Steeping Tin Leaves (Ficus carica) Improves Sperm Quality of Male Mice (Mus musculus) Exposed to Lead Acetate

by Emi Kusumawardani

Submission date: 19-Aug-2022 11:14AM (UTC+0800)
Submission ID: 1884196114
File name: artikel-Steeping_Tin_Leaves.pdf (715.44K)
Word count: 3671
Character count: 19896

Steeping Tin Leaves (*Ficus carica*) Improves Sperm Quality of Male Mice (*Mus musculus*) Exposed to Lead Acetate

Emi Kusumawardani[1], Budi Santoso[2], Widjiati[3], Hari Basuki Notobroto[4], Heru Santoso Wahito Nugroho[5]

[1]Doctoral Student at Faculty of Public Health, Universitas Airlangga, Indonesia, [2]Lecturer, Faculty of Medicine, Universitas Airlangga, Indonesia, [3]Lecturer, Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia, [4]Lecturer, Faculty of Public Health, Universitas Airlangga, Indonesia, [5]Researcher, Poltekkes Kemenkes Surabaya, Indonesia

Abstract

Introduction: Lead can induce lipid oxidation in cell membrans, thus forming free radicals. The process of imbalance of free radicals and antioxidants will disrupt the normal function of cells, causing cell death and decreased sperm quality. **Purpose**: The purpose of this research is to explain the mechanism of steeping tin leaves (*Ficus carica*) increase sperm quality in male mice (*Mus musculus*) exposed to lead acetate. **Methods:** This type of research was true experimental design with posttest only control group design with the number of replications of 10 male mice, the experimental unit will be distributed proportionally to 5 groups. Steeping tin leaves will be given with a dose of Pb + Tin Leaf 1.664 mg (P1) and Pb + Tin Leaf 3.328 mg (P2), while for lead acetate dose 0.5 mg and quercetin dose 0.7 mg. The analyzed variables included spermatozoa motility, spermatozoa morphology, and spermatozoa concentration. Data analysis was conducted including the Shapiro-Wilks normality test, and the homogeneity test used the Levene test. If the data were not homogeneous, the group average test would use the Brown-Forsythe test, then continued with a different test for each group using the Post Hoc Games-Howell test. If the Levene test data were homogeneous, the group average test would use Oneway Anova. **Findings**: The results showed that giving of steeping tin leaves with a dose of Pb + Tin leaves 3.328 mg (P2) is able to increase spermatozoa motility and spermatozoa morphology. **Conclusion**: The steeping tin leaves increase sperm quality.

Keywords: *tin leaves (Ficus carica), sperm quality, motility, morphology, concentration*

Introduction

Male reproductive function has declined since World War II in many countries. Several studies suggest that the incidence of testicular cancer, hypospadias, and cryptorchidism has increased and sperm quality has decreased in the last 50-60 years [(1)]. Several studies have identified men in North America, Europe, Asia and Africa, who show that there has been a significant reduction of 57% in mean sperm concentration over the past 35 years [(2)]. Rolland, et al [(3)] also reported a 32% decrease in the number of spermatozoa in men from 1989 to 2005. Rolf et al [(4)] reported that as many as 14% of men over the age of 40 had reproductive tract infections and lower sperm counts. than a 20 year old man.

Common causes of male reproductive disorders are impaired sperm production, blocked sperm transport system, health conditions and environmental conditions, as well as heavy metal contamination. [(5)] Some chemicals in the environment are known as Endocrine Disrupting Chemicals (EDCs), which are chemicals. which can interfere with endocrine function in the body [(6)]. Lead has the strongest influence on endocrine disorders in humans [(7)].

