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Effects of administration of hydro-alcoholic extract of ginger (*Zingiber officinale*) on blood serum cations (Mg, Na^+, K^+, Ca^{2+}) and the sex ratio of male and female newborn Wistar rats

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ABSTRACT

Ginger is a medicinal herb rich in potassium that has the most anti-oxidants (vitamins E, C, B) and promotes sex. The aim of this study was to examine the effect of hydro-alcoholic extract of ginger on serum level of cations and sex ratio in newborn rats. In this study, 160 female adult rats and 84 male Wistar rats were used. Male rats were divided into 3 groups-controls, sham, maximum dose (2000mg/kg/day). Female rats were divided into 5 groups-control, sham, minimal (500mg/kg/day), medium (1000mg/kg/day) and maximal experimental (2000mg/kg/day). All experimental groups received the extract. Control group received extraction solvent and Sham group received an equal amount of water and alcohol. Injections were performed as Gavage for a period of 30days. After the period was completed, blood was collected. The rest of the male and female rat's in-group and out-group were associated and sex ratio of male to female was measured. The results evaluated through one-way analysis of variance, T test and ANOVA using 20SPSS software. Results indicated a significant accumulation of sodium and potassium ions in the blood serum of male and female rats that received a ginger extract compared to that of the control groups. Moreover, there was a significant difference between the amount of sodium and potassium ions in the blood serum of female rats at least with maximum and medium groups. The number of male newborn rats that received extract was higher than that of females. The number of male newborn rats in the experimental group at a minimum dose was higher than that of the other groups. The results indicated that ginger extract alters the function of the reproductive system in male and female rats and increases sex ratio of male and the chance of male birth. This is due to the change in serum ion balance and consequently an electrolyte change of the internal environment of the uterus and discharges of the fallopian tubes that effect on the acceptability of X-, Y- bearing sperms in egg and alters the sex ratio of newborns.

Key words: Ginger, Blood Cations, Sex Ratio, Rat

1. INTRODUCTION

While every couple dreams of having a healthy child, knowing the sex of the future baby is very pleasant and has always been the aspiration of human being (1). Thus having a healthy child with gender preference is highest in demand. There are many methods of sex selection such as: the consumption of particular foods, the use of various vaginal douches, the timing of intercourse in relation to ovulation, sperm sorting, pre-implantation genetic diagnosis (PGD), selective abortion, infanticide, pre-conceptual methods and post-conceptual methods (2, 3). In general, among gender selection methods, the role of diet, especially medicinal herbs, is a simple, cheap and safe option. Most importantly, with respect to the belief-cultural context in Asian countries, especially the Muslim community, this method has the more generalized capability compared to other methods. Therefore, more research is needed in this area

(2). Ginger is the rhizome of the plant *Zingiber officinale*. It has been practiced since the ancient time as a medicine. It is abundant in areas ranging from eastern parts of Asia to the tropical regions of Australia (4).The ginger effects on the body include reduction of pain, rheumatoid arthritis treatment (5, 6), anti-inflammation (5, 6), anti-tumor (6-8), anti-oxidant, free radical elimination (4, 8), stimulating menstruation, relieving menstrual irregularities, increasing spermatogenesis and sex (9, 10). It is used for the treatment of nausea, flatulence, dyspepsia, gastric ulcer (11). It is also used to remove cholesterol and reduce blood pressure (12,13). The main ingredients of ginger include shogavel, Jeranul, Jynkl, Jranyl, sesquiterpene, ginger and Pyrogalol, theYnjyern, Arkokormen, betacescopi Flandren, betabizabolen, vitamins and minerals (14). The hot taste of ginger is due to the presence of gingerol that belongs to the oleoresin alcoholic group. Every 100 grams of ginger rhizome contains 13mg sodium, 415mg potassium, 16 mg calcium, 43mg magnesium (15). Biological effects of sodium, potassium, magnesium, calcium ions on sex determination were specified (16). Ions such as sodium, magnesium, calcium are essential for embryo implantation and normal growth. In various mammals and men, change in ion concentration of female animal dietary (17). Before intercourse can affect the sex ratio of birth, increase in sodium, potassium, and decrease in calcium and magnesium in the body, for those who want male fetus. Moreover, it is opposite for those who want female embryo. It is effective and useful (15,17). The best hypothesis about the mechanism of ion is selective fertility assumptions because of effects on the metabolism and intracellular PH. Changes in mineral balance disrupted the ratio of ions in the ovaries and eggs. Which led to potential changes, causing chromosomal changes in membrane components and structure of the sperm receptor sites on the transparent membranes of ovule, influencing certain type of sperm (16, 18). As mentioned above, it seems like extract of ginger probable changes in electrolyte conditions of discharges of fallopian tubes, through effect on the metabolism of the egg, increasing the egg tendency to combine with the sperm containing the Y chromosome. The aim of this study was to examine the effect of hydro-alcoholic extract of ginger on serum level of cations and sex ratio in newborn rats.

