THE EFFECTS OF HYDROALCOHOLIC EXTRACT OF *ACTINIDIA CHINENSIS* ON SPERM COUNT AND MOTILITY, AND ON THE BLOOD LEVELS OF ESTRADIOL AND TESTOSTERONE IN MALE RATS

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**Background:** Foods contain nonnutritional phytochemicals that may affect human health. The effect of phytochemicals on sperms is controversial.

**Objective:** To determine whether kiwi (*Actinidia chinensis*) extract can change sperm quality and the blood level of sex hormones.

**Methods:** 40 male rats were divided into 4 groups; 3 experimental groups were fed with 75, 100, or 150 mg/kg of hydroalcoholic extract of kiwi and the control group was fed with the solvent for 50 days each. Blood samples were taken and the levels of estradiol and testosterone were measured. The number of sperms and the percentage of motile sperms in semen samples were also calculated.

**Results:** The level of testosterone and sperm count were decreased significantly in groups treated with 150 mg/kg of kiwi extract. The estradiol level also was decreased in groups treated with 100 and 150 mg/kg at the extract.

**Conclusion:** It seems that hydroalcoholic extract of kiwi has deleterious effects on spermatogenesis. It is therefore, recommended to use it with caution if there is a reproductive problem.

**Keywords:** *Actinidia chinensis* • phytoestrogen • serotonin • spermatogenesis • testis

**Introduction**

There is scarce information on the influence of phytochemicals on male reproductive pathophysiology. Declines in human sperm counts and motility over the recent decades may be attributed to increased exposure to environmental endocrine disruptors. The potential reproductive benefits and/or toxicity of most of the environmental agents to which animals and humans are daily exposed is unknown. *Actinidia chinensis* (kiwi) is a fruit that has been extensively used in Iran during recent decades. It is rich in vitamin C and E, fructose and galactose, minerals, isoflavones, flavonoid, and serotonin.

Two of the major classes of phytoestrogens are flavonoid and isoflavones, which are among important ingredients of kiwi. Phytoestrogens are naturally-occurring, plant-derived compounds that are functionally and structurally similar to estrogen, such as endogenous estradiol. Various studies have demonstrated the health benefits of phytoestrogens for various conditions including vasomotor symptoms, postmenopausal health risks, as well as their promising anticarcinogenic, neuroprotective and cardioprotective activities, prostatic health, and bone promoting properties. In males, estrogen is present in low concentrations in blood, but it can be found in

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extraordinarily high concentrations in semen and rete testis fluid. It is well known that male reproductive tissue express estrogen receptors.14 Serotonin is found in gonads and accessory reproductive organs of several species.15 The regulatory roles of serotonin in gonadal testosterone production and LH and FSH concentration have also been studied by many investigators.16 – 23 Kiwi, with its serotonin content, may therefore have some effects on sex hormone production as well as on sperm counts and motility.

We conducted this study to examine the effect of hydroalcoholic extract of kiwi on sperm count and motility, and on the blood level of sex hormones in male rats.

**Material and Method**

Forty Sprague-Dowley male rats were obtained from the animal house of Shiraz University of Medical Sciences. The animals were acclimated to the laboratory for one week prior to beginning the experiments. Animals were maintained at a controlled temperature (22 – 24°C) and a period of 14 hours of light plus 10 hours of darkness. Rats had free access to food and tap water.

Kiwi extract was obtained through the percolation method. For hydroalcoholic extraction of kiwi, fresh fruit was obtained from a commercial supplier during summer, peeled, sliced, and dried in an open shadow. The dried fruit was then powdered. One hundred grams of powder was put into a percolator and 800 mL of 50% ethanol was added to the powder over three days. The flow rate of solvent was 5 mL/kg/min. The extract solution was collected and the solvent was evaporated. Four hundred and seventy-nine of semisolid extract was obtained and the appropriate amount were mixed with saline to obtain different concentrations.

All rats were given daily gavage administration of 1 mL of solvent (water), 75, 100, or 150 mg/kg/day of hydroalcoholic extract of kiwi fruit for 50 days. Blood samples were taken from the tail vein before starting the experiment, at day 25 and at the end of the experiment. Blood samples were centrifuged and their plasma portions were separated. The concentration of testosterone and estradiol was determined using immunoradioassay kits (Spectria).

**Sperm collection method**

Sperm specimens were collected using the
The aspiration method involves making a small cut in the cauda of the right ductus deferens and aspirating 1.5 µL of semen into a polyester capillary tube. The sample was then flushed into an aliquot of 1 mL of normal saline. Attempts were made to keep the temperature of the instruments and liquid at about 37°C during the sperm collection and analysis phases.

