

The Effectiveness of Zinc Micronutrients From Pumpkin (*Cucurbita moschata D*) Extract on the Testosterone Levels of Mice (*Mus musculus L*)

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Abstract

Various factors cause disharmony in the household. One of them is erectile dysfunction. This can be treated by providing aphrodisiacs and some minerals such as zinc to increase libido. One of the plants that contain zinc is pumpkin (*Cucurbita morchata D*). This study was conducted to determine the differences in giving pumpkin skin, meat, and seed extracts to testosterone levels. This study uses a laboratory experimental research type. This study, using extract variables from the skin, meat and seeds of pumpkin (*Cucurbita moschata D*) and levels of the hormone testosterone. The sample used was male mice (*Mus musculus*) divided into control groups and three treatment groups. Data were analyzed using the One-way ANOVA statistical test. There were differences in testosterone levels in mice between the control group and the group of mice given extracts of meat, skin and seeds (p-value <0.05). The highest testosterone level in mice was in mice given pumpkin seed extract.

Key words: Zinc Micronutrients, *Cucurbita moschata D*, Extraction, Testosterone Levels, *Mus musculus L*

Introduction

Multiple factors cause disharmony in the household. One of them is erectile dysfunction and premature ejaculation. The incidence of erectile dysfunction increases with age, namely 12 cases per 1,000 people aged 40-49 years, 30 cases per 1,000 people aged 50-59 years, and 46 cases per 1,000 people aged 60-69 years¹. In a study in the United States, there was a 5% incidence of erectile dysfunction². There is no factual data on the incidence

of sexual dysfunction in Indonesia, but it is suspected that less than 10% of married men experience erectile dysfunction³. according to the data in the Congress of Urology Asia IV in Singapore revealed that 14% of men aged over 18 years who underwent outpatient in Asia turned out to suffer from erectile capacity, and of that amount, the most experienced impotence is male urban China 25%, followed by Indonesian men 21% (second place).

From studies in the Journal of the American Health Association, three out of 10 men experience sexual problems. Generally, among others, complaints in the form of 21% premature ejaculation, 5% erectile dysfunction, and 5% low sexual desire. The proportion of cases of erectile dysfunction of all cases of sexual dysfunction is 50%. The prevalence of erectile dysfunction in men aged 40-50 years is 40-50% and increases with age. Erectile dysfunction is a

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complex and multifactorial problem ⁴.

Domestic harmony is closely related to sexual activity or quality conjugal relations with adequate quantity. However, from the above explanation, it turns out that data on cases of erectile dysfunction (premature ejaculation) occurs not only in Indonesia but also become a global problem. The ability of an erection has a linear correlation with libido and the hormone testosterone. Both are related to various things, especially food consumption such as protein, vitamins and minerals.

There are various forms of the term aphrodisiac stimulant or stimulants that bus a libido or sexual desire in biology. Aphrodisiacs themselves can be grouped into two. The first one affects physically and psychologically, for example, through sight, taste, smell and impressions such as perfume. Second, that affects from within the body, for example, food, drink, medicine or spices ⁵.

Aphrodisiacs (aphrodisiacs) are substances or drugs that arouse sexual desire or libido ⁶. Aphrodisiac plants contain saponin derivative compounds, alkaloids, tannins, and other compounds from several studies, which physiologically can improve circulation in the central nervous system (cerebral) or peripheral blood circulation (peripheral). This increase in blood circulation will indirectly enhance body tissues' activity to improve organ function. Several types of plants with aphrodisiac content include earth peps, clove flower buds, ginseng, stoic bamboo shoots, cinnamon, lavender, chocolate and pumpkin, etc. Apart from aphrodisiac ingredients, several minerals such as zinc also play a role in increasing libido. Yellow pumpkin is an example of plant foods with the highest zinc content. Meanwhile, from animal protein, oysters are foods that are high in zinc content.