The human body naturally has a defense system against free radicals, namely intracellular endogenous antioxidants consisting of enzymes synthesized by the body such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) [8]. If the production of ROS exceeds the existing antioxidant capacity, it will lead cells to oxidative stress, apoptosis or necrosis [9]. Therefore, the body needs an important substance, namely antioxidants that are able to capture these free radicals, so that these radical compounds become stable and cannot cause oxidative stress (imbalance between pyroxides and antioxidants) [10]. The antioxidant mechanism can occur through the binding of metal ions, oxygen scavenger, hyproperoxide decomposition into non-radical forms, absorbing ultraviolet light or deactivating singlet oxygen. A preventive defense system, the formation of ROS compounds and free radicals is inhibited by binding to metals or damaging their formation. This metal binding system occurs in the extracellular fluid. On the other hand, in intracellular fluid, ROS compounds and free radicals are damaged by enzymes [11].

Antioxidants of flavonoid compounds can donate hydrogen atoms to free radicals so as to produce stable low-energy radicals that come from flavonoid compounds that lose hydrogen atoms [12].

Ficus carica Linn is a plant that is rich in polyphenol antioxidants such as flavonoids, especially in fruit and leaves to prevent free radical damage, besides being a source of minerals, high in fiber, low in sodium and free of fat or cholesterol [13].

Material and Methods

Making Tin Leaf Steeping

Tin leaves were obtained from cultivating the tin plant "Bumi Tin" in Ngrawe Hamlet, Morosunggingan Village, Peterongan District, Jombang Regency, Indonesia. Tin leaves taken were 3-6 pieces below the shoot and dark green. Plant identification was carried out at the Indonesian Institute of Sciences (LIPI), Purwodadi Botanical Garden. A total of 70 plucked tin leaves were cleaned, washed, dried and kept out of the sun for 1 week. Then mashed in a blender to obtain 104.5 g of dry tin leaf powder. Diluted simplicia 104.5 g of dried tin leaves with 1400 ml of water, then bring to a boil and obtain a solution of 950 ml of tin leaves. After that, freeze dry was placed for 2x24 hours and obtained 18 g of tin leaf extract.

Preparation of Experimental Animals

Adult male mice were taken from mice stock developed by outbreeding at the Center for Veterinary Medicine, Surabaya, with the criteria of being healthy, 2-2.5 months old, 30-35 g body weight, then adjusted to the environment for 1 (one). week, given to eat pellets at the place to eat and given a drink. After that, mice were grouped randomly into 4 (four) groups, namely:

a. KN group: The normal control group consisted of 10 mice without steeping tin leaves and without any exposure to lead.

b. Group K: The negative control group consisted of 10 mice without steeping tin leaves and exposed to lead acetate at a dose of 0.5 mg / 0.01 kgBW.

c. Group P1: The treatment group consisted of 10 mice which were given tin leaf infusion at a dose of 1.664 mg / 0.01 kgBW per day and exposed to lead acetate at a dose of 0.5 mg / 0.01 kgBW

d. Group P2: The treatment group consisted of 10 mice that were given tin leaf infusion at a dose of 3.328 mg / 0.01 kgBW per day and exposed to lead acetate at a dose of 0.5 mg / 0.01 kgBW

Spermatozoa Motility

The sperm motility examination was done by dropping 1 drop of sperm suspension on a glass object, then examining it under a microscope with a magnification of 100 times. In each field of view the individual movement patterns of each spermatozoa

were observed. With a hand counter, out of 100 spermatozoa, the percentage of each movement pattern was calculated, especially progressive motion. Movement of spermatozoa was observed and categorized as follows [14]:

a. +++ = If the sperm is moving fast and straight forward (forward motion is very good)

b. ++ = If the motion is slow or difficult to advance straight or move not straight (weak motion)

c. + = If not moving forward

d. N = If the sperm does not move (Necrozoospermia)

Spermatozoa Morphology

Spermatozoa shape was called abnormal when there is one or more abnormal spermatozoa parts (head, midpiece, circular tail, small head, double tail), and the result was expressed as a percent. Normal mouse spermatozoa consist of a head (caput) that forms a hook-like tip, a short middle piece, and a very long cauda. The results obtained are calculated in value by the formula,

$$\frac{a}{a+b} \times 100\%$$

Information: a = number of normal morphology b = number of abnormal morphology [15]

Spermatozoa Concentration

Calculation of the spermatozoa concentration was done by taking spermatozoa from the cauda epididymis. Spermatozoa were put into the counting chamber of the haematometer until the room was evenly filled. Then count the number of spermatozoa in one of the counting rooms and then determine the dilution to be carried out and the number of squares to be counted. Calculation of spermatozoa concentration (million / ml) can be seen in table 1.

