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Ameliorative Potentials of Methanol Extract and Chloroform Fraction of *Drymaria cordata* on MSG-induced Uterine Hyperplasia in Female Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author AOO designed the study, wrote the protocol, carried out the hormonal assay, managed the analysis of the study, performed the statistical analysis and wrote the first draft of the manuscript. Author OOA worked on the histology and did the histomophometry. Author ETH fed, treated and took the care of the animals and also did the literature searches. Author OOO designed, approved the study and read through the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Uterine fibromyomas are non-cancerous or benign growth of the uterus. They are sensitive to changes in levels of oestrogen and progesterone which affect the size of the fibroid. It has been well established that glutamate, a naturally occurring amino acid induces uterine fibroid in rat by increasing the levels of estradiol. *Drymaria cordata* is used traditionally for the shrinkage and destruction of uterine fibroid. The biochemical basis of this effect is unknown.
Methodology: The effect of the crude Methanol Extract (MEDC) and Chloroform Fraction (CFDC) of the methanol extract of *Drymaria cordata* on Monosodium Glutamate (MSG)-induced hyperplasia in female wistar rats were investigated. Thirty six mature virgin female rats were randomly divided

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into six study groups; A(Control), B(MEDC (200 mg/kgbdwt)), C(CFDC (100 mg/kgbdwt)), D(MSG (200 mg/kgbdwt)), E (MSG + MEDC) and F(MSG + CFDC). The administration was carried out as a single daily dose by oral galvage for 28 days. The animals were sacrificed 24 hrs after the final exposure. Blood was collected by cardiac puncture into EDTA-sterilized sample bottles. Total estradiol (estrogen), progesterone and cholesterol were determined according to standard procedures. The uteruses were harvested and subjected to histological examination.

Results: The results showed that co administration of both MEDC and CFDC reversed MSG-induced uterine hyperplasia observed in the myometrium of the uteruses of the animals with CFDC having higher effect. In addition, the increase in levels of total progesterone, cholesterol and estrogen in the MSG-treated animals were ameliorated by both MEDC and CFDC.

Conclusion: These findings suggest that MEDC and CFDC have the ability to prevent and reverse the development of fibroids. This shows that certain bioactive components present in the extract and fraction may prove useful in the treatment of uterine fibromyomas.

Keywords: Drymaria cordata; fibroid; Monosodium glutamate; hyperplasia; oestrogen.

1. INTRODUCTION

Uterine fibroids are benign tumors of smooth muscle cell and fibrous connective tissue that develop within the wall of the uterus or on the outer wall [1]. Uterine leiomyomas (fibroids) are the most common benign tumors in women of reproductive age [2]. Leiomyomas are associated with significant symptoms, such as anemia, excessive vaginal bleeding, pelvic pain, pressure-related bowel and bladder dysfunction, recurrent miscarriage, and preterm labor. Leiomyomas are also associated with infertility and recurrent abortion [3].

The initiating factors that lead to the development of fibroids are not well understood. However, ample evidence supports that ovarian steroids, such as estrogen and progesterone are important factors for leiomyoma growth [4]. High level of estrogen has been reported to be the most common cause of fibroid and painful menstruation [5,6].

Monosodium Glutamate (MSG) is a salt of glutamate, synthesized from L-glutamic acid and used as a flavour enhancer in foods. Studies have also shown that MSG induces uterine fibroid in rats by increasing the levels of total protein, cholesterol and estradiol [7].

Though MSG improves taste stimulation and enhances appetite, studies have shown that it is toxic to human and experimental animals [8]. MSG has a toxic effect on the testis by causing a significant oligozoospermia and increase abnormal sperm morphology in a dose-dependent manner in male Wistar rats [9]. It has been implicated in male infertility by causing testicular hemorrhage, degeneration and alteration of sperm cell population and morphology [10]. MSG has also been reported to have neurotoxic effects resulting in brain cell damage [11], retinal degeneration, endocrine disorder and some pathological conditions such as addiction, stroke, epilepsy, brain trauma, neuropathic pain, schizophrenia, anxiety, depression, Parkinson's disease, Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis [12]. In testicular tissues ascorbic acid content is reduced by MSG [13]. The degenerative and atrophic changes in the fallopian tubes are induced by MSG when administred in higher dosage and for prolonged period [14].

