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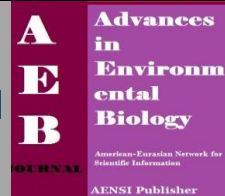
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The Effect of Halcoholic Extract of Celery Leaves on the Delivery Rate (Fertilization and Stillbirths), the Number, Weight and Sex Ratio of Rat off Spring

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ABSTRACT

Objectives: Infertility is one of the most common health problems in the world. Different plants are used in traditional medicine to treat infertility. Celery is rich in flavonoids and vitamins E and C. The present study investigated the effect of hydro-alcoholic extract of celery leaves on the delivery rate, number, weight and sex ratio of rat offspring. **Materials and Methods:** In this experimental study, 45 Wistar rats were randomly divided into three groups of fifteen (five male and 10 female rats per group). The control group received 1 ml of distilled water, and experimental groups received respectively doses of 100 and 200 mg/kg/BW celery extract for five weeks by gavage. During the fifth week each male rat was mated with two female rats from the same group in a separate cage. After a week, every female rat was kept in the separate cages. Females received celery extract during pregnancy and until childbirth. Finally, the number of the delivery females, number, weight and sex ratio of offspring were recorded. The data were analyzed by one-way ANOVA test using SPSS15 software. **Results:** The number of the delivery rats in the experimental groups was not significantly different than the control group ($P > 0.05$). The number of newborns was significantly increased in each experimental group compared to the control group ($P < 0.05$), but the weight of infants in the experimental group receiving doses of 200 mg/kg showed a significant decrease compared to the control group ($P < 0.05$). No significant difference was existed between the experimental and control groups in terms of sex ratio ($P > 0.05$). **Conclusions:** Celery improved fertility due to have flavonoid and antioxidant activity, and its consumption in both sexes can be useful in the treatment of infertility.

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INTRODUCTION

Fertility is the natural and biological ability of men and women for childbirth, and the total fertility rate is the average number of children that would be born to a woman during her fertility and it represents the reproductive function of women, which is one of the most important demographic and health indicators [1]. While the biggest dreams for each couple is having a healthy child, knowing the future baby's gender for parents is very pleasant. Selecting favorite son has long been one of human aspirations; families with the hope of having one of the two sexes have had a lot of pregnancy and one after another with a little interval but without the desired result [2]. In traditional medicine various plants have been considered to increase fertility [2] or decline fertility [3] as well as determine the sex of the baby. However, different amounts of minerals in food can affect fetal sex determination [4]. Celery (*Apium graveolens*) of the family Apiaceae plant belonging to the parsley descent (Umbelliferae) herbaceous, biennial, branched stems with a height of 20 up to 60 cm [5]. Celery leaves and stalks contain phenol. The majority of flavonoid in celery leaves is apigenin [6]. Celery due to high calcium in the treatment of high blood pressure is worthwhile [7]. Celery extract has an anti-inflammatory and analgesic

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effect [8]. Recently, physiological activity of celery against insects, mites and fungi has been considered [9]. Celery seed can separate incompatible and non-complementary base pairs [10]. Sperm plasma membrane due to large amounts of unsaturated fatty acids is susceptible to oxidative damage, the damage led to a decrease in sperm motility and viability [11]. The use of antioxidant compounds enhances sperm's function and fertility.

Celery due to having flavonoids has the ability to collect a free radical and antioxidant properties [12]. Flavonoids are not made by the human body and must be received through diet. Phenolic compounds and flavonoids have pharmacological features such as anti-oxidants, anti-bacterial, anti-mutagenic, anti-diabetic, anti-thrombosis, anti-inflammatory, anti-cancer, and hyperlipidemia [13- 17]. Celery is one of the plants that are rich in flavonoid, such as apigenin and apiin [18,19].

In this respect, it has been reported that the removed OH and DPPH radicals [20], the testes protected against Sodium valproate [21], the DI -2-ethylhexyl phthalate [22] protected by celery and the inhibited liposomal peroxidation are due to being antioxidant compounds in this plant. Celery is also noted in traditional medicine as an appetite stimulant and sexual power booster and its effect on abortion and menstruation [23]. Due to the flavonoid compounds and antioxidant properties of celery, this herb can be very effective on fertility rate. On the other hand, there are minerals in its compounds and it has a high pH [4], which probably may also affect the baby's gender determination; so, we decided to conduct an experimental study of the possible effects of hydro-alcoholic extract of celery leaves on the delivery rate (fertilization and stillbirths), the number, weight and sex ratio of rat offspring.

