Protective effect of Aqueous Extract of *Melissa officinals* and Ziziphus jujube against Mice's Testis and Epididymis Lead Poisoning

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Abstract

Introduction: Some of the natural and synthetic compounds with antioxidant properties play an important role to protect testis and epididymis against destructive factors. The purpose of this study was to investigate the protective effect of aqueous extract of *Melissa officinals* in combination with *Ziziphus juju be* against the testis and epididymis lead poisoning in mice. **Methods:** A sum of 40 male mice with an average weight of 35-30 g and then randomly divided into four equal groups Compatible courses, Include: (1) Control group: saline recipient. (2) Sham group1: received lead acetate for 7 days and after 25 days of normal diet. (3) Sham group2: received formulations for 25 days with a dose of 200 mg/kg. (4) Intervention group: This received a solution of lead acetate in drinking water at a dose (ppm1000) for 7 days and 25 days post -dose formulation 200 mg/kg, the results of the statistical program SPSS software, one way ANOVA and T-test range test were used for statistical analysis at 0.05% probability level. **Results:** With formulation prescription to the testis and epididymis tissues, the intervention group had less morphological changes than in the first treatment group. Control group's testis and epididymis tissues were normal seen and morphologic changes in second treatment group were close to the control group. **Conclusion:** This study showed that formulation has a significant protective effect in the reducing of lead poisoning.

Keywords: Lead, extract Melissa officinalis and Ziziphus jujube, Mice, Testis and Epididymis

INTRODUCTION

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Issues related to Fertility and Infertility is one of the most complex issues in Medicine. In every community almost 13% of persons are infertile that among this the most common cause of infertility in men, is their inability to produce enough healthy and active sperm.^[1] Spermatogenesis in the, which are controlled by testosterone which is secreted by testicles and secretary activity of testicles is under control the axis of hypothalamic-pituitary-testis.^[2] Considering to

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Month of Submission: 06-2017Month of Peer Review: 07-2017Month of Acceptance: 07-2017Month of Publishing: 08-2017

the negative effects and side effects of chemical drugs, nowadays using of traditional medicine especially herbal therapy is considered. In recent years great attention has been paid to study the effects of different plants on fertility of laboratory Mammals and the results obtained from these studies provide valuable information.^[3] Lead is a gray metal with bluish tint, due to frequency of its use in the industry in the world today is one of the environmental pollutants. The metal to the form of particles with smoke and dust suspended in the air and entered to daily water and food of man. The most harmful effect of lead is in the children under 6 years and more serious, are less than 3 years. ^[4] Studies have shown that lead has adverse effects on different parts of the Animal's body including: nervous system, blood, kidneys, blood circulation system, reproductive system (testicles).^[2] Lead causes damages in the structures inside the cell, the genetic structures such as DNA and free radical production in the cell and causes

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to reduce available antioxidant for cell. And also cuts off enzyme function and reduces mineral absorption. Lead bands with Sulfhydryl protein and causing the disturbance of calcium homeostasis and reduces the amount of stored antioxidant Sulfhydryl in the body.^[3] One of the main sources contaminated with lead can be noted to the air that lead particles can enter into the body from both the food and the air. Lead in the air up to 50 percent is absorbed to blood from inhalation, While the intestinal absorption of lead in food and water, and is about 11 percent. Empty stomach and fatty foods also speed up the absorption of lead. Intestinal absorption of children is more than adults and can reach 75 percent of consumed lead. In individuals with nutritional defect and iron and calcium deficiency, lead absorption intensity is higher. Fetal and neonatal poisoning occurs through the bloodstream and contaminated breast milk.^[5] Lead has different effects on the blood, nervous system, kidneys, reproduction and bone and symptoms of lead poisoning include gastrointestinal symptoms, weight loss, colic, anemia, brain damage to the, loss of memory and learning, male infertility and sperm damage and defects, abortion, Infant insufficiency and damage to the kidney.^[6-7] Jujube fruit with a scientific name as Ziziphuse jujube that belongs to the (Lamiaceae) Lamiace Ian has effective compounds such as phenol, flavonoid, saponin, vitamins A, Group B and C and has minerals such as calcium, phosphor, magnesium, sodium and potassium and has been proven that these materials can be useful for reducing and excreting the effects of lead in the body. Jujube contains thiamin vitamins and folic acid that has been shown in studies on laboratory animals these two vitamins have a massive effect on the excretion of lead in the body, particularly from the brain tissue.^[8] Studies have shown that B6 vitamin which is in this fruit can reduce lead damage and increase tissue restructuring.^[9] Studies have shown that minerals such as calcium that is in this fruit prevents from destructive effects of lead in the body by this mechanism that calcium can be replaced with lead in the tissues.^[8-9] Lemon balm with the scientific name of Melissa officinals is one of the herbal pharmaceutical and belongs to the (Lamiaceae) Deadnettle family that in all classifications of medicinal plants is introduced as green and first class plants. The most important constitutive compounds of Decadal essence including: Linoleic acid, Carnosic, Ursonic, Rosmarinic acid, Citral, Citronellal, Geraniol and Linalool.^[8] Since in this examination the therapeutic assessment for formulation of aqueous extract of Melissa officinals and Ziziphus jujube was done on lead, and it was according to standard protocol that is based on earlier work; so that seven days before starting prescription the formulation of aqueous extract of Melissa officinals and Ziziphus jujube, solved lead in drinking water were given to 2 and 3 groups in order to induct lead poisoning.