Fibroids growth depends on estrogen, reaching their peak during ovulating and just before the commencement of menstrual period and also increases during pregnancy when Gonatrophins-Releasing Hormones (GnRH) is at its highest [15,16]. High level of estrogen has been reported to be the most common cause of fibroid and painful menstruation [17]. Estrogens exhibit their effects through binding to one of two variants of ERs, ERα or ERβ [18]. Upon binding of estrogen, the ER dimerizes and binds to the estrogen-response element (ERE), causing transcription of estrogen dependent genes [19]. Modulation of estrogen exposure as a treatment for breast cancer began as early as the late nineteenth century when complete ovariectomy was noticed to have a favorable effect on cancerous progression [20]. While ovarian ablation (through surgery, irradiation, or medication) is still utilized clinically for some pre-menopausal breast cancer patients [21,22], extensive research has been performed to modify estrogen exposure pharmacologically. Modulation of estrogens and ERs can be accomplished by inhibiting ER

binding, by downregulating ERs, or by decreasing estrogen production [23,24,25].

In recent years, research work threw light on the effect of several kinds of plants on the health of man and animals where it is commonly used for treatment of certain ailments. Medicinal plants continue to be an important source in search of a suitable active principle(s), wherein they are currently being investigated for their potential pharmacological properties [26]. *Drymaria cordata* (Linn.) Willd (Caryophyllaceae) is a weak spreading herb found widely dispersed in damp places all over the tropics of Africa, Asia and the Americas. It is a sprawling herb with procumbent and more or less ascending branched stems, often rooting at the lower nodes, quadrangular, glabrous or papillose especially in the upper internodes, which are slender, generally 2-6 cm long. In tropical Africa, *D. cordata* preparations are used for the treatment of diverse ailments including cold, headache, coryza, bronchitis, as poultice on sore (to treat aching, inflamed or painful parts), leprosy, tumors, as fumigant for eye troubles, as cerebral stimulant and antifebrile agent [27]. Extracts of *D. cordata* have previously been reported to possess antitussive [28], anti-bacterial [29] anti-inflammatory [30], anxiolytic [31], cytotoxic [32] and analgesic activities [33]. In Nigeria, *D. cordata* (chick weed; "Calabar woman's eye") is used in folk medicine to treat sleeping disorders, convulsions, and febrile conditions in children [34]. Previous work in our laboratory showed that the solvent fractions of methanol extract of *Drymaria cordata* (MEDC) causes induction of mitochondrial permeability transition (mPT) pore opening and the release of cytochrome C with chloroform fraction (CFDC) being the most potent [35]. This current study therefore focused on the the modulatory effect of chloroform fraction (CFDC) and methanol extract (MEDC) of *Drymaria cordata* on MonoSodium glutamate-induced uterine hyperplasia.

2. MATERIALS AND METHODS

2.1 Monosodium Glutamate

MSG was obtained from Sigma Aldrich Chemical Co. St Louis USA and a stock solution was prepared by dissolving 20 g of MSG in 200 mls of distilled water. Based on the weight of the animals, 200 mg/kg dosages of MSG were administered to the group taking MSG alone and the groups taking co administration of MSG with CFDC and MEDC, respectively.

2.2 Collection of Fresh *Drymaria cordata*

The whole plant of *Drymaria cordata* were freshly harvested and obtained from Department of Botany, University of Ibadan, Nigeria. Samples were authenticated and identified at the Herbarium, Department of Botany, University of Ibadan, Ibadan, Oyo State and a specimen Voucher No.UIH-22555 was deposited in the Herbarium. The plants were washed, air-dried for three weeks in the laboratory after which they were powdered with industrial machine and weighed.