2. MATERIALS AND METHODS

This studies an experimental one in which all the animals complied with the ethical principles.

2.1. Preparation of extract

To prepare ginger extract through Percolation method, 3kg of fresh ginger rhizome was obtained from Herbal Research of Fars Research Center for Agriculture and Natural Resources Center. Experts approved it and number 24999 was attributed for each barium. They were dried in shade in 20-25 c and then grinded to fine powder. Then 100gm of the resulting powder was dissolved in 800 ml of 70% alcohol. The resulting solution was maintained at room temperature in percolator for 72 hours then. The funnel performed further extraction. The extract was placed in bonMari for 24 hours. Than in desiccators 24hr to evaporate extra water and alcohol by a vacuum pump, and eventually for preparation of extracts of the study 500, 1000, 2000 mg / kg of dry powder were dissolved in 1000 ml of distilled water. To prevent contamination the extract was kept in a refrigerator.

2.2. Animals

In this study 84 male adult rats and 160 female Wistar adult rats weighing approximately180-200gm and almost 12 weeks of age was purchased from Razi Institute for Serum Vaccine Research. Plan site was located in Animal House of Islamic republic university of Jahrom and animals were reserved in house to habituate the new environment for 2 weeks. 23 ±1° ambient temperature and light / dark cycle (12:12h) were selected. Standard food Livestock, Poultry Company and drinking water during experiment were used. Cage floor was covered with sawdust, wood chips were washed and disinfected twice a week. Male and female rats were placed in separated cages. At two stages of pregnancy and after pregnancy, they were studied as follows. Before pregnancy- male rats were divided in to three groups (control, sham, maximum dose) and female rats were classified in five groups (control, sham, minimum, Medium, maximum) according to the following table1.

Table 1: Classification of rats for mating

Group combination		Group number
control Female	control Male	Group 1
sham female	control Male	Group 2
female with minimum dose	control Male	Group 3
female with medium dose	control Male	Group 4
female with maximum dose	control Male	Group 5
control Female	sham male	Group 6
sham female	sham male	Group 7
female with minimum dose	sham male	Group 8
female with medium dose	sham male	Group 9
female with maximum dose	sham male	Group 10

control Female	maximum dose male	Group 11
Shame female	maximum dose male	Group 12
female with minimum dose	maximum dose male	Group 13
female with medium dose	maximum dose male	Group 14
female with maximum dose	maximum dose male	Group 15

Male rats received 2000mg/kg of ginger and female rats received 500-1000-2000mg/kg/day of ginger by gavage. In this period, the control group (male and female) received no drug. In addition, female and male rats of sham group received the equal amount of water and alcohol by gavage. The duration of gavage was 30 days. Following the end of the gavages females and males were divided into 2 groups: a group that was ready to blood sample and another group that was ready for mating. Females and males of the group that were ready formatting were put together in cages to mate and then embryo was born. Classification at this point was completely random and groups were classified as follows: After the last gavage, eight rats were selected for taking blood and were anesthetized. Blood samples were collected and kept at laboratory temperature for 20 minutes and then were centrifuged 3000 rpm for 15 min and the serum was separated. To evaluate and compare the extract performance compared to other groups, serum calcium, magnesium, potassium and sodium concentrations were measured through enzyme and colorimetric method using test kits provided by the biorexom company, which was made in England. The results were analyzed by SPSS software version 20 and one-way ANOVA and T test were done. A P value of ≤0.05 was considered as statistically significant.

3. RESULTS AND DISCUSSION

3.1. Effect of ginger extract on blood cations levels

Comparing the results of calcium, magnesium, potassium and sodium ions in male and female rats in Tables 2 and 3, we found that sodium and potassium ions in groups receiving ginger especially the one with the minimum dosage have increased significantly (P<0.05) in comparison with the control group. Results indicate a significant increase in serum levels of sodium and potassium ions in Group of female rats receiving minimum (500mg / kg / day), medium (1000 mg/kg/day), maximum doses of (2000mg / kg/day) ginger extract compared to that of the control group. In addition, there was a significant difference between the amount of sodium and potassium ions in the blood serum of female rats in moderate (1000mg/kg/day) and maximum dose (2000mg/kg/day) (table 2). These results may suggest a dose-dependent effect of ginger extract on serum levels of potassium and sodium ions. The results also indicate a significant accumulation of sodium and potassium ions in the blood serum of male rats receiving a maximum dose (2000mg/kg/day) of ginger extract compared to that of the control group (table 3).

