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Fertility Outcome of Aqueous and Ethanolic Extracts of Rizhomes of *Zingiber Officinale* in Female Wistar Albino Rats: A Plant Based Approach Towards Contraception

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ABSTRACTS: Pharmacological properties of ginger include anticancer, antiulcer, spermicidal, antidiabetic, antioxidant, antibacterial, anti-neuroinflammatory, and anti-vomiting effects when used in chemotherapy. In this work, female Wistar albino rats were used as test subjects to examine the antifertility effects of an ethanolic and aqueous extract of *Zingiber officinale* rhizomes. Anti-fertility activity of both the extracts were carried out in female Wistar rats at the both doses (200mg/kg & 400mg/kg) by evaluating number of implants on day 10, number of pups born and level of hormones (LH, FSH).To know the exact mechanism of action uterotropic assay was carried out in which, the estrogenic effects of EEZO were further observed by administering it to the female immature Wistar albino rats and parameters evaluated were vaginal cornification, uterine weight, body weight, histopathology of uterus and biochemical parameter(Estrogen). Alkaloids, flavonoids, steroids, terpenoids, and resins are among the phyto-constituents found in the two extracts, according to phytochemical research. The number of implants ($P<0.001$) and pups born ($P<0.0001$) significantly decreases as a result of the anti-fertility activity. The greatest 50% reduction in pregnancies was seen in rats treated with EEZO.The most powerful extract, EEZO, was investigated for its oestrogenic effects; results included increased body and uterine weight, vaginal cornification, and uterine proliferation. Hormonal and biochemical tests confirmed the previous findings demonstrating the oestrogenic potential of EEZO, which may be connected to the phytoestrogen (quercetin) content of the plant.It was observed that *Zingiber officinale* extracts had a strong oestrogenic and abortifacient effect. This could be because the extract contains zingiberene, a sesquiterpene called phytoestrogen (quercetin).

KEYWORDS: Anti-fertility; *Zingiber officinale*; abortifacient; Anti-Implantation; Estrogenic agent

INTRODUCTION

The population expansion in emerging nations like India is a scourge and a threat to the nation's and its society's progress. Rapid population growth reduces the amount of resources available per person and increases poverty, making it more difficult for humans to survive. The developing world already has limited resources, which are made even more scarce by the population's increased demand. In the last fifty years, India's population has increased by 650 million, and in the next century, it may reach 1.5 billion[1].

Drugs that have the ability to induce pregnancy termination are known as antifertility agents. The need for new contraceptive agents that are safe, effective, and provide maximal protection has been highlighted by the global population explosion. When using current synthetics for extended periods of time, their adverse effects on the typical

human body become much more aggressive and unexpected. The current climate is thus warning us to consider herbal product-based contraceptive options[2].

Ginger is herbaceous rhizomatous perennial, reaching up to 90 cm in height under cultivation. The underground stem or rhizome has a fleshy, are aromatic, thick lobed, pale yellowish, interior with secretory cells and ethereal oils. Phytochemical studies show that ginger rhizome contains a wide variety of biologically active compounds which impart medicinal property. *Z. officinale* is reported to possess essential oils, phenolic compounds, flavonoids like quercetin[3,4], carbohydrates, proteins, alkaloids, glycosides, saponins, steroids, terpenoids and tannin as the major phytochemical groups. The volatile oil consists mainly of the mono terpenes; and sesquiterpenes; camphene, β-phellandrene, curcumene, cineole, geranyl acetate, terpineol, terpenes, borneol, geraniol, limonene, βelemene, zingiberol, linalool, α-zingiberene, β-sesquiphellandrene, β-bisabolene, zingiberenol and α-farnesene. Zingiberol is the principal aroma contributing component of ginger rhizome.[5]The therapeutic use of ginger is described in ancient Ayurvedic texts as a remedy for a plethora of ailments including arthritis, digestive maladies, cardiovascular complaints and respiratory illness.[6]In Papua New Guinea, rhizome used to treat cough, influenza, sore throat, digestive system disorders, to heal wounds and as contraceptive. Zingiberene has a wide range of therapeutic application such as absorption promoter for transdermal pharmaceuticals, antifertility agent[7] and insecticidal agent[8].