Table 1. Spermatozoa concentration formula

No	Number of boxes counted	Spermatozoa concentration formula
1	5	n x 10,000 x 50 x 5 x 0.5
2	10	n x 10,000 x 20 x 2.5 x 0.5
3	25	n x 10,000 x 10 x 1 x 0.5

Statistical Analysis

The data obtained were processed with the SPSS 17.0 for windows program. The data analysis in this study included the normality test using the Shapiro-Wilks test, and the homogeneity test using the Levene test. For non-homogeneous data, the Brown-Forsythe test was used to determine the mean difference between groups. To find out the difference in the mean between groups, a comparison test was carried out using the Post Hoc Games-Howell test. For homogeneous data, the mean differences between groups were tested by Anova.

Findings

Figure 1 shows that the highest spermatozoa motility was in the normal group, namely 2.25, followed by the Pb + Tin leaves 3.328 mg (P2) group at 1.55, followed by the Pb + Tin leaves 1.664 mg (P1) group at 1.22, and the lowest at the Pb + Aquabidest group of 0.62. Statistical analysis showed that there was an effect of infusion of tin (*Ficus carica*) leaves on the sperm motility of male mice ($p < 0.05$). The results of the analysis showed that the spermatozoa

motility data were not normally distributed and homogeneous, then Anova test was performed, which showed differences in spermatozoa motility between groups (p <0.05).

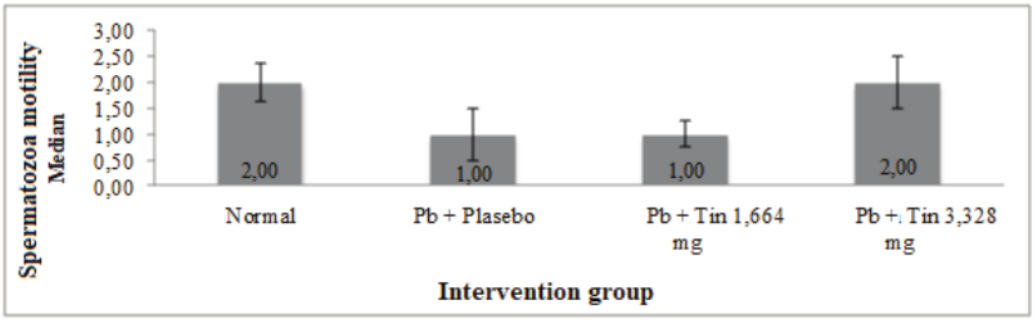

Figure 1. Mean spermatozoa motility in cauda epididymis of male mice (*Mus musculus*)

Figure 2 shows that the highest mean spermatozoa morphology was in the normal group, namely 77.00, followed by Pb + Tin Leaves 1.664 mg (P2) at 69.11, followed by Pb + Tin Leaves 1.664 mg for 62.66, and the lowest at the Pb + Aquabidest group of 45.12. The analysis showed that the spermatozoa morphology was normally distributed and homogeneous between groups. Anova test results showed that there were differences in spermatozoa morphology between groups (p <0.05).