2.2.1 Preparation of crude methanol extract and chloroform fraction of *Drymaria cordata*

Six (6) kilogramme of air-dried, whole plant of *Drymaria cordata* were extracted with sufficient methanol (Sigma Aldrich Chemical Co. St Louis USA) in all- glass jars at room temperature for seventy-two hours. The filtrate was decanted, filtered and concentrated under reduced pressure using a rotary evaporator (Stuart). The crude methanol extract was heated over a water bath at 40°C to obtain a solvent free extract. The crude methanol extract was further partitioned between n-hexane, chloroform, ethylacetate and methanol using vacuum liquid chromatography technique. All these fractions were concentrated to dryness under pressure using rotary evaporator at 40°C to obtain the n-hexane (HF), chloroform (CF), ethylacetate (EF) and the methanol (MF) fractions.

2.3 Experimental Animals and Ethical Considerations

Thirty six mature virgin female Wistar strain rats ranging from between 170g-200 g in weight were obtained from the Pre-clinical Animal House, University of Ibadan, Ibadan, Nigeria. The animals were allowed to acclimatize for 14 days in cages in the Animal House of the Department of Biochemistry, University of Ibadan. The animals had access to water and chow *ad libitum* and were kept under standard conditions of temperature and humidity. The rats used in this study showed regular oestrous cycle length (4 to 5 days). The oestrous cycles of the animals were assessed by observing the vaginal smear in the morning according to the procedure described by Solomon et al. [34]. Ethical approval for this study was obtained from the University of Ibadan Animal Care & Use Research Ethics Committee (ACUREC). Rules

guiding animal studies as stipulated by the Ethical Committee of University of Ibadan were strictly followed. These rules are similar to international guidelines on animal handling.

2.4 Animals Groupings, Treatment and Sample Collections

Rats were divided into 6 groups: A (control group), B (CFDC: 100 mg/kgbdwt), C (MEDC: 200 mg/kgbdwt), D (MSG only: 200 mg/kgbdwt), E (MSG: 200 mg/kgbdwt + CFDC: 100 mg/kgbdwt) and F (MSG: 200 mg/kgbdwt +MEDC: 200 mg/kgbdwt). The administrations were carried out as a single dose daily by oral gavage for 28 days. One day after the final exposure, the animals were sacrificed. Blood was collected by cardiac puncture into EDTA sample bottles. Plasma was prepared by centrifugation (3000xg, 20 min) and used for the analysis of total estradiol (estrogen), progesterone and total cholesterol.

2.5 Histological Study

The uteruses were harvested, dehydrated in an ascending grade of (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of 5 microns thick were obtained using a rotatory microtome. The deparaffinised sections of the uterus were stained routinely with Masson's Trichome stain, a differential stain for connective tissue and the cellular precursors. The Histological pictures were taken with a Digital Microscope, VJ-2005 DN MODEL BIO-MICROSCOPE®. The morphometrical analyses of the density of spindle shaped cells within the endometrial submucosa were done using TS View CX Image® Software, File version 6.2.4.3 Motic Image 2000 (China).

2.6 Statistics

Data were expressed as a mean ± standard deviation. The mean and standard deviation (SD) were calculated for serum estrogen, progesterone levels and total cholesterol of control and experimental groups. Comparison of the variables were made using the ANOVA and *P-value* of < 0.05 was considered as statistically significant.