An ethno-medical survey revealed that the rhizomes of *Zingiber officinale* is used to interrupt pregnancy in females. However, despite the Anti-Implantation claim of *Zingiber officinale* in folklore medicine, there is no published scientific evidence that has either substantiated or refuted this claim. Therefore, this research work was carried out to provide scientific evidence to the claimed antifertility potential of rhizomes of *Zingiber officinale* in pregnant rats.

MATERIALS AND METHODS

Plant collection and identification:

The rhizomes of *Zingiber officinale* were collected from the local market of Kalaburagi, Karnataka, India. The plant was authenticated by Dr. Pratibha Sanghapurkar, HOD, and professor. Dept. of Botany, H.K.E.S's Veeramma Gangasiri College for Women, kalaburagi. 585102, Karnataka. The rhizomes of *Zingiber officinale* was cut into small pieces and dried under ceiling fan for 5 to 6 days. The dried ginger was ground in an electronic grinder and powder was collected and stored in an airtight container for studies.

Preparation of different extracts:

Ethanolic extract of *Zingiber officinale*: The powder of *Zingiber officinale* rhizome was extracted with Ethanol (50g of powder was extracted in 250mL of ethanol) by continuous hot percolation method using Soxhlet apparatus at 40˚ C for 48 h to obtain the ethanolic extract of the *Zingiber officinale* (EEZO).

Aqueous extract of *Zingiber officinale*: About 200 grams of powder was taken in a round bottom flask and macerated by simple maceration process using 1000 ml of distilled water and 10 ml of chloroform (preservative) for 24 hrs with occasional shaking in a closed vessel. Then the marc was removed by filtering the extract, and then it was concentrated on a water bath at 40˚C to get a semi-solid mass. The obtained extracts were concentrated under vacuum using Rota flash evaporator and then dried. The percentage yield was calculated and reported.[9]

Phytochemical analysis:

Preliminary phytochemical tests for different extracts of *Zingiber officinale* were carried out for alkaloids, flavonoids, steroids, carbohydrates, resins and oils[10].

Preparation of test samples:

From previous literature on acute toxicity, it was found that both extracts of *Zingiber officinale* were found to be safe at a dose of 2000 mg/kg.[11] So in the present study, we have selected 1/10th of 2000mg i.e.,200 mg/kg and double the dose, i.e., 400 mg/kg doses for aqueous extract and ethanolic extract.

The weighed amounts of ethanolic extract was suspended in 1% sodium methyl cellulose to formulate the ethanolic test sample and aqueous extract was prepared by dissolving the weighed proportion in water for injection.

Experimental animals:

Female immature Wistar albino rats weighing between 50-55 g body weight and also adult Wistar albino rats of either sex weighing between 180-250 g body weights were used. The animals were procured from animal house of MR medical college, Kalaburagi. They were acclimatized for one week. The animals were maintained in standard conditions of light, temperature, and humidity; Rats were fed on standard rat diet and water was supplied ad libitum. The animals were fasted 12h prior to the experiment. The study protocol was carried after duly approved by the Institutional Animal Ethical Committee (IAEC), Ref. No. HKES's MTRIPS/ IAEC/100/2018-19.

