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Improvement The Quality of Sperm In Mice (Mus Musculus) Exposed To Paraquat dichloride (Herbicide) Using Sunflower Seed Extract (Helianthus annuus L.)

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ABSTRACT

Background

One of the causes of infertility in men is due to exposure to substances that are cytotoxic. One of the cytotoxic substances is herbicide (paraquat dichloride). These herbicides can form Reactive Oxygen Species (ROS). ROS contains highly reactive oxygen which turns into free radicals. Sunflower seeds contain vitamin E and selenium and phytochemical content, namely alkaloids, glycosides, saponins, flavonoids, terpenoids, and phenolic which are effective antioxidants as antidotes to free radicals.

Objective

This study aims to determine improvement the quality of sperm in mice (Mus musculus) exposed to paraquat dichloride (herbicide) using sunflower seed extract (Helianthus annuus L.).

Method

This study is a true experimental study with a posttest only with control group design. Samples of 28 male mice were divided into 4 groups. The K- group was given standard feed for 28 days; the K+ group was given exposure to paraquat dichloride 3 mg / kg; group K1 was given exposure to paraquat dichloride 3mg / kgbw and sunflower seed extract 0.18gr / 20grbw for 28 days; group K2 was given 3mg / kgbw paraquat dichloride exposure and 0.36gr / 20grbw sunflower seed extract for 28 days. Sperm quality was observed with a 400X magnification microscope. Data analysis with the Anova test was continued with the Pos Hoc test.

Results

Giving sunflower seed extract can increase and significantly influence the motility of spermatozoa with p value = 0,000, the concentration of spermatozoa with p value = 0,000, and the viability of spermatozoa p value = 0,000 in mice exposed to herbicide (paraquat dichloride).

Conclusion

Giving sunflower seed extract can increase and significantly influence the motility, the concentration, and the viability of spermatozoa in mice exposed to herbicide (paraquat dichloride).

Keywords: Sunflower seed extract, Sperm quality, The motility of spermatozoa, The concentration of spermatozoa, The viability of spermatozoa and the morphology of spermatozoa

INTRODUCTION

More than half of the causes of infertility in men are unknown (idiopathic) and can be congenital or acquired. Male infertility can be diagnosed initially by semen analysis [1]. One of the causes of infertility in men is due to exposure to cytotoxic substances [2]. Exposure to cytotoxic substances one of which is herbicide (paraquat dichloride) [3]. The herbicide can form Reactive Oxygen Species (ROS). ROS contains highly reactive oxygen which turns into free radicals. The use of herbicides in Indonesia in agriculture and plantations in terms of cost and labor has proven to be very efficient. Paraquat dichloride known simply as Paraquat is a type of herbicide with the trademark gramoxone® [4].

Paraquat dichloride is carcinogenic because it disrupts the normal activity of the endocrine system through its antiestrogenic, antiandrogenic, antithyroid and antigonadotropic effects. The chemicals in herbicides cause a decrease in testosterone levels, free androgen index, erectile function, sperm count and fertility [5, 6]. In one study it was proven to cause a decrease in semen quality in oil palm farmers, and a lack of the hormone testosterone resulted in erectile dysfunction in men [7, 8].

When there is an increase in ROS levels, the body will respond by producing enzymes CAT, GPx, and Superoxide Dismutase (SOD) to neutralize ROS. Even so, there will still be ROS left over, if there is a lot of ROS production. To reduce the remaining ROS it is necessary to provide additional anti-oxidants such as vitamin C, vitamin E, uric acid, polyphenols (flavonoids), etc. to minimize the effects of the ROS [9, 10].

This study uses extracts from sunflower seeds (Helianthus annuus L.). In half a cup (64 gm) of dried roasted sunflower seeds there are 17 mg of vitamin E, and 51 mcg of selenium. Vitamin E and selenium are nutrients that can ward off free radicals in the body that are exposed to toxins [11]. The phytochemical content of sunflower seeds is alkaloid 1.23%, glycosides 0.04%, saponins 1.46%, flavonoids 0.03%, terpenoids 0.64% and phenolic 0.34%. Flavonoids are known to be very effective antioxidants as antidotes to free radicals, for anti-cancer, hypo lipidemic, antiageing, and anti-inflammatory. In addition, the protective effect of flavonoids in biological systems is associated with their capacity to bind to free radicals, metal chelate catalysts, activate antioxidant enzymes, reduce alpha tocopherol radicals and inhibit oxidase [12].

