International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2020.267.275

Research Article
Zingerone Enhances Fertility Markers in Both Male and Female Rats and Increases Aryl Hydrocarbon Receptor Expression

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Abstract

Background and Objective: Recently, the major active constituents of ginger were subject to extensive research. Zingerone is a major active ingredient of ginger, sharing many of its therapeutic effects. This study investigated the fertility enhancing activity of zingerone in male and female Wistar rats as well as the relationship between zingerone and the aryl hydrocarbon receptor, that is actively involved in genital physiology. **Materials and Methods:** Morphometric values including body weights and gonadal weights, in addition to sperm counts were measured. Furthermore, indirect immunohistochemical methods were applied using peroxidase enzyme activity to assess the expression and tissue localization of the proliferation marker Ki67 and the aryl hydrocarbon receptor in gonadal and uterine specimens. **Results:** The results revealed a marked dose-dependent enhancement of fertility in male and female rats. This was evidenced by increments in gonadal weights and sperm counts, body weights showed a significant increase in male rats but not in female ones. Immunohistochemical studies revealed elevated cellular expression of Ki67 proliferation marker protein and the aryl hydrocarbon receptor in gonads after zingerone oral administration. **Conclusion:** Therefore, zingerone is a potent fertility boosting compound that could be exerting its pharmacologic effects through modulation of the aryl hydrocarbon receptor as an exogenous ligand.

Key words: Zingerone, fertility, ginger, Ki67 proliferation marker, aryl hydrocarbon receptor, Wistar rats

Citation: Basma Ghazi Eid, Abeer Hanafy, Atif Hasan and Thikryat Neamatallah, 2020. Zingerone enhances fertility markers in both male and female rats and increases aryl hydrocarbon receptor expression. Int. J. Pharmacol., 16: 267-275.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Zingerone one of active ingredients present in ginger has long been used as a medicine and as a spice. It was widely used to relieve a number of ailments including gastrointestinal disorders, arthritis and colds among others[1,2]. The major ingredients of ginger including: zingerone, gingerdiol, zingiberene, gingerols and shogaols are believed to under lie these observed effects[3]. Zingerone was first produced in 1945, by drying of ginger and by heat treatment of gingerol, it represents about 9.25% of ginger[4]. It belongs to methoxyphenols, which have a methoxy group united with the benzene ring of a phenol moiety[4]. It generally has no toxic effects and is an inexpensive compound, it has variable pharmacological activities including anti-inflammatory[5], antioxidant[6], antimicrobial[7] and anticancer properties[8]. Recently, drugs of natural sources including ginger have gained wide attention. In males, ginger is used medicinally for its androgenic activity[9]. It was observed that the oral administration of ginger elevated both plasma and semen testosterone hormone levels and stimulated spermatogenesis[10]. In females, ginger was traditionally used to reduce pain which accompanies menses and primary dysmenorrhea[11]. Furthermore, it was found that ginger exerts supportive effects on folliculogenesis and implantation processes[12].

The Aryl Hydrocarbon Receptor (AhR) is part of the basic helix-loop-helix/PER-ARNT-SIM family and serves as a transcription factor[13]. It is activated by a several planar aromatic hydrocarbons and polychlorinated biphenyls[14]. A number of consumable flavonoids serve as AhR agonists[15] as well as various drugs, such as; omeprazole and ketoconazole[16,17]. Previous studies pointed to the involvement of the AhR in the physiology of female genital organs in animals as well as human subjects[18-20]. Studies by Bidgoli *et al.*[21] have linked the AhR to male infertility in humans. Shogaol which is a major active ingredient of ginger was found to activate AhR and its gene battery in HepG2 cell lines[22]. It is worthy to note that both shogaol and zingerone contain a benzene ring and phenolic moiety in their structure. The fertility enhancing effects of zingerone on the genital organs in both males and females has not been extensively studied before. Therefore, the fertility boosting effects of zingerone in both sexes of Wistar rats were evaluated in this study, as well as the role of the AhR on the observed zingerone biological activity.

MATERIALS AND METHODS

This study was carried out in the Department of Pharmacology and Toxicology, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia (From September, 2018 until July, 2019).

Animals: The animal study methodologies were ethically approved by the Research Ethics Committee of the Faculty of Pharmacy, King Abdulaziz University, Kingdom of Saudi Arabia (Approval No. "PH-108-40").

Animal treatment: A total of 36 (18 male and 18 female), 7 week old male and female Wistar rats (King Abdulaziz University, Kingdom of Saudi Arabia) were kept in separate cages for one week prior to the experiment under controlled environmental conditions with 12/12 h light/dark cycle, 30-70% relative humidity and a temperature of 25°C. The animals were given water and food *ad libitum*. In order to synchronize the female estrous cycles, females were placed in mature rats' soiled sheets, 3 days prior to the start of the experiment. Vaginal smear tests were used to confirm the estrous stage. After that, each sex was divided randomly to three groups (n = 6 per group). The first group (G1): Rats received 1 mL distilled water/rat/day orally for an 8 week duration. Group 2 (G2): Rats were given zingerone (20 mg/kg/day) orally for an 8 week duration. Group 3 (G3): Rats were given zingerone (40 mg/kg/day) orally for an 8 week duration. Zingerone (Sigma-Aldrich, St. Louis, MO, USA) was freshly prepared daily by dissolving it in distilled water to be ready for oral administration to the rats.

Tissue sampling: Rats were weighed at the end of the protocol. Urethane (1 g kg^{-1}) was given intraperitoneally to anesthetize the rats and blood from the retro-orbital plexus was obtained and centrifuged at 4000 g and 4°C for 20 min. Serum was separated and kept frozen at -80°C for hormonal assays. Testes and ovaries were then weighed after excision. Almost 10% formalin solution was used to fix a part of the tissues for 24 h for histological and immunohistochemical examination.