Experimental design:

Screening of Antifertility Activity:

Female Wistar albino rats, weighing 180-250 g were be used. The rats which were found in pro-estrous phase of estrous cycle were caged overnight for mating with adult male rats of known fertility in the ratio 2:1. Vaginal smear of those female rats were examined the following morning for evidence of copulation. The presence of motile sperm or thick clump of spermatozoa in vaginal smear indicated pregnancy and that day was designated as 'day 1' of pregnancy[12]. The pregnant rats were separated out for testing antifertility activity. Those pregnant rats were grouped in five groups and each group containing 6 animals. Group I- received vehicle served as control, Group II &Group III- received test dose of AEZO Group IV & Group V- received dose of EEZO at level of 200 mg/kg and 400 mg/kg respectively. All the doses were administered orally, by means of oral feeding needle from day 1 to 7 of pregnancy. The animals were laprotomized under light ether anesthesia on day 10 of pregnancy. Implantation sites on both horns of uterus were recorded. The abdominal wound was sutured layer by layer, and the animals were allowed to go term. After delivery, the number of pups born were noted. The pups born were observed for 1 month for any evidence of gross teratogenicity.[13]The parameters considered were percent reduction in pregnancy & pregnancy index (PI) and calculated by these formulae. Blood was collected on the 8th day of pregnancy from retro orbital puncture. The blood was centrifuged to separate serum. Blood serum was further processed for the estimation of biochemical parameters such as LH and FSH by fully automated immunoassay analyzer.[14]

1. $\boldsymbol{Pregnancy\ index}\ (PI) = (\text{No. of pups delivered})/(No.of\ implants) \times 100$

2. **Percent reduction in pregnancy (%)**

$$= 100 - \left[\frac{No.of\ rats\ showingimplants}{No.of\ rats\ showing\ spermatozoa}\right] \times 100$$

Uterotropic assay:

For estrogenic activity female immature Wistar albino rats (50-55g) were used. The bilateral ovariectomy(Figure 1.) was performed under light anesthesia and sterile conditions. The uterine horns were exteriorized and ovaries were excised by laparotomy incision. After one week the animals were divided into four groups containing six animals in each. Group I was received vehicle which served as control. Group II was received 17-α-ethinylestradiol at a dose of 1µg/rat/day suspended in olive oil, which served as positive control. Group III and Group IV received 200mg/kg and 400 mg/kg test dose of EEZO respectively. All the extracts and vehicle were administered orally for 7 consecutive days. On the last day of treatment, the blood was withdrawn from each animal of all groups by retro orbital puncture and processed for biochemical analysis (Estrogen). On the 8th day, all the animals will be sacrificed under high dose anesthesia. The final body weight, uterine weight, vaginal opening and cornification of all the animals were observed. Then surrounding tissues were removed from the dissected uteri, and uteri were blotted on filter paper, weighed quickly on electronic balance. The uterine horn from the control, all group treated animals were stored in 10% of formalin for up to 24hrs and histological examination was carried out [14]

Histological assessment of uterus:

The uterine horn was preserved in 10% formalin, and two sections from each uterine cornua were removed and fixed in 10% formalin. The sample was dehydrated by immersing it in xylene three times for one hour each, and then two hours

each in alcohol at 70%, 90%, and 100% strength. One-hour treatments with paraffin wax were used to carry out the infiltration and impregnation twice. Melted wax was used to create blocks using a "L" block field. Using a microtome, sections 3-5 µm thick were cut, and then the sections were mounted on glass slides coated in glycerin/egg albumin. The sections underwent microscopic investigation after being stained with hematoxylin and eosin dye.

Estimation of Serum estrogen level:

The blood was centrifuged to separate the serum. The serum estrogen was analyzed by clia (chemi-luminoscence immunoassay): immulite fully automated immunoassay analyzer (mini vidas), Biomerieux, USA[15].

STATISTICAL ANALYSIS

Data was expressed as Mean ± S.E.M. and statistical analysis was carried out by One-way ANOVA followed by Dunnett's test using Graph Pad Prism version 5.00 for Windows Vista TM BASIC, Graph Pad Software, San Diego California USA. P value < 0.05 was considered as significant.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis:

The percentage yield of EEZO and AEZO were 8.75% and 12% respectively. Preliminary phytochemical study found that both the extracts contains alkaloids, flavonoids, resins, steroids, carbohydrates and terpenoids.