3. RESULTS

Fig. 1 showed the results of the effect of chloroform fraction (CFDC) and methanol extract (MEDC) of *Drymaria cordata* on oestrogen level of the female rat treated with monosodium glutamate. The investigation showed that the animals treated with MSG has significant ($p<0.05$) increase (87.5%) in the levels of estrogen (estradiol) in the animals treated with 200 mg/kgbdwt MSG when compared with the oestrogen levels in the control group. The groups co-treated with both MEDC and CFDC were able to ameliorate the oestrogen level to about 52.25% and 25.5% respectively, when compared with the MSG-treated group. This implies that both CFDC and MEDC contain bioactive agents that can reduce the levels of oestrogen implicated in the development of uterine fibroid. The figure also showed that CFDC is more potent in the reversal of the elevated estrogen level found in the MSG-treated group. Also, the value of progesterone level in the MSG-treated groups was significantly increased by about 59.0% when compared with the control group while the co-administration with the extract and fraction reduced the progesterone level to about 36.4 and 27.2% respectively, when compared with the MSG-treated group. Fig. 3 depicts the level of total cholesterol which also showed a similar pattern. There was increase in the level of total cholesterol in the MSG-treated group and this was significantly reduced both in the MEDC and CFDC group when compared with the MSG-treated group ($P<0.05$). Fig. 4 showed the cell count density obtained from the myometrium of the uterus of the control and treated female rats. The histology results of the uterus using histomorphometric technique showed the cell counts of stained spindle shaped connective precursor cells elevated in the MSG-treated group when compared with the control group. In contrast, the cell counts in the co-administered groups showed a mitigated level when compared with the MSG-treated group. The cell counts density recorded in the MSG-treated group was 3.6 folds compared with the control group. This was significantly reduced by the co-administration with MEDC and CFDC having 39% and 56% reduction respectively when compared with the MSG-treated group ($P<0.05$). Figs. 5a-5f showed the photomicrograph of the masson trichrome-stained sections of myometrium of the connective tissue and precursor cells within the endometrial submucosa and the endometrial glands. Fig. 5a which is the control uterine section showed normal architecture of the connective tissues and the precursor cells. Similar pattern of results were noticed in the CFDC and MEDC treated groups. However, in

Fig. 5d which is the group treated with 200 mg/kgbdwt MSG, there was a severe hyperplasia of spindle shaped precursor cells. The hyperplasia was mitigated in the group that received co administration of CFDC and MEDC as shown in Fig. 5e and 5f.

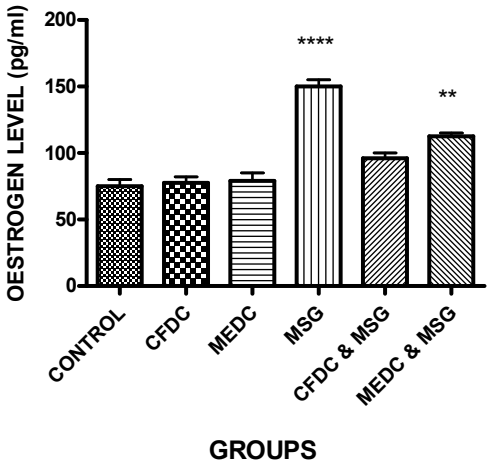

Fig. 1. Effects of methanol extract and chloroform fraction of *Drymaria cordata* on oestrogen level of MSG-treated female rats

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

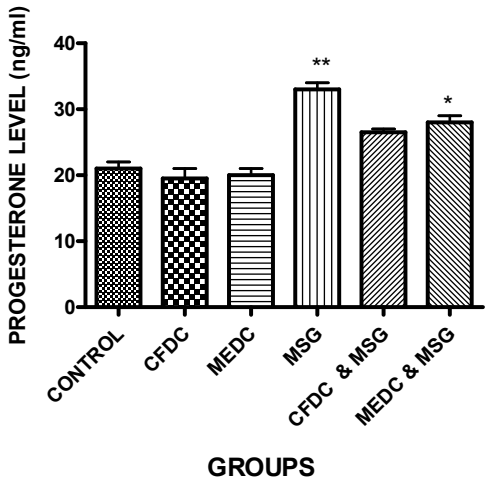

Fig. 2. Effects of methanol extract and chloroform fraction of *Drymaria cordata* on progesterone level of MSG-treated female rats