Testosterone assay: Testosterone levels in the serum of male rats was determined using Testosterone ELISA Kit (ab108666) from abcam®, Cambridge, MA, USA.

Estradiol assay: Estradiol levels in the serum of female rats was assayed by 17 beta Estradiol ELISA Kit (ab108667) from abcam®, Cambridge, MA, USA.

Sperm count: Semen in the epididymis was squeezed and diluted with semen dilution fluid (sodium bicarbonate 5 g, formalin 1 mL, distilled water 99.0 mL). Sperm were counted as described by World Health Organization[23] using a Neubauer's haemocytometer under a light microscope at 400x magnification and expressed as million mL^{-1}.

Immunohistochemical protocols: Samples from testes, ovaries and uteri were routinely processed and embedded in paraffin wax blocks to be ready for sectioning[24]. By using a rotatory microtome, 5 µm thick sections were prepared and used in immunoreactions. The expression and tissue localization of two proteins were investigated. The first was Ki67 (proliferation marker) by using of rabbit polyclonal anti-Ki67 antibody (ab15580); the second was aryl hydrocarbon receptor (it is an intracellular multifunctional protein involved in biology of genital organs) which was localized by rabbit polyclonal anti-aryl hydrocarbon receptor antibody (ab84833). The immunohistochemical protocol which was described by Hasan and Fischer[19] was applied, this method uses the peroxidase enzyme activity with diaminobenzidine (DAB) as a chromogen substrate. Antibodies and Normal Goat Serum (NGS) were diluted in 3% Bovine Serum Albumin (BSA) dissolved in Phosphate Buffer Solution (PBS). Sections which were destined to be used with Ki67 protein were subjected to heat pre-treatment (95°C-30 min) in citrate buffer solution for the purpose of antigen retrieval. The 3% H_2O_2 in methanol was used to block endogenous peroxidase by incubation for 30 min in a dark box. Unspecific reactions were blocked by 10% NGS in PBS (ab7481). Primary antibodies (Anti-Ki67 and Anti-AhR) were diluted at 1:150 while secondary antibody (Goat anti-rabbit-ab205715) was used at a 1:500 dilution in BSA. Washing of tissue sections was in PBS with tween 20 (PBST). Immuno-positive reactions were visualized by DAB (ab 64238). Control negative tissue slides were included and they were always immune-negative. Immuno-positive results were photographed by a digital camera under light microscope.

Statistical analysis: The ANOVA test was utilized for comparison of data in the control group and experimental groups. Results were expressed as mean±standard deviation (SD). The p value <0.05 were considered to be statistically significant.

RESULTS

Effect of zingerone on the morphometric parameters: In male rats, there was a significant increase ($p<0.05$) in body weight, testicular weight and sperm count in both G2 and G3 which were treated with zingerone when compared with the rats of the control group. G3 showed a significant increase ($p<0.05$) in these parameters when compared with G2 (Table 1 and Fig. 1).

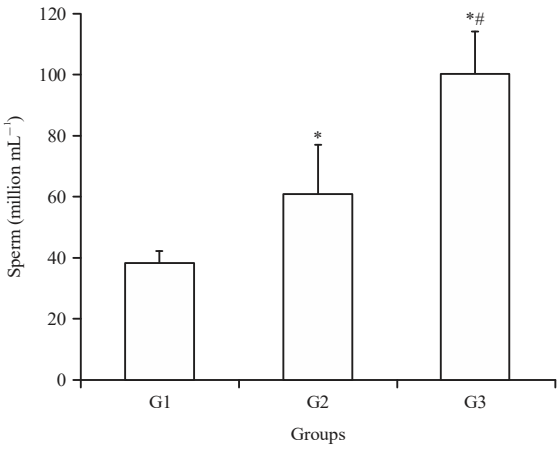

Fig. 1: Sperm count in control and zingerone treated male rats
G1: Control male rats, G2: Treated male rats with 20 mg kg^{-1} zingerone, G3: Treated male rats with 40 mg kg^{-1} zingerone. Data are expressed as Mean±SD of the mean, *p<0.05 in comparison to group 1, #p<0.05 in comparison to group 2

Table 1: Effect of zingerone on body weights and testicular weights of male rats (g)

Parameters	Group 1	Group 2	Group 3
Body weight	245.00±7.1	288.50±18.4*	300.00±23.8*#
Testicular weight	1.38±0.13	1.64±0.73*	1.80±0.74*#

Data are expressed as Mean±SD of the mean, *p<0.05 in comparison to group 1, #p<0.05 in comparison to group 2

Table 2: Effect of zingerone on body weight and ovarian weight of female rats (g)

Parameters	Group 1	Group 2	Group 3
Body weight	207.80±11.9	213.50±6.4	220.00±3.3
Ovarian weight	0.52±0.012	0.73±0.001*	0.74±0.002*

Data are expressed as Mean±SD of the mean, *p<0.05 in comparison to group 1

In female rats, there was a marked but not significant increase in body weight of the treated groups. However, ovarian weight in both treated groups showed a significant increase ($p<0.05$) in comparison to the control group (Table 2).

Effect of zingerone on serum level of hormones: In male rats, there was a significant increase ($p<0.05$) in the level of testosterone in zingerone-treated rats of G2 and G3 if compared with the control rats in G1. Moreover, G3 showed a significant increase ($p<0.05$) in serum testosterone when compared with G2 (Fig. 2).

In female rats, there was a significant increase ($p<0.05$) in the level of estradiol in rats of G2 and G3 if compared with the control rats in G1. The G3 showed a significant increase ($p<0.05$) in serum estradiol when compared with G2 (Fig. 3).