Table 1: Phytochemical constituents of both extracts of *Zingiber officinale*.

Sr. No.	Plant constituent	EEZO	AEZO
1.	Carbohydrate	+	+
2.	Alkaloid	+	+
3.	Flavonoids	+	+
4.	Resins	+	+
5.	Oils	+	+
6.	Steroids &Terpenoids	+	+

Effect on fertility outcome:

Female rats were laparotomized on the 10th day of pregnancy, and the anti-implantation impact was quantified as a percentage of animals showing absence of implantation in uteri. The anti-implantation impact of the treatment on pregnant rats was observed to be dose-dependent (Table 2).The pregnancy index, mean number of implants, and number of litters born were all significantly ($P < 0.001$)reduced by the extracts as compared to rats in the control group(Table 3, Figure 1). The reduction in pregnancy was found to be 0%, 16.67%, 33.37% and 50% in control, AEZO & EEZO (200mg/kg), AEZO(400mg/kg) and EEZO (400mg/kg) ($P < 0.001$) respectively (Table 2). Effect of EEZO and AEZO on the biochemical parameters has been presented in (Table 4). The EEZO at both doses showed a significant decrease in the level of LH and FSH in serum as compared to that of control.

Table 2: Fertility outcome of AEZO & EEZO in female Wistar albino rats

Sr. No.	Treatment	No. of rats showing sperms	No. of rats showing implants day 10	Total no. of implants	Total no. of litters	% reduction in pregnancy	Pregnancy Index %
1.	Vehicle	6	6	63	62	0	98.41
2.	200mg /Kg AEZO	6	5	37	12	16.66	32.43
3.	400mg /Kg AEZO	6	4	31	9	33.37	29.03
4.	200mg /Kg EEZO	6	5	32	13	16.66	40.62

5.	400mg /Kg EEZO	6	3	27	7	50.00	25.92

Table 3: Effect of AEZO & EEZO on implantation sites and number of pups born. (Mean± SEM, n=6)

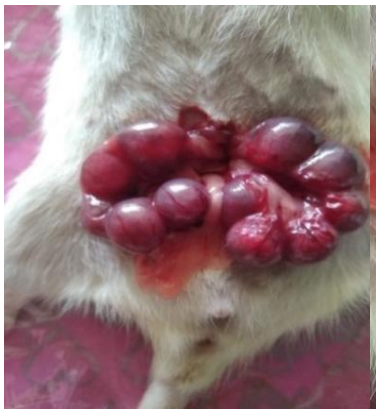
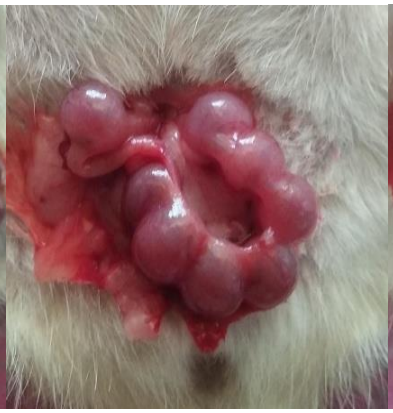
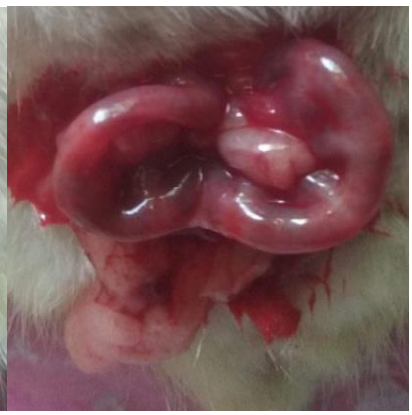
Groups	Treatments	No. of mean implantation on 10^{th}day	Mean no of pups born
I	Vehicle	10.50± 0.4282	10.33±0.4216
II	200mg /Kg AEZO	6.167± 1.302**	2.000±0.4472***
III	400mg /Kg AEZO	5.167± 1.276**	1.500±0.6191***
IV	200mg /Kg EEZO	5.333 ± 1.308*	2.167±0.7491***
V	400mg /Kg EEZO	4.500± 1.118**	1.167±0.5426***

Note: *P < 0.05, **P< 0.01, ***P < 0.001 compared to control group.

