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ORIGINAL ARTICLE

Effects of Sunflower (Aspillia Africana) Leaves on the Serum Total Protein Levels of MSG-Induced Fibroids in Adult Female Wistar Rats

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ABSTRACT

Monosodium glutamate (MSG) flavor enhancing properties has projected its wide use in the food industry, yet it is taught to be linked to uterine fibroids .This study was undertaken to determine the effects of sunflower (Aspila africana) leaves extracts on serum total protein levels of MSG-induced fibroids in female wistar rats.Preparation of aqueous extract of Aspillia africana was done by mimicking indigenous medical practitioners. Fifteen rats were grouped randomly into three groups. Rats in group 1 severed as control and received only distilled water. Group 2 received 750 mg MSG /kg bodyweight only for 28 days, while group 3 received 750 mg MSG/Kg bodyweight for 28 days then was treated with 350 mg extract/kg bodyweight for another 28 days. Samples were collected weekly via ocular puncture and assayed for total protein. However, a day after final exposure, rats were euthanized by inhalation of chloroform. Blood was then collected by cardiac puncture and total protein levels evaluated. the result shows that MSG alone increase the total protein levels.Treatment with Aspillia africana showed no significant difference in the first three weeks ($p = 0.638$, $p = 0.615$ and $p = 0.058$). However, in the fourth week we observed a significant decrease ($p=0.011$). MSG possess degenerative effects in the female reproductive system and suggest that prolong exposure to Aspillia africana could be an effective herbal therapy for patients with fibroids without need for surgery.

Keywords Aspilla africana, leiomyoma, Fibroids, Food addictive, Leiomyoma, Wistar rats, Monosodium glutamate

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INTRODUCTION

Monosodium glutamate is the sodium salt glutamic acid, a non-essential amino acid often found biological substances [1].Glutamate has flavor enhancing effect[2], thus is often added to foods as purified monosodium salt. Estimated average daily consumption of MSG

in Europe is about 30 mg/kg bodyweight/day which usually proportional to concentration of MSG in food and individual preferences[3].Similar data for Africa is limited.

The safety of MSG has continue to be controversial. Studies have reported negative effects of MSG to experimental animals and even humans [3], [4], [5], [6].Eweka et al. [7] reported degenerative and atrophic changes in the fallopian tubes after prolong exposure of rats to high concentration of MSG. MSG has also been associated with the 'Chinese Restaurant Syndrome' character- ized by numbness, weakness, flushing, sweating, dizziness and headaches [4], MSG is taught to stimulate the expression of several genes linked to adipocytes differentiation, increase levels of serum free fatty acids, triglycerides, insulin and bile biosynthesis [8]. It elevates aspartate aminotransferase, alanine aminotransferase in adult rats leading to degenerative changes on the liver and dilation of the central veins [9]. Reports have also indicate MSG increases oxidative stress

[10], [11] and the levels of total protein, cholesterol and estradiol [12], [13] which were taught are part of complex mechanisms in uterine fibroids.

Fibroids, leiomyoma and myoma are synonymous and are the most gynecological tumours of the female genital track with prevalence of 70 % - 80 % of women in their mid-fifties [14]. They are more frequent in black women[14], [15]. They are the most leading cause of hysterectomy in the US and most common cause for surgery for women after caesarean section[16]. Leiomyomas are benign turmours of smooth muscles, the type of muscles found in the heart and uterus [17]. They can grow enough to obstruct the uterus and compress the great vessels [18]. Studies have not yet fully elucidate the cause of fibroids. Somatic mutations such as translocations, duplications and deletions have been identified in almost one half of leiomyoma studied by cytogenic analysis[18].The most frequent cytogenic changes involved chromosome bands 12q1415 and 7.q22[17]. Elevated levels of estrogen and progesterone has also been implicated[18], [1], [11]. Risk factors includes, early menarche [19], [20], age [15], [21], ethnical differences [22], [23], [24], parity and pregnancy [19], [22], caffeine intake [21] and obesity, among others[25]. Uterine fibroids are often asymptomatic but they could cause a multitude of symptoms such as abnormal uterine bleeding, pelvic pressure, pain, urinary incontinence or retention, which has been link with reproductive problems such as infertility and miscarriage [26], [14], [18]