MATERIALS AND METHODS

Preparation of Crude Extract

This experimental study was performed in Kermanshah University of Medical Sciences (KUMS) at 2015.At the first jujube fruits and Melissa officinalswere prepared from the medicinal plant expert and after separating the jujuba's coretheir fleshy part was dried in the shadow and by using crusher they changed into tiny pieces. These materials were mixed with distilled water and were boiled for half hour. After cooling, this mixture was centrifuged with four thousand rpm for twenty minutes. The supernatant was collected and in low temperature and low pressure converted to the powder form. The powder was held in -20°C until they were used. With using the obtained powder by using dosage of 200 mg per kg of normal saline solution was prepared for gavage.First 50 grams of the Melissa officinalspowder was added to a solution of 1 liter of 75% alcohol and placed in the dark for 48 hours. The resulting solution was passed twice through the filter paper and obtained substance was put in rotary device (vacuum distillation) at 38 ° degrees Celsius in order to remove its alcohol. Then remaining liquid in the bottom of the dish was placed in the incubator at 38 ° degrees Celsius to dry.^[10] Finally, after scraping that amount, 45/11 grams of the extract was obtained.

Animals

In This experimental study we use sum of 40 adult male mice weighing 30 grams average weight were obtained from Medical College of Kermanshah University, they were housed under standard conditions of temperature and they rats maintained at $22\pm3^{\circ}$ C, 30 to 55% of relative humidity on standard protocol 12 hours day and night cycle was maintained food and water was provided ad libitum.^[11] The handling of the animals was in accordance with the standard principles of laboratory animal care of the United States National Institutes of Health.^[12] Gavage for all groups was done for a period of 21 days.

Experimental Groups and Protocol

After a period of adaptation for a week, they were randomly divided into four groups in terms of the number and weight so that each group had 10 mice. Include: (1) Control group: saline recipient. (2) Sham1 group: received lead acetate for 7 days and after 25 days of normal diet. (3) Sham 2 group: received formulations for 25 days with a dose of 200 mg/kg. (4) Intervention group: This received a solution of lead acetate in drinking water at a dose (ppm1000) for 7 days and 25 days post-dose formulation 200 mg/kg.

Blood and Tissue Collection

At the end of this work 12 hours before taking blood samples, mice's food was removed and mice's bed was Alimoradi, et al.: Aqueous Extract of Melissa Officinals and Ziziphus Jujube Against Mice's Testis and Epididymis Lead Poisoning

cleared and they were gone under deep anesthesia with ether and the blood sample was taken by syringe from the heart and (cardiac puncture) and centrifugation was done with1500 rpm for 10 minutes.

Tissue Preparation

Immediately the tissues of testis and epididymis were separated and the tissues were kept in ten percent formalin solution until Histopathological examination. Tissues afterfixing by different degrees of Ethylic alcohol, respectively (Ethylic alcohol 70% for 24 hours, Ethylic alcohol 90% for 3hour, Ethylic alcohol 90% for 2 hours and Ethylic alcohol 96 % for 12 hourswere dehydrated. Then samples were dehydrated by chloroform and in order to mold they were placed slowly in liquid paraffin. Then paraffin blocks were cut after cooling by the microtome device to the size of 5-6 microns and sectional samples were free from paraffin by xylazine and after exposure in water, they were studied immediately by optical microscope and were painted by Hematoxylin&Eosin method (H & E).

Statistical Analysis

SPSS software, one way ANOVA and T-test range test were used for statistical analysis at 0.05% probability level.

RESULTS

Results showed that control groups testicular had normal tissue and seminiferous tubes were seen which have spermatogenesis cells types from spermatogonia (A) to spermatozoid in their sidewall and in their