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Original Research Article

ADENO-HYPOPHYSEAL CONSEQUENCE, HISTOLOGICALLY, UPON THE INDUCTION OF UTERINE FIBROID VIA MONOSODIUM GLUTAMATE AND THE PROTECTIVE, PROPHYLACTIC AND PREVENTIVE EFFECTS OF GINGER EXTRACT ON THE INDUCED TUMOR

Abstract:

Background: Uterine fibroids, also referred to as uterine myomas, leiomyomas, myomatas, or simply fibroid are benign soft-tissues tumors that arise from uterine smooth muscle tissue (myometrium). They have been described numerously to be hormone-dependent and uterine structure-damaging, but the present study reports the implication of this soft-tissue tumor to the functioning and eventual anatomical change in the pituitary gland and the potential role of ginger extract in reversing the damages

Aim: To understand uterine fibroids at the level of the pituitary gland, while studying the effects that aqueous extract of ginger will play in the MSG-induced uterine fibroid

Study design: Experimental

Place and Duration of Study: Department of Anatomy, Benjamin S. Carson School of Medicine, Babcock University, Ilishan-Remo, Ogun State, Nigeria between January 2017 and May 2017

Methodology: Acclimatization lasted for 10 days following procurement, after which oral administration of Monosodium Glutamate (MSG) and Aqueous ginger extract ensued to determine the prophylactic, protective and curative effects of ginger on MSG-induced uterine fibroid in adult female wistar rats. Administration lasted for 50 days, after which the experimental animals were sacrificed via cervical dislocation, blood samples were collected for LH and FSH hormone level determination and the pituitary gland was fixed in 10% formosaline for histological analysis

Results: Results showed that MSG-induced uterine fibroid had abnormal effects on the pituitary gland histology and an abnormality in the hormone LH and FSH levels, while ginger reversed this effect.

Conclusion: The results of this study may contribute greatly to knowledge and may offer a non-surgical therapy of treating women with fibroids

1. INTRODUCTION

Uterine fibroids are considered as hormone dependent. They are tumors that develop in the uterus but depend mostly on estrogen hormone which has cranial control from pituitary hormones including LH and FSH. The hypothalamus and pituitary gland (also referred to as the master gland) regulate the reproductive

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39 hormones. The hypothalamus releases the GnRH which, in turn, stimulates the
40 pituitary gland to produce FSH and LH. At the command of FSH and LH, estrogen
41 and progesterone are released from the ovaries.

42 ## 2. Methodology

43 After receiving ethical approval from Babcock University Health Research Ethics
44 Committee (BUHREC), Forty nine (49) healthy, non-pregnant adult female wistar
45 rats (Rattus novergicus) weighing between 190-230g were obtained from, housed,
46 and cared for at the Babcock University animal house, Babcock University, Ilishan-
47 Remo, where a good housing measure was observed as recommended by the
48 Animal Research Review Panel (ARRP, 2002). MSG, which was used to induce
49 uterine fibroid, was obtained from a major seasoning shop at Agric Bus-stop,
50 Ikorodu, Lagos State, dissolved in distilled water to produce the desired
51 concentration of 900mg/kg body weight and stored in the refrigerator below (-4ºC).
52 The median lethal dose of MSG is 15,000mg/kg (Walker *et al.,* 2000). Ginger extract
53 was used as a prophylactic, protective and curative agent in the present study. Fresh
54 ginger rhizomes were purchased and washed in distilled water and ground in distilled
55 water for 1 minute using a kitchen blender. The ground mixture was filtered through
56 a fine cotton cloth and the aqueous extracts was administered orally to the animals
57 in various grades (i.e., 500mg/kg, 900mg/kg, 1,700mg/kg) as instructed by the
58 groupings

59 After the animals were allowed to acclimatize for a period of 10 days with free
60 access to feed and water, administration began thus:

61 Group A (Control group): Rats were given dry pelletized feed and clean water ad
62 libitum.

63 Group B (Negative control group): Rats were given oral treatment of 900mg/kg body
64 weight of MSG for 25 consecutive days

65 Group C (Positive control group): Rats were given oral treatment of 900mg/kg body
66 weight of ginger extract for 25 consecutive days

67 Group D (Prophylactic group): Rats were given oral treatment of 900mg/kg body
68 weight of ginger extract for 25 consecutive days, followed by oral treatment of
69 900mg/kg body weight of MSG for 25 consecutive days