Until recently, medical management options for uterine leiomyoma aside surgery, have been of limited value. Novel therapies at the receptor and gene levels are undergoing inves- tigations and may eventually offer better long term management options. In Nigeria many indigenous plants are used in herbal medicine to cure diseases and heal injuries. One of such plants used is Aspilla africana. This plant also known as wild sunflower, is a common weed of field crops in West Africa[27].The phytochemical study of Aspillia africana leaves has revealed the presence of saponins, tannis, alkaloids, flavonoids, terpe- niod and phenols with the absence of steroids, phylobatamin and cardiaglycoside[28]. The plant contains ascorbic acid, riboflavin, thiamine and niacin[29].Its antifungal, antiulcer, anticoagulant, antibacterial, and antimalarial effects

have been reported[30], [31]. Methanolic extracts of Aspillia africana leaves have been reported to have negative effects on the estrous cycle and uterine tissues of wistar rats suggesting its negative influence on reproductive health of animals [32]. Thus, the study aims to take advantage of these effects to extenuate the growth of uterine fibroids and also indirectly mitigate the degenerative influence of MSG on the female reproductive system by monitoring the weekly concentration of serum total protein. It was intended at reducing the burden of fibroids following oral intake of Aspillia africana by mimicking traditional methods rather than surgical procedures. On the sidelines, this study was meant to create more awareness, on the negative effects of some food additives, in particular monosodium glutamate to the female reproductive system.

MATERIALS AND METHODS

Collection of plant materials and monosodium glutamate

In March 2016, fresh but matured leaves of Aspillia africana were harvested from a local farm at Amuri, Nkanu West Local Government Area of Enugu state, Nigeria. They were conveyed to the Applied Biochemistry store in a brown polythene bag. The plants were authenticated by Professor C. S. Eze, a Botanist at the Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology. The leaves were rinsed severally with clean tap water then distilled water to remove dust particles and debris and thereafter allowed to completely drain. Synthetic glutamate (MSG) was obtain from a major Ajino Motto distribution shop at Ogbette main market (Enugu, Nigeria) for the use of the study.

Preparation of plant extracts

The air-dried plant samples were grinded to reduce the surface area and expose the cells. The grounded samples were weighed and kept prior to extraction. For extraction, 520g of the plant sample was weighed into a 5 liters capacity aluminum pot. Four hundred (400) ml of distilled water was added and allowed to boil for 25 minutes. It was filtered using a muslin cloth. The filtrate is allowed to concentrate using a water bath at 70 ^{0}c. The extracts were refrigerated at 2 – 8 ^{0}C until use. This procedure was done to mimic local methods for the preparation of traditional remedies.

Experimental studies

Thirty four albino rats (females only) of wistar strain weighing about 130-180 grams were obtained from the Department of Veterinary Science, Faculty of Medicine, University of Nigeria, Nsukka. The animals were allowed to acclimatize for two weeks in the animal house. Animals were housed in well ventilated cages (aluminum bottom and wire mesh top) and kept under ambient environmental conditions of temperature, relative humid-

ity and 12 hour light/dark cycle. The animals were maintained on palletized grower feed obtained from Vital Feeds Jos, Plateau State, Nigeria. Both feed and water were provided ad libitum[26], [11].

Experimental Design

3.4.1 Preliminary phase: Procedures as described by [11], for the induction of uterine fibroid using MSG were adhered to with modifications. Due to perceived slight variation in the concentration of synthetic MSG from production lines, a preliminary test was done to ascertain the concentration of MSG needed for the induction of uterine fibroid. Eight rats were randomly grouped into four distinct cages marked A, B, C, and D. They received 350 mg, 750 mg, 850 mg, and 1050 mg MSG/kg body weight respectively. Warm water was used to dissolve synthetic MSG, which was administered daily via oral gavages between the hours of 11am -12pm Nigerian time and lasted for a period of 28 days [26 27] **.** Animals in group D all died after the first week. Thus, 1050 mg MSG/kg body weight was regarded lethal dose (LD 50) while 750 mg MSG/kg body weight was seen as an effective dose [4],