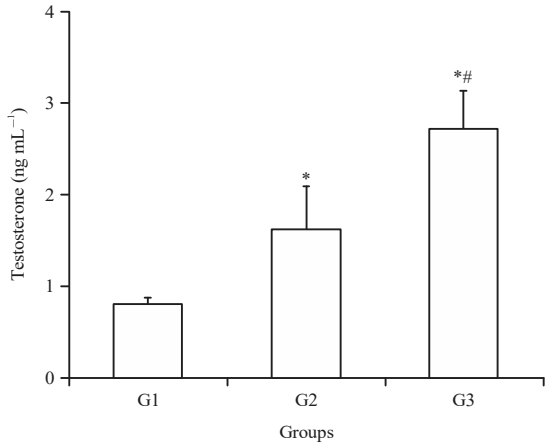

Fig. 2: Effect of treatment on serum testosterone levels (ng mL^{-1})
G1: Control male rats, G2: Treated male rats with 20 mg kg^{-1} zingerone, G3: Treated male rats with 40 mg kg^{-1} zingerone, data are expressed as Mean±SD of the mean, *p<0.05 in comparison to group 1, #p<0.05 in comparison to group 2

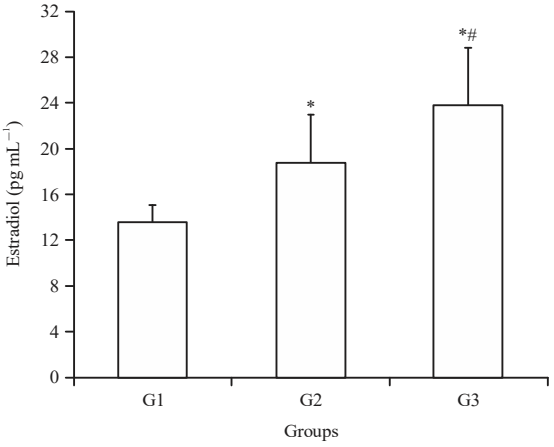

Fig. 3: Measurements of serum estradiol levels in control and zingerone treated female rats
G1: Control female rats, G2: treated female rats with 20 mg kg^{-1} zingerone, G3: Treated female rats with 40 mg kg^{-1} zingerone, data are expressed as Mean±SD of the mean, *p<0.05 in comparison to group 1, #p<0.05 in comparison to group 2

Immunohistochemical results

Expression of proliferation marker Ki67: Cellular proliferation in genital organs was detected by Ki67 proliferation marker (nuclear protein) in specimens from testes, ovaries and uteri and the following results were obtained.

In the testes, Ki67 protein was expressed in a limited number of gonadal cells which occupy the outer most layer in the seminiferous tubules (spermatogonia) in control rats (Fig. 4a-b). This expression pattern was changed in treated rats to a double layer of cells including larger number of spermatogonia and spermatocytes (Fig. 4c-d) in G2. A Larger number of gonadal cells and a multilayer of stained cells was recorded with G3 (Fig. 4e-f). Neither sertoli cells nor interstitial cells expressed the proliferation marker Ki67.

Expression of Ki67 in ovaries of control female was restricted to theca cells (Fig. 5a) and few follicular cells (Fig. 5b) but not in primary oocytes (Fig. 5a-b). In treated rats, higher expression was recorded with G2 zingerone dose, it was present in both follicular and theca cells of developing follicles (Fig. 5c-d). It was also expressed in primary oocytes (Fig. 5c), interstitial cells and corpus luteum (Fig. 5d). More cells in ovaries of G3 dose rats were expressing Ki67 like follicular theca cells and interstitial cells (Fig. 5e), the oocyte was also immune-positive (Fig. 5e). In the corpus luteum, many luteal cells were expressing Ki67 (Fig. 5f).

In the uterus; in control rats (G1), Ki67 was expressed in the epithelium of uterine glands (Fig. 6a-b), while in the endometrium, it was expressed in limited number of luminal epithelial and stromal cells (Fig. 6a-b). In G2 treated rats, a higher expression of Ki67 was recorded in the proliferated epithelium of both uterine glands and endometrium (Fig. 6c-d). In addition, an increased number of endometrial stromal cells were immunopositive (Fig. 6d). In G3 treated rats, higher expression of Ki67 was found in uterine glands epithelium, endometrial epithelium and stromal cells (Fig. 6e-f).

Expression of aryl hydrocarbon receptor (AhR): Expression of AhR receptor in control and treated rats was detected by a polyclonal rabbit anti aryl hydrocarbon antibody, significant results were detected in the testis and uterus (Fig. 7a-f).

In the testis, AhR was expressed in a few number of seminiferous tubules (Fig. 7a) of control rats (G1), while treated rats (G2, G3) showed higher expression in larger number of seminiferous tubules (Fig. 7c, e). Higher magnifications revealed that AhR showed cell specific expression, in control rats, AhR was localized in spermatogonia and interstitial cells but not in sertoli cells (Fig. 7b), the reaction was diffuse cytoplasmic and nuclear. In treated rats, higher expression of AhR was recorded with both doses of zingerone (G2, G3), in addition, sertoli cells were immuno-positive showing nuclear and diffuse cytoplasmic staining (Fig. 7d, f), moreover, spermatogonia showed cytoplasmic staining while spermatocytes till spermatids showed nuclear staining (Fig. 7d, f). Interstitial cells in treated rats (G2, G3) were immuno-positive showing nuclear and diffuse cytoplasmic staining (Fig. 7d, f).

