



Original Research Article (Experimental)

Protective effects of alpha stone on monosodium glutamate-induced uterine hyperplasia in female wistar rats

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ABSTRACT

Background: Uterine leiomyomas (fibroids), a menace of the reproductive age, is characterized by proliferation of smooth muscle cells (hyperplasia) of the uterus. Alpha Stone Decoction is a poly-herbal formulation that is used for the shrinkage and prevention of uterine fibroids in folkore medicine.

Objective: We investigated the efficacy and safety of Alpha Stone Decoction (ASD), on monosodium glutamate (MSG) induced uterine hyperplasia.

Materials and methods: Twenty-eight mature virgin female rats were randomly divided into four study groups: A-control B- MSG (200 mg/kgbw), C- MSG + ASD (100 mg/kgbw) and D- ASD 100 mg/kgbw alone. The administration was carried out by as a single daily dose via intraperitoneal route for 14 days. Total protein, triglycerides, estradiol (estrogen), progesterone, and total cholesterol levels in sera were determined using appropriate kits. Uterine hyperplasia was assessed via histomorphometric method using the mitotic image plus software to compute the fibroblast cell count density while the uteri and ovaries of animals were stained with mason-tricon stain for histological examination.

Results: Administration of MSG for 14 days resulted heavy deposits of collagen connective tissue within the myometrium layers of the uteri. ASD significantly ($p < 0.05$) reduced fibroblast cell count in MSG-treated animals and also protected against MSG-induced damage observed in the myometrium of the uteri and ovaries of the animals. Significant increases ($p < 0.05$) in levels of total protein; triglycerides, progesterone, cholesterol and estrogen in the MSG-treated animals were ameliorated following administration of ASD.

Conclusion: These findings suggest that ASD contains bioactive agents which reversed MSG-induced uterine hyperplasia. It may therefore be useful in reducing the proliferation of fibroblast cells and managing other symptoms associated with uterine myoma.

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1. Introduction

Poly herbal formulation is a traditional therapeutic strategy that takes advantage of the combination of several medicinal herbs to achieve enhanced therapeutic effects against a disease [1]. The polyherbal therapy has been used in China and India for treatment of diseases for ages and it is gaining acceptance across the world, especially in Africa due to its advantages over single herbs [2]. Research has shown that when compatible herbs are combined in a

formulation, they produce better efficacy at low doses as a result of different phytoconstituents acting in synergy [3]. Besides, it is believed that the herb–herb formulation offers a combination of active ingredients which may reduce the toxicity of other more potent herbs while taking care of all symptoms of the disease.

Uterine fibroids otherwise referred to as leiomyomas are an important public health issue and has been tagged as an African problem in women [4]. They represents the most common category of solid pelvic tumors in women, occurring in up to 70–80% women in their reproductive age [5] and has a cumulative life time risk of 70%. These benign tumors originate from the uterine smooth muscle cells and consist of large amounts of extracellular matrix that contain collagen, fibronectin, and proteoglycan [6].

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The major symptom associated with leiomyomas is uterine bleeding which usually results in anaemia [7]. Others include back pain, recurrent pregnancy loss, frequent urination and infertility [5]. Although, the major causes of uterine fibroids are poorly understood, risk factors include ethnicity, genetics, and hormonal factors among others, however the risk factor with is strongest evidence is the black race [8]. Treatment of uterine fibroids includes myomectomy, hysterectomy, hormone replacement therapy and uterine artery embolization. Given the risks, health care cost and side effects associated with these procedures, besides the fact that reoccurrence of the fibroids usually occurs after a period of time, women are interested in non-surgical interventions with less risks and adverse effects in managing this problem of the reproductive age.

Monosodium glutamate is a flavour enhancer and a popular food additive [9,10]. However, several studies have shown the adverse effects of MSG on the female reproductive system [10,11]. Few studies have used MSG to create an animal model of obesity and uterine hyperplasia [12,13].