3.4.2 Treatment phase: While [11] used 100 mg MSG/kg body weight for 60 days, we adopted to make use of 750 mg MSG/kg body weight for 28 days [4]. Fifteen rats were randomized into 3 groups of five rats each. Group one received only distilled water throughout the entire experimental period. Group 2 received only 750 mg MSG/kg body weight without treatment (for 28 days), while group 3 received 750 mg MSG/kg body weight for 28 day and 350 mg extract/kg body weight for another 28 days period. All administration were done via oral intubation and were administered between the hours of 11 am – 12 pm daily. Blood was collected weekly by ocular puncture and assayed for serum total proteins. However, a day after the final exposure, the animals were euthanized by inhalation of overdose of chloroform. Blood was collected by cardiac puncture into EDTA sterilized sample bottles. Serum was prepared by centrifugation (6000×g, 30 min) and used for analysis of serum total protein levels.

Biochemical Assay

Determination of total protein: Serum total protein was determined by Biuret method describe by [28], Five dilutions of Bovine Serum Albumin (BSA) (0.5g BSA in 5 ml distilled water) were prepared within the range 0 - 10 mg/mL by successively pipetting 0.2, 0.4, 0.6, 0.8 and 1 mL of the BSA, into test tubes 1 to 5. The total volume of each test tube was made up to 1 mL by adding the corresponding amount of distilled water. Into another test tube marked blank, 1 mL of distilled water is added and placed on the rack. In the test tube marked sample, 0.5 mL of serum was added. Two mL of biuret reagent (1.5 g CuSO4, 6 g sodium potassium tartrate, 500 mL distilled water and 300 mL of 10 % NaOH) was added to each test tube and allowed to stand for 20 min, at 37 ^{0}C for the purple colour to fully develop. Using a spectrophotometer the absorbance was read against the blank at 540 nm.

Statistical Analysis

All data are presented as mean ± standard deviation (SD). The results were analyzed for statistical significance by analysis of variance (ANOVA) (two-factor wit replication) using SPSS statistical programme of MS excel program. P values < 0.05 were considered significant.

RESULTS AND DISCUSSION

isplays the total protein levels of groups for four consecutive weeks. The mean total protein levels for the rats groups in ascending other of magnitude were as follows:

In the first week; group 1 (5.75), group 2 (5.82) and group 3 (5.91). In week 2; group 1 (5.73), group 3 (5.83) and group 2 (6.12). In week 3; group 1 (5.76), group 3 (5.95), and group 2 (6.12). In week 4; group 1(5.74), group 3 (5.96) and group 2 (6.22). There was no significant total protein difference between the rats for week 1 (p =.638), week 2 (p = .625) and week 3 (p = .058). This implies that the total proteins were quite similar for all groups within the first three weeks of study. However, in week four, there was significant difference among groups. Group 2 was significantly higher compared to other groups (p = .011).

Table 1. Comparison of Total Protein Levels between Groups

Groups	Week 1 (mg/ml)	Week 2 (mg/ml)	Week 3 (mg/ml)	Week 4 (mg/ml)
Groups 1	5.75±0.18	5.73±0.18	5.76±0.17	5.74±0.14^{a}
Groups 2	5.82±0.13	5.95±0.18	6.12±0.15	6.22±0.16^{c}
Groups 3	5.91±0.29	5.83±0.43	5.95±0.24	5.96±0.20^{ab}
CV%	0.06549	0.089938	0.089938	0.089938

Keys:

Group 1 = No MSG + no treatment;

Group 2 = 750 mg MSG/kg body weight + no treatment

Group 3 = 750 mg MSG/kg body weight + 350 mg Aspillia africana /kg body weight.

