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STUDY OF THE EFFECTS OF ZINGIBER OFFICINALE (GINGER) ON SPERMATOGENESIS IN MICE

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ABSTRACT
The aim of the present study was to determine the influence that Zingiber officinale (ginger) has on the development of sperm of the male mice. There were used 10 male mice which were treated with ginger, each receiving about 0,026 g natural product on the basis of ginger. From the group of 10 male mice, 8 males were sacrificed for the analysis of sperm cells, and the other 2 were put at one part with 5 females each. Sperm study was done under a microscope, quantitative determination was made by using the Macklercamera, and the qualitative with colorants hematoxylin and eosin. Analysis of sperm cells shows us that, on the whole, ginger does not have a positive effect on spermatogenesis in males mice of line SWISS. Compared with other studies, our results are contradictory.
KEY WORDS: *ginger, male mouse, spermatogenesis*

INTRODUCTION

Genus *Zingiber* belongs to the family Zingiberaceae and includes about 85 species of aromatic herbs. The tuberous rhizome of the *Zingiber officinale* (ginger) plant is a specialized segmented stem structure that grows horizontally just under the soil surface. In the first year, a green, erect reed like stem about 60 cm high grows from this rhizome. The plant has narrow lanceolate to linear-lanceolate, 15-30 cm long leaves which die of each year. Ginger produces clusters of white and pink flower buds that bloom into yellow flowers. Adventitious roots and lateral growing points emerge from the nodes of the rhizome stem. In ginger, the roots emerge from the lower rhizome sections. For commercial purposes, ginger is grown as an annual crop, the rhizomes are harvested after seven to nine months (Wilson & Ovid, 1993; Kikuzaki & Nakatani, 1996*;* Chrubasik *et al*, 2005; Ianovici, 2006; Ianovici, 2010; Ghosh, 2011; Zadeh & Kor, 2014).

Already known in ancient times, at the same time spice and medicine, ginger has found the road from India to Europe, from the time of ancient Greece. Arabs knew him since 650 BC. Ginger also has a role in traditional Ayurvedic medicine. In Western Europe, ginger was used extensively: was known as a drug against plague. The powder was sprinkled over beer in saloons in England during the 19th century. The traditional remedies are using ginger to combat fever, pain and nausea. Today, there are already countless proven therapeutic effects, and ginger penetrated

successfully also in cosmetics. Responsible for this success is first and foremost the content of gingerol, shogaol and zingerol of the rhizomes, which stimulates blood circulation, activates metabolism, helps burn fat and skin (Wang *et al*, 2014). At the same time, ginger is invigorating, vasodilator, activates peripheral blood circulation, eliminating the symptoms of cold feet and hands, prevents the risk of blood clots and cardiovascular diseases, has soothing effect, in combat fatigue. It is used against stomach pains, facilitates digestion, in the case of vomiting, sensations of evil air or sea, the abdominal pain and bloating but also for reducing cholesterol levels and a significant reduction in pain in the case of rheumatoid arthritis (Bhandari *et al*, 1998; Christen & Christen, 2001; Checiu, 2003). Ginger tea, ginger powder added to a cup of warm milk, is an ideal remedy for colds, to relieve inflammations of the throat, respiratory congestion, sinusitis and hoarseness, for lowering fever and against the removal of the best effects are obtained with tincture of ginger. Depending on the amount consumed, ginger may have constipant or laxative effect. If you eat more than 10 g of ginger during the meal, effect is constipant, while a smaller amount is laxative. Ginger has antibacterial effect (Akoachere *et al,* 2002) and helps hair care. Relieves menstrual cramps. Ginger paste is used against headaches. Ginger is known for his property to relieve physical and sexual fatigue. In addition increases male fertility by stimulating spermatogenesis and sperm mobility (D'Cruz *et al,* 2010).

The main pharmacological actions and compounds isolated there from include anti-tumorigenic, immuno-modulatory, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, anti-lipidemic and anti-emetic actions. Ginger is a strong anti-oxidant substance and may either mitigate or prevent generation of free radicals (Fuhrman *et al,* 2000; Ahmad *et al,* 2006; Ali *et al*, 2008). Although several studies have mentioned antidiabetic activity of *Zingiber officinale*. It is also known for its antiallergic characteristics (Andallu *et al,* 2003; Ianovici, 2007; Rani *et al,* 2011). The radioprotective effect of the hydroalcoholic extract of ginger rhizome was studied (Jagetia *et al,* 2003). Dietary ginger phytochemicals target cholesterol metabolism and fatty acid oxidation in mice, with anti-obesogenic but also hypercholesterolemic consequences (Beattie et al, 2011). It is considered a safe medicinal plant with only few and insignificant adverse effects (Zadeh & Kor, 2014; Ianovici *et al,* 2010; Spinella, 2001). Here it is noted that herbal products are not regulated and rigorous tested for purity and potency. The medicinal plants can contain complex mixture of 400 or more chemicals. On the other hand, some of the adverse effects could be caused by impurities (e.g., allergens, pollen and spores) or batch-to-batch variability (Johns Cupp, 1999; Philomena, 2011).

