USA), with a phenyl column (Zorbax, 3.0 x 150 mm x 3.5 microns) and methanol:water (50:50) as the stationary phase and mobile phase respectively. A 20 μl quantity of the sample was analyzed isocratically at a wavelength of 278 nm (flow rate of 1 ml min^{-1}) and a chromatogram obtained.

2.2.6 Animals and husbandry

Female Sprague-Dawley rats (non-pregnant) weighing 180-240 g were obtained from the Animal House of the Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, Ghana. They were relocated from the Animal Rearing Section to the Experimental Study Section of the Animal House a week before the study to make them adapt to the new environment. They were housed in groups of eight (8) in stainless steel cages with wood shavings as the bedding material and a wire screen top. The cages were adequately ventilated and kept at a room temperature and relative humidity of 24-28°C and 60-70% respectively, with a natural light-dark cycle. Good hygiene was maintained. The animals were fed with commercial pellet rat feed (GAFCO, Tema, Ghana) and clean water *ad libitum*.

2.2.7 Dosing of experimental animals

Doses of EBU in this study (i.e. 50 and 100 mg kg^{-1}) were selected based on a preliminary study and acute toxicity study conducted. Sprague-Dawley rats were dosed by gavage. Dosing was once daily over the experimental period at a volume of 2 ml kg^{-1} body weight. Individual dose volumes were calculated based on the animal's most recent recorded body weight. The oral route of administration was used because it is the intended human exposure route.

2.2.8 Experimental procedure

2.2.8.1 Preventive study

Female rats were put into seven groups of five. Group A was the no treatment group (Control). Groups B and C were treated with either 600 or 800 mg kg^{-1} MSG only. Groups D, E, F, and G were treated concomitantly with MSG and EBU as in Table 1. MSG doses were selected based on preliminary studies using the limit of ingestion of the chemical written on the product package. All treatments were conducted for 30 days after which total plasma cholesterol and plasma estradiol were determined. The uterus was harvested, photographs taken, and uterus weight-to-body weight ratios were determined.

2.2.8.2 Curative study

Female rats were put into seven groups of five. Group A was the no treatment group (Control). Groups B and C were treated with only 600 or 800 mg kg⁻¹ MSG respectively. Groups D-G was pretreated with either 600 or 800 mg kg⁻¹ followed by either 50 or 100 mg kg⁻¹ EBU as shown in Table 1. Treatment with MSG was conducted for 30 days followed by EBU for 30 days after which total plasma cholesterol and plasma estradiol were determined. The uterus was harvested, pictures taken, and uterus weight to body weight were determined

Table 1. Grouping for preventative and curative study

	Preventive	Curative
A	No Treatment	No Treatment
B	600 mg kg ⁻¹ MSG only	600 mg kg ⁻¹ MSG only
C	800 mg kg ⁻¹ MSG only	800 mg kg ⁻¹ MSG only
D	600 mg kg ⁻¹ MSG + 50 mg kg ⁻¹ EBU	600 mg kg ⁻¹ MSG pre-treated/50 mg kg ⁻¹ EBU
E	800 mg kg ⁻¹ MSG + 50 mg kg ⁻¹ EBU	800 mg kg ⁻¹ MSG pre-treated/50 mg kg ⁻¹ EBU
F	600 mg kg ⁻¹ MSG + 100 mg kg ⁻¹ EBU	600 mg kg ⁻¹ MSG pre-treated/100 mg kg ⁻¹ EBU
G	800 mg kg ⁻¹ MSG + 100 mg kg ⁻¹ EBU	800 mg kg ⁻¹ MSG pre-treated/100 mg kg ⁻¹ EBU