Alpha stone is a combination of acacia honey and different parts of plants with claims with high medicinal values from the eastern part of the world. It is popularly used in the treatment of several diseases including tumours, pile, infections, ulcers, fibroids, malaria, high blood pressure, asthma and rheumatism. The polyherbal formulation consists of leaves and acorns of Palestine oak (*Quercus calliprinos*) shoots of terebinth (*Pistacia palestine*), Golden chamomile, Carob (*Ceratonia siliqua*), leaves of Dominican sage (*Salvia dominica*), leaves of field marigolds (*Calendula arvensis*), Tulips (*Tulipa systola*), shoots of the white broom (*Retama raetan*), the fruit of the bitter almond tree (*Amygdalius arabica*), seeds of fennel (*Foeniculum vulgare*) and shoots of the leafless ephedra (*Ephedra foeminea*) which is very rich in phenolics and flavonoids [14]. A recent study showed that essential oil from *Pistacia palaestina* inhibited the *in vitro* proliferation of human leukemic K562 cells [15] while *C. siliqua*, commonly known as the carob tree (St John's-breador locust tree [16]) has been shown to have several pharmacological activities including anti-inflammatory, antimicrobial, antioxidant, anti-ulcer [17]. Recently, *Ephedra foeminea* has been described as a wonder plant due to its cytotoxic activity of chemotherapy agents (cisplatin and carboplatin) on breast cancer cell cultures [18]. Studies reveals that the antiproliferative, and anticancer effects of honey are mediated via diverse mechanisms, including induction of mitochondrial outer membrane permeabilization, apoptosis, modulation of oxidative stress and reversal of angiogenesis in cancer cells [19]. Although, ASD is used to manage uterine leiomyoma traditionally, the scientific basis for this effect is lacking. This study therefore investigated the efficacy and safety of ASD on MSG-induced uterine hyperplasia in female reproductive function of wistar rat models.

2. Materials and methods

2.1. Chemicals

Monosodium glutamate and other chemicals were obtained from Sigma Aldrich Chemical Co. (St Louis USA) except where otherwise stated. Cholesterol, triglyceride, total protein, estrogen and progesterone kits were obtained from Randox Laboratories, London, England.

2.2. Experimental animals

Virgin female wistar strain rats (twenty-eight) weighing between 100 and 120 g were obtained from the pre-clinical animal house, University of Ibadan, Ibadan, Nigeria. The rats were allowed

to acclimatize for 14 days in animal house, Department of Biochemistry, University of Ibadan. They were kept in ventilated cages with 12 h light/dark cycling and were given chow and water *ad libitum* and were kept under standard conditions of temperature and humidity. All the rats used in this study showed regular oestrus cycle length (4–5 days). The oestrus cycle of the animals were assessed by observing the vaginal smear in the morning.

2.3. Preparation of alpha stone decoction

Alpha Stone Decoction was formulated from equal proportion of the aqueous extracts of the plants with enough quantity of Acacia honey. Alpha stone was obtained from a local distributor in Ibadan, Oyo State. It was pulverized to a fine powder using a mortar and pestle. The powdered alpha stone was stored at room temperature in a clean bottle.

2.4. Phytochemical screening

Alpha stone was subjected to phytochemical screening in accordance with the standard procedures described by Sofowora [20], and Trease and Evans [21].

2.5. Ethical approval

Experimental protocols were carried out according to the approval and guidelines given by the University of Ibadan Ethical Committee which conformed to the acceptable guidelines on the ethical use of animals in research. These rules are similar to international guidelines on animal handling.

2.6. Dosing of experimental animals

Doses of MSG (200 mg/kgbw) and ASD (100 mg/kgbw) were selected based on a preliminary study. Dosing was once daily via the intraperitoneal route over the experimental period (14 days).

2.7. Experimental design

Twenty-eight female rats were randomly divided into 4 groups with seven animals in each group; A (Control), B (MSG only: 200 mg/kgbw), C (MSG: 200 mg/kgbw and ASD: 100 mg/kgbw) and D (ASD only: 100 mg/kgbw). The administration was carried out by as a single daily dose via inter-peritoneal route in solution (water used as vehicle/solvent) for 14 days, after which they were sacrificed.

2.8. Preparation of serum

Blood (3 ml) was collected by cardiac puncture into EDTA sterilized sample bottles. Serum was prepared by centrifugation (3000 rpm, 20 min) and subjected to analysis of total estradiol (estrogen), total protein, triglycerides, progesterone, and cholesterol were determined according to manufacturer instruction (Randox Kits).

2.9. Determination of serum estradiols (Estrogen) and progesterone

Levels of estradiols and progesterone and was determined with modification of Enzyme linked Immunosorbent Assay (ELISA) method described by Meyer et al. [35].

2.10. Determination of serum total cholesterol

Serum total cholesterol and triglycerides were determined with the method of Brown and Goldstein [22] and Sullivan et al., [23].

Briefly, cholesterol was extracted from the serum with ethanol. The extract was then reacted with solution of FeCl₃ dissolved in phosphoric acid and the resulting colour was read in a spectrophotometer at 550 nm against a reaction blank.