Yellow pumpkin is a local food ingredient and is very easy to obtain. It contains a very suitable protein for consumption, has a digestibility of 99%, and beta carotene is relatively high, which is 1.569 µg / 100 g. Carotenoid levels, especially β carotene,

β cryptoxanthin, lutein and zeaxanthin ⁷. Carotene is known as a potential reducer of singlet oxygen species (ROS). Consumption of antioxidants such as carotenoids, polyphenols and tocopherols can prevent oxidative stress ⁸.

Seeds of *Cucurbita moschata*, also known as pumpkin seeds, watermelon seeds, and delicious snack, also have properties to prevent the prostate gland's benign enlargement. These seeds also contain mineral elements Zn (zinc) and Mg (Magnesium), which are very important for reproductive organs' health, including the prostate gland. 100 g of *Cucurbita moschata* seeds contain the mineral Zn of 6.5 mg ⁹.

Yellow pumpkin (*Cucurbita morchata D*) contains many vitamins and minerals, nutrients in every 100 g of 34 cal, pumpkin, 1.1 protein, 0.3 fat 08 minerals, 45 mg calcium and 0.3 mg zinc. Zinc deficiency can cause impaired growth of reproductive organs, especially testes in men and sexual maturity, impaired seminal and prostate vesicle secretion, seminal and prostate vesical secretion disorders. Impaired function of Leydig cells can cause a decrease in the production of androgens, mainly testosterone. Fertility disorders due to zinc deficiency can be in low semen volume due to decreased seminal vesical secretion, impaired sperm quality and reduced sperm quantity due to reduced testosterone secretion, which plays a role in the spermatogenesis process and sperm maturation in the epididymis ¹⁰.

The research from S. Aghaei et al. suggests the protective effect of pumpkin seed extract on the characteristics, biochemistry and histology of male rat sperm by giving cyclophosphamide. Stated that pumpkin seeds are rich in oxidants, reduce the toxicity of cyclophosphamide, and significantly increase rats' antioxidant levels. Yellow pumpkin contains alkaloid compounds useful for stimulating the nervous system, raising or lowering blood pressure and fighting microbial infections. Yellow pumpkin contains natural phenolic compounds that have potential as antioxidants and have medicinal bioactivity. This

compound can be found in stems, leaves, flowers and fruit. Flavonoids in the human body function as antioxidants, protect cell structures and increase anti-inflammatory and antibiotic vitamin C¹¹.

Decreased testosterone levels due to impaired Leydig cell function will cause a decrease in the frequency of sexual intercourse. Besides, it will affect sexual response because decreased libido causes the phase of sexual response is not optimal. That sexual dysfunction can arise, which can be erectile dysfunction, ejaculatory dysfunction and orgasm dysfunction¹⁰.

This study will focus on the pumpkin as an aphrodisiac and zinc source because similar studies have not been widely conducted. This study is based on data on increasing reports of erectile dysfunction and premature ejaculation among men. Erectile dysfunction is caused by many things, one of which is inadequate food intake. Foods containing zinc and aphrodisiac ingredients can increase sexual desire and libido and reduce erectile dysfunction. One of the foods that are included in the aphrodisiac ingredients with the highest zinc content is pumpkin. Therefore, this study was conducted to determine the differences in the administration of extracts of skin, meat, and pumpkin seeds to the hormone testosterone levels in mice.

Materials and Methods

Plant preparation and extraction

The pumpkin (*Curcubita moschata* D) was identified in the plant taxonomy manual. The pumpkin fruit that has been identified is then separated based on the parts to be extracted, namely the skin of the fruit, the flesh, the seeds. The pulp, skin, and seeds extraction process, which already had constant weight, was macerated with methanol-water (4: 1) at room temperature (25-28 ° C) for 10 hours, respectively. The maceration results were filtered with Whatman 41 paper, and the filtrate was centrifuged for 10 minutes. Then the solvent was evaporated using a

rotary evaporator at a temperature of 65 ° C to obtain a thick solution of the extract of the flesh of the fruit, skin and pumpkin seeds.