70 Group E (Protective group): Rats were given 900mg/kg body weight of MSG orally
71 with simultaneous oral administration of 900mg/kg of ginger extract for 25
72 consecutive days

73 Group F (Curative group 1): Rats were given oral treatment of 900mg/kg body weight
74 of MSG for 25 consecutive days, followed by oral treatment of lower dose 500mg/kg
75 body weight of ginger extract for 25 consecutive days

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76 Group G (Curative group 2): Rats were given oral treatment of 900mg/kg body
77 weight of MSG for 25 consecutive days, followed by oral treatment of higher dose
78 1,700mg/kg body weight of ginger extract for 25 consecutive days

79 For each of the animals in groups A, F and G, blood tests were carried out on the
80 25th day to confirm the presence of fibroids before administration of ginger extract.
81 Biochemical assays on serum estradiol, serum progesterone were carried out as
82 they have been confirmed to be notable markers for uterine fibroid (Obochi *et al*.,
83 2009; Zia *et al*., 2012; Koffour *et al*., 2013).

84 One day after the last administration, the experimental animals were weighed and
85 then sacrificed by cervical dislocation after blood collection through the orbits for
86 hormone level determination of LH and FSH. The brain was excised and the pituitary
87 gland was fixed in 10% formosaline for histological demonstration.

88 Procedure for Haematoxylin and Eosin (H & E) for general histoarchitecture of the
89 Pituitary glands

90 1. The harvested tissue samples were immersion-fixed in 10% formo-saline at
91 room temperature
92 2. The tissues were then dehydrated in ascending grades of alcohol (70%, 95%,
93 100%, and 100%)
94 3. The tissues were cleared with two changes of xylene for one hour, 30 minutes
95 each
96 4. They were then transferred into two changes of molten paraffin wax I and II
97 for one and half hour each and wax- III for overnight in an oven at 65°C for
98 infiltration.
99 5. Thereafter, the tissue blocks were serially sectioned at 6μm thickness using a
100 microtome. Strips of sections were gently lowered into the surface of a warm
101 water bath at 40℃.
102 6. The floated sections were mounted on egg albumin-coated microscopic
103 slides, and put in an oven maintained at 60℃ for 30 minutes to fix the tissue
104 firmly on the slide.
105 7. The slides were dewaxed with two changes of xylene and hydrated with
106 decreasing alcohol concentration and then immersed in water for 5 minutes.
107 8. The sectioned tissues were then stained regressively with Ehrlich's
108 hematoxylin and counter stained with Eosin.
109 9. After staining with eosin, tissues were washed in tap water and dehydrated by
110 rinsing in increasing concentration of alcohol and then xylene-I. They were
111 then placed in xylene-II until mounting.
112 10. Finally, a drop of mountant DPX (A mixture of Distyrene, a Plasticizer, and
113 Xylene) was placed on top of the sections and the cover slip was applied.

114 **SERUM FSH and SERUM LH (Elabscience, 2015)**

115 After fasting morning serum sample was obtained following blood collection from the
116 animals though ocular puncture in plain red-covered, clot-activator sample bottles,

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117 the blood was allowed to clot and then centrifuged at 3000rpm for 15 minutes to
118 separate serum from the cells, Samples were refrigerated at -7°C for 4 days

119 1. All reagents and samples were brought to room temperature before use; the
120 samples were centrifuged after thawing before and the reagents were mixed
121 thoroughly by gentle swirling before pipetting as foaming was avoided. The
122 samples and standards were assayed in duplicate.
123 2. 100µL of standard, blank, or sample per well was added according to the
124 assigned well. The blank well was added with Reference Standard & Sample
125 diluent and solutions were added to the bottom of micro ELISA plate well, and
126 inside wall touching and foaming were avoided. After mixing, the plate was
127 covered with the sealer and incubated for 90 minutes at 37℃.
128 3. The liquid of each well was removed, after which 100µL of Biotinylated
129 Detection Ab working solution was added immediately to each well after which
130 they were covered with the Plate sealer. The plate was tapped gently to
131 ensure thorough mixing and incubation ensued for 1 hour at 37℃.
132 4. Each well was then aspirated and washed by filing with approximately 350ul,
133 this was repeated three times, it is essential to not that complete removal of
134 liquid was ensure at each step. After the last wash, remaining wash buffer
135 was removed by aspirating. The plate was then inverted and patted against
136 thick clean absorbent paper.
137 5. 100µL of HRP Conjugate working solution was added to each well and
138 covered with the plate sealer, thereafter they were incubated for 30 minutes at
139 37℃ and the wash process was Repeated for five times as conducted earlier
140 6. 90µL of Substrate Solution was also added to each well and covered with a
141 new plate sealer and Incubated for about 15 minutes at 37℃, while being
142 protected from light. The reaction time of 30 minutes was shortened with
143 observance of colour change. With appearance of apparent gradient in the
144 standard wells, the reaction was terminated.
145 7. 50µLof Stop Solution was added to each well in the same order the substrate
146 solution was added, and an immediate colour change to yellow was observed.
147 8. The optical density (OD value) of each well was determined at once using a
148 micro-plate reader set to 450 nm.