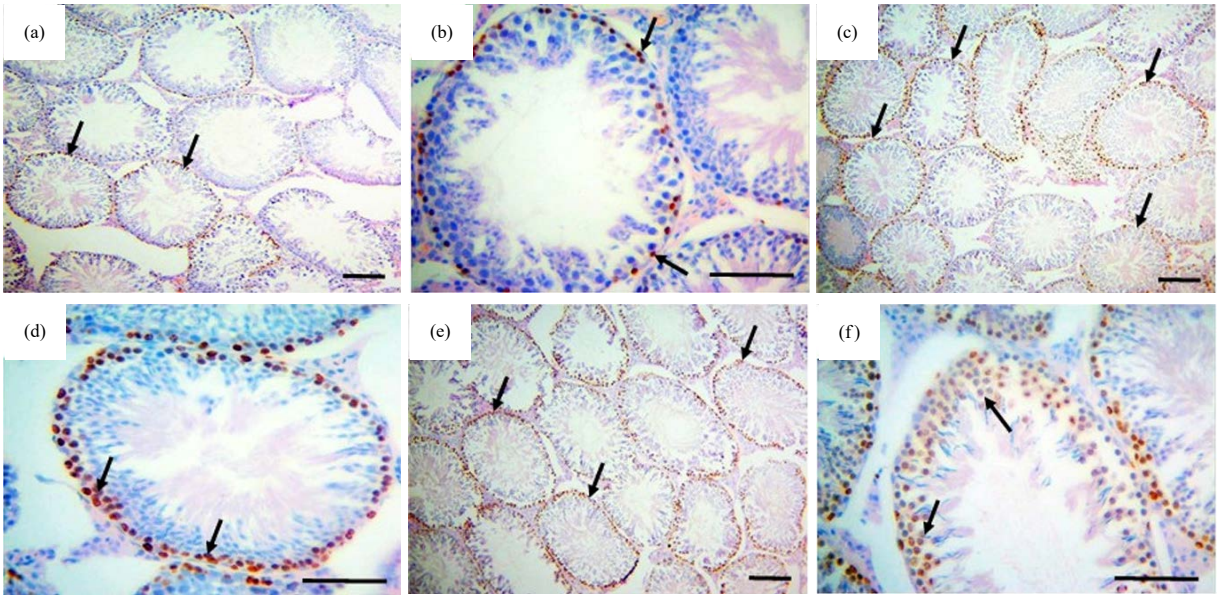

Fig. 4(a-f): Photomicrographs showing expression of Ki67 in Control-G1 and Zingerone treated-G2 and G3 on testis of male rats, (a) G1, Ki67 protein was expressed in seminiferous tubules (ariel view), (b) G1, Ki67 protein was expressed in spermatogonia, (c) G2, Ki67 was expressed in seminiferous tubules (ariel view), (d) G2, Ki67 was expressed in seminiferous tubules, (e) G3, Ki67 was expressed in seminiferous tubules (ariel view) and (f) G3, Ki67 was expressed in seminiferous tubules

G1: Arrows show being localized in nuclei of spermatogonia, G2 (c-d) and G3 (e-f): (c and e, arrows); larger numbers of spermatogonia and spermatocytes were immunopositive, (d, arrows), in addition to different stages of developing spermatocytes in G3 (f, arrows), bars = 40 μm

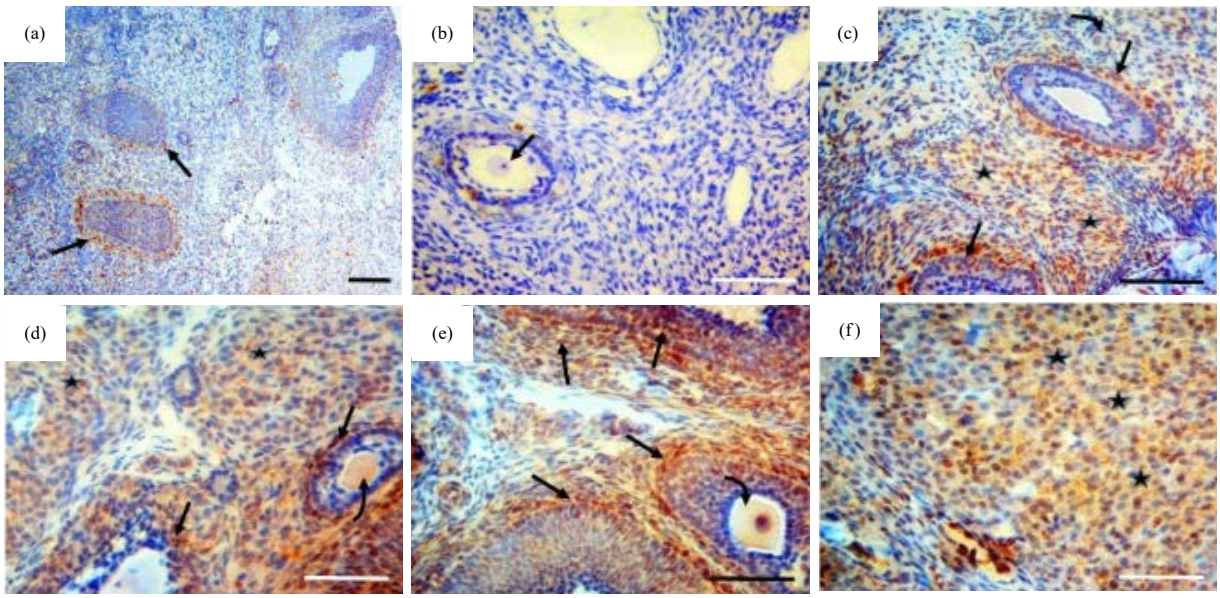

Fig. 5(a-f): Photomicrographs showing expression of Ki67 in Control G1 and Zingerone treated-G2 and G3 on ovary of female rats, (a) G1, Ki67 was expressed in theca cells (control) (b) G1, Ki67 was expressed in theca cells having primary oocytes, (c) G2, Ki67 expressed in larger number of theca cells, (d) G2, Ki67 expressed in larger number of granulosa cells, (e) G3, Ki67 expressed in follicular cells and (f) G3, Ki67 expressed in follicular cells having corpus luteum