Animal groupings and treatment

This study used 30 male mice obtained from PUSVETMA Surabaya. 30 male mice were grouped into 4 groups, namely control, seed treatment, skin treatment and meat treatment, each of which was 3 replications. Group, I was a control group given distilled water. Group II was a group given pumpkin skin extract. Group III was a group given pumpkin seed extract, and Group IV was a group given pumpkin seed extract. Oral administration of the extract solution using a needle with a dose of 2gr / kg BW and a volume of 0.2 ml is given for 36 days according to the time needed in the spermatogenesis cycle.

Mice Blood Collection

Blood collection of mice was carried out on day 36, the mice were euthanized using chloroform, then the abdominal part of the mice was surgically removed, and 1 ml of blood was taken intracardiac. Then the blood is put into a vacutainer, left for 24 hours until the blood plasma is separated from the erythrocytes and coagulants.

Testosterone Level Analysis

Blood plasma that has been separated from the coagulant is taken using a micropipette, then the blood plasma is inserted into the microtube, centrifuged for 10 minutes at a speed of 3500 rpm. Test of testosterone levels was carried out in the Laboratory of the Tropical Disease Center, Airlangga University using the ELISA method.

Statistical Analysis

Values are presented in form of mean ± Standard Error of Mean and were analyzed in SPSS by One Way Analysis of Variance (ANOVA). A significant difference was recorded at $p < 0.05$.

Results

The process of analyzing the testosterone hormone must be carried out as a whole. Simultaneously, the sample results in the form of blood serum must be stored in a freezer at a temperature of -50 ° C so that

the hormones contained therein are not damaged. ELISA using Testosterone Mouse Kit from China. The results of the analysis of blood oxytocin levels from various treatment doses using ELISA can be seen in Figure 1

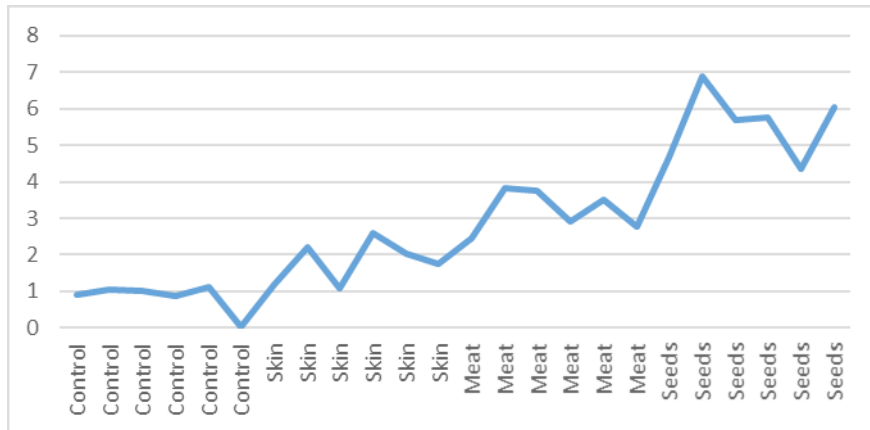


Fig 1: Testosterone levels in the treatment group of mice (Mus Musculus)

The results of the study on the calculation of testosterone levels in male mice treated with extracts of pumpkin skin, fruit and seeds, showed a difference, the group that showed the highest increase in testosterone levels was in the group treated with pumpkin seed extract, which was an average of 5.573 ng / ml, while the control group was the group with the lowest testosterone level at 0.837 ng / ml, then the pumpkin skin extract group was 1.814 ng/ml, and the pumpkin pulp extract was 3.202 ng/ml, respectively.