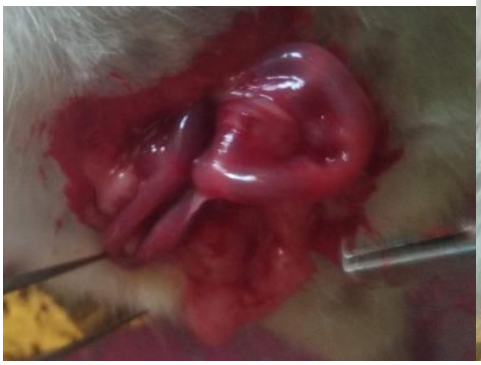
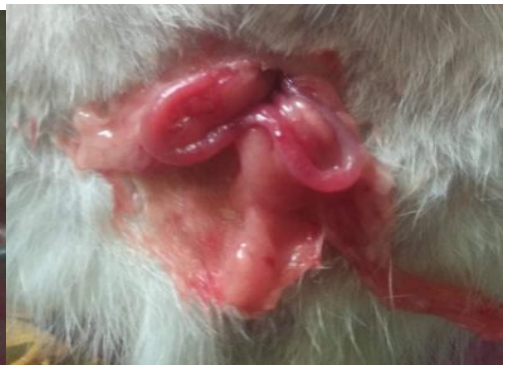
Table 4: Effect of AEZO and EEZO on biochemical parameters (mean ± SEM, n=6)

Parameters	Control	AEZO(200)	AEZO(400)	EEZO(200)	EEZO(400)
LH (ng/mL)	34.60±1.20	32.50±0.42	31.00±0.63	29.58±1.12**	26.30±1.41***
FSH (ng/mL)	125.5±1.14	122.0±0.57	120.8±1.10*	119.4±0.82**	106.6±1.32***

Note: *P< 0.05, **P< 0.01, ***P < 0.001 compared to control group.

Photograph of Control **Photograph of AEZO 200mg/kg** **Photograph of AEZO400mg/kg**

Photograph of EEZO 200mg/kg **Photograph of EEZO 400mg/kg**

Figure 1. Photographs of implants in different groups

Effect on vaginal cornification, body weight, uterine weight and biochemical parameter (Estrogen):

Vaginal opening and cornification were observed in immature rats treated with EEZO (200 and 400 mg/kg). In the vaginal smear, there were significantly less cornified cells than in the ethinyl estradiol (+++) group, but a significant increase (from + to ++) was seen compared to the control group. Oral EEZO administered at 200 and 400 mg/kg body

weight to ovariectomized rats resulted in a significant ($P < 0.01$) increase in body weight and uterine weight relative to the control group showing estrogenic potential of EEZO (Table V). The extract's estrogenic action was further supported by a noteworthy increase in blood estrogen levels in immature ovariectomized rats at both doses as compared to the control group.