2.2.8.3 Determination of total plasma cholesterol

A day after the experimental period (30 days for preventive treatment and 60 days for curative treatment), the animals were euthanized. Blood was collected from the jugular vein into MediPlus K3 EDTA tubes (Sunphoria Co. Ltd., Taiwan). Plasma was obtained by centrifugation (Hettich Zentrifugen, Germany) at 3220 rpm for 20 min. Total cholesterol was assayed using Cromatest[®] (LiNEAR Chemicals, Spain). All reagents, standards and plasma were brought to room temperature and 1 ml of Monoreagent pipetted into test tubes labeled blank, samples and standard. 10 µl of samples and standard were pipetted and added into respective labeled test tubes, mixed well and incubated for 5 minutes at 37°C. The absorbance of the standard and the samples were read at 500 nm against the reagent blank. The total cholesterol concentration of the sample was calculated as follows:

$$\text{Total Cholesterol (mg dl}^{-1}\text{)} = (A_{\text{sample}}/A_{\text{standard}}) \times C_{\text{standard}}$$

Where A = Absorbance; and C = Concentration

2.2.8.4 Determination of estradiol (estrogen)

A day after the experimental period (30 days for preventive treatment and 60 days for curative treatment), the animals were euthanized. Blood was collected from the jugular vein into MediPlus K3 EDTA tubes (Sunphoria Co. Ltd., Taiwan). Plasma was obtained by centrifugation (Hettich Zentrifugen, Germany) at 3220 rpm for 20 min. 17β-estradiol was assayed using the fortress 17β-estradiol assay kit (Fortress Diagnostics Limited, UK).

Plasma, controls and all reagents were brought to room temperature, 25 µl of plasma references and controls were dispensed into their respective wells. 50 µl of estradiol biotin reagent was added to all wells and swirled gently for 30 s to mix. 50 µl of estradiol enzyme reagent was added to all wells and mixed after incubating at 28°C for 30 min. The wells were covered with a foil and incubated at 28°C for 90 min. When incubation had been complete,

the foil was removed, and the content of wells aspirated using an automatic washer (Rayto RT-3100, China) with each well washed three (3) times with 350 μ l wash Buffer. After this 100 μ l of working substrate solution was dispensed into each well in the same order and incubated for 20 min at room temperature. 50 μ l stop solution was added into all wells in the same order, swirled gently to mix for 20 s. The absorbance of the specimen was measured at 450 nm using a microplate reader (Rayto RT-2100C, China) within 30 min after addition of the stop solution. The values of the samples were obtained from a graph constructed using the standards.

2.2.8.4 Determination of uterus weight to body weight ratio

The female rats were weighed before they were sacrificed and dissected to obtain the intact uterus, fallopian tubes and ovaries. The wet organs weights were taken and the organ weight to body weight ratio was calculated

2.2.9 Acute and delayed toxicity studies

EBU, reconstituted in distilled water, was administered orally to 5 groups of rats ($n = 6$) at dose levels of 10, 50, 100, 500 and 1000 mg kg^{-1} . Control group received 2 ml/kg Distilled water *per os*. The animals were observed for up to 24 hours (acute) and subsequently for 14 days to observe possible delayed toxicity. The time of onset, intensity, and duration of these symptoms, if any, was recorded.

2.2.10 Data analysis

Bar graphs were obtained by the software Graph Pad Prism for Windows version 5 (GraphPad Software, San Diego, CA, USA) were subjected to One-Way Analysis of Variance (ANOVA) with Dunnet's *post hoc* test. $P \leq .05$ was considered statistically significant in all analysis.

3. RESULTS AND DISCUSSION

3.1 Survey on Artificial Food Seasonings

Of the 21 sampled artificial food seasonings on the Ghanaian market, 18(85.7%) were identified to contain MSG while 3 (14.3%) had no ingredient inscriptions on them; "no ingredient inscription" does not mean MSG is not present (Table 2). This reveals the abundance of MSG containing artificial food seasonings being consumed by Ghanaian. This may be a contributory factor to the high prevalence of the development of uterine leiomyoma (fibroids) in women in Ghana [5].