^{a}= Significantly different to group 2

^{c}= Significantly different to all groups

^{ab} = Significantly similar to group 1 but significantly different to group 2

CV% = Coefficient of variation

Also, a bar chart (Figure 2) was used to illustrate the variations in concentration of serum total protein among study groups. The results shows that MSG alone significantly increased the total protein which was same with the findings of [9,12].The increase in the total protein levels may be due to the degenerative effects of MSG which led to the proliferation of proteins from various tissues and organs into the blood[4, 10]

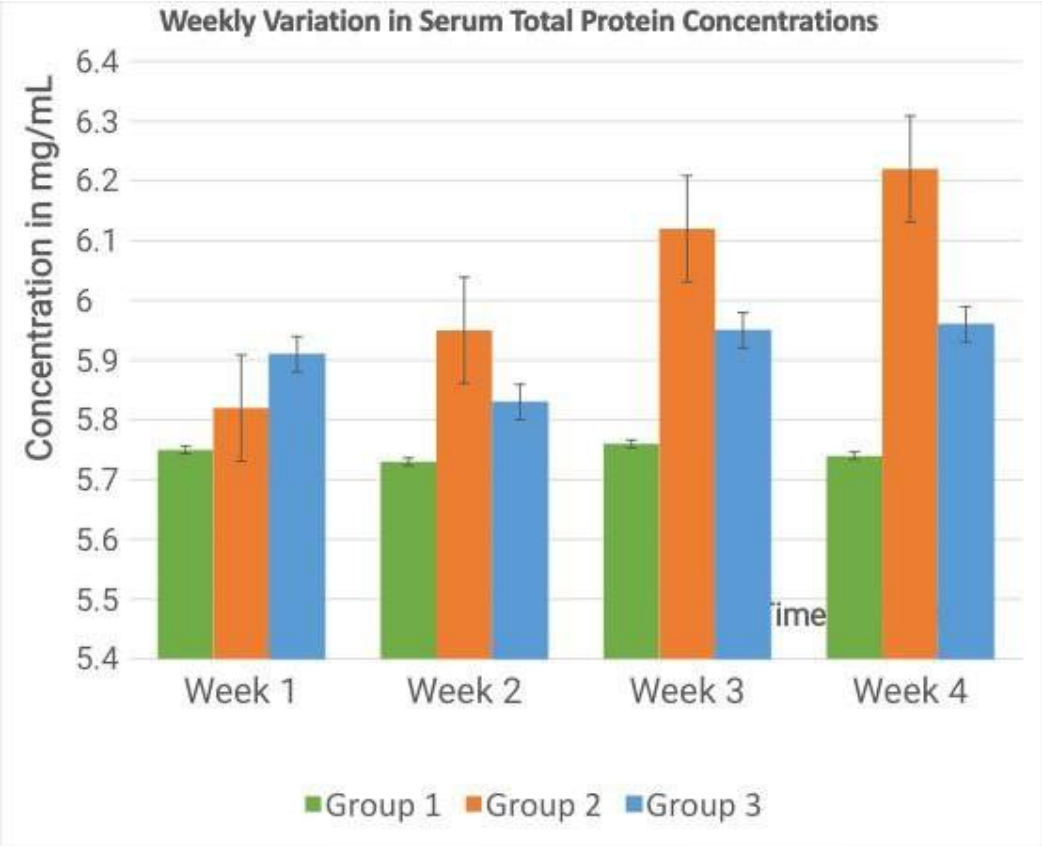

Chart 1: Graphical representation of the changes in serum total proteins of various groups.

The effects of MSG on the total protein levels could be attributed to the activation of transcriptional promoter and enhancer elements used for the control of gene expression, which promoted the ability of RNA polymerase to recognize the nucleotide at the initiation stage, thereby increased protein synthesis [12], [36] Olubukola et al. (2020) reported heavy deposits of collagen connective tissue within the myometrium layers of the uteri. They also notice significant increase in the levels of total proteins. Thus the increase in total pro- teins resulting from the effect of MSG, might have led to increased production of estro- gen leading to hormonal imbalance [8], [36],[38] ,[39], [40] reported signifi- cant increase in serum estrogen and progesterone levels in estrogen-induced rat models of uterine leiomyoma with similar features in human uterine leiomyomas. Rats treated with Aspillia africana showed a significant reduction of total proteins compared to non-treated rats against untreated rats during the fourth week of exposure (p= .011). This result correlates with findings of [11], [12] ,[38],and[6]. It seems that Aspillia africana assisted in the removal of metabolic wastes, promoted sloughing of the endometrium and estrogen hemostasis [12]. It also seems to hinder tumour development by inhibiting the proliferation of diseased cells in the pituitary gland and tumour growth factors [17],[41]. Aspilla africana might have reduced the effects of MSG by supporting the immune system with a multitude of antioxidants, thus suppressing the oxidative effects of MSG[10], [11].