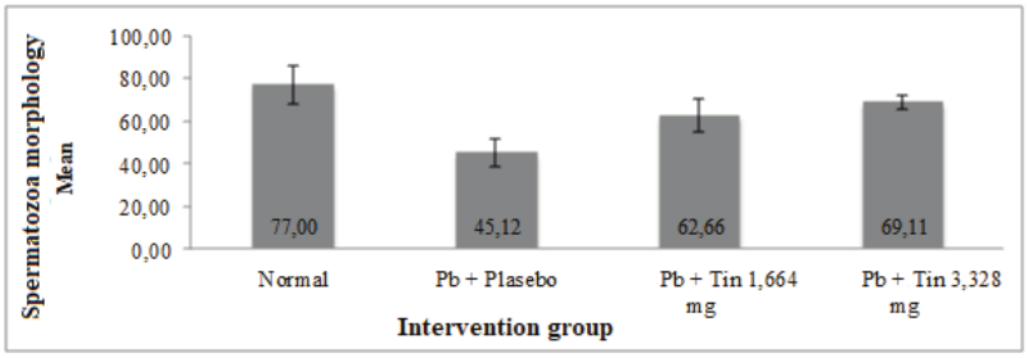

Figure 2. Mean spermatozoa morphology in cauda epididymis of male mice (*Mus musculus*)

Figure 3 shows that the highest mean spermatozoa concentration was in the normal group, namely 2.31, followed by the Pb + Tin Leaves 1.664 mg (P1), followed by the Pb + Tin Leaves 3.328 mg (P2) group of 1.44, and the lowest was the Pb + Aquabidest group of 1.26. The analysis showed that the spermatozoa concentrations were normally distributed and homogeneous between groups. Anova test results showed that there were differences in spermatozoa concentration between groups (p <0.05).

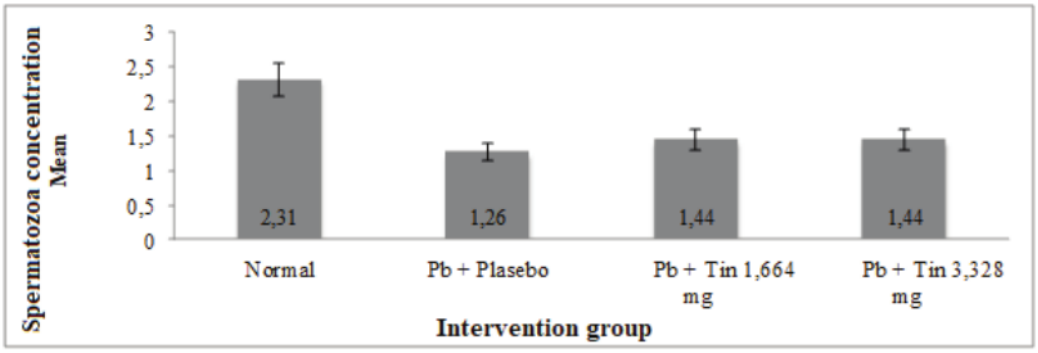

Figure 3. Mean spermatozoa concentration in cauda epididymis of male mice (*Mus musculus*)

Discussion

Spermatozoa Motility

Lead as a free radical can interfere with the activity of ATP-ase in the cell membrane. ATP-ase is located in the middle of the tail and functions to maintain internal homeostasis of the cell membrane [16]. Spermatozoa motility is highly dependent on ATP which is produced from oxidative phosphorylation in the mitochondral sheath. The movement of spermatozoa requires a certain amount of ATP which is used to move the flagellum apparatus. Impaired mitochondrial respiration function can lead to decreased motility and fertility [17].

In the biochemical system there is a balance between prooxidants and antioxidants, so that the body's tissues are protected from damage due to ROS [18]. When there is an increase in ROS levels, the body will respond by producing CAT, HPx and SOD enzymes to neutralize ROS. However, there is still some ROS left, especially if the ROS production is excessive. To reduce the remaining ROS, it is necessary to provide additional antioxidants such as vitamin C, vitamin E, uric acid, polyphenols (flavonoids), and others in order to minimize the effects of these ROS [19-21].

Polyphenol compounds have activity as antioxidants, as direct scavenger of free radicals. There are several components in tin leaves, and it is reported that caffeoylmalic acid (CMA) is the most abundant polyphenol in tin leaves, which exhibits antioxidant activity similar to vitamin C or catechins. Other antioxidants such as ubiquinone and beta carotene are fat-soluble antioxidants that will trap radicals on the lipoprotein plasma cell membrane. Apart from fat-soluble antioxidants, there are also a variety of water-soluble antioxidants such as ascorbic acid, uric acid, and polyphenol derivatives of plant origin [22].