3.2 Phytochemical Analysis

The phytochemical screening aimed at identifying the secondary metabolites within the stem barks of *Blighia unijugata* was carried out to set the tone for further investigation into finding the specific secondary metabolites responsible for the therapeutic activity of the plant under investigation. Triterpenoids and glycosides were present in EBU, ABU and PBU while alkaloids, tannins and flavonoids were absent in all. Only EBU and ABU contained saponins while phytosterols were found present in only EBU and PBU. EBU however had glycosides, phytosterols, saponins and triterpenoids (Table 3). EBU thus had more phytochemicals

which could possibly give it better therapeutic value than ABU and PBU. EBU was therefore selected for this study.

Table 2. Artificial food seasonings and the presence or absence of MSG

Product name	MSG(+ or -)
Adjo spice seasoning powder	+
Benny-chicken and beef seasoning	+
Big mama tomato seasoning powder	+
Calhort seasoning	+
Chasito seasoning	+
Cito seasoning	+
Euroman seasoning	+
For you tomato stew seasoning	+
Heena	-
Jumbo cubes	+
Larsor seasoning	+
Maggi-cube & seasonings	+
Milan kwality prawn flavor	+
Nappa valley-beef flavor	+
Onga –cubes and seasoning	+
Royco cube	-
Super A-1	+
Super Mona	-
Tak tak tak seasoning	+
Tasty cubes	+
Xinle msg	+

(-): No ingredient written on package (+): MSG present

Table 3. Phytochemical screening of EBU, ABU and PBU

Phytochemicals	Characterization test results		
	EBU	ABU	PBU
Alkaloids	-	-	-
Flavonoids	-	-	-
Glycosides	+	+	+
Phytosterols	+	-	+
Saponins	+	+	-
Tannins	-	-	-
Triterpenoids	+	+	+

(-): absent; (+): present.

3.3 Thin Layer and High Performance Liquid Chromatography

Five separated spots were identified after TLC analysis with the highest and lowest R_f values of 0.88 and 0.13 respectively (Table 4). Two peaks which were not distinct were observed for EBU under the stated HPLC parameters. The HPLC analysis was performed using a UV detector at wavelength 278 nm. The chromatogram (Fig. 1) suggests the presence of secondary metabolites with chromophoric moieties in the extract. This chromatogram was developed at ambient temperature using a mobile phase of methanol: water (50:50) at 1 ml

min⁻¹. The chromatogram obtained can be employed as a qualitative tool for the quality control of the ethanolic stem bark extract of *Blighia Unijugata* (Sapindaceae). TLC and HPLC standardize the extract and give an indication that the extract is phytochemically active.

Table 4. R_f values obtained for EBU after TLC analysis

Spot	R _f values
A	0.13
B	0.25
C	0.65
D	0.78
E	0.88

Solvent system EtOAc: CHCl₃: Glacial CH₃COOH (3:1:1), visualizing under UV 254 nm

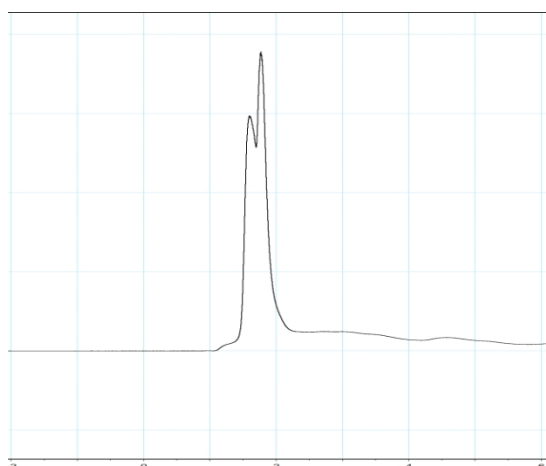


Fig. 1. HPLC Chromatogram of EBU. Stationary phase: SB phenyl column; Mobile phase: CH₃OH: H₂O (50:50); $\lambda = 278$ nm, Flow rate: 1 ml min⁻¹; Temperature: Ambient