Table 1: One-way ANOVA test results

Group		Group	Mean Difference	Std. Error	Sig.
Lsd	Control	Skin	-.97650*	0.37629	0.017
		Meat	-2.36483*	0.37629	0
		Seeds	-4.73617*	0.37629	0
	Skin	Control	.97650*	0.37629	0.017
		Meat	-1.38833*	0.37629	0.001
		Seeds	-3.75967*	0.37629	0
	Meat	Control	2.36483*	0.37629	0
		Skin	1.38833*	0.37629	0.001
		Seeds	-2.37133*	0.37629	0
	Seeds	Control	4.73617*	0.37629	0
		Skin	3.75967*	0.37629	0
		Meat	2.37133*	0.37629	0

The highest testosterone levels in mice were in mice given pumpkin seed extract (mean = 5.5732). There was a difference in testosterone levels of mice between the control group and the group of mice given extracts of meat, skin and seeds. This can be seen from the significance value of the p-value (0.000), which is more than α (0.05), which means that there are differences from 4 groups (control, meat, skin and seeds).

From table 1, it can be concluded that there are at least two different groups. However, it turns out that all groups have significant differences with the group. The difference between the control group and the skin group has a p-value (0.017) less than α (0.05). The difference between the skin group and the meat group had a p-value (0.001) less than α (0.05). The difference between the control group and the seed group has a p-value (0.000) less than α (0.05).

Discussion

In this study, the extract from the skin, fruit pulp, and pumpkin seeds was given orally so that it went through several stages of absorption to respond to increase testosterone levels. The process of zinc absorption is similar to iron in the body, where absorption requires transportation. This process occurs in the small intestine (duodenum); zinc is transported by albumin, and transferrin enters the bloodstream and is carried to the liver. Excess zinc is stored in the liver in the form of metallothionein; otherwise, it is brought to the pancreas and other body tissues. In the pancreas, zinc is used to make digestive enzymes released into the digestive tract at mealtime. Thus, the digestive tract receives zinc from two sources: food and digestive juices from the pancreas.

Zinc absorption is regulated by metallothionein, which is synthesized in the cell wall of the gastrointestinal tract. When the consumption of zinc is high in the gastrointestinal wall cells, some are converted into metallothionein as storage so that absorption is reduced. The amount of zinc that is absorbed ranges from 15-40%. Zinc absorption is

affected by body zinc status. If the more zinc is needed, the more zinc is absorbed. Zinc is excreted mainly through faeces. Besides, zinc is excreted through urine, and body tissues are passed, such as skin tissue, small intestinal wall cells, menstrual fluids and sperm.

According to research conducted by Hayati, it was shown that the morphology of spermatozoa increased after giving the ethanol extract of pumpkin seeds¹². The statistical analysis results showed that the treatment group experienced a significant increase in spermatozoa morphology ($p < 0.05$). Widowati stated that giving the mineral Zn caused a rise in spermatogenic cells due to increased testosterone⁹. Testosterone from the seminiferous tubules is bound by protein binding antigens and transported by seminiferous tubular fluid. Testosterone reaches the epididymis. While in the epididymis, the spermatozoa undergo a maturation process. In the epididymis, testosterone is converted by the five reductase enzyme into DHT, which functions to remove cytoplasmic remains that stick to the spermatozoa after leaving the seminiferous tubules. The increase in DHT causes the mechanism of eliminating cytoplasmic remains to be more effective, resulting in improved spermatozoa's typical morphology.

Widowati stated that giving the mineral Zn caused increased spermatogenic cells due to increased testosterone⁹. This mineral Zn is also contained in pumpkin seed extract, so providing the extract can increase testosterone. Testosterone in the epididymis is converted into DHT by the enzyme five reductases. This DHT hormone functions to remove cytoplasmic droplets in spermatozoa. The spermatozoa released from the epididymis have high motility. If the testosterone and DHT in the epididymis increase, the process of eliminating cytoplasmic droplets will be better, and the motility of spermatozoa that comes out of the epididymis will also increase.