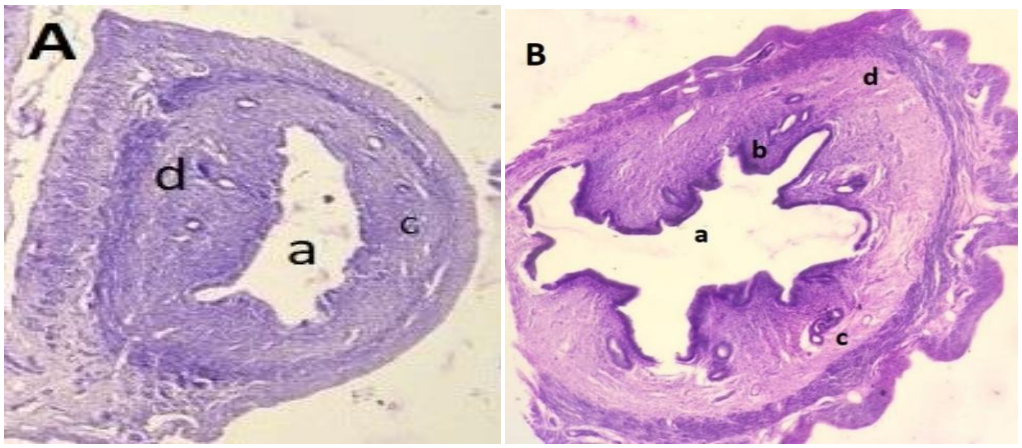
Table 5: Effect of EEZO on uterine weight, body weight and biochemical parameter (estrogen) (Mean ± SEM, n=6)

Group	Treatment	Uterine weight(mg)	Body weight gain (mg)	Serum estrogen level (pg/mL)
I	Vehicle	90.50±8.793	16.40±1.030	64.38±5.658
II	17-α-Ethinyl estradiol (1µg)	404.5±34.73***	31.60±1.327***	161.4±7.624***
III	200mg /Kg EEZO	159.8±5.209*	24.60±2.400*	103.1±2.641**
IV	400mg /Kg EEZO	170.0±4.568*	26.80±2.177**	147.8±7.435***

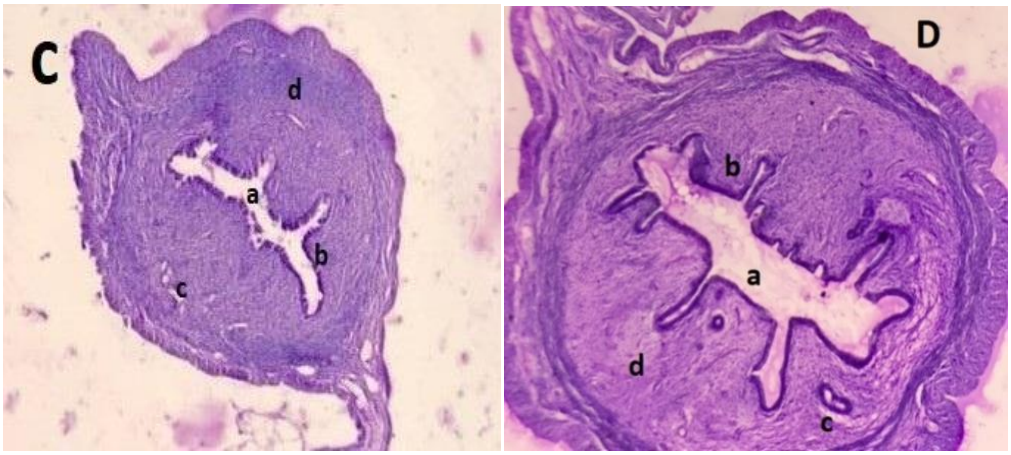
Note: *P< 0.05, **P< 0.01, ***P < 0.001 compared to control group.

Effect on histopathology of uterus:

An overview of the uteri's histological examination was provided in Figure 2. The control group's uterus had a lumen that resembled a slit and was tiny and inactive. Compaction of the stromal cells was also seen. There were a few small-diameter glands seen in the stroma. The layers of the myometrium's circular and longitudinal muscles as well as the perimetrium were thin. Treatment with estradiol resulted in a substantial proliferation of endometrial glands, many folds in the uterus lumen, increased endometrial and myometrial thickness, normal cells, and no inflammation. There was sparse gland proliferation and numerous lumen folds, endometrial and myometrial hyperplasia, normal cellular organization without inflammation, in both EEZO dosages.