3.4 Effect of EBU on MSG-treated Rats (Preventive and Curative)

3.4.1 Total plasma cholesterol

There was very significant elevation 31.57% ($P \leq .001$) and 31.58% ($P \leq .001$) in cholesterol level by treating normal female rats with 600 and 800 mg kg⁻¹ MSG respectively. A preventive treatment (100 mg kg⁻¹ EBU in the 600 mg kg⁻¹ MSG-treated, and 50 mg/kg EBU in the 800 mg/kg MSG-treated rats) caused significant ($P \leq .001$) lowering of elevated plasma cholesterol. Levels however did not reduce below normal (Control). All doses of EBU in curative treatment however decreased elevated plasma cholesterol to normal and further decreased significantly ($P \leq .01$) levels to below normal in both the 600 and 800 mg kg⁻¹ MSG-treated rats (Figs. 2 and 3). This indicates that there is a drastic reduction in plasma cholesterol associated with a curative treatment (than a preventive treatment) with EBU.

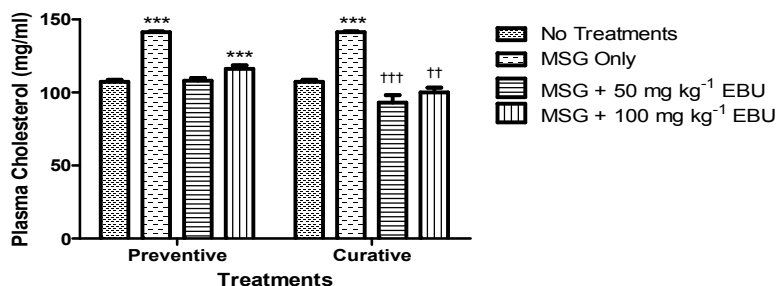


Fig. 2. The preventive and curative effects of the ethanolic extract of *Blighia unijugata* (EBU) on total plasma cholesterol in female Sprague- Dawley rats pre-treated with 600 mg kg⁻¹ MSG

Test drugs: significant from normal control, Increments: *** $P \leq .001$; For decrements below values observed in control group: †† $P \leq .01$, ††† $P \leq .001$

Mean \pm S.E.M = Mean values \pm Standard error of means of five experiments

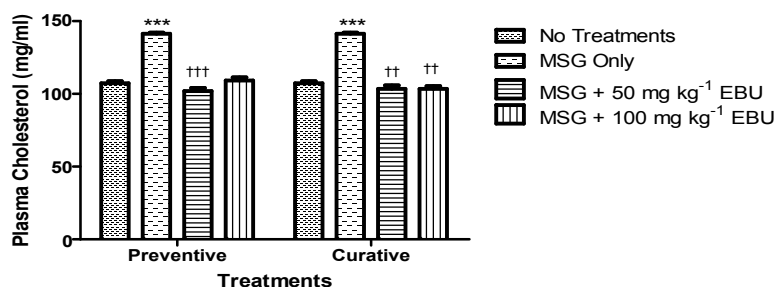


Fig. 3. The preventive and curative effects of the ethanolic extract of *Blighia unijugata* (EBU) on total plasma cholesterol in female Sprague-Dawley rats pre-treated with 800 mg kg⁻¹ MSG

Test drugs: significant from normal control, Increments: *** $P \leq .001$; For decrements below values observed in control group: †† $P \leq .01$, ††† $P \leq .001$

Mean \pm S.E.M = Mean values \pm Standard error of means of five experiments

A rise in total plasma cholesterol is usually attributed to the activation of the enzyme 3-hydroxyl-3-methoxylglutamyl-COA reductase (HMGR). By covalent modification from its phosphorylated (inactive) state to dephosphorylated (active) state [21], HMGR catalyzes the conversion of HMG-COA to mevalonate, the rate limiting step of cholesterol synthesis. The activation of HMGR further increases insulin levels which stimulate the removal of phosphates from the cells leading to increase activity of HMGR and resultant increase in cholesterol synthesis [21]. The cholesterol lowering effect of EBU could possibly be attributed to decreased levels of dephosphorylated HMGR (active form). EBU could also be activating glucagon and epinephrine that negatively affects cholesterol biosynthesis.