Vitamin E is a well-documented fat-soluble antioxidant and has been shown to inhibit free radical damage that induces sensitive cell membranes. In one study, lipid peroxidation in seminal plasma and spermatozoa were estimated by the concentration of malondialdehyde (MDA). Oral supplementation with vitamin E significantly decreases MDA concentration and increases sperm motility. The recommended nutritional adequacy of vitamin E is 15 mg/day. Selenium supplements have been shown to be beneficial if taken by men 200mcg/day in improving sperm characteristics in subfertility men [13]. Se and vitamin E supplements can improve semen quality and are useful as protection, especially in sperm motility [14].

In previous studies using sunflower seed extract with 2 doses, the first dose was 0,09gr / 20grbw, and the second dose was 0,18gr/20grbw on the diameter of the seminiferous tubules of mice exposed to cigarette smoke, but the results of the extract with 2 doses were not yet optimum to the diameter of the seminiferous tubules [15]. Previous studies also used ethanol extracts of red ginger to be given to mice exposed to [16]. However, in red ginger there is a special content of arginine which is a precursor of endogenous Nitrite Oxide (NO). The nature of NO as an oxidant, which if the levels are excessive in the body and cannot be compensated, and the function turns to endanger the body cells including spermatozoa [17]. The chemical composition in red ginger only contains vitamin E as much as 0, 02 mg and selenium as much as 0, 04 mcg [18]. Meanwhile, clinical trials giving kola leaf extract (Cola nitida) on the quality of human sperm show that kola leaf extract has no effect on the viability of spermatozoa.

This study uses sunflower seed extract with the same dose as previous studies, namely dose 1 of 0,18gr / 20grbw and dose 2 increases from the previous dose of 0,36gr / 20grbw, 0,18gr / 20grbw dose is still used to compare with the results of previous studies. Based on these preliminary studies, the authors conducted an experimental study administering extracts of sunflower seeds (Helianthus annuus L.) on sperm quality in mice (Mus musculus) exposed to paraquat dichloride (herbicides).

OBJECTIVE

This study aims to determine improvement the quality of sperm in mice (Mus musculus) exposed to paraquat dichloride (herbicide) using sunflower seed extract (Helianthus annuus L.).

MATERIALS AND METHODS

This research is a true experimental study with laboratory experiments that use these using experimental animals as research objects and post-test research design only with control group design. Researchers used 2 control groups and 2 intervention groups. The control group in the study consisted of 2 types, namely the negative control group and the positive control group.

The process of making extract requires 1.5 kg of sunflower seeds. Refined flower seeds are mixed with ethanol with a volume of 1.5 liters. After soaking ethanol for 3 days then filtered using filter paper to move the liquid. The next extract fluid uses Rotary Evapolator for 8 hours in a temperature of 50^0C. This process causes the ethanol solution to evaporate, leaving only the extract of sunflower seeds. Making extracts with 500 grams of sunflower seeds can produce ± 57 grams of sunflower seed extract.

The negative control group is normal male mice and the positive control group is male mice exposed to the Paraquat dichloride with the trademark gramoxone® containing 276 mg / ml paracetate as much as 3 mg / kg body weight orally. is given 2 times a week for 28 days [19, 20].

The intervention group in the study was male mice exposed to Paraquat dichloride given sunflower seed extract at different doses. This study uses a dose of sunflower seeds based on previous research [15]. Doses given were 0,18g / 20grbw and 0,36g / 20grbw dissolved in aquabides for a maximum of 1 mL for 28 consecutive days. One day after the treatment ended, the test animals were dissected then spermatozoa were taken from the cauda epididymis, then a suspension of spermatozoa was made and analyzed. Weight of testicle, diameter of testicle and volume of testicle were measured. Sperm quality was observed with a 400X magnification microscope. Data analysis with Anova test.