MATERIALS AND METHODS

Animals:

In this experimental study 45 Wistar rats, including 15 male rats (170-220 g) and 30 female rats (200-250 g) were obtained from the animal house center of Ahwaz Jundishapur University of Medical Science (AJUMS). Animals were maintained in plastic cages with 12/12 h light/dark cycle at 21 ± 2 °C. All animal experiments were carried out in accordance with Ahwaz University Ethical Committee. All rats were fed a standard diet and water.

Preparation of extract:

To prepare the extract, leaves of celery prepared from agricultural lands in Dezful (Khuzestan Province) then were specified by an expert in the pharmacognosy department of pharmacy faculty of AJUMS. The leaves were shade dried and milled to a fine powder using a mechanical grinder. The powder (50-g) was soaked in 200 ml of 70% ethanol for three days with occasional shaking. It was passed through filter paper, and the solution was spread on the glass surface at room temperature to evaporate from the solvent [22]. The extract was diluted in distilled water and prepared according to the required concentration (200 and 100 mg/kg) [24].

The experimental groups and procedure:

Animals were randomly divided into three groups of 15 rats and in each group of five male and 10 female rats were used. The control group received 1 ml of distilled water (as a solvent) and experimental groups 1 and 2 received respectively doses of 100 and 200 mg / kg / BW hydro-alcoholic extract of celery leaves daily for five weeks by gavage method. During the fifth week each male rat was mated with two female rats from the same group in a separate cage. After one week, female rats with vaginal plaque were kept in separate cages until their delivery time. Female rats also received celery extract during the period of pregnancy and until giving birth. At the end, the number of the female rats that had the delivery, and the number and neonatal weight were recorded; moreover, thirty days after birth by determining the urogenital distance, babies' sex was specified and their sex ratio (male/female) was recorded [2, 25].

Statistical analysis:

Data were analyzed with SPSS15 and analysis of variance one-way ANOVA and backup test of LSD. The differences were considered as significant if a $P < 0.05$.

Results:

Number of births and stillbirths:

The number of the delivery in the control group was eight (80%), in the group receiving doses of 100 mg/kg of hydro-alcoholic extract of celery was eight (80%) and in the group receiving doses of 200 mg/kg of hydro-alcoholic extract of celery was nine (90%). No statistically significant difference was existed between the experimental and control groups ($P > 0.05$); also no stillbirths were observed in all groups studied.

Number, weight and sex ratio of offspring:

In the control group, 63 infants were born (33 female infants; 30 male infants). Within the groups receiving doses of 100 mg/kg of hydro-alcoholic extract of celery 73 infants were born (39 female infants; 34 male infants) and in the group receiving a dose 200 mg/kg of celery 79 infants were born (41 female infants; 38 male

infants). The number of newborns has significantly increased in each experimental group 1 and 2 (9.12 ± 0.29 , 8.77 ± 0.14 respectively) compared to the control group (7.87 ± 0.39) ($P < 0.05$) (figure 1). No significant difference existed between the groups in terms of sex ratio ($P > 0.05$) (figures 2 and 3). The weight of infants in the group receiving a dose of 200 mg/kg of celery extract (5 ± 0.17) compared to the control (5.98 ± 0.2) group showed a significant reduction ($P < 0.05$) (figure 4).

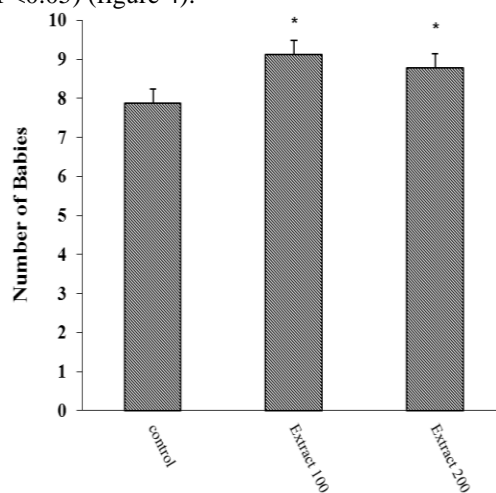


Fig. 1: Comparison of MEAN \pm SE of number of babies between the experimental groups and control group; * indicating significant differences between experimental and control groups ($P < 0.05$).