G1: (a, arrows), primary oocytes were immunonegative (b, arrow), G2: (c and d, arrows) were immunopositive; primary oocytes (c, curved arrow) and interstitial cells (c and d, stars) were immunopositive, G3 (e, arrows), primary oocyte (e, curved arrow) and corpus luteum (f, stars), bars = 40 μm

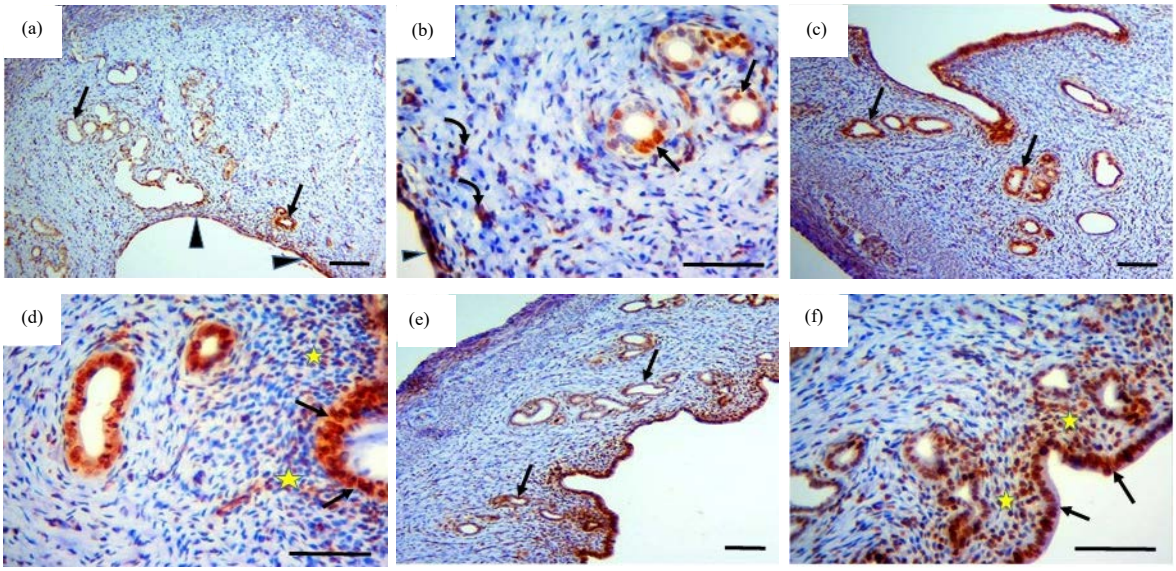

Fig. 6(a-f): Photomicrographs showing expression of Ki67 in Control-G1 and Zingerone treated-G2 and G3 uterus, (a), G1: Ki67 was expressed in uterine glands having luminal epithelium, (b), G1: Ki67 was expressed in uterine glands having stromal cells, (c), G2: Ki67 was expressed in uterine glands and epithelium (ariel view), (d), G2: Ki67 was expressed in uterine glands and epithelium, (e) G3: Ki67 was expressed in uterine glands, endometrial epithelium and (f) G3: Ki67 was expressed in uterine glands, endometrial epithelium and stromal cells

G1: (a and b, arrows), luminal epithelium (a and b, arrowheads) and stromal cells (b, curved arrows), G2: and G3 (c and e, arrows), luminal epithelium (d and f arrows) and many stromal cells (d and f-stars), Bars = 40 μm