149 Calculation of results

150 Cholesterol conc. of unknown (ng/ml) = Cholesterol conc. of∆control x 450 of
151 unknown
152 ∆ 450 of control

153 Precautions:

154 • It was ensured that Substrate Reagent wasn't kept at -20℃
155 • Exposure of reagents to strong light was avoided in the process of incubation
156 and storage

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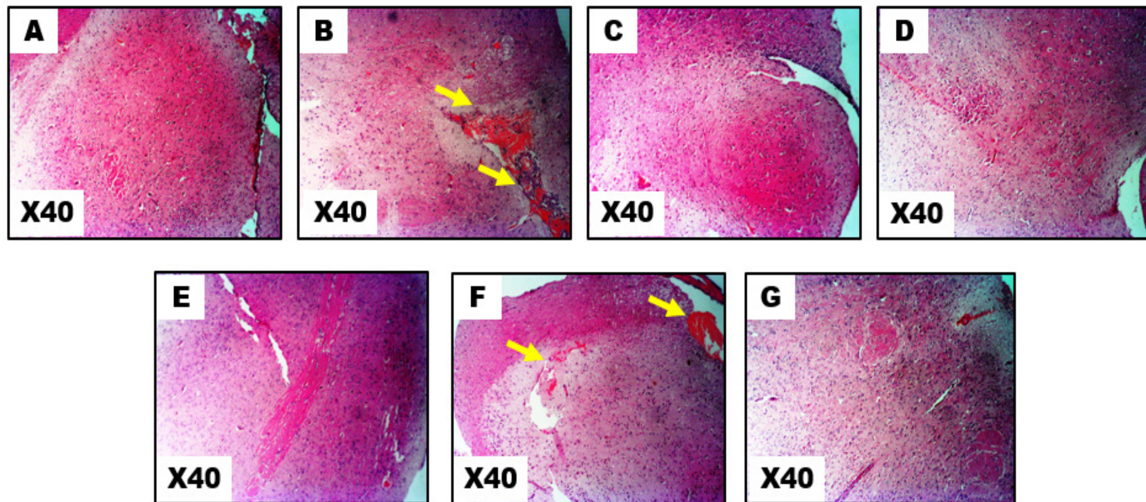
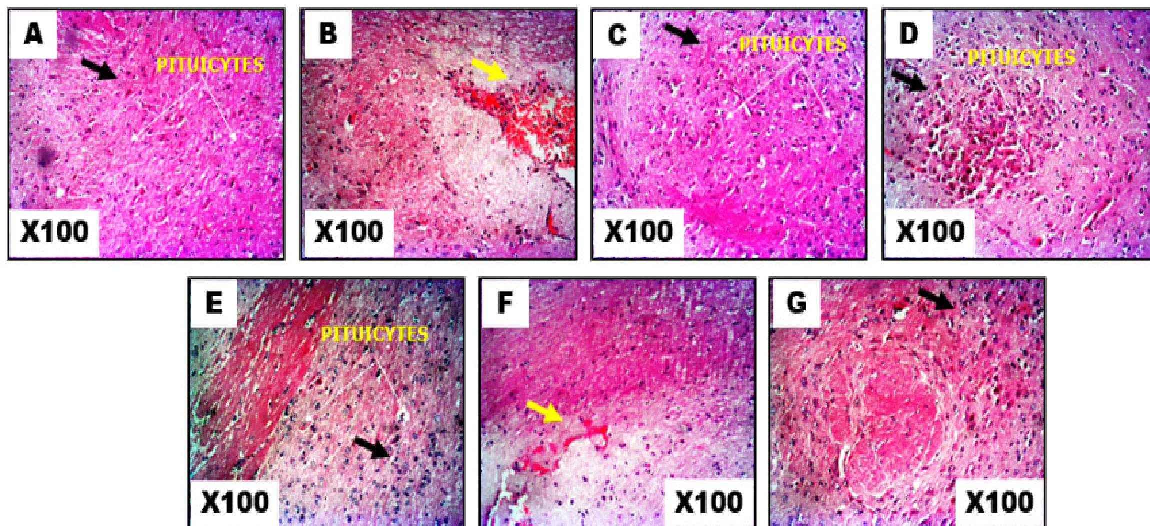
- It was also ensured that all the taps of reagents were tightened to prevent evaporation and microbial contamination.
- Hemolysis was avoided during serum aspiration
- The micro-plate reader should be opened in advance, the instrument preheated, and the testing parameters set.