CONCLUSION

This study shows that MSG, a common food additive possess some degenerative effects in the female reproductive system as it significantly increase the serum total protein levels of rats under study. Abnormal increase in total protein levels when compared to controls are mostly associated with incidences of malignancies. Thus, its use as food enhancer be reduced or more regulated. Aspillia africana significantly decreased the serum total protein levels in treated groups as compared to non-treated groups, suggesting that prolong exposure to Aspillia africana could be an effective herbal therapy for patients with myomas.

DECLARATIONS

1. All authors contributed equally to the paper, with tasks divided collaboratively, including research and writing. Each author shares equal responsibility for the content and conclusions.

2. Conflict of interest

The authors declare no conflict of interest

3. Ethical approvals

(Institutional ethical approvals and informed consent)

This research does not conflict with our university's ethical standards, nor with any known ethical criteria.

REFERENCES

1. Zia MS, Qamar K, Hanif R, Khalil M. Effect of monosodium glutamate on the serum estrogen and progesterone levels in female rat and prevention of this effect with diltiazem. Journal of Ayube Medical College Abbottabad. 2014;26(1):18–20.
2. Aahaolu JO, Ukwenye VO, Okonoboh AB, Ghazal OK, Jimoh AAG. Effects of monosodium glutamate on hematological parameters in wister rats. International Journal of Medicine. 2011;3(6):219–222.
3. Geha RS. Double-blind placebo controlled multiple challenge evaluation of reported reaction to MSG. Journal of Allergy and Clinical Immunology. 2000;106(5):973–980. https://doi.org/10.1067/mai.2000.110794.
4. Eze-Steven PE, Udedi SC, Ude CM. Effects of Spondias mombin and Aspilia Africana aqueous extracts on rats with monosodium glutamate-induced leiomyoma. Cross Current International Journal of Medical and Bioscience. 2019;1(1):2663–2446.
5. Oyebode OT, Obiekwe ME, Olorunsogo OO. Protective effects of alpha stone on monosodium glutamate-induced uterine hyperplasia in female wistar rats. Journal of Ayurveda and Integrative Medicine. 2020;11(3):217–223.

https://doi.org/10.1016/j.jaim.2019.05.001.