Although a possible pharmacological mechanism has been speculated, EBU was found to phytochemically contain phytosterols, glycosides, saponins and triterpenoids. Studies have shown that phytosterols and glycosides reduce plasma cholesterol [22]. The European Foods Safety Authority (EFSA) concluded that blood cholesterol can be reduced on average by 7 to 10.5% within the first 2–3 weeks if a person consumes 1.5 to 2.4 g of plant sterols and stanols every day [23]. It has also been established that phytosterols reduce cholesterol absorption [24,25] and plasma LDL cholesterol [26]. A number of studies have shown that

different kinds of saponins lower serum cholesterol levels in a variety of animals and human subjects [27-30]. This effect augurs well for the management of estradiol-induced hyperplasia of the uterus as it reduces total plasma cholesterol; the precursor for the synthesis of estradiol.

3.4.2 Total plasma estradiol

There was significant elevation (16.30%; $P \leq .01$) of plasma estradiol on treating normal female rats with 600 mg kg⁻¹ MSG, but elevation was very significant (59.23%; $P \leq .001$) with 800 mg kg⁻¹ MSG. This showed dose-dependency. Preventive treatments with EBU (50 and 100 mg kg⁻¹) caused significant ($P \leq .001$) lowering of elevated plasma estradiol. Again, as seen for cholesterol, estradiol levels did not reduce below normal (Control). All doses of EBU in curative treatment however decreased elevated plasma estradiol to normal and further decreased significantly ($P \leq .01$) levels to below normal in both the 600 and 800 mg kg⁻¹ MSG-treated rats (Figs. 4 and 5). Steroid hormones in mammals are biosynthesized from cholesterol which in turn is made *in vivo* from acetyl-CoA by mevalonate pathway. Subsequently, by the action of the enzyme aromatase on androstenedione or testosterone, estrogens including estradiol are synthesized [31]. Elevated plasma cholesterol would therefore lead to elevated plasma estradiol.

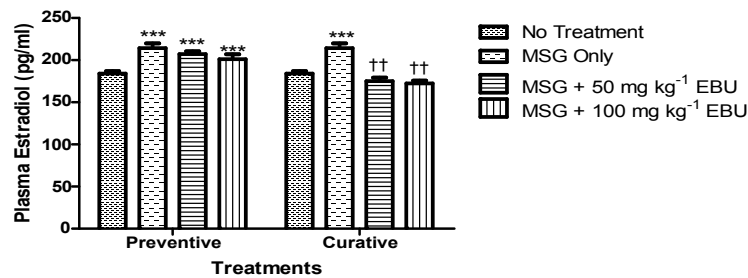


Fig. 4. The preventive and curative effects of the ethanolic extract of *Blighia unijugata* (EBU) on plasma estradiol in female Sprague-Dawley rats pre-treated with 600 mg kg⁻¹ MSG

Test drugs: significant from normal control, Increments: *** $P \leq .001$; For decrements below values observed in control group: †† $P \leq .01$

Mean \pm S.E.M = Mean values \pm Standard error of means of five experiments

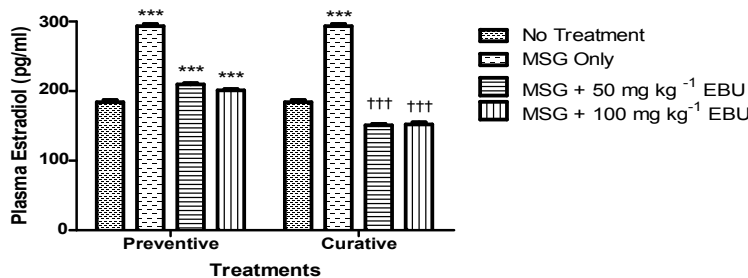


Fig. 5. The preventive and curative effects of the ethanolic extract of *Blighia unijugata* (EBU) on plasma estradiol in female Sprague-Dawley rats pre-treated with 800 mg kg⁻¹ MSG