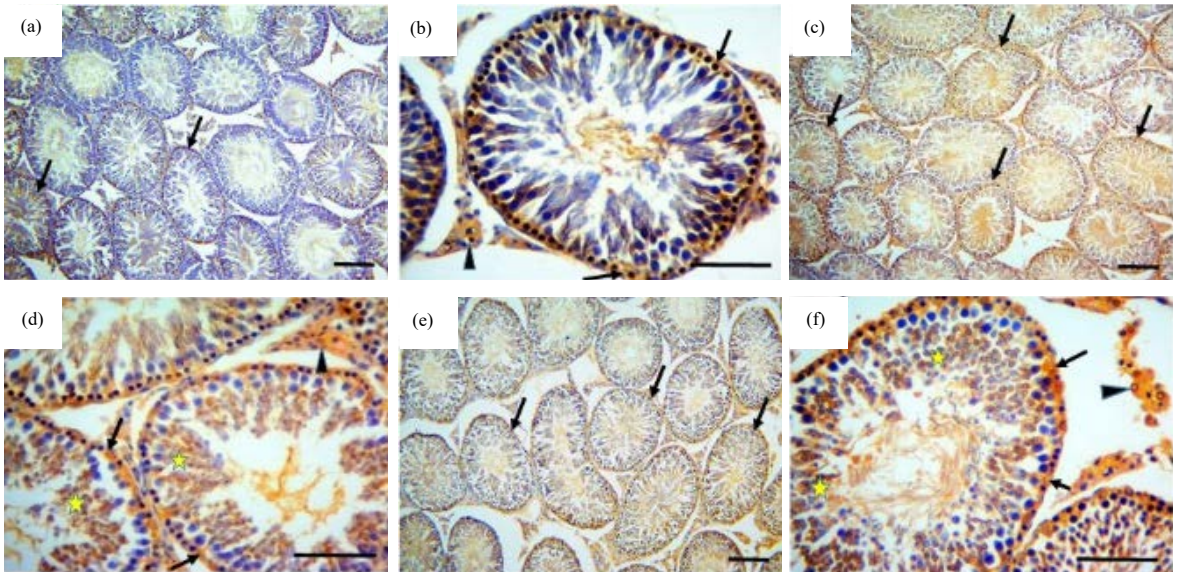

Fig. 7(a-f): Photomicrographs of rat testis Control-G1 and Zingerone treated-G2 and G3 showing ARh expression, (a) G1: Control rat testis (photomicrographs), (b) G1: AhR was expressed in seminiferous tubules, (c) G2: Zingerone treated AhR expression, (d) G2: AhR expressed in more seminiferous tubules in interstitial cells, (e) G3: Zingerone treated AhR expression (cytoplasmic and nuclear in sertoli cells and (f) G3: AhR expressed in more seminiferous tubules showing developing spermatocytes

G1: (a, arrows), the reaction was diffuse cytoplasmic in spermatogonia (b, arrows) and interstitial cells (b, arrowheads), G2 and G3: More seminiferous tubules were expressing AHR (c and e, arrows), the reaction was diffuse cytoplasmic and nuclear in sertoli cells (d and f, arrows) and interstitial cells (d and f, arrowheads), different stages of developing spermatocytes showed nuclear staining (d and f, stars), Bars = 40 μm

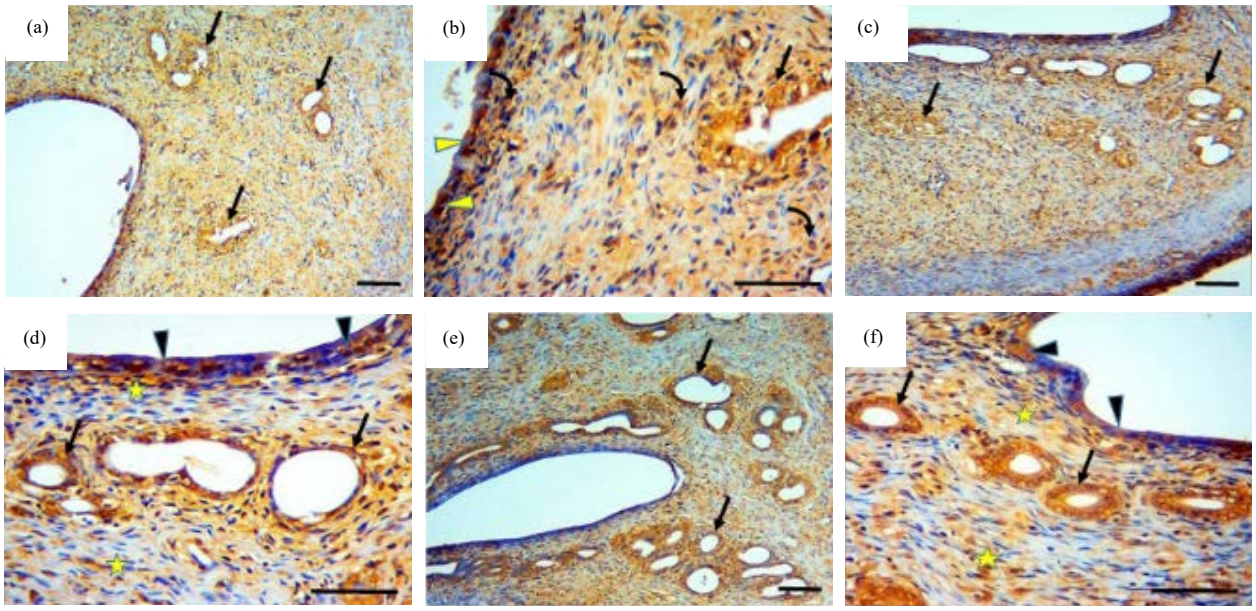

Fig. 8(a-f): Photomicrographs of rat uterus expression, (a) Photomicrographs of rat uterus (control), (b) AhR was expressed in uterine glands, (c) Photomicrographs of rat uterus (treated), luminal epithelial, (d) Proliferated endometrium expressed AhR in uterine glands, (e) Photomicrographs of rat uterus (treated group 3) and (f) Proliferated endometrium expressed AhR in uterine glands diffuse cytoplasmic

G1: AhR was expressed in the uterine glands (a and b, arrows), few luminal epithelial cells (b, arrowhead) and stromal cells (b, curved arrows), G2 and G3: Proliferated endometrium expressed AhR in uterine glands (c and f, arrows), luminal epithelium (d and f, arrowheads) and stromal cells (d and f-stars), the reaction was always diffuse cytoplasmic and nuclear, bars = 40 μm

In the uterus, the expression of AhR in endometrium of all control and treated rats, showing higher expression in treated rats (Fig. 8a-f). In control rats (G1), AhR was localized in luminal epithelium, uterine glands and endometrial stromal cells (Fig. 8a, b), the reaction was diffuse cytoplasmic ad nuclear (Fig. 8b). In treated rats, endometrial proliferation was evident in G2 treated rats and increased with G3 treated rats (Fig. 8c, e). The AhR expression followed endometrial proliferation and increased in luminal epithelium, uterine glands and stromal cells (Fig. 8d, f), the reaction was always nuclear and diffuse cytoplasmic.