Control β-estradiol group



EEZO (200 mg/kg) EEZO (400 mg/kg)

Figure 2. Histo-architecture of uterus of different groups (a. Lumen of the uterus; b. Folds of lumen; c. endometrial glands; d. Myometrium thickness.)

DISCUSSION

Plant source has been widely studied for their therapeutic importance among them several plants have been studied so far showing promising anti-implantation or causes detachment of the already implanted embryos; they act as abortifacient and some plants show disruption of the estrous cycle and inhibition of ovulation, hence exhibits their contraceptive effect. The present study showed that both the high and low doses of EEZO and AEZO have shown 0%, 16.67%, 33.37% and 50% in control, AEZO & EEZO (200mg/kg), AEZO(400mg/kg) and EEZO (400mg/kg) reduction in the pregnancies respectively compared to control group. Both the extracts showed significant (P<0.001) reduction in no. of implants and also in the number of pups born compared to control.

The hormonal data of reduction in serum LH and FSH in the EEZO treated group indicates that estrogen might inhibit the release of these hormones from adenohyposis by negative feedback mechanism. Therefore, an attempt was made to carry out the uterotropic assay of the potent extract, EEZO for estrogenic activity. Estrogenic activity results indicated that EEZO(400 mg/kg) treated rats had cornified cells in vaginal smear indicating the disruption of estrous cycle, where the rats remains in estrous cycle of increased period of time, conforming its contraceptive action. EEZO at both the doses causes significant (P<0.01) increase in body and uterine weight in the ovariectomized rats.

Estrogen causes a rise in the uterine weight, fluid retention thereby causing ballooning of the uterus, cornification and vaginal opening, hence leading to a non-receptive condition and altering the harmone level in the uterus. The decrease in the FSH is an indication of disturbed estrus cycle and ovulation. The changes in the serum LH level are associated with physiological process of luteolysis preceding parturition. EEZO showed reduction in LH and FSH, at the same time showed increase in serum estrogen. Histopathological study (Figure 2) also showed endometrial proliferation (increase in no of endometrial glands, many folds of lumen and myometrial hyperplasia) in EEZO treated rats confirming its estrogenic potential.

Phytochemical analysis showed presence of flavonoids in the extract which might act as Sphytoestrogen and responsible for its antifertility and estrogenic activity. Also the putative rise in serum estrogen by EEZO might be due to phytoestrogens that are present in EEZO getting converted into estrogen in the body. Earlier researchers have also reported that flavonoids have antifertility activity[16,17,18,19]. Priyanka Sharma *et al.* and Ghasemzadeh A *et al* proved that quercetin[3,4], a flavonoid was present in *Zingiber officinale.* Snehendu Bhattacharya and some other researcher have been confirmed antifertility activity of quercetin[16,17,18]. Literature claims that zingiberene, a sesquiterpene found in *zingiber officinale*, has antifertility properties as well. Therefore, the extract's quercetin or zingiberene content may be the cause of AEZO and EEZO's antifertility effect, or it may be the result of their synergism.

CONCLUSION

The present study concluded that both the extracts of *Zingiber officinale* have shown dose-dependent inhibition of pregnancy and possess significant abortifacient potential. EEZO exhibited potent abortifacient potential that can be

attributed to its estrogenic activity and inhibitory effect on LH and FSH which may be due to presence of phytoestrogen such as flavonoid (Quercetin) or zingiberene a sesquiterpene in the extract. In conclusion, this study provides novel evidence in support of continuing the traditional use of *Zingiber officinale* in contraception.