6. Eweka AO, Eweka A, Om'iniabohs FAE. Histological studies of the effects of monosodium glutamate on the fallopian tubes of adult female wistar rats. North American Journal of Medical Sciences. 2010;2(3):146–149. 10.4297/najms.2010.3146.
7. Collision KS, Maqbool ZM, Inglis AL, Markhu NJ, Saleh SMA, Bakheat RH, et al. Effects of dietary monosodium glutamate on HFCS-induced hepatic steatosis: expression profiles in the liver and versceral fat. Obesity (Sliver Spring). 2010;18:105–111. org/10.1038/oby.2009.502.
8. Eweka AO, Igbigbi PS, Ucheya RE. Histochemical studies of the effect of monosodium glutamate on the liver of adult rats. Annals of Medical and Health Science Research. 2011;1:3507088–3507088.
9. Hashen HE, El-Din S, Algaidi S. The effects of monosodium glutamate on the cerebellar cortex of male albino rats and the protective role of vitamin C (histological and immunohistochemical study). Journal of Molecular Histology. 2012;43(2):179–186. https://doi.org/10.1007/s10735-011-9380-0.
10. Abdulghani MAM, Alshehade SA, Kamran S, Alshawsh MA. Effect of monosodium glutamate on serum sex hormones and uterine histology in female rats along with its molecular docking and in-silico toxicity. Heliyon. 2022;8(10). DOI: 10.1016/j.heliyon.2022.e10967.
11. Obochi GO, Malu SP, Obi-Abang M, Alozie Y, Iyam M. Effects of garlic extracts on MSG-induced fibroids in wistar rats. Pakistan Journal of Nutrition. 2009;8:970–976.
12. George AK, Kofi A, James OK, Hope KF, Ernest E. Effects of ethanolic stem bark of Blighia unijugata on glutamate-induced uterine leiomyoma in Sprague-Dawley rats. British Journal of Pharmaceutical Research. 2013;3(4):880–896. DOI: 10.9734/BJPR/2014/5402.
13. Baird DD, Dnson DB, Hill MC, Cousin D, Schetman JM. High cumulative incidence of uterine fibroids in black and white women: ultrasound evidence. American Journal of Obstetrics and Gynecology. 2003;188:1000–107. https://doi.org/10.1067/mob.2003.99.
14. Wise LA, Palmer JR, Stewart EA, Rosenberg L. Age specific incidence rate for self-reported uterine leiomyoma in the Black women's health study. Journal of Obstetrics and Gynecology. 2005;105(3):563–568. 10.1097/01.AOG.0000154161.03418.e3.
15. Paker WH. Etiology, symptomatology, and diagnosis of uterine myomas. Fertility and Sterility. 2007;87(4):725–736. https://doi.org/10.1016/j.fertnstert.2007.01.093.
16. Parazzini F, Neggri E, La-Vecchia C, Chatenoud L, Ricci E, Guarnerio P. Reproductive factors and risk of uterine fibroids. Epidemiology. 1996;7(4):440–442.
17. Sato F, Miyake H, Nishi M, Kudo R. Early normal menstrual cycle pattern and the development of uterine leiomyoma. Journal of Women's Health Gender Based Medicine. 2000;9(3):299–302. https://doi.org/10.1089/152460900318489.
18. Laughlin SK, Schroeder JC, Baird DD. New directions in the epidemiology of uterine fibroids. Seminars in Reproductive Medicine. 2010;28(3):204–217. DOI: 10.1055/s-

0030-1251477.

19. Wise LA, Palmer JR, Harlow BL, Spiegelman D, Stewart EA, Admas CL. Reproductive factors, hormonal contraception, and risk of uterine leiomyomata in African-American women: a prospective study. American Journal of Epidemiology. 2004;159(2):113–123. https://doi.org/10.1093/aje/kwh016.
20. Wei J, Chiriboga L, Arslan AA, Melamed J, Yee H, Mittal K. Ethnic differences in expression of the deregulated protein in uterine leiomyomata. Human Reproduction. 2006;21(1):57–67.
21. Wang T, Zhang X, Obijuruetal L. A micro-RNA signature associated with race, tumour size and target gene activity in human uterine leiomyoma. Genes Chromosomes and Cancer. 2007;46(4):336–347. https://doi.org/10.1002/gcc.20415.
22. Lippman SA, Warner M, Samuels S, Olive D, Vercellini P, Eskenazi B. Uterine fibroids and gynecologic pain symptoms in a population-based study. Fertility and Sterility. 2003;80:2207–2213. https://doi.org/10.1016/S0015-0282(03)02207-6.
23. Okokon JE, Nwidu LI, Essiet G. Evaluation of in-vitro antiplasmodial activity of Aspillia africana. International Journal of Pharmacology. 2006;2(3):348–351.
24. Souza JM, Chang MR, Brito DZ, Farias KS, Damasceno-Junior GA, Turatti IC, et al. Antimicrobial activity of Aspilia latissima (Asteraceae). Brazilian Journal of Microbiology. 2015;46(4):1103–1110. https://doi.org/10.1590/S1517-838246420131281.
25. Ewekaa AO. Histological studies of the effects of oral administration of Aspilia africana (Astaeraceae) leaf extract on the ovaries of female wistar rats. African Journal of Complementary and Alternative Medicine. 2009;6(1):57–61. 10.4314/ajtcam.v6i1.57074.
26. Wheatley JL. A gavage dosing apparatus with flexible catheter provides a less stressful gavage technique in the rat. Laboratory Animal. 2002;(7):53–56.
27. Atcha Z, Rourke C, Neo AH, Goh CW, Lim JS, Aw CC, et al. Alternative method of oral dosing for rats. Journal of the American Association for Laboratory Animal Science. 2010;49(3):335–343.
28. Gornall AG, Bardawill CJ, David MM. Determination of serum proteins by means of the biuret reaction. Journal of Biological Chemistry. 1949;177:751–766. https://doi.org/10.2508/chikusan.67.382.
29. Levy G, Hill MJ, Beall S, Zarek SM, Sergars JH, Catherino WH. Leiomyoma genetics, assisted reproduction, pregnancy and therapeutic advances. Journal of Assisted Reproduction and Genetics. 2012;29:703–712. https://doi.org/10.1007/s10815-012-9784-0.
30. Agbadua OG, Idusogie LE, Chukwuebuka A, Nnamdi CS, Sylvester S. Evaluating the protective and ameliorative potential of unripe palm kernel seeds on monosodium glutamate-induced uterine fibroids. Open Access Library Journal. 2020;7. DOI: 10.4236/oalib.1106461.
31. Chen HG, Lan ZHU, Cui QC, Lang JH, Li B. Estrogen induced rat model of uterine leiomyoma. Zhongguo yi xue ke xue yuan xue bao. Acta Academiae Medicinae Sinicae. 2011;33(4):408–411. https://doi.org/10.3881/j.issn.1000-503x.2011.04.012.