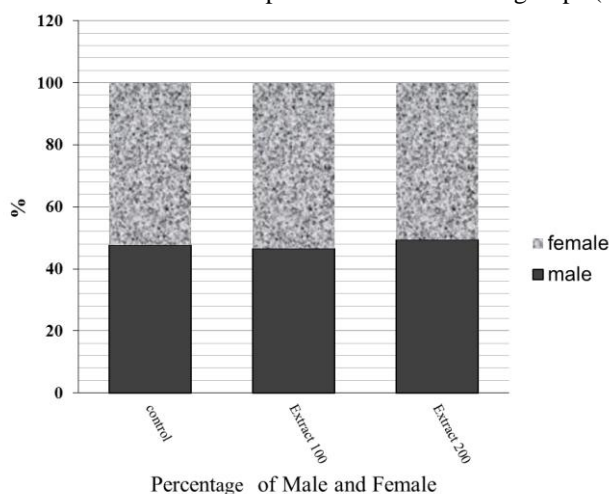


Fig. 2: Percentage of male and female infants in the experimental and control groups.

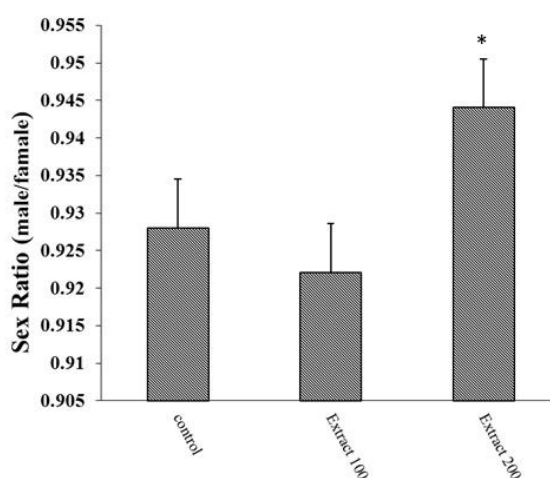


Fig. 3: Comparison of MEAN \pm SE of sex ratio between the experimental groups and control group; * indicating significant differences between experimental and control groups ($P < 0.05$).

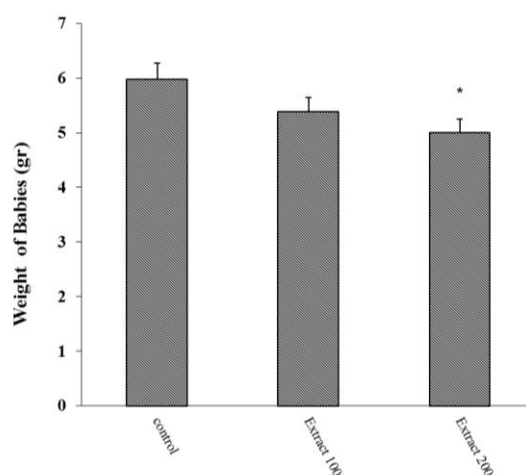


Fig. 4: Comparison of MEAN \pm SE of baby's weight between the experimental groups and control group; * indicating significant differences between experimental and control groups ($P < 0.05$).

Discussion:

Infertility is one of the most common health problems in the world that affects approximately 15% of couples [26]. Several studies have been conducted in order to find an effective, less complicated and less costly drug in the infertility treatment. Among these, during experimental research herbs as the first stage of the health care system have extensively proven their effectiveness in many parts of the world [27]. According to the findings of the present study, using hydro-alcoholic extract of celery leaves did not have an effect on the delivery rate. So far the experimental research in this field has not yet been carried out but the result of a study showed that administration of celery extract in male rats does not have any effect on the delivery rate in female rats [28], also the result of other study showed that celery did not have anti-implantation effect [29]. As mentioned, so far no experimental or clinical examination has been conducted on celery plant's effect on the rate of delivery. But other experimental studies have examined the effect of the other herbs such as chicory and dill on the amount of delivery, which have reported similar results [2]. According to the result of the present study, celery extract consumption in both sexes (male and female rats) increased the number of babies. Kerishchi *et al.*'s research showed that to a certain dose (200 mg/kg) of celery extract can have an additive effect on spermatogenesis and sperm motility. The increased testosterone level in the dose was significant as well [30]. Flavonoids are secondary metabolites of plant compounds that have antioxidant properties due to removing harmful free radicals. Celery is one of the plants that is rich in flavonoids such as apigenin and apiin [18, 19]. This herb also contains vitamins E and C. During the experimental study it was determined that these two vitamins improve sperm parameters (count, motility) against oxidative stress induced by cadmium in rat [31, 32]. In a study Hamza *et al.* showed that celery in addition to protecting the testes is capable to increase spermatogonium, spermatozoid, and the primary spermatocyte [21]. Thus, the findings of previous studies are consistent with the result of the present study.