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

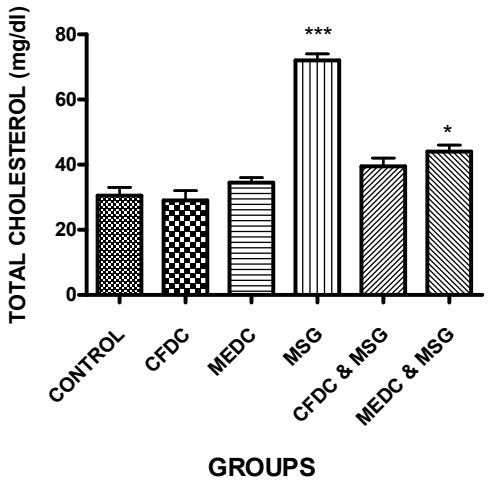

Fig. 3. Effects of methanol extract and chloroform fraction of *Drymaria cordata* on total cholesterol level of MSG-treated female rats

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

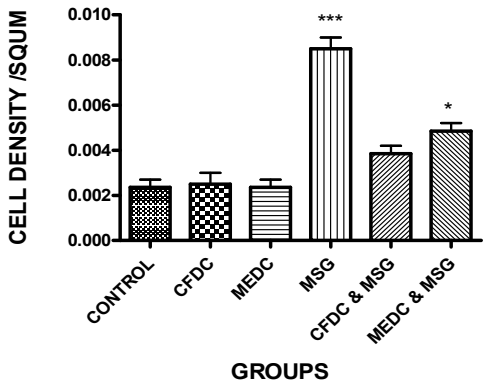

Fig. 4. Cell count density obtained from the myometrium of uterus of female wistar rats treated with MSG , MEDC and CFDC

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

4. DISCUSSION

Uterine fibroids are monoclonal tumors that arise from the uterine smooth muscle tissue (i.e., the myometrium) [36] Histologically, fibroids are benign neoplasms composed of disordered smooth-muscle cells buried in abundant quantities of extracellular matrix. A striking feature of uterine fibroids is their dependency on the ovarian steroids estrogen and progesterone [37]. Ovarian activity is essential for fibroid

growth. A large body of experimental data and circumstantial evidence suggests that estrogen stimulates the growth of uterine fibroids through estrogen receptor α [38]. The primary roles of estrogen and estrogen receptor α in fibroid growth are permissive in that they enable tissue to respond to progesterone by inducing the expression of progesterone receptor [40]. Fibroid tissue is exposed to ovarian estrogen and to estrogen produced locally through the aromatase activity in fibroid cells [41]. In fibroid tissue, multiple promoters controlled by a diverse set of transcription factors contribute to the expression of a single aromatase protein that converts circulating precursors into estrogens [40]. These observations suggest that the inhibition of aromatase in fibroid tissue is a key mechanism in hormone-dependent shrinkage of fibroid growth.

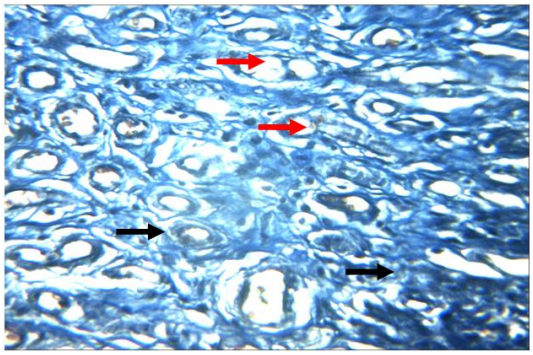

Fig. 5A. Control section showing the connective tissue and precursor cells within the endometrial submucosa (black arrows) and the endometrial glands (red arrow) MT (Mag. x400)