RESULTS

Based on the process of making sunflower seed extract, if 500 grams of sunflower seeds produce approximately 57 grams of sunflower seed extract, then the dose of 0.18gr/20grbw if converted in the form of sunflower seeds becomes approximately 1.5 grams, and 0, 36gr/20grbw to be approximately 3 grams of sunflower seeds. Doses of sunflower seed extract 0.18gr/20grbw if converted into a human dose with body weight 70kg becomes 19, 94mg/kg, and 0.36gr/20grbw becomes 39, 88 mg/kg [21]. The dose of 19, 94 mg/kg is converted into the form of sunflower seeds becomes approximately 166 grams, and the dose of 39,88 mg/kg becomes approximately 332 grams.

Table 1. The Quality of Sperm

No	Groups	Motility		Concentration		Viability		Morphology	
		Mean±SD	*P Value*	Mean±SD	*P Value*	Mean±SD	*P Value*	Mean±SD	*P Value*
1	K-	51,67±14,72		35,67±5,31		83,17±10,22		91.33±3,44	
2	K+	15,00±8,36	0,000	17,08±1,4	0,000	56,67±4,32	0,000	86.17±7,13	0,350
3	K1	55,00±10,48		30,08±2,57		75,17±8,37		88.00±4,05	
4	K2	45,00±10,48		32,00±5,50		80,50±6,12		90.17±5,45	

Info: The K- group was given standard feed; the K+ group was given exposure to paraquat dichloride 3 mg / kg; group K1 was given exposure to paraquat dichloride 3mg / kgbw and sunflower seed extract 0.18gr / 20grbw; group K2 was given 3mg / kgbw paraquat dichloride exposure and 0.36gr / 20grbw sunflower seed extract , p value and post hoc test: significant <0.05.

Table 2. The Morphometry of Testicle

No	Groups	Testicle Diameter		Testicle weight		Testicle Volume	
		Mean±SD	***P Value***	**Mean±SD**	***P Value***	**Mean±SD**	***P Value***
1	K-	4.61±0,35		0.34±0,023		0.12±0,025	
2	K+	4.85±0,75	0,619	0.36±0,045	0,520	0.15±0,054	0,271
3	K1	4.56±0,30		0.35±0,020		0.12±0,025	
4	K2	4.83±0,31		0.35±0,008		0.15±0,044	

Info: The K- group was given standard feed; the K+ group was given exposure to paraquat dichloride 3 mg / kg; group K1 was given exposure to paraquat dichloride 3mg / kgbw and sunflower seed extract 0.18gr / 20grbw; group K2 was given 3mg / kgbw paraquat dichloride exposure and 0.36gr / 20grbw sunflower seed extract, p value and post hoc test: significant <0.05.

Explanation in table 1 of motility of spermatozoa in the dose 1 group had a greater mean of 55.00 and dose 2 of 45.00 compared to the positive group of 15.00. This proves that the effect of giving sunflower seed extract both at dose 1 and dose 2 on the motility of spermatozoa, this is reinforced by the Anova test results that p value of 0,000 (P value <0.05). However, the results of the Post Hoc test (LSD) showed no significant difference between dose 1 with dose 2, namely p value of 0.139 (P value> 0.05). Even so from the results of the Post Hoc test (LSD) also proved no significant difference in the negative control group (normal mice) with a dose of 1 namely p value of 0.614 (P value> 0.05) and dose 2 of p value of 0.317 (P value> 0.05), which means that both doses 1 and 2 can restore or equal the motility of spermatozoa in mice exposed to herbicides to normal.

The concentration of spermatozoa in the dose group 2 had a greater mean of 32.00 million / ml and dose 1 of 30.08 million / ml compared to the positive group of 17.08 million / ml. This proves that the effect of giving sunflower seed extract both at dose 1 and dose 2 on the concentration of spermatozoa, this is reinforced by the Anova test results that p value of 0,000 (P value <0.05). Post Hoc test results (LSD) prove a real difference in the negative control group (normal mice) with a dose of 1 that is p value of 0.029 (P value <0.05) which means that dose 1 has not been able to reach the negative group average or equal the concentration of spermatozoa in normal mice. In the dose group 2 the Post Hoc (LSD) test result, which is a p value of 0.137 (P value> 0.05), explains that dose 2 can restore or equal the concentration of spermatozoa in mice exposed to herbicide returning to normal.