Spermatozoa Morphology

Lead has a tendency to catalyze oxidation reactions leading to the formation of reactive oxygen species (ROS). ROS can inhibit the production of sulfhydryl antioxidants, inhibit the production of heme antioxidants, damage nucleic acids, and inhibit DNA repair. ROS also induces lipid peroxidation reactions in cell membranes [23]. Oxidative damage to spermatozoa cell membrane lipids will change the fatty acid composition of the spermatozoa cell membrane, resulting in increased membrane permeability and damage to the spermatozoa membrane resulting in low spermatozoa membrane integrity [24]. Damage to the spermatozoa plasma membrane will result in disruption of the active transfer of substances that are a source of spermatozoa such as glucose, amino acids and fatty acids. As a result of the disruption of this mechanism, the spermatozoa will lack energy so that their vitality will decrease, as well as their motility.

Damage to the plasma membrane will also disrupt the balance of ions which are essential for spermatozoa [25]. In addition, lipid peroxidation can destroy the structure of the lipid matrix in the spermatozoa membrane and cause loss of motility and damage to the integrity of the spermatozoa membrane, decreased levels of ATP that reduce viability, causing axonal damage and increasing morphological defects in the mid-piece [26].

Tin leaf steeping contains polyphenols and tannins [27]. Tannin compounds have a role in phenolic compounds that produce antioxidant effects and protect lipids [28]. Polyphenol compounds can also increase the work of antioxidant enzymes in the body such as GSH which can convert H_2O_2 molecules and lipid peroxide into H_2O. The GSH enzyme in the cytoplasm will act on the phospholipid membrane which is oxidized by free radicals [29].

Spermatozoa Concentration

Every day hundreds of millions of spermatogonia cells are formed into spermatozoa in the testes through the process of spermatogenesis. Some of the spermatogonia cells are successfully processed into viable spermatozoa and intake, while some others fail to process, so they remain immature, defective or undergo apoptosis [18, 20]. As a result, the remaining spermatozoa concentration in the ejaculate is only half, or even less, with deformities or motility disorders, so it is called oligoasthonoteratozoosperm (OAT). Such failure of spermatogenesis is mainly due to oxidative stress, as a result of increased ROS or decreased antioxidants. Under normal circumstances, certain levels of ROS are needed to promote spermatozoa function such as hyperactivation, capasitation, acrosome reactions, and fertilization [30].

Tin leaves contain phenolic or polyphenol compounds which consist of several other types of compounds, namely simple flavonoids, phenolic acids, complex flavonoids and colored anthocyanins. Polyphenol compounds are able to inhibit oxidation reactions by donating one electron to an unpaired electron in free radicals so that the number of free radicals is reduced. Phenolic compounds include a variety of compounds derived from plants, which have the same characteristic, namely aromatic rings containing one or two hydroxyl groups. Flavonoids are good reducing compounds, inhibiting many oxidation reactions both enzymes and nonenzymes [31]. Flavonoids act as good reservoirs for hydroxy and superoxide radicals, thus protecting membrane lipids against damaging reactions [32].

To maintain balance so that hemeostasis is maintained, both the reproductive tract and the ejaculate are equipped with a system to counteract the increased production of excessive ROS. A system consisting of enzymatic and non-enzymatic antioxidants functions to maintain an optimal and sensitive balance between antioxidants and prooxidants [33].

Conclusion

Steeping tin leaves (*Ficus carica*) has the potential to increase spermatozoa motility, prevent spermatozoa morphological damage, and increase the concentration of spermatozoa in the cauda epididymis of male mice (*Mus musculus*) exposed to lead acetate.

Conflict of Interest : There is no conflict of interest of any kind associated with this research and publication.

Source of Funding : All funding required in this research and publication comes from the researchers themselves.

Ethical Clearance : This research has received ethical approval from the Health Research Ethics Commission, Faculty of Public Health, Universitas Airlangga, with number: 514-KEPK.

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