In the study probably by stimulating the secretion of testosterone the celery has strengthened the reproductive parameters and spermatogenesis in male rats. The beneficial effect of celery on fertility in the female gender probably is due to the existence of phytoestrogens and inhibition of decomposition of progesterone, which causes the endometrial boost and keep the beneficial effect of progesterone [33]. On the other hand, one of the most important flavonoids quercetin celery is studied within several researches.

Morovvati *et al.*'s study showed that quercetin improves the thickness of the endometrium in of the two dense and sponge layers as well as the number and density of cells and glands in the endometrium in the ovariectomized rats [34]. Thus, strengthening effect of celery on fertility in female rats (due to compounds such as quercetin) is justified. According to the finding of the present study, consumption of hydro-alcoholic extract of celery leaves in both sexes (male and female rats) was not effective in the sex ratio of newborns. There are several conditions to determine gender and it is done based on the Y chromosome [35]. Some researchers believe that if the mother eat salty foods during pregnancy the possibility of getting male of her child is more, and if at this time she use the sweet foods, the odds that the girl child is born will be the most, thus recent research showed the mineral nutrient levels are effective in fetal sex determination, feeding up to 75% can be involved in determining the gender, but the dietary intake of at least one month before pregnancy should be observed [36]. Other factors such as: the time interval of fertilization from the time of ovulation, fertilization age, maternal age, and the amount of vaginal pH are affecting the sex determination [35]. To enter the sperm into the oocyte, it should pass a transparent protein membrane of the protein called the zona pellucida that has embraced around the oocyte and reach the oocyte genetic content, while the research shows the ratio of

potassium (K^+) to sodium-calcium-magnesium ($Na^+ - Ca^{2+} - Mg^{2+}$) with impact on receptors that affect zona pellucida levels, which can affect the possibility of sperm entry contains X or Y; therefore it is more likely mineral content of food can be effective on the baby's sex determination. Sodium and potassium found in celery would probably increase on the motion speed of the Y chromosome sperm to the oocyte [35]. Probably consumed extract in female rat affect the zona pellucida around oocyte and creates a disorder in the process and accordingly can prevent an impact on certain gender. Definitely, if the period of prescription of celery was considered over a month, or separately checked in both sexes, then more precise results were seen. No similar experimental study exists in the field, but different experimental studies investigated the effects of herbs on the sex ratio. During a study of the chicory, fennel and dill plants conducted by Fallah Hasani *et al.*, similar results were obtained [2]. In another study aimed at investigating the effect of apples diet on the sex ratio, the results showed that the fruit does not have any effect on the sex ratio [25]. According to the findings of this study, celery extract in high doses reduces the weight of babies. Birth weight of infant, health indicators in any society and, it is considered as the only means of assessing the outcome of the pregnancy. Birth weight depends on many factors such as genetic factors, the status of mother's nutrition, baby gender and failure on the mother's weight gain during pregnancy. These risk factors can have correlation, and one of them can be hardly introduced as the main factor [36]. No many experimental studies exist in this field. In an experimental study it became clear that food rations contain high amounts of coumarin and karvon and limonan compounds reduces the weight of baby rat [37]. Since these compounds are in celery, there are also probably effective in reducing weight. Also, celery will probably reduce the weight of the newborn during pregnancy by the disruption in a weight gain of the mother.

Conclusion:

The present study confirms the effect of the hydro-alcoholic extract of celery leaves in improving the fertility parameters. One of the new findings of the present study was the increased number of infants and being a natural amount of labor after taking the extract of the celery leaves. Since in this study, healthy rats have been studied, so be the effectiveness of the celery plant on a certain type of infertility cannot be confirmed. Probably due to having flavonoids, and antioxidant properties, the celery has caused improvement of the sperm parameters (the mobility and number) in male sex as well as boost of the hormone progesterone in the female sex. Obviously, reducing the weight of babies after consumption high concentration of celery is a serious warning for the proper and correct use of this medicinal herb in the diet. More experimental and clinical studies on the total extract of this plant and their effects on the health or the occurrence of embryonic abnormalities can be proposed.

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Conflict of interest statement:

The authors declare that there are no conflicts of interest

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