The mechanism of spermatogenesis in the testes depends on the harmonious action of the

hypothalamic-pituitary-testis axis. Local factors and sometimes immunological factors are essential in this mechanism. Among them, nutrients and supplements such as zinc come from factors that can affect reproductive hormone secretion. Therefore, the presence of zinc in food can affect reproductive parameters. The hormone-releasing gonadotropins from the hypothalamus affect the anterior pituitary and cause increased secretion of LH, FSH, and then simulate testosterone's secretion. Simultaneous increase of FSH and LH in serum by the mineral zinc 100 ppm affects the hypothalamus-pituitary-testis axis. This can occur due to a rise in LH and FSH secretion from the front pituitary and following an increase in serum testosterone levels¹³. Overall, it can be seen that adding organic or mineral zinc can affect hormone levels in both dependent and independent reproductive systems.

Zinc is involved in more than 90 enzymes related to carbohydrate and energy metabolism, protein degradation/synthesis, nucleic acid synthesis, heme biosynthesis, CO₂ transport (carbonic anhydrase) and other reactions. The most obvious effect is on metabolism, function and maintenance of the skin, pancreas and male reproductive organs, especially in converting testosterone to active dihydrotestosterone. In the pancreas, zinc has something to do with the amount of protease secretion needed for digestion. It also has to do with insulin, although it does not play a direct role in insulin activity.

Zinc has a direct effect on the maturity of sperm cells and maintains the germinal epithelium of the male sex glands. It also plays a role in the growth and development of male sexual organs and their biological activities. Zinc deficiency causes delays in testicular growth and development and inhibits the spermatogenesis process. Zinc supplements are useful in the reproductive cycles of many species and are essential for the synthesis of spermatogenesis. The effect of zinc on the prostate gland is apparent. Zinc deficiency reduces testosterone discharge and prevents spermatogenesis.

According to research from Azadeh, Relatively testicular weight increases with zinc supplementation¹⁴. In general, the weight and size of the testicles are directly related to their performance. Therefore, the increase in weight has a positive effect on the spermatogenesis activity, and the production of androgen hormones in the testes and the increase in testosterone increases testicular weight. The increase in testicular weight in this study can be ascribed to a rise in stem cells and primary spermatocytes. Lydig cells make androgen hormones which are transferred to blood cells and Sertoli. Part of the testosterone that reaches the Sertoli cells is converted into estradiol by the aromatase enzyme. Zinc deficiency in male goats causes smaller testes and causes decreased libido¹⁵. Lack of nutrition indirectly affects fertility in men. This nutritional effect results from metabolic effects. So, according to this study, an increase in serum testosterone levels synergizes with an increase in metabolic activity and an increase in testicular weight, influenced by the intake of nutrients derived from zinc.

Previous research conducted by Taufiqurrachman states that giving 50 mg of pumpkin extract can increase LH and testosterone hormones levels compared to the control group¹⁶. According to Taufiqurrachman, the increase in LH levels is caused by the pumpkin's sitosterol content, which stimulates the anterior hypnosis gland without affecting FSH secretion. Another study conducted by Juniarto, by administering 25mg / 2ml of pumpkin and 25mg / 2ml of earth peg to Sprague Dawley rats for 53 days was able to increase the number of spermatozoa significantly differently from the control group. According to Juniarto, the increase is due to the content of eurikomolactone and amarolinda, which can increase LH and FSH's secretion by improving the affinity of the anterior pituitary cell receptor membrane. This component also enhances the cell receptor membrane's association and even to the 5- α reductase enzyme, which plays a role in converting testosterone to dehydrotestosterone¹⁷.

In this study, the difference in the results from the testosterone levels analysis indicated that pumpkin seed extract administration affected the increase in testosterone levels of male mice.

Conclusion

There was a difference in testosterone levels of mice between the control group and the group of mice given extracts of meat, skin and seeds (p-value 0.000). The group that showed the highest increase in testosterone levels was treated with pumpkin seed extract, with an average of 5,573 ng/ml. In contrast, the control group was the group with the lowest testosterone level, namely 0.837 ng/ml, then the pumpkin skin extract group. 1,814 ng / ml and extract of pumpkin pulp 3,202 ng / ml respectively.

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Conflict of Interest: Nil

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