The concentration of the unknown was calculated using ratio formula:

$$(A_{550nm} \text{ unknown} : A_{550nm} \text{ Standard} \times \text{Conc. of Std.} \times 100)$$

$$\left(\frac{A_{550nm}}{A_{550nm} \text{ Standard}} \times \text{Conc. of Std.} \times 100 = \frac{\text{mg}}{\text{dl}} \right)$$

2.11. Determination of serum total protein

Serum total protein was determined by the Biuret method described by Gornall et al., [24].

2.12. Tissue preparation for histological examination

The tissues were harvested, rinsed in phosphate buffered saline and thereafter used for histopathology study. They were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of 6 microns thick were obtained using a rotatory microtome. The deparaffinised sections of the uteri were stained routinely with Masson's tricon, a differential stain for connective tissue and the cellular precursors [39]. The Histological pictures were taken with a Digital Microscope, VJ-2005 DN MODEL BIO-MICROSCOPE®. The morphometrical analyses of the fibroblast count density (**fibroblast count/μm²**) of spindle shaped cells within the endometrial submucosa were done by modification of the method of Mutter et al., [25] using TS View CX Image® Software, File version 6.2.4.3 Motic Image 2000 (China).

2.13. Statistical analysis

Bar graphs were obtained by using the software Graph Pad Prism (Windows Version 5) and subjected to One way Analysis of Variance (ANOVA). Data were expressed as a mean ± SD and $p < 0.05$ was considered statistically significant in all analysis.

3. Results

3.1. Phytochemical constituents

The result from the phytochemical screening of ASD revealed the formulation contains flavonoids, phenols, saponins, steroids and tannins. The formulation was stable at room temperature.

3.2. Effect of intraperitoneal administration of ASD on levels of hormones in MSG-treated animals

Fig. 1a and b depict the variation in the levels of serum progesterone and estrogen following MSG administration and during treatment with ASD (100 mg/kgbw). The data reveals that intraperitoneal administration of MSG (200 mg/kgbw) for 14 days in female rats resulted in elevation in the levels of serum progesterone and estrogen when compared with control animals. However, co-administration of MSG with ASD resulted in significant ($p < 0.05$) lowering of elevated levels of these hormones. Furthermore, there was no significant difference in the levels of progesterone and estrogen in control animals compared with those exposed to ASD alone.

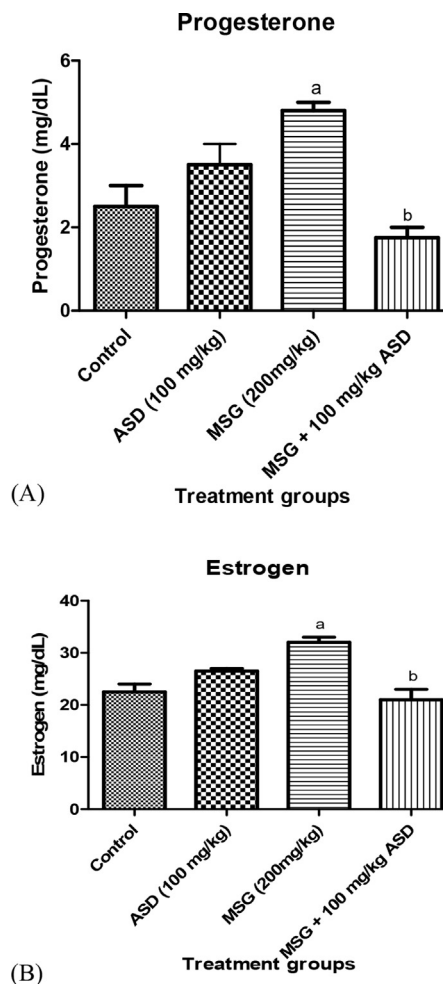


Fig. 1. Effects of administration of Alpha Stone Decoction on progesterone and estrogen levels in female rats. (MSG-Monosodium Glutamate, ASD-Alpha Stone Decoction). Values are mean ± SD, $p < 0.05$ compared with control (a), $p < 0.05$ compared with MSG alone (b).

3.3. Effects of administration of ASD on lipid profile and total protein levels in MSG-treated female rats

The effects of ASD on serum levels of total protein, triglycerides and total cholesterol following MSG pre-treatment in female rats are shown in Fig. 2. The data show that there were significant ($p < 0.05$) increases in triglycerides, total protein and cholesterol levels in groups that received 200 mg/kgbw MSG only when compared with the control group after 14 days of intraperitoneal administration. However, this increase was ameliorated ($p < 0.05$) by treatment with 100 mg/kgbw of ASD.