32. Lee EJ, Kong G, Lee SH, Rho SB, Park CS, Kim BG. Profiling of differentially expressed genes in human uterine leiomyoma. International Journal of Gynecological Cancer. 2005;15:146–154. DOI:10.1136/ijgc-00009577-200501000-00022.
33. Wheatley J. L. (2002). A gavage dosing apparatus with flexible catheter provides a less stressful gavage technique in the rat. Laboratory Animal, 31(7), 53–56. https://doi.org/10.1038/5000176
34. Atcha, Z., Rourke, C., Neo, A. H., Goh, C. W., Lim, J. S., Aw, C. C., Browne, E. R. & Pemberton, D. J. (2010). Alternative method of oral dosing for rats. Journal of the American Association for Laboratory Animal Science, 49(3), 335–343. https://pubmed.ncbi.nlm.nih.gov/20587166/
35. Gornall, A. G., Bardawill, C. J. & David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. Journal of Biological Chemistry, 177, 751-766. https://pubmed.ncbi.nlm.nih.gov/18110453/
36. Levy, G., Hill, M. J., Beall, S., Zarek, S. M., Sergars, J. H. & Catherino, W. H. (2012). Leiomyoma genetics, assisted reproduction, pregnancy and therapeutic advances. Journal of Assisted Reproduction and Genetics. 29, 703-712. https://doi.org/10.1007/s10815-012-9784-0
37. Olubukola T. O., Martin, E. O., Olufunso, O. O. (2020). Protective effects of alpha stone on monosodium glutamate-induced uterine hyperplasia in female wistar rats. Journal of Ayurveda and Integrative Medicine, 11, 217-223 https://doi.org/10.1016/j.jaim.2019.05.001
38. Agbadua, O. G., Idusogie, L. E., Chukwuebuka, A., Nnamdi, C.S. & Sylvester, S. (2020). Evaluating the protective and ameliorative potential of unripe palm kernel seeds on monosodium glutamate-induced uterine fibroids. Open Access Library Journal, 7, e6461. https://doi.org/10.4236/oalib.1106461
39. Ohiagu, F. and Agada, A. B. (2023). Changes in the reproductive hormone levels of male and female rats consuming monosodium glutamate and soybean extracts. World News of Natural Sciences, 51(70), 71-82. https://doi.org/10.13140/RG.2.2.12048.38406
40. Chen, H. G., Lan, Z. H. U., Cui, Q. C., Lang, J. H., & Li, B. (2011). Estrogen induced rat model of uterine leiomyoma. Zhongguo yi xue ke xue yuan xue bao. Acta Academiae Medicinae Sinicae, 33(4), 408-411. https://doi.org/10.3881/j.issn.1000-503X.2011.04.012
41. Lee, E. J., Kong, G., lee, S. H. Rho, S. B. park, C. S. & Kim, B. G. (2005). Profiling of differentially expressed genes in human uterine leiomyoma. International Journal of Gynecological Cancer, 15, 146-154. https://doi.org/10.1111/j.1048-891x.2005.15016.x