DISCUSSION

This study experimentally investigated the fertility boosting effects of zingerone in both male and female adult rats. The results revealed a direct enhancement effect on gonadal activity in both sexes. It is thought that, zingerone as an active ginger ingredient exerts its action through its antioxidant activity[4]. The administration of ginger has improved testosterone levels and stimulated spermatogenesis in rats[10,25] and earlier studies mentioned that ginger has some androgenic activity[9]. However, no direct studies have explored the effects of zingerone on male genital organs.

The results demonstrated a significantly increased body and gonadal weight in male rats. This result was not recorded before and may be due to the anabolic effect of elevated testosterone. However, the exact mechanism through which zingerone exerts its anabolic effect is a matter of further investigation. Studies have reported that zingerone possesses anti-inflammatory, antioxidant and antimicrobial therapeutic actions[5-7]. Sperm count studies showed a dose-dependent increase in sperms numbers when compared with control rats and these findings were compatible with previous studies where ginger was administrated in rats[26]. Furthermore, earlier studies reported that ginger is helpful in male infertility problems resulting from metabolic diseases[27,28]. In female rats, zingerone significantly elevated plasma estrogen hormone levels and increased ovarian weight in a dose-dependent manner. It was found that ginger has positive effects on folliculogenesis in rats[12], a recent study on humans stated that ginger relieves pain which accompanies primary dysmenorrhea in females[11].

Shogaol which is a major ginger active ingredient was found to activate AhR and its gene battery in HepG2 cell line[22]. Both shogaol and zingerone contain a benzene ring and phenolic moiety in their structure. Many AhR exogenous ligands contain a benzene ring and are aromatic in nature[29-31]. Therefore, zingerone could possibly be exerting its effects via modulation of the AhR. It has been postulated that the multifunctional AhR is involved in genital physiology. This receptor was expressed in the human endometrium[18]. Studies on the rabbit uterus showed high regulation of the AhR during the preimplantation period and its involvement in pro-gestational proliferation[19]. In addition, AhR was expressed in steroid secreting cells in the rabbit and chicken ovaries[20]. In the current study, signs of proliferation were evident in the rat testis and uterus after oral administration of zingerone in a dose-dependent manner. Furthermore, testosterone and estrogen plasma levels were elevated. The AhR receptor expression showed higher regulation concomitantly in same tissues in the testis and uterus indicating that AhR could possibly be involved in spermatogenesis and uterine proliferation. The AhR was also expressed in the testicular endocrine (Leydig) cells.

The results showed that tissue expression of AhR in the testis and uterus was highly upregulated after zingerone administration. This clearly indicates that zingerone could be exerting its activating effects through AhR modulation. Therefore, zingerone could be exerting its effects through a mechanism involving the AhR and may act as an AhR exogenous ligand, but further studies are necessary to prove this.

CONCLUSION

It can be concluded that, zingerone is a fertility boosting active ingredient of ginger which is suitable for oral administration. It exerts it biological effects through a mechanism involving the aryl hydrocarbon receptor and it may acts as an AhR exogenous ligand.

SIGNIFICANCE STATEMENT

The study highlights the potential use of zingerone as a beneficial agent for fertility. A link between the aryl hydrocarbon receptor and the improvement in fertility markers was found, which could help researchers uncover new treatment targets for treating fertility disorders. Therefore, zingerone may be acting as an AhR ligand that could potentially be used to ameliorate fertility in human subjects. This provides a novel role for AhR and opens new areas of research and therapeutic potentials.

ACKNOWLEDGMENTS

This project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, under grant No. G-412-249-1439. The authors, therefore, acknowledge with thanks DSR for technical and financial support.

REFERENCES

1. Ferreira, F.M.D., E.Y. Hirooka, F.D. Ferreira, M.V. Silva, S.A.G. Mossini and M. Machinski Jr., 2018. Effect of *Zingiber officinale* Roscoe essential oil in fungus control and deoxynivalenol production of *Fusarium graminearum* Schwabe *in vitro*. Food Addit. Contam.: Part A, 35: 2168-2174.
2. Yamamoto-Ribeiro, M.M.G., R. Grespan, C.Y. Kohiyama, F.D. Ferreira and S.A.G. Mossini *et al*., 2013. Effect of *Zingiber officinale* essential oil on *Fusarium verticillioides* and fumonisin production. Food Chem., 141: 3147-3152.
3. Attari, V.E., A.M. Mahdavi, Z. Javadivala, S. Mahluji, S.Z. Vahed and A. Ostadrahimi, 2018. A systematic review of the anti-obesity and weight lowering effect of ginger (*Zingiber officinale* Roscoe) and its mechanisms of action. Phytother. Res., 32: 577-585.
4. Ahmad, B., M.U. Rehman, I. Amin, A. Arif and S. Rasool *et al*., 2015. A review on pharmacological properties of zingerone (4-(4-Hydroxy-3-methoxyphenyl)-2-butanone). Scient. World J., Vol. 2015. 10.1155/2015/816364.
5. Kim, M.K., S.W. Chung, D.H. Kim, J.M. Kim and E.K. Lee *et al*., 2010. Modulation of age-related NF-κB activation by dietary zingerone via MAPK pathway. Exp. Gerontol., 45: 419-426.
6. Rajan, I., N. Narayanan, R. Rabindran, P.R. Jayasree and P.R.M. Kumar, 2013. Zingerone protects against stannous chloride-induced and hydrogen peroxide-induced oxidative DNA damage *in vitro*. Biol. Trace Element Res., 155: 455-459.
7. Kumar, L., S. Chhibber and K. Harjai, 2013. Zingerone inhibit biofilm formation and improve antibiofilm efficacy of ciprofloxacin against *Pseudomonas aeruginosa* PAO1. Fitoterapia, 90: 73-78.
8. Vinothkumar, R., R. Vinothkumar, M. Sudha and N. Nalini, 2014. Chemopreventive effect of zingerone against colon carcinogenesis induced by 1,2-dimethylhydrazine in rats. Eur. J. Cancer Prev., 23: 361-371.
9. Kamtchouing, P., G.Y.M. Fandio, T. Dimo and H.B. Jatsa, 2002. Evaluation of androgenic activity of *Zingiber officinale* and Pentadiplandra brazzeana in male rats. Asian J. Androl., 4: 299-301.