3.4. Histological evaluation of the effect of intraperitoneal administration of ASD on uteri and ovaries of MSG-treated female rats

Fig. 3 shows the photomicrographs of the ovarian sections stained by massion trichrome in MSG-treated female rats. The ovarian sections of the control animals (A) showed normal deposits of collagen connective tissues in an ovarian section with the vessels appearing normal. Female rats administered ASD alone also showed normal deposits of collagen connective tissues with no indication of fibrosis while ovarain sections of animals intraperitoneally treated with MSG (200 mg/kgbw) alone showed moderate

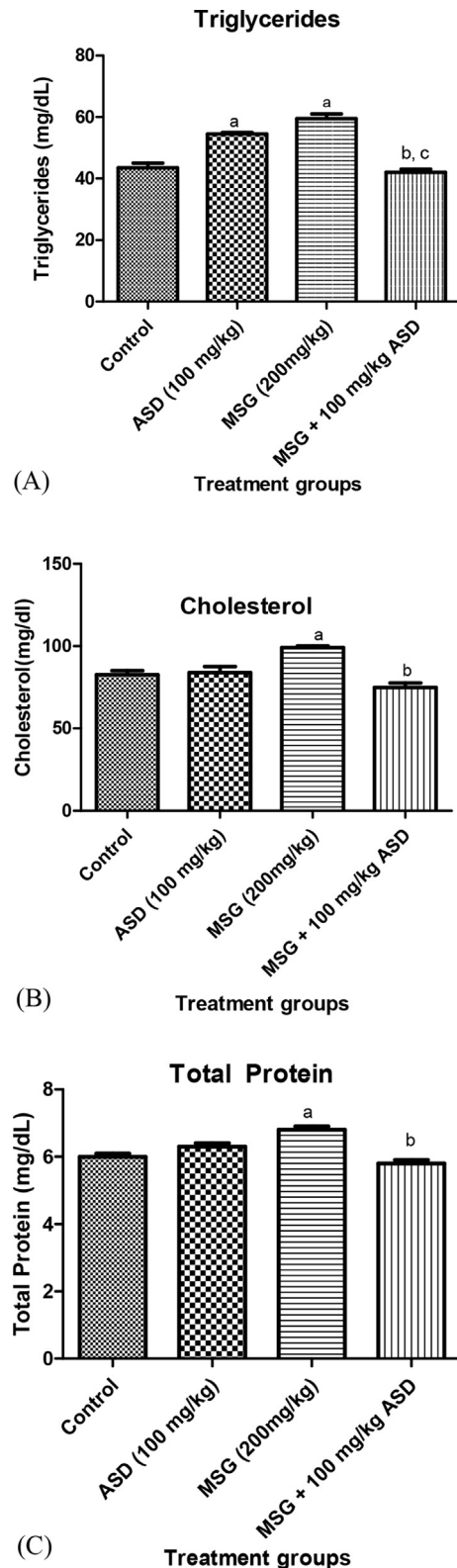


Fig. 2. Effects of Alpha Stone Decoction on lipid and profiles and total protein levels in MSG pre-treated female rats. Values are mean \pm SD, $p < 0.05$ compared with control (a), $p < 0.05$ compared with MSG alone (b).

to severe deposits of collagen connective tissue with the ovarian stroma showing fibrosis. Interestingly, animals co-administered with ASD (100 mg/kgbw) showed normal deposits of collagen connective tissues and normal vessels.

Photomicrographs of masson trichrome-stained sections of myometrium of the connective tissue and precursor cells within the endometrial submucosa and the endometrial glands of MSG-treated female rats were depicted in Fig. 4. The uteri of the control animals showed normal connective tissues within the endometrium layer (white arrow) with the muscle within the myometrium appearing normal (blue arrow). The endometrium layer of female rats administered ASD (100 mg/kgbw) had mild deposition of connective tissues (collagen fibre) within the myometrium indicating normal connective tissues. However, animals administered MSG (200 mg/kgbw) had heavy deposits of collagen connective tissues within their endometrium (white arrow) and myometrial layers (slender arrow). Besides, the endometrial layer appeared thickened (black arrow). Interestingly, mild deposits of collagen connective tissues involving all layers of the uteri including endometrium layer and myometrial layer were observed in animals administered MSG and ASD (100 mg/kgbw). However, there were moderate inflammatory cells seen in the endometrial layer (slender arrow).