Table 2. The comparison of female rats in terms of Na, K, Ca, Mg ions

P value	Medium male	P value	Medium male	P value	Minimum male	P value	Sham male	P value	Control male	Group parameter
0.54	10/660±0/250	0.57	**10/700±0/094**	0.55	**10/610±0/159**	0.4	**11/220±0/182**	0.48	**11/060±0/347**	**Ca**
0.48	2/774±0/053	0.37	**2/738±0/145**	0.59	**2/456±0/129**	0.44	**2/60±0/085***	0.31	**2/808±0/211**	**Mg**
0.003*	5/900±0/203*	0.002*	**6/06±0/100***	0.0001**	**6/50±0/089****	0.41	**5/060±0/067**	0.4	**5/00±0/106**	**K**
0.002*	151/200±1/067*	0.001*	**154/600±0/400***	0.0002**	**159/200±1/11****	0.33	**140/400±1/288**	0.2	**143/200±1/113**	**Na**

Table 3. The comparison of male rats in terms of Na, K, Ca, Mg ions

P value	Maximum male	P value	Sham male	P value	Control male	Group Parameter
0.50	9.533±0.545	0.31	**10.333±0.218**	0.45	**9.966±0.417**	**Ca**
0.44	2.516±0.524	0.29	**2.860±0.057**	0.30	**2.693±0.208**	**Mg**
0.004*	6.43±0.317*	0.3	**5.00±0.100**	0.34	**5.033±0.088**	**K**
0.003*	158/333±0.666*	0.47	**143.666±0.881**	0.50	**142±0.577**	**Na**

**Indicates a significant difference at p<0.01
* Indicates a significant difference at p<0.05

According to Figures 1, 2 and 3, the frequency of male infants had a significant increase in comparison with that of the female infants in the group receiving ginger. Especially the female group with the minimum dosage in comparison with the control group ($p \leq 0.05$). Infant's sex ratio (the number of male to total new born) for mating groups in which female and male rats have received extract group showed a significant difference compared with only one male or female rat. These results indicate the effectiveness of ginger extract on male and female rats. The highest number of male new born for mating group receiving a minimal dose of extract (500mg/kg/day) has been observed. Therefore, the effect of ginger extract on the sex ratio of new borns is probably dose-dependent (Figures 1, 2, 3).

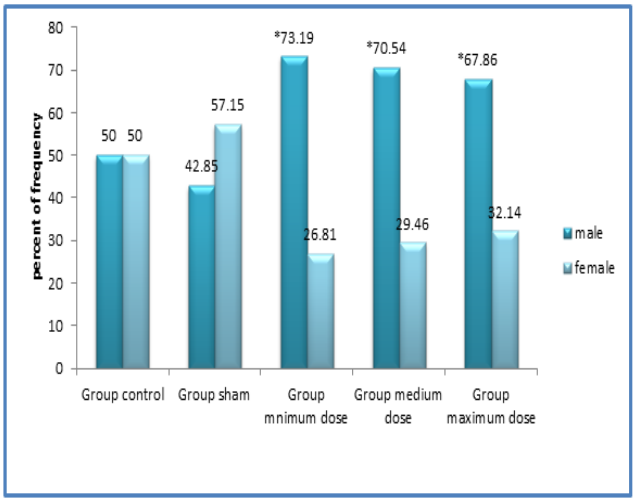

Figure1. Percent of frequency of group in terms of sex (male control)

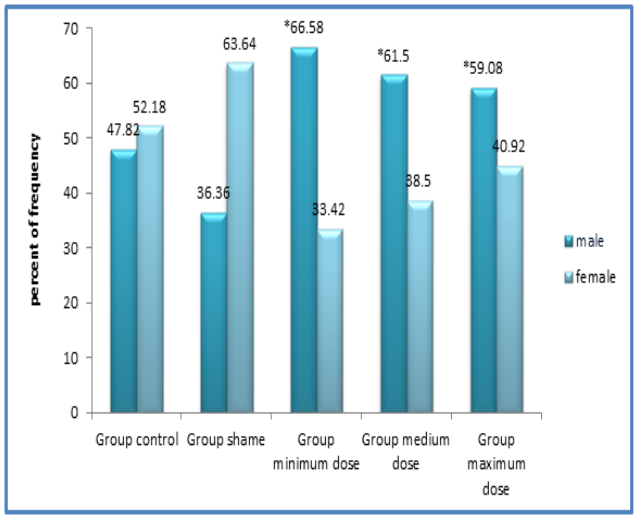

Figure 2.percent of frequency of group in terms of sex (male sham)