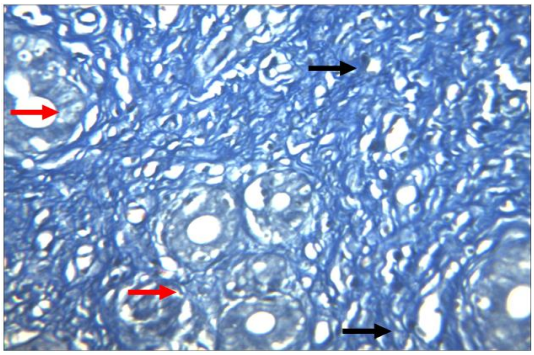

Fig. 5B. Treatment section of the myometrium (MEDC) showing the connective tissue and precursor cells within the endometrial submucosa (black arrows) and the endometrial glands (red arrows) MT (Mag. x400)

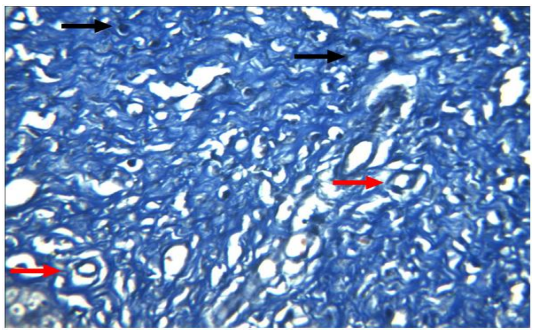

Fig. 5C. Treatment section of the myometrium (CFDC) showing the connective tissue and precursor cells within the endometrial submucosa (black arrows) and the endometrial glands (red arrows) MT (Mag. x400)

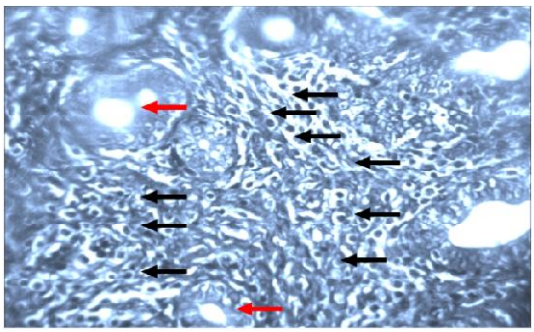

Fig. 5D. Treatment section of the Uterus (200 mg/kgbdwt MSG) showing the connective tissue and precursor cells within the endometrial submucosa (black arrows) and the endometrial glands (red arrows) MT (Mag. x400), There is a severe hyperplasia of spindle shaped precursor cells

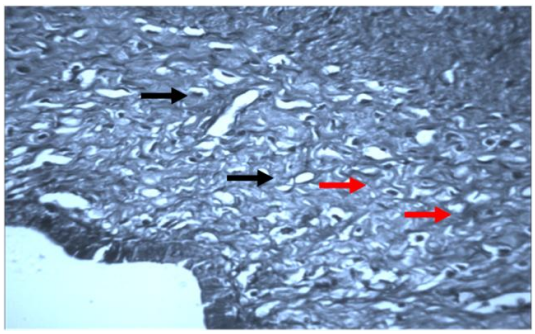

Fig. 5E. Treatment section of the endometrium (200 mg/kg MSG & 100 mg/kg CFDC) showing the connective tissue and precursor cells within the endometrial submucosa (black arrows) and the endometrial glands (red arrows) MT (Mag. x400), There is a reduction of hyperplasia compared with MSG-trated group

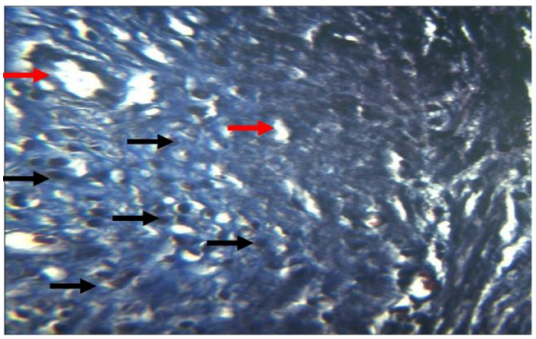

Fig. 5F. Treatment section of the endometrium (200 mg/kg MSG & 200 mg/kg MEDC) showing the connective tissue and precursor cells within the endometrial submucosa (black arrows) and the endometrial glands (red arrows) MT (Mag. x400), There is a reduced hyperplasia of spindle shaped precursor cells