3.5. ASD reduces fibroblast cell count in myometrium of MSG pre-treated female rats

Fig. 5 shows the fibroblast count density obtained from the myometrium of the uteri of the control and treated female rats. Using histomorphometric technique, the fibroblast cell counts of stained spindle shaped connective precursor cells was significantly ($p < 0.05$) elevated in the MSG-treated group when compared with the control group. In contrast, the cell counts in the animals that have been treated with ASD following exposure to MSG decreased significantly ($p < 0.05$) when compared with those in the MSG-treated group.

4. Discussion

Uterine fibroids represent the most common tumour in women. The toxicity of monosodium glutamate has been well documented including its implication in the development of uterine hyperplasia in rats [13]. ASD is a formulation made up of acacia honey and extracts of certain Israeli plants. It is used traditionally for the management of various diseases including fibroids. This study investigated the effects on ASD a poly herbal phytomedicine on MSG-induced hyperplasia and exposure with ASD in female rats.

The observation that the levels of total protein, total serum cholesterol of MSG-treated animals were significantly elevated ($p < 0.05$) when compared with control animals and those that received both MSG and alpha stone decoction ($p < 0.05$) suggests that ASD may contain agents that could trigger cholesterol biosynthesis resulting in elevated cholesterol synthesis in the MSG-treated rats. Similarly, marked increase in levels of triglycerides in MSG administered animals is in consonance with the observations of Lorden and Caudle [26] that consumption of MSG was associated with increasing levels of plasma triglycerides. Besides, Mariyamma et al. [27] had proposed that a shift in glucose metabolism towards lipogenesis might account for the hyperlipidaemia in MSG-treated rats.

Similarly, there was significant ($p < 0.05$) elevation in the levels of estrogen and progesterone in MSG-treated female rats when compared with control. However, significant reduction ($p < 0.05$) in the hormonal levels in female rats co-administered with ASD was observed. These results were in consonance with the findings of Reiss et al., [28] which showed that estradiol and progesterone are major players in the transformation of myometrial cells into leiomyoma cells. Given that the human body is incapable of making hormones without cholesterol, this serves as a pointer to the fact that elevated levels of total cholesterol (a precursor of steroid hormones) in MSG-treated animals is a factor in the enhanced

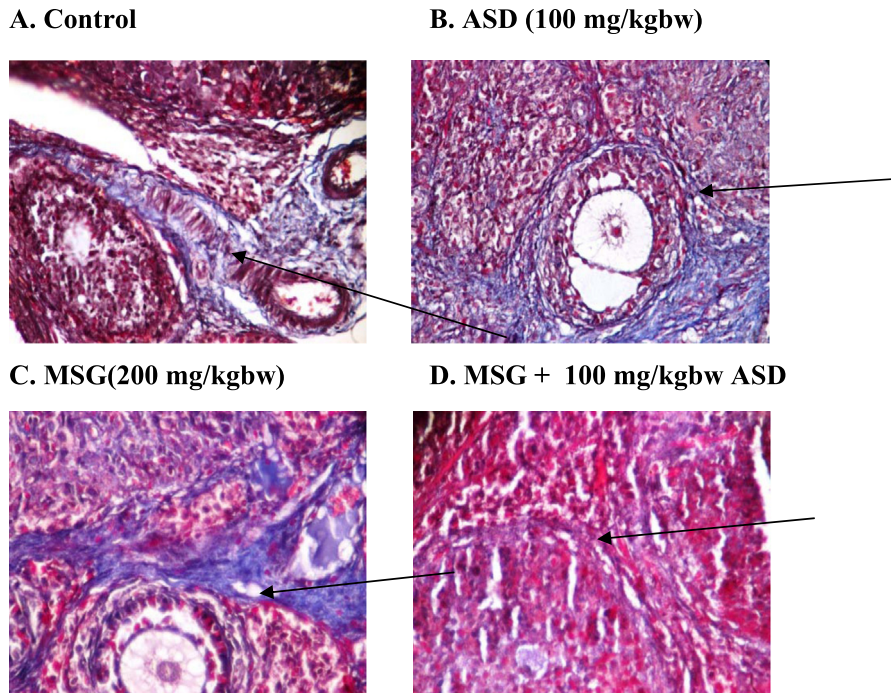


Fig. 3. Photomicrographs of ovarian sections stained by masson trichrome in MSG-treated female rats (X 400) (collagen appears blue; muscles and red blood cells appear red; nuclei -black) (a) Normal ovarian section with normal deposits of collagen connective tissues. (b) Normal deposits of collagen. (c) Moderate to severe deposits of collagen tissue with fibrosis observed in ovarian stroma. (d) Normal deposits of collagen connective tissue with vessels appearing normal.

concentrations of estrogen and progesterone in the MSG-treated groups when compared with the control and co-administered groups.