The viability of spermatozoa in the dose 2 group had a greater mean of 80.50% and dose 1 of 75.17% compared to the positive group of 56.67%. This proves that the effect of giving sunflower seed extract both at dose 1 and dose 2 on the viability of spermatozoa, this is reinforced by the results of the Anova test that is p value of 0,000 (P value <0.05). However, the results of the Post Hoc test (LSD) showed no significant difference between dose 1 with dose 2, namely p value of 0.624 (P value> 0.05). The results of the Post Hoc test (LSD) also proved that both doses 1 and 2 can restore the viability of spermatozoa in mice exposed to herbicide back to normal, where the dose group 1 is p value of 0.292 (P value> 0.05) and dose 2 is p value equal to 0.928 (P value> 0.05) to the negative control group (normal mice), meaning that there was no significant difference in the dose 1 and dose 2 groups in the negative control group (normal mice).

The morphology of spermatozoa in the dose 2 group had a greater mean of 90.17% and dose 1 of 88.00% compared to the positive group of 86.17%. The mean in the dose 2 group was almost equal to the average in the negative group (normal mice), which was 91.33%. But this does not prove that the effect of giving sunflower seed extract both at dose 1 and dose 2 on the morphology of spermatozoa, this is reinforced by the Anova test results that p value of 0.350 (P value> 0.05).

Explanation in table 2 the diameter of the testicle in the positive control group is greater than the negative control group (normal mice), because of the enlargement of the testicles in one of the mice. Similarly, the mean diameter of the testicle in the 2 dose group was greater than the negative control group (normal mice) and the positive control group. In the positive control group the weight of the testicle was greater than the negative control group (normal mice). This also happened to the mean diameter of the testicle in the dose 2

group which was greater than the negative control group (normal mice), but smaller than the positive control group. In the positive control group the testicular volume was greater than the negative control group (normal mice). The average testicular volume in the dose 2 group was greater than the negative control group (normal mice) and as large as the positive control group.

DISCUSSION

Since herbicides play an important role in agricultural life and human plantations, the resulting toxicity makes human attention due to a number of diseases that arise affecting humans, one of which is fertility. Herbicides are organophosphate-dicocol groups that cause both intra and extracellular free radicals that can reduce sperm count and infertility [6]. Lack of knowledge in oil palm farmers who do not use personal protective equipment when spraying herbicides makes it easy to be exposed to inhaled or swallowed [22]. These herbicides (paraquat dichloride) are what disrupt normal endocrine system activity through antiestrogenic, antiandrogenic, antithyroid, and antigonadotropic effects [23].

Endocrine system disorders, especially sex hormones, are very influential on male sexual function [24]. Infertility in men can be diagnosed initially by semen analysis. Male infertile seminograms can reveal many abnormal conditions, which include azoospermia, oligozoospermia, teratozoospermia, pyospermia, asthenozoospermia, and necrospermia [1]. Spermatozoa abnormalities are caused by failure of spermatogenesis caused by various things. One of them is because it is obtained, namely exposure to cytotoxic substances such as exposure to herbicides (paraquat dichloride) [2]. This study proves the smallest mean of spermatozoa motility, spermatozoa concentration, spermatozoa viability, and morphology found in the positive control group that is mice exposed to herbicide (paraquat dichloride) compared to the negative control group (normal mice).

Increased motility, concentration, viability, and morphology of spermatozoa that occur in doses 1 and 2 groups caused by the content of vitamin E and selenium in sunflower seed extract can counteract free radicals in the body that are exposed to toxins [11]. One study states that taking supplements containing Se and vitamin E can improve semen quality and is useful as a protection, especially in sperm motility [14]. The phytochemical content in sunflower seeds, saponins, which are classified as steroids and flavonoid compounds can increase sperm motility, and contribute to improving sperm quality [25, 26]. Antioxidant effects from flavonoids which provide protection for the plasma membrane and reduce the amount of excessive free radicals in rat sperm [27].