Fig. 5. Photomicrograph of Collagen Masson Trichome staining of the myometrium of the control and the treated rat uteruses

Studies have shown that during fibroid growth, there is increase in collagen fiber. Masson Trichome which can stain collagen fiber cells was employed in this study to stain and differentiate between the collagen cells of the uterus of the control and treated animal. The findings in this study show increase in the precursor cell density (hyperplasia) in the MSG-treated group when compared with the control and the co administered groups. The hyperplasia noticed in the MSG-treated group could be as a results of cellular proliferation due to prolonged intake of MSG. Also, the oestrogen level in the MSG-treated group was significantly higher compared with the control group (P<0.05) and the co administered groups (P<0.05). The effects of MSG on estradiol (estrogen) levels could be attributed to the activation of the enzyme, aromatese, which catalyzed the conversion of testosterone to β-estradiol and aromatization of ring A of β-estradiol, which increased the activity of the enzyme, resulting in increased estradiol synthesis. However, treatments with methanol extract and chloroform fraction of *drymaria cordata* mitigated the hyperplasia and the elevated oestrogen level that have been induced by the MSG treatment. These findings suggest that MEDC and CFDC may have inhibitory effect on the aromatase enzyme activity, thereby reducing the levels of oestrogen implicated in the development of uterine tumor and thus reduce the uterine hyperplasia in the co administered groups. Similar pattern of result was found in the case of progesterone. The progesterone levels were also significantly higher in MSG- treated group compared to control group (P<0.05) and the group that received co administration with MEDC and CFDC. These results are in consonant with the findings of Muhammad et al. [41] who found increased levels of progesterone in the plasma of MSG-treated animals, which were significantly higher than those found in control animals. The reason for increase in progesterone levels in the MSG-treated rats was probably due to increased levels of luteinizing hormone as a result of MSG treatment. The significant increase in the total cholesterol level noticed in the MSG-treated group when compared with the control group (P<0.05), indicates that MSG could cause disorder in lipid metabolism in rats if they are exposed to MSG-treatment for long time. The elevated serum total cholesterol was significantly lowered (P<0.05) by MEDC and CFDC co administered groups with CFDC being more potemt. This is similar to our previous findings in our laboratory showing the potency of CFDC over MEDC with respect to mitochondrial Permeability Transition Pore (mPT) opening [35].

The results of this study suggest that both MEDC and CFDC contain bioactive agent that can ameliorate MSG-induced uterine hyperplasia/tumor. The study also suggests that the possible mechanism by which both MEDC and CFDC ameliorated the MSG-induced hyperplasia is via inhibition of aromatase enzyme activity. It may be inferred from the present results that higher dose and prolonged administration of MSG resulted in hyperplasia observed in the uterus of the MSG-treated groups and this was significantly (P<0.05) mitigated in the MEDC and CFDC co administered groups with CFDC having a higher potency in the amelioration of the MSG-induced hyperplasia in the treated female rats. This study justifies the folkloric use of *Drymaria cordata* in the treatment of uterine fibroid. It is recommended that further studies be carried out to corroborate these findings.

5. CONCLUSION

Administration of MSG results in an increase in serum levels of estrogen, progesterone and total cholesterol in rats while co-administration of MEDC and CFDC ameliorated this effect. Also MSG-induced uterine hyperplasia was reversed in the co administered groups. The results of this study show that MEDC and CFDC contain certain bioactive agents that can mitigate uterine-

induced hyperplasia and also protect against MSG-induced increase in the levels of hormones that are associated with the development of uterine fibroid in rats. This study justifies the folkloric use of *Drymaria cordata* in the treatment of uterine fibroid.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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