3. Results and Discussions





Photomicrographs showing panoramic views of hypophyseal general histomorphological presentations in Adult female Wistar rats across the various groups A-G. H&E stain (*x40 & x100*). Neurohypophysis is well demonstrated while adenohypophysis is not conspicuous at this cortical section, pars distalis, pars intermedia and pars nervosa are the various parts

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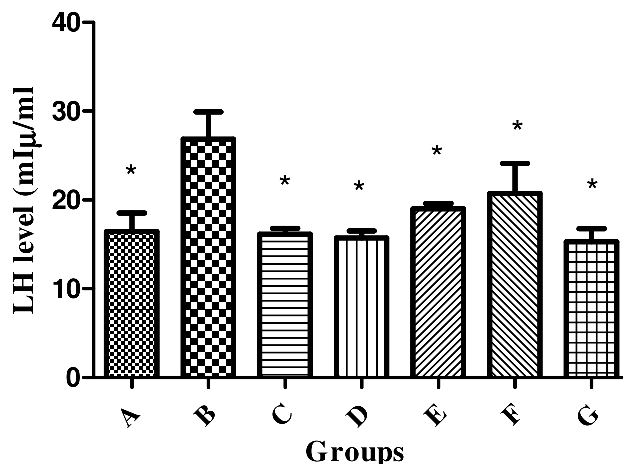
172 **of the hypophysis associated with different secretions. Across the groups,**
173 **degenerative changes as well as presentation of red and inflammatory cells**
174 **are seen present in treated group B & F (yellow arrows) as against the control**
175 **group A & C with well outlined panoramic cytoarchitectural presentation (black**
176 **arrows). Treatment received by groups D, E & G showed a mild degenerative**
177 **change which is not that conspicuous. MSG treatment induces degenerative**
178 **changes in the cytoarchitectural presentation of the pituitary gland as**
179 **demonstrated by H&E stain.**

180 Luteinizing Hormone (LH) level

181 Group A: Control
182 Group B: MSG only
183 Group C: Ginger only
184 Group D: Ginger → MSG
185 Group E: Ginger + MSG
186 Group F: MSG → Ginger (Low dose)
187 Group G: MSG → Ginger (High dose)



188
189 Fig 1.1. Bar graph showing LH levels (mIµ/ml) of control and experimental groups
190 **=P<0.05 when compared with Group B*
191 As shown in Fig 1.2., there was a statistically significant increase in the LH level of
192 group B (26.84±3.088) in comparison with that of group A (16.44±2.067). Also, when
193 compared to group B, there was a significant decrease in the LH levels of groups D
194 (15.72±0.8120), E (19.01±0.6106), F (20.71±3.410), G (15.30±1.458), as these
195 groups held no statistical significant difference when compared to group A
196 (16.44±2.067). Also, in comparison with group A, Group C (16.17±0.6548) held no
197 significant statistical difference.

198 It is also depicted in Fig 1.2. that group B (26.84±3.088) had the highest LH level
199 while group G (15.30±1.458) had the lowest.