Taking into account the experience gained in our institution in the field of embryology (Checiu *et al,* 2003; Checiu *et al,* 2006; Checiu *et al,* 2007; Checiu *et al,* 2008; Huţanu, 2011; Ungurean, 2013), in order to better observe the effect that this plant, I considered it useful to investigate the influence of ginger extract on the

vertebrates of the lower classes of mammals (Sharma *et al,* 1996; Wilkinson, 2000; Amin & Hamza, 2006; Khaki *et al*, 2009; Nassiri *et al,* 2009; Hafez, 2010). For this purpose, we investigated the effects of this extract on the sperms of ads from the SWISS. The objective of the study is to investigate the possible effects of ginger extract on the sperm of the male ads in line with SWISS and reproductive females from the same line, but not treated with ginger.

MATERIALS AND METHOD

The experiments have used white mice, males of the SWISS, coming from stockfarm Biology Department, Faculty of Chemistry-Biology-Geography, University of the West of Timişoara. Mice had an initial mass of about 30 grams, and were fed with oats, sunflower, maize, milk, nuts, apples, alfalfa and drank water from the tap. The farm has respected the 12 hours light and 12 hours of darkness. At the commencement, on March 3, 2012, mice were weighing and before each slaughter has been repeated weighing. They were given a capsule of 0.26 grams per day in mice (the drug being packaged in the form of capsules). A mouse has received approximately 0,026 grams of the drug. The mice consisted of 20 individuals from the SWISS: 10 mice for experimentation: 8 were slaughtered and 2 were put with 5 females and 10 witnesseswich was done the same. For mating were put in a cage five female and one male from the same line, the SWISS, in the evening, at 21. The next morning, at 9, females have been checked for the presence of vaginal plug. At a distance of 30-40 days after the end of natural product administration, mice have been slaughtered.

RESULTS AND DISCUSSIONS

Comparing the weight of mice before starting the experiment, eight mice from experimental group have been weighing at the analytical balance. Before each slaughter, mice have been weighing again. Their weight is put on the table below (table 1). From the analysis we see a decrease in the weight of the animals during the period as they ate ginger.

After coloration with hematoxylin and eosin, sperm were observed under a microscope, have submitted changes to different parts of the body. Some sperm have presented different shape of the head, the other a drop of cytoplasm in the neck, sperm were reduced in size, others with very small and sharp head (fig. 1), or conical, head bowed, the two ends (fig. 2), a tail with loops, huge round head (fig. 3). Such changes were reported after administration of Bisphenol A to laboratory mice (Zhang *et al,* 2013). All these changes can be further examined.

Table No. 2 is showing us an average of each category of sperm: good mobile, asthenic and immovable for every male in the part from treated group. In Table No. 3 we also present an average of each category of sperm for every male in the part of the control group. Following the comparison made between the table with the number 2

and number 3, I noticed a significant decline at mobile sperm and an increase at the asthenic sperm. Total number of sperm per individual also has dropped considerably. In all three types, the values obtained are significantly different.