Sperm count was determined by counting the sperm using a hemacytometer. Ten fields of the microscope were randomly selected and the sperm motility of 10 sperms was assessed on each field. Therefore, the motility of 100 sperms was assessed randomly. Sperms were labeled as motile, sluggish, or immotile. The percentage of motile sperms was defined as the number of motile sperms divided by the total number of counted sperms (i.e. 100).

**Statistical analysis**

Data were analyzed with one-way analysis of variance (ANOVA). More analysis was done for the level of hormones within the groups by Friedman test and between the groups by LSD. Sperm count and motility were analyzed using the Student’s t-test.

**Results**

High concentration of hydroalcoholic extract of kiwi had some effects on blood levels of sex hormones. Serum testosterone and estradiol levels were reduced through feeding of the kiwi extract (Figures 1 and 2). Serum testosterone in the 150 mg/kg and estradiol in the 100 and 150 mg/kg-treated groups were significantly ($P < 0.05$) decreased as compared to blood hormone levels in blood samples taken last time from the control rats. Hormone levels were also significantly different from those of sampled at the beginning of the experiment in the same group. There were no significant changes in blood hormone levels of the group that was fed with 75 mg/kg of kiwi extract.

The extract had some deleterious effects on sperms; sperm count and motility were decreased significantly ($P < 0.05$) in the 150 mg/kg-treated group as compared to the control group (Figures 3 and 4).

**Discussion**

Kiwi is rich in vitamin C, fructose, isoflavones, flavonoids, and serotonin. There is some evidence, which shows the effects of ascorbic acid and fructose on sperm count and motility. It has been shown that seminal fructose levels give no information on clinical use in defective sperm formation but that intraperitoneal injections of vitamin C can improve sperm count and morphology in the murines.

**Figure 2.** Blood testosterone level (ng/mL) at the beginning (B0), on day 25 (B1), and at the end of the experiment (B2) in all groups. * = significantly different from B2 of control group ($P < 0.05$); † = significantly different from B1 of the same group ($P < 0.05$); + = significantly different from B0 of the same group ($P < 0.05$); П = significantly different from B0 of the same group ($P < 0.05$).
Regarding the decrease in sperm count and motility that was induced by exposure to kiwi extract, other phytochemicals other than vitamin C and fructose in the extract may exert the effects. The influence of phytoestrogen on sperm characteristics is controversial. Some evidence indicates that phytoestrogen has no effect on sperm characteristics. On the other hand, there are some studies, which show that sperm motility and numbers are decreased in a dose-dependent manner by phytoestrogens. The difference between the various studies may be attributed to the difference in the species or the age of animals used and the type of phytoestrogens studied in each project.

Our data suggested that high concentrations of kiwi extract decreases the blood levels of testosterone and estradiol. It seems that phytoestrogens, such as genistein, can decrease the serum testosterone level in the rat. Meanwhile, coumestrol, a phytoestrogen, had no significant effects on testosterone level. Kiwi is rich in serotonin too. It has been shown that in vitro basal and HCG-stimulated testosterone production was significantly inhibited in the presence of physiological concentrations of serotonin. Serotonin causes a significant reduction in serum concentration of LH and intratesticular testosterone. The serum concentrations of testosterone and FSH were unaffected. As a consequence, the kiwi-induced reduction in serum testosterone levels should be due to action of phytoestrogen and not serotonin.

Previous studies have shown that phytoestrogens had no effect on serum estradiol levels. The effect of phytoestrogen on LH and FSH in rats is controversial. If it is postulated that the effect of kiwi extract is due to its isoflavon and flavonoid contents, our data would confirm the previous studies about testosterone but not estradiol. It has been suggested that the effects of dietary phytoestrogen are independent from changes in the pituitary-gonadal axis. Therefore, it may have a direct effect on the gonad to change the hormonal level. It is observed that some kind of phytoestrogens such as zearalenone has competitive effects but the others such as genestein had additive effects. The differences between our results and others regarding estradiol levels may be attributed to the type of phytochemical studied. It is possible that the decreased estradiol level was due to serotonin.

Decreasing serum testosterone and estradiol may explain why the sperm count and motility were also reduced by kiwi extract. Estrogen regulates the reabsorption of luminal fluid in the head of the epididymis. Therefore, semen is diluted rather than concentrated. Testosterone is important in spermatogenesis as well. In this way, the reduction in hormone levels can influence sperm count.

In conclusion, kiwi extract can decrease the sperm quality in rats. However, to shed light over the mechanism by which kiwi exerts its effects we have to study more. The effects of kiwi extract on human reproductive activity and fertility are unknown. Nevertheless, considering our findings in this animal model, it is recommended that those men with infertility or reproductive problems abstain from eating kiwi during the treatment period.

**Acknowledgment**

The authors wish to thank the Research Deputy.
of Shiraz University of Medical Sciences for offering the grant for this project and Miss Pirsalami and Ms Arasteh for their excellent technical supports.

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The effects of hydroalcoholic extract of *Actinidia chinensis*