Test drugs: significant from normal control, Increments: *** $P \leq .001$; For decrements below values observed in control group: ††† $P \leq .01$

Mean \pm S.E.M = Mean values \pm Standard error of means of five experiments

3.4.3 Uterus weight-to-body weight ratio

A 600 and 800 mg kg⁻¹ MSG treatment of rats caused significant, dose-dependent increases (60.67%, $P \leq 0.001$, and 71.55%, $P \leq 0.001$ respectively) in uterus weight-to-body weight ratio (Figs. 6 and 7). Preventive and curative treatments with 50 and 100 mg kg⁻¹ EBU significantly decreased ($P \leq 0.001$) organ weight to body weight ratio. Uterus size also increased very significantly with MSG treatment as seen in photographs of isolated uteruses (treated and untreated) taken during the study (Table 5). An increase in weight and size of an organ usually indicates hyperplasia (cell proliferation). Endometrial hyperplasia may represent an early neoplastic process which can lead to uterine leiomyoma.

MSG treatment resulted in an increase in cholesterol and estradiol. Estradiol is specific in uterine cell proliferation [31]. It binds to ER α receptors that are abundant in the uterus, forming a complex that interacts with DNA of the nucleus to activate transcriptional promoter and enhancer elements responsible for control of gene expression.

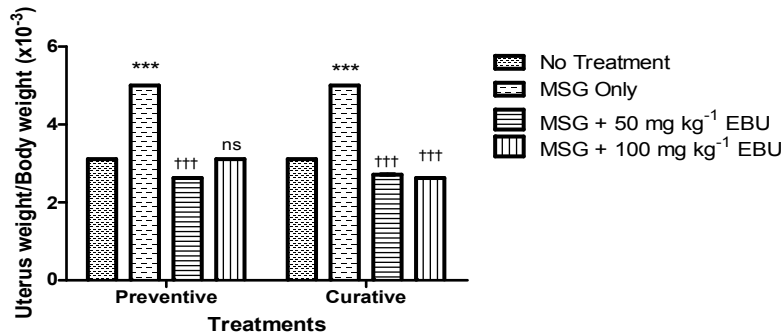


Fig. 6. The preventive and curative effects of the ethanolic extract of *Blighia unijugata* (EBU) on uterus weight in female Sprague-Dawley rats pre-treated with 600 mg kg⁻¹ MSG

Test drugs: significant from normal control, Increments: *** $P \leq .001$; For decrements below values observed in control group: ††† $P \leq .01$

Mean \pm S.E.M = Mean values \pm Standard error of means of five experiments

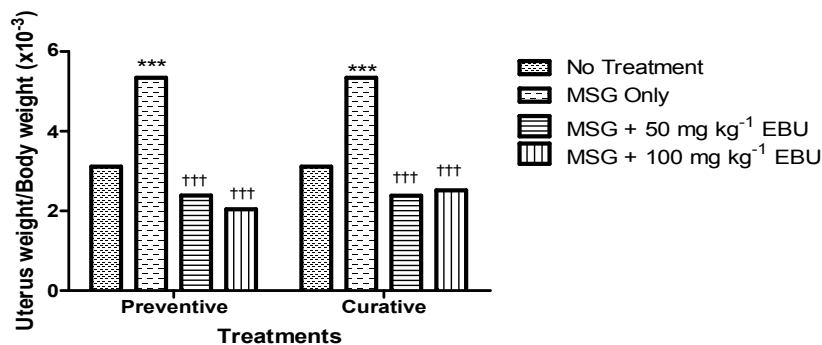


Fig. 7. The preventive and curative effects of the ethanolic extract of *Blighia unijugata* (EBU) on uterus weight in female Sprague-Dawley rats pre-treated with 800 mg kg⁻¹ MSG

Test drugs: significant from normal control, Increments: *** $P \leq .001$; For decrements below values observed in control group: ††† $P \leq .01$