REFERENCES

1. Cassen R, Visaria P. India: looking ahead to one and a half billion people. BMJ 1999; 319: 995-997.
2. Farnsworth N R, Bingel A S, Cordell G A. Potential values of plants as a source of new antifertility agents I [J]. J Pharm Sci 1975; 64 (4): S535-598.
3. Sharma P, Verma P K, Sood S. Protective Effect of Quercetin and Ginger (*Zingiber officinale*) extract against Dimethoate Potentiated Fluoride-Induced Nephrotoxicity in Rats. Foods 2003; 12(9): 1-17.
4. Ghasemzadeh A, Hawa J, Rahmat A. Identification and Concentration of Some Flavonoid Components in Malaysian Young Ginger (*Zingiber officinale* Roscoe) Varieties by a High Performance Liquid Chromatography Method, Molecules 2010; 15(9): 6231-6243.
5. Govindarajan V S. Ginger: Chemistry, technology, and quality evaluation: Part 2, Crit Rev Food Sci Nutr 1982; 17(3): 189-258.
6. Remadevi R, Surendran E, Ravindran P. Properties and Medicinal Uses of Ginger. In: Ravindran, P and Babu, K (Eds.) Ginger: The Genus Zingiber. Florida, USA: CRC Press 2005; 489-508.
7. Wang Y, Du A. Isolation of Zingiberene from Ginger Essential Oil by two-step intermittent Silica Gel Column Chromatography, Adv. Mater Res.2012; 550: 1666-1670.
8. Marg K S, Wealth of India, Raw materials. First Supplement Series, R-Z. 5th ed., National Institute of Science Communication and Information Resources (CSIR). New Delhi, India. 2008; 400-418.
9. Mukherjee, P K. Quality control of herbal drugs.1st ed.,Business Horizons: Pharmaceutical Publishers, New Delhi, 2002 ; 399–407.
10. Khandelwal K R. Practical pharmacognosy techniques and experiments.15th ed., Nirali Prakashan, Pune: Pragati Books Pvt. Ltd., 2008; 149–156.
11. Torre V V, Flores J E, Silva Correa C R, Pena A A, Villalaz C L, Sanchez C D *et al*. Evaluation of the acute toxicity of the ethanolic extract of the rhizome of *Zingiber officinale* Roscoe in rats, Pharmcogn. J. 2024;16 (2): 323-331.
12. Marcondes F K, Bianchi F J, Tanno A P. Determination of the estrous cycle phages of rats: some helpful consideration, Braz. J. Biol 2002; 62 (4A): 609-614.
13. Reda H E, Azza A A. Ginger causes subfertility and abortifacient in mice by targeting both estrous cycle and blastocyst implantation without teratogenesis, Phytomed 2018; 50 : 300-308.
14. Sharma P, Manjusha, Rani S, Malhotra H, Nitesh, Deswal S, Singh S. Antifertility potential of hydroalcoholic extract of *Cordiadichotoma*, G Forst leaves: A folklore medicine used by Meena community in Rajasthan state in India, Asian pac.J. Reprod. 2015 ; 4(2): 100-105.
15. Aswar U M, Bodhankar S L., Mohan V, Thakurdesai P A. Effect offurostanol glycosides from *Trigonella foenum*-graecum on the reproductive system of male albino rats, Phytother. Res.2010; 24(10): 1482–1488.
16. Bhattacharya S, Avtar C R, Samanta J. Antifertility activity of *Thevetia peruviana* (Pers.) K. Schum leaf in female Sprague Dawley rat, Indian J. Pharmacol.2016; 48(6): 669-674.
17. Neeru V, Sharma S. Post-coital antifertility activity of *Hibiscus rosasinensis* Linn. Roots, eCAM 2008;5: 91-94.
18. Keshri G, Lakshmi V, Singh M M. Pregnancy interceptive activity of *Melia azedarach* Linn. In adult female Sprague-Dawley rats,Contraception 2003; 680 : 303-306.
19. Malpani A, Mahurkar N, Aswar U. Phytochemical analysis and antifertility potential of Cynodon dactylon in female Wistar rats: A herbal approach towards contraception, CHM 2020; 12 : 281–288.