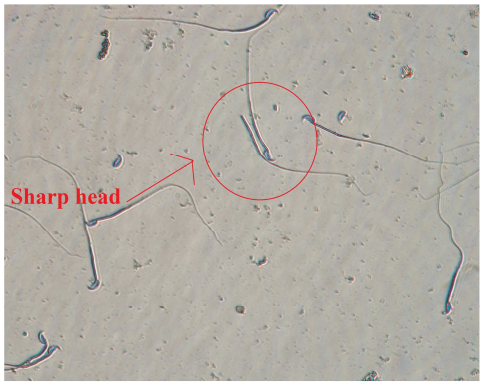

FIG. 1Sperm with a small and sharp head. Hematoxylin coloration. 200X

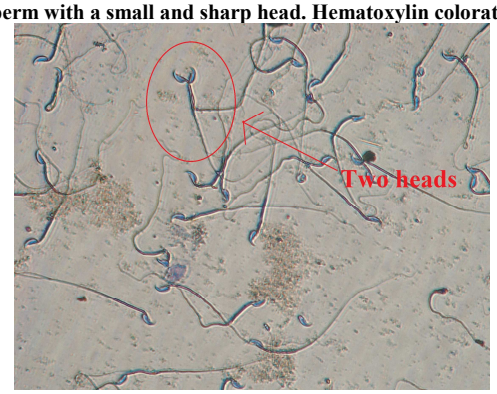

FIG.2Sperm with two heads. Hematoxylin coloration. 200X

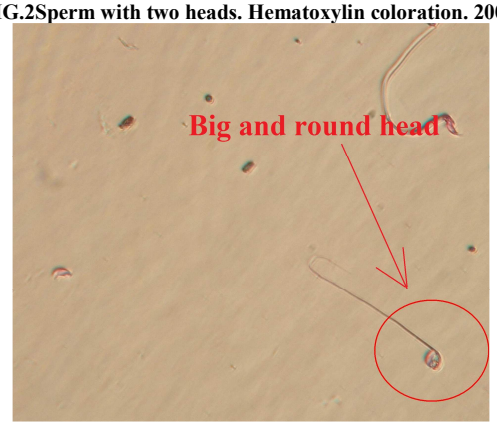

FIG. 3 Sperm with round and big head. Eosin coloration. 200X

TABLE.1 Weight of mice

	Mouse 1	Mouse 2	Mouse 3	Mouse 4	Mouse 5	Mouse 6	Mouse 7	Mouse 8
Initial weight (g)	29	26	28	27	26	30	32	35
Final weight (g)	25	22	26	25	23	21	24	35

TABLE. 2 Average sperm for treated group

n	Mobile sperm	Asthenic sperm	Immovable sperm
1	2,4	0,2	3
2	2,8	1	3,8
3	7,2	1,2	2,2
4	3,8	1	2
5	10,6	3,4	5
6	1,6	1,2	2,2
7	6,6	5,8	4,6
8	7,4	0,6	3

TABLE. 3 Average sperm for witness group

n	Mobile sperm	Asthenic sperm	Immovable sperm
1	8,8	3,2	3
2	26,8	2	2,8
3	9,2	4,6	5,4
4	3,4	0,4	6
5	11,4	1,4	13,4
6	12	2,6	4,2
7	16,6	1,4	3,4
8	22,8	2,6	4,4

TABLE. 4 The arithmetic mean of sperm per male

	Sperm average mouse1	Sperm average mouse 2	Sperm average mouse 3	Sperm average mouse 4	Sperm average mouse 5	Sperm average mouse 6	Sperm average mouse7	Sperm average mouse8
Witness batch	12,4	34	19	9,8	26,4	22,3	28	14,2
Treated batch	4,8	7,6	10,6	6,8	19	5,2	21	11

On the basis of determinations made in Mackler Chamber, the preparations of batch experimental compared to preparations of batch witness, were obtained following data shown in table no. 4: each column is presented for each male in hand,

an average of the number of sperm each mouse testicles, first for the control group, then for the experimental lot. The results are significantly different (p=0,008419). Average was higher in untreated mice.

We have used 2 males of mouse in the control group and two males from the treated group for reproduction. The two males were mated with 5 females. They were fed with the same food as the others 8 and were given the same treatment with ginger in the same amount. From the data obtained after reproduction between these two males and the 5 females, we have achieved an inventory (table no.4 and 5) and wrote observations regarding the condition of infants at birth. We consider inconclusive results. From this results that is not a positive effect of chronic intake of ginger on reproduction. Are necessary for a good coverage of new statistical investigations. In the control group were born fewer mice, a female and some young mice died. In the treated group were born more mice, but two females not became pregnants.

TABLE 5. Results obtained in the wake of the treated batch reproduction with 5 females

Females of SWISS	Date of birth	Comments
Female nr.1	20.05.2012	Born 10 live young mice
Female nr.2	20.05.2012	Born 5 live young mice
Female nr.3	20.05.2012	Born 10 live young mice
Female nr.4	-	Was not pregnant mice
Female nr.5	-	Was not pregnant mice

TABLE 6. Results obtained in the wake of the witness batch reproduction with 5 females

Females of SWISS	Date of birth	Comments
Female nr.1	20.05.2012	Born 7 live young mice
Female nr.2	21.05.2012	Born 7 live young mice
Female nr.3	24.05.2012	The female died
Female nr.4	30.05.2012	Born dead
Female nr.5	-	-

CONCLUSIONS

As a result of the tests performed and the analysis of microscopic Mackler chamber results were obtained showing that mice who took ginger treatment had a significantly lower number of sperm versus mice from of the witness batch. According to the data, ginger in experimental animals inhibit mobility and decreases the number of sperm well. The weight of the experimental lot decreased as a result of chronic consumption of ginger. Three of the females that have been reproduced with the batch treated males born 25 live young. Two of females mating with males from the control group have borned 14 live young mice. This is a preliminary study. In the future it is necessary to use numerous statistically significant batches of mice. The powder will be

administered in different doses. It is possible that the administered dose in this study has been too high relative to the weight of the mice.

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