Mean \pm S.E.M = Mean values \pm Standard error of means of five experiments

This allows binding of RNA polymerase II and subsequent initiation of transcription which produces proteins that leads to increase proliferation of the cells of the uterus and ovaries [18]. The dose dependent hyperplasia effect suggests that increased MSG produces a corresponding increase in synthesis of these biochemical markers that enhance proliferation process of the uterus. The reversal of MSG induced hyperplasia by EBU could be due its activity of reducing cholesterol and biochemical markers of protein synthesis. Uterine proliferation has also been linked to periods of estrogen secretion because of their increase response to estradiol [32]. Decreased levels of estradiol by effect of EBU would decrease uterine cell proliferation. Phytosterols have exhibited encouraging results in inhibiting cancer. There have been positive results – even on estrogen-dependent cancer lines. The evidence that sitosterols and stigmasterols have anti-estrogenic activity is building up. A study found estrogen receptor expression to be down-regulated with doses of beta-sitosterol glycosides (BSSG) compared to estradiol control [33, 34].

Table 5. Photographs of a Sprague-Dawley rat uterus before, and after treatment with MSG and MSG-treated uterus treated with 50 and 100 mg/kg of the ethanolic extract of *Blighia unijugata* (EBU)



Plate A: Normal uterus of a Sprague-Dawley rat



Plate B: 800mg/kg MSG-treated Sprague-Dawley rat uterus



Plate C: 800mg/kg MSG-Treated Sprague-Dawley rat uterus treated with 50 mg/kg EBU



Plate D: 800mg/kg MSG-Treated Sprague-Dawley rat uterus treated with 100 mg/kg EBU

Photographs are in JPEG Format

3.5 Acute and Delayed Toxicity Test

No mortality occurred during the study. Daily clinical observations recorded were considered common findings in laboratory rats which could not be associated to EBU treatment. There were no secretions from the eye, ear, nose, anus, and external genitalia, no “wasting”,

audible “chattering”, alopecia, and pallor in the eyes. The mice were not lethargic, they fed well and had normal formed stool. There were no ocular findings, decreased motor activity and neurological conditions. There was no significant test article effect on body weight

Observations made at 1-2 hour intervals for 24 hours and then daily for 14 days makes the study convenient for an acute and delayed toxicity study because most of the observable symptoms of acute toxicity occur within the first 1-2 hours of drug administration [35]. The “no effect on body and skin” observed suggests that EBU may not have caused hypersensitization and neurogenic inflammation which results in rats scratching, licking, or bite their skin in response to the allergy [36, 37]. The product does not cause autonomic nervous system hyperreflexia because lacrimation, miosis, rhinorrhoea, salivation, urination, defecation, and labored breathing which are signs of muscarinic hyperactivity [38].

Observations showed no CNS excitation or depression, muscle relaxation effects (noticed as a decrease in locomotory activity) as well as pain and inflammatory effects (realized as writhing, change in gait and body posture and decreased locomotory activity). The product did not seem to have any “wasting effect”. There was no pallor in the eyes (symptom of anaemia). No death recorded implies that the lethal dose was less than 1000 mg kg⁻¹ when given as a single dose.

4. CONCLUSION

Monosodium Glutamate is present in almost all artificial food seasonings in Ghana. Monosodium Glutamate treatment elevates total plasma cholesterol, plasma estradiol and uterus size and weight. The ethanolic stem bark extract of *Blighia unijugata* significantly decreases elevated levels of plasma cholesterol, estradiol, as well as uterus size and weight suggesting its efficacy as an anti- fibroid agent. The lethal dose was less than 1000 mg kg⁻¹ *per os*.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by Committee on Animal Research, Publication and Ethics (CARPE) of the Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, Ghana; Ethics Reference №: FPPS/PCOL/0018/2012. Laboratory study was carried out in a level 2 biosafety laboratory. All the technical team observed all institutional biosafety guidelines for protection of personnel and laboratory.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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