The antioxidant effect of sunflower seeds can counteract free radicals also because of the additional role of Superoxide Dismutase (SOD) in counteracting free radicals in the body. That happens when the body responds to the presence of ROS in the body, so the body produces enzymes CAT, HPx, and Superoxide Dismutase (SOD) to neutralize ROS [9, 28].



Figure 1. Testicles of mice exposed to herbicides

In figure 1, the enlargement of the testicle experienced by mice in the positive control group or called varicocele occurs due to the influence of exposure to paraquat dichloride (herbicide). Hormonal disorders and the presence of oxidative stress are important mediators that have an impact on infertility due to varicoceles. [29] While the increase in diameter, weight and volume of the testicle in the dose 2 group occurs because the vitamin E content in sunflower seed extract has an

effect on increasing testicular weight, sperm count, motility sperm, and estrogen production, as well as increasing the survival and development of mouse sperm. [30] The correlation results show an association of testicular weight, testicular diameter and testicular volume. So that if the weight of the testicles increases, it is followed by the weight and volume of the testicles which increase.

Doses of sunflower seed extract 0.18gr/20grbw if converted into a human dose with body weight 70kg becomes 19, 94mg/kg, and 0.36gr/20grbw becomes 39, 88 mg/kg [21]. The dose of 19, 94 mg/kg is converted into the form of sunflower seeds becomes approximately 166 grams, and the dose of 39, 88 mg/kg becomes approximately 332 grams. At 166 grams of sunflower seeds contain approximately 43 mg of vitamin E and around 153 mcg of selenium. At 332 grams of sunflower seeds contain approximately 85 mg of vitamin E and around 255 mcg of selenium.

The Institute of Medicine recommends that vitamin E be safe for consumption up to 1000 mg / day at the age of 19 and above and selenium as much as 55 mcg/day to 280 mcg/day [31].Chinese scientists estimate the dose of selenium poisoning if its consumption is more than 900 mcg/day. Based on research results prove that the intake of selenium 724 mcg in adults is still at a safe level. Therefore the safe maximum dose level is 400 mcg [32, 33].

Based on the results of this study it can be seen that the fact the administration of sunflower seed extract has not been able to protect the morphology of Spermatozoa, and Morphometry of testicular absolutely and completely from oxidative stress caused by toxic herbicides (paraquat dichloride). Damage to spermatozoa or cells in the testicle may still occur, but the damage can be reduced by the presence of antioxidants and vitamins from sunflower seed extract. So, it is possible that if we give a higher dose of sunflower seed extract, it will be able to improve the morphology of spermatozoa, and the Morphometry of the testicle is equivalent even greater than that of mice that are not exposed to herbicides (paraquat dichloride).

CONCLUSION

Giving sunflower seed extract can increase and significantly influence the spermatozoa motility, spermatozoa concentration, and viability of spermatozoa in mice exposed to herbicide (paraquat dichloride). Doses of sunflower seed extract 0.18gr/20grbw if converted into a human dose with body weight 70kg becomes 19, 94mg/kg, and 0.36gr/20grbw becomes 39, 88 mg/kg [21]. The dose of 19, 94 mg/kg is converted into the form of sunflower seeds becomes approximately 166 grams, and the dose of 39, 88 mg/kg becomes approximately 332 grams.

RECOMMENDATION

1. It is expected can be benchmarks in consuming sunflower seeds to improve the quality of spermatozoa. It is expected to be a reference material in the prevention of infertility in men.
2. It is hoped that similar studies can be carried out by adding the duration of exposure to herbicides and applying sunflower seed extract to each treatment group.
3. Expected to increase the dose of sunflower seed extract to find the optimum dose of spermatozoa and testicle of mice exposed to herbicides.
4. It is expected to conduct research on the effect of sunflower seed extract on testicular histology, and Superoxide Dismutase (SOD) of mice [1].

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