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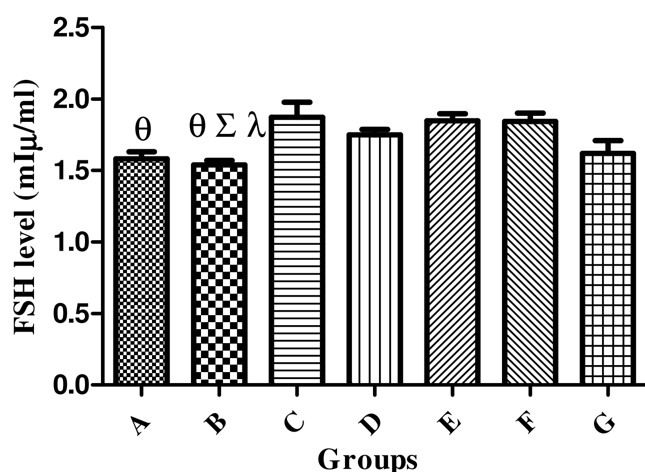
200 LH levels were more pronounced in uterine fibroid animals than in the control
201 animals. Also there was a decrease in the level of LH in the ginger-administered
202 groups.

203 FOLLICLE-STIMULATING HORMONE (FSH) LEVEL
204 Group A: Control
205 Group B: MSG only
206 Group C: Ginger only
207 Group D: Ginger → MSG
208 Group E: Ginger + MSG
209 Group F: MSG → Ginger (Low dose)
210 Group G: MSG → Ginger (High dose)



211
212 Fig 1.2. Bar graph showing FSH levels (mIμ/ml) of control and experimental groups
213 *θ=P<0.05 when compared with Group C*
214 *λ=P<0.05 when compared with Group F*
215 *Σ=P<0.05 when compared with Group E*
216 FSH levels were slightly decreased in uterine fibroid animals than in the control
217 animals. Also there was an increase in the level of FSH in the ginger-administered
218 groups.

219 Conclusion: Ginger has fibroid-preventing and fibroid-reducing properties at the level
220 of the pituitary gland.

221 References:

222 1. Simoni M., Gromoll J., and Nieschlag E. (1997). The follicle-stimuating
223 hormone receptor: biochemistry, molecular biology, physiology and
224 pathophysiology. Endocr. Rev., 18, 739-773
225 2. Grumbach M, Kaplan S. The neuroendocrinology of human puberty: an
226 ontogenetic perspective. In: Grumbach M, Sizonenko P, Aubert M, editors.
227 Control of the onset of puberty. Baltimore: Williams & Wilkins; 1990.

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228 3. Schwanzel-Fukuda M, Pfaff DW. Origin of luteinizing hormonereleasing
229 hormone neurons. Nature. 1989;338(6211):161–4.
230 4. Silverman AJ, Jhamandas J, Renaud LP. Localization of luteinizing hormone-
231 releasing hormone (LHRH) neurons that project to the median eminence. J
232 Neurosci. 1987;7(8):2312–9.
233 5. Yahalom D, Chen A, Ben-Aroya N, Rahimipour S, Kaganovsky E, Okon E, et
234 al. The gonadotropin-releasing hormone family of neuropeptides in the brain
235 of human, bovine and rat: identi fi cation of a third isoform. FEBS Lett.
236 1999;463(3):289–94.
237 6. White RB, Eisen JA, Kasten TL, Fernald RD. Second gene for gonadotropin-
238 releasing hormone in humans. Proc Natl Acad Sci USA. 1998;95(1):305–9.
239 7. Serin IS, Tanriverdi F, Ata CD, Akalin H, Ozcelik B, Ozkul Y, et al. GnRH-II
240 mRNA expression in tumor tissue and peripheral blood mononuclear cells
241 (PBMCs) in patients with malignant and benign ovarian tumors. Eur J Obstet
242 Gynecol Reprod Biol. 2010; 149(1):92–6.
243 8. Poon SL, Klausen C, Hammond GL, Leung PC. 37-kDa laminin receptor
244 precursor mediates GnRH-II-induced MMP-2 expression and invasiveness in
245 ovarian cancer cells. Mol Endocrinol. 2011; 25(2):327–38.
246 9. Chou CS, Beristain AG, MacCalman CD, Leung PC. Cellular localization of
247 gonadotropin-releasing hormone (GnRH) I and GnRH II in fi rst-trimester
248 human placenta and decidua. J Clin Endocrinol Metab. 2004;89(3):1459–66.
249 10. Siler-Khodr TM, Grayson M. Action of chicken II GnRH on the human
250 placenta. J Clin Endocrinol Metab. 2001;86(2):804–10.
251 11. Messinis IE, Vanakara P, Zavos A, Verikouki C, Georgoulias P, Dafopoulos K.
252 Failure of the GnRH antagonist ganirelix to block the positive feedback effect
253 of exogenous estrogen in normal women. Fertil Steril. 2010;94(4):1554–6.