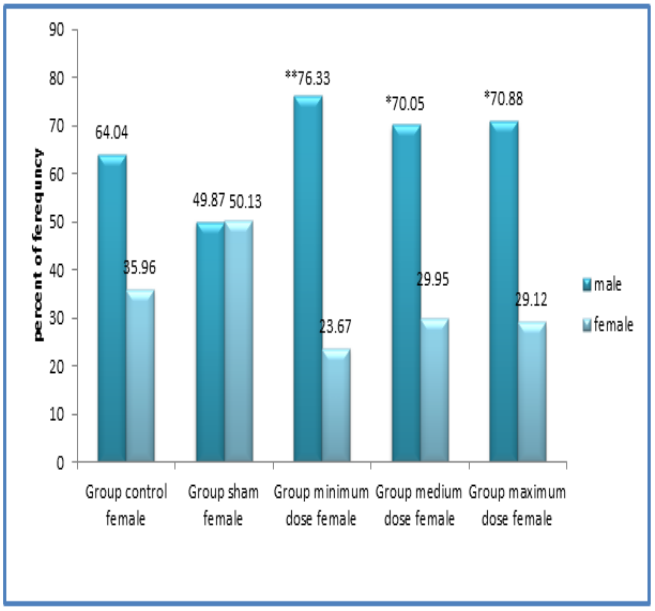

Figure 3.percent of frequency of group in terms of sex (male with maximum dose)

These findings are consistent with the view of many researchers about sex determination through a diet method. In method of determining the sex through diet for the birth of male gender, it is recommended that women use food rich in sodium and potassium (15). High levels of potassium and sodium ions in blood possibly change egg metabolic rate and especially the outer layer of the egg in such a way that Y-chromosome-bearing sperm is attracted to the egg and as a result, the compound of sperm having a Y chromosome, egg, and male fetuses is created. The results from the study by Stolkowski showed that a high ratio of potassium and sodium to magnesium, calcium ions in food of large animals, especially cow, lead to the birth of more male calves (15). Although the results of assessing the amount of magnesium and calcium ions in the blood of male and female rats in groups receiving different doses of ginger extract and their statistical analysis did not show any significant differences between the experimental and control groups. However, it is possible that the effects of calcium and magnesium ions on the egg metabolism are in contrast with sodium and potassium ions. In addition, the comparison of ratio of ions (potassium and sodium) /(magnesium and calcium) in male and female rats receiving ginger extract showed a significant increase in the proportion of ions in female than in male rats ($p<0.05$). Some studies have shown that female rats consume more sodium chloride than male rats (19). And the effect of sodium ions probably is on the expression of genes (16). Sex determining gene (TDF) was recognized in 1990 and was called SYR in man. It is called SRY in mice (20). Definitely, other genes are involved in the regulation of this gene and factors such as PH, temperature and ions can have an impact on the expression of these genes. Environmental factors such as temperature and PH on sex determination in animals are clear and indicated possible impact on gene expression, but this impact needs to be

assessed more and more accurately (16, 21) .

3.2. Relationship between level of cations and sex ratio

The results of the study indicate that the increase in the sex ratio of new born (the number of male to total new born) is highly correlated with an increase in blood levels of ions. Assuming the composition of the diet, the condition of electrolyte and the serum level of ions in terms of the diet are affected. It can be concluded that a person who receives food rich in calcium or devoid of sodium, and potassium, serum ratio of ions is affected. *Stalkowsky and Chokron* reported that about 80 to 84 percent of success in their subjects by administering food rich in potassium and sodium ions (compared with calcium and magnesium) (2, 22). Overall, we can conclude that the results of the effect of hydro-alcoholic extracts of ginger are consistent with the findings of previous researchers, and advocates of sex determination using the diet (2).Yet these results are inconsistent with Stolkowski's report since he stated that majority of rats receiving a food rich in calcium will give birth to a male infant (15).

4. CONCLUSION

Although ginger extract has no effect on serum levels of calcium and magnesium ions but serum level of sodium and potassium ions increase since ginger contains a lot of sodium and potassium. These two mineral shave an effect on vaginal PH and alkaline vaginal environment. Therefore, Y sperm motility accelerates in this environment and can influence the sex of the fetus. Which means it changes the ionic balance of serum. Moreover, probable change in electrolyte conditions of discharges of fallopian tubes, through effect on the metabolism of the egg, increases the egg tendency to combine with the sperm containing the Y chromosome.

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AUTHORS CONTRIBUTION

This work was carried out in collaboration between all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

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