10. Mohammadi, F., H. Nikzad, M. Taghizadeh, A. Taherian, A. Azami-Tameh, S.M. Hosseini and A. Moravveji, 2014. Protective effect of *Zingiber officinale* extract on rat testis after cyclophosphamide treatment. Andrologia, 46: 680-686.
11. Rad, H.A., Z. Basirat, F. Bakouei, A.A. Moghadamnia and S. Khafri *et al*., 2018. Effect of Ginger and Novafen on menstrual pain: A cross-over trial. Taiwanese J. Obstet. Gynecol., 57: 806-809.
12. Yilmaz, N., B. Seven, H. Timur, A. Yorganci and H.A. Inal *et al*., 2018. Ginger (*Zingiber officinale*) might improve female fertility: A rat model. J. Chin. Med. Assoc., 81: 905-911.
13. Marlowe, J.L. and A. Puga, 2005. Aryl hydrocarbon receptor, cell cycle regulation, toxicity and tumorigenesis. J. Cell. Biochem., 96: 1174-1184.
14. Tuomisto, J., 2005. Does mechanistic understanding help in risk assessment—the example of dioxins. Toxicol. Applied Pharmacol., 207: 2-10.
15. Nguyen, L.P. and C.A. Bradfield, 2008. The search for endogenous activators of the aryl hydrocarbon receptor. Chem. Res. Toxicol., 21: 102-116.
16. Dzeletovic, N., J. McGuire, M. Daujat, J. Tholander and M. Ema *et al*., 1997. Regulation of dioxin receptor function by omeprazole. J. Biol. Chem., 272: 12705-12713.
17. Tsuji, G., M. Takahara, H. Uchi, T. Matsuda and T. Chiba *et al*., 2012. Identification of ketoconazole as an AhR-Nrf2 activator in cultured human keratinocytes: The basis of its anti-inflammatory effect. J. Invest. Dermatol., 132: 59-68.
18. Kuchenhoff, A., G. Seliger, T. Klonisch, G. Tscheudschilsuren and P. Kaltwaßer *et al*., 1999. Arylhydrocarbon receptor expression in the human endometrium. Fertil. Steril., 71: 354-360.
19. Hasan, A. and B. Fischer, 2001. Hormonal control of arylhydrocarbon receptor (AhR) expression in the preimplantation rabbit uterus. Anat. Embryol., 204: 189-196.
20. Hasan, A. and B. Fischer, 2003. Epithelial cells in the oviduct and vagina and steroid-synthesizing cells in the rabbit ovary express AhR and ARNT. Anat. Embryol., 207: 9-18.
21. Bidgoli, S.A., M. Karimi, Z. Asami, H. Baher and M.D. Zavarhei, 2011. Association between testicular aryl hydrocarbon receptor levels and idiopathic male infertility: A case-control study in Iran. Sci. Total Environ., 409: 3267-3273.
22. Yoshida, K., H. Satsu, A. Mikubo, H. Ogiwara, T. Yakabe, T. Inakuma and M. Shimizu, 2014. 6-Shogaol, a major compound in ginger, induces aryl hydrocarbon receptor-mediated transcriptional activity and gene expression. J. Agric. Food Chem., 62: 5492-5499.
23. World Health Organization, 2001. [Laboratory manual of the WHO for the examination of human semen and sperm-cervical mucus interaction]. Annali dell'Istituto Superiore di Sanità, 37: 1-123, (In Italian).
24. Bancroft, J.D. and A. Stevens, 1996. Theory and Practice of Histological Techniques. 4th Edn., Churchill Livingstone, New York, USA., ISBN-13: 9780443047602, Pages: 766.
25. Morakinyo, A.O., O.S. Adeniyi and A.P. Arikawe, 2008. Effects of *Zingiber officinale* on reproductive functions in the male rat. Afr. J. Biomed. Res., 11: 329-334.
26. Fathabad, A.A., S. Shekarforoush, M. Hoseini and Z. Ebrahimi, 2018. Attenuation of sulfite-induced testicular injury in rats by *Zingiber officinale* Roscoe. J. Dietary Suppl., 15: 398-409.
27. Ghlissi, Z., R. Atheymen, M.A. Boujbiha, Z. Sahnoun and F.M. Ayedi *et al*., 2013. Antioxidant and androgenic effects of dietary ginger on reproductive function of male diabetic rats. Int. J. Food Sci. Nutr., 64: 974-978.
28. Khaki, A., A.A. Khaki, L. Hajhosseini, F.S. Golzar and N. Ainehchi, 2014. The anti-oxidant effects of ginger and cinnamon on spermatogenesis dys-function of diabetes rats. Afr. J. Tradit. Complement. Altern. Med., 11: 1-8.
29. Hahn, M.E., 1998. The aryl hydrocarbon receptor: A comparative perspective. Comp. Biochem. Physiol. Part C: Pharmacol. Toxicol. Endocrinol., 121: 23-53.
30. Fischer, B., 2000. Receptor-mediated effects of chlorinated hydrocarbons. Andrologia, 32: 279-283.
31. Pocar, P., B. Fischer, T. Klonisch and S. Hombach-Klonisch, 2005. Molecular interactions of the aryl hydrocarbon receptor and its biological and toxicological relevance for reproduction. Reproduction, 129: 379-389.