The observed reduction in fibroblast count of endometrium of uteri of female rat may be due to reduced cell proliferation resulting from growth hormone deprivation. The ECM collagens are known to

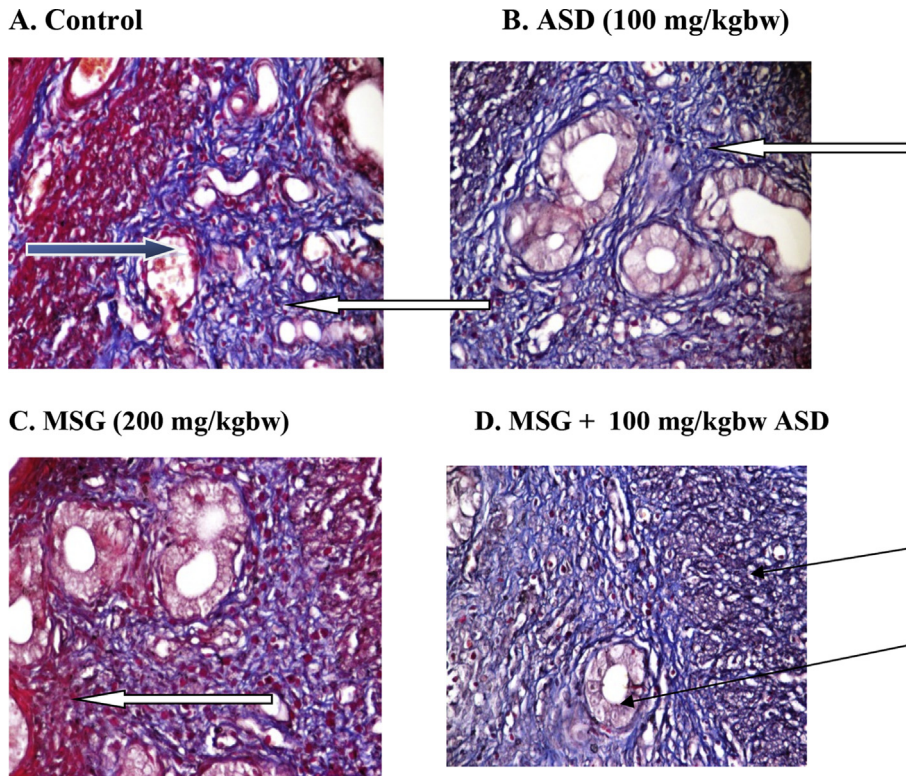


Fig. 4. Photomicrograph of uterine sections stained by masson trichrome in MSG-treated female rats. (collagen appears blue; muscles and red blood cells appear red; nuclei -black) (X 400). (a) Normal connective tissues within the endometrium layer (white arrow) with the muscle in the myometrium appearing normal (blue arrow). (b) Mild deposition of connective tissues (collagen fibre) within the endometrium layer (white arrow), the myometrium show normal connective tissues. (c) Heavy deposit of collagen connective tissues within the endometrium layer (white arrow) and myometrial layer (slender arrow), the endometrial layer appear thickened (black arrow). (d) moderate deposit of collagen connective tissues involving all layers of the uterus including endometrium layer and myometrial layer. A moderate inflammatory cells seen in the endometrial layer (slender arrow).

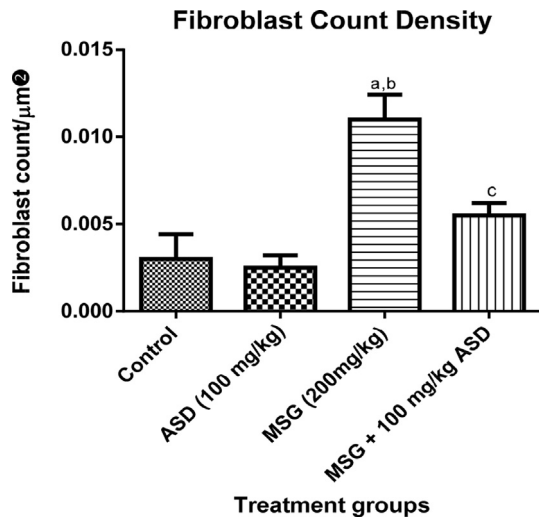


Fig. 5. Cell count density obtained from the myometrium of uteri of female wistar rats in MSG pre-treated rats. Mean \pm Standard deviation ($n = 5$). $p < 0.05$ compared with control (a), $p < 0.05$ compared with ASD alone (b), $p < 0.05$ compared with MSG alone (c).

both maintain cellular morphology and they connect extracellular stimuli and cells by regulating several processes including proliferation of cells [29]. However, fibrotic tumors have been shown to contain an abundance of disorganized ECM collagen [30,31]. Masson's trichrome stain is used to express collagen in tissues [32] and was employed in this study to differentiate between the collagen cells of the uteri of the rats. The photomicrographs presented showed that formation of collagens were upregulated in uteri of MSG-treated animals compared to normal myometrium (control animals). Heavy deposits of collagen are an indication of fibrosis. It may be inferred from the present study that high dose of MSG resulted in hyperplasia observed in the uteri of the MSG-treated groups, which was as a result of increase in the average fibroblast cell density (hyperplasia) in the MSG-treated group when compared with the control and the co-administered group.

Put together, the results of this study show that ASD contains certain bioactive agents that ameliorate uterine-induced hyperplasia and also protect against MSG-induced increase in the levels of hormones that were associated with the development of uterine fibroids in rats. This study justifies the folkloric use of ASD in the management of uterine hyperplasia and opens a window for ascertaining the nature of the chemical components present in ASD that have the potential for use as an anti-tumour agent in fibroid treatment.

5. Conclusion

Administration of MSG increased the levels of estrogen (estradiol), progesterone, triglycerides and total cholesterol in female wistar rats which led to increased proliferation of cells of the myometrium as indicated in the rise in fibroblast cell count. However, treatment with ASD mitigated these effects by reduction of levels of ovarian hormones (estrogen and progesterone) as well as levels of lipid profile.

These findings suggest that ASD may possess anti-fibrotic property which may prove useful in the management of fibroids. Further work should include identifying potential targets relevant for the initiation and development of tissue fibrosis in myometrium for screening and therapeutic purposes.

Sources of funding

None.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jaim.2019.05.001>.

References

- [1] Che C, Wang Z, Chow M, Lam C. Herb-herb combination for therapeutic enhancement and advancement: theory, practice and future perspectives. *Molecules* 2013;18:5125–41.
- [2] Srivastava S, Klal V, Pant W. Polyherbal formulations based on Indian medicinal plants as antidiabetic phytotherapeutics. *Phytopharmacology* 2013;2:1–15.
- [3] Parasuraman S, Thing G, Dhanara S. Polyherbal formulation: concept of ayurveda. *Phcog Rev* 2014;8(16):73–80.
- [4] Eltoukhi Heba M, Modi Monica M, Weston Meredith, Armstrong Alicia Y, Stewart Elizabeth A. The health disparities of uterine fibroids for African American women: a public health issue. *Am J Obstet Gynecol* 2014 Mar;210(3):194–9. <https://doi.org/10.1016/j.ajog.2013.08.008>.
- [5] Parker W. Etiology, Symptomatology and diagnosis of uterine myomas. *Fertil Steril* 2007;87(4):725–36.
- [6] Sankaran S, Manyonda I. Medical management of fibroids. *Best Pract Res Clin Obstet Gynaecol* 2008;22(4):655–76.
- [7] Puri K, Famuyide AO, Erwin PJ, Stewart EA, Laughlin-Tommaso SK. Submucosal fibroids and the relation to heavy menstrual bleeding and anemia. *Am J Obstet Gynecol* 2014;210:38.e1–7.
- [8] Stewart EA, Cookson CL, Gandolfo RA, Schulze-Rath R. Epidemiology of uterine fibroids: a systematic review. *BJOG* 2017;124:1501–12.
- [9] Yamaguchi S, Ninomiya K. Umami and food palatability. *J Nutr* 2000;130(4):921–6.
- [10] Miškowiak B, Kesa B, Limanowski A, Partyka M, Filipiak B. Long-term effect of neonatal monosodium glutamate (MSG) treatment on reproductive system of the female rat. *Folia Morphol (Warsz)* 1999;58(2):105–13.
- [11] Mondal M, Sarkar K, Nath P, Paul G. Monosodium glutamate suppresses the female reproductive function by impairing the functions of ovary and uterus in rat. *Environ Toxicol* 2017;8:1–11.
- [12] Gobatto CA, Mello MA, Souza CT, Ribeiro IA. The monosodium glutamate (MSG) obese rat as a model for the study of exercise in obesity. *Res Commun Mol Pathol Pharmacol* 2002;111(1–4):89–101.
- [13] DwiWahyun E, Situmorang C, WisnuBarlianto C, Dwijayasa P. Combination of vitamin C and E modulated monosodium glutamate-induced endometrial toxicity in female Wistar rat. *Asian Pac J Reprod* 2014;3:106–9.
- [14] Saïda I, Emin S. Chemical composition of various *Ephedra* species. *BJBMS* 2015;15(3):21–7.
- [15] Lampronti A, Saab R, Gambari. Medicinal plants from Lebanon: effects of essential oils from *pistacia palaestina* on proliferation and erythroid differentiation of human leukemic k562 cells. *Minerva Biotec* 2005;17:153–8.
- [16] Rehm S, Espig G. "The cultivated plants of the tropics and subtropics: cultivation, economic value, utilization" - Weikersheim (DE). 1991. Margraf, -viii,552. p. 220.
- [17] Rtibi K, Selmi S, Grami D, Amri M, Eto B, El-Benna J, et al. Chemical constituents and pharmacological actions of carob pods and leaves (*Ceratonia siliqua* L.) on the gastrointestinal tract: a review. *Biomed Pharmacother* 2017;93:522–8.
- [18] Ben-Arye E, Mahajna R, Aly M, Ali-Shtayeh S, Bentur Y, Lev E, et al. Exploring an herbal "wonder cure" for cancer: a multidisciplinary approach. *J Cancer Res Clin Oncol* 2016;142(7):1499–508.
- [19] Erejuwa O, Sulaiman S, Mohd S, Wahab M. Effects of honey and its mechanisms of action on the development and progression of cancer. *Molecules* 2014;19:2497–522.

- [20] Sofowora AO. Medicinal plants and traditional medicine in Africa. 2nd ed. University of Ife Press; 1993. p. 320.
- [21] Trease G, Evans W. A textbook of pharmacognosy. 13th ed. London: Bailliere Tinnall Ltd.; 1989.
- [22] Brown M, Goldstein J. How LDL receptors influence cholesterol and atherosclerosis. *J Am Sci* 1984;251:58–66.
- [23] Sullivan D, Kruijswijk Z, West Z, Kohlmeier C, Katan M. Determination of serum triglycerides by an accurate enzymatic method not affected by free glycerol. *Clin Chem* 1985;7:1227–8.
- [24] Gornall A, Bardawill C, David M. Determination of serum proteins by means of the biuret reaction. *J Biol Chem* 1949;177:751–66.
- [25] Mutter G, Kauderer J, Baak J, Alberts D. Biopsy histomorphometry predicts uterine myoinvasion by endometrial carcinoma: a gynecologic oncology group (GOG) study. *Hum Pathol* 2008;39(6):866–74.
- [26] Lorden J, Caudle A. Behavioural and endocrinological effects of single injection of MSG in the mouse. *Toxicol Teratol* 1986;8:509–49.
- [27] Mariyamma T, Sujatha KS, Sisilamma G. Protective effect of Piper longum (Linn.) on monosodium glutamate induced oxidative stress in rats. *Indian J Exp Biol* 2009;47(3):186–92.
- [28] Reis F, Bloise E, Ortega-Carvalho T. Hormones and pathogenesis of uterine fibroids. *Best Pract Res Clin Obstet Gynaecol* 2015. <https://doi.org/10.1016/j.bpobgyn.2015.11.015>.
- [29] Pickering J. Regulation of vascular cell behavior by collagen: form is function. *Circ Res* 2001;88:458–9.
- [30] Leppert P, Baginski T, Prupas C, Catherino WS, Pletcher S. Comparative ultrastructure of collagen fibrils in uterine leiomyomas and normal myometrium. *Fertil Steril* 2004;82:1182–7.
- [31] Rogers R, Norian J, Malik M, Chrisman G, Abu-Asab M. Mechanical homeostasis is altered in uterine leiomyoma. *Am J Obstet Gynecol* 2008;198(474):1–11.
- [32] Cook DJ. Cellular pathology: introduction to techniques and applications'. 2nd ed. Bloxham: Scion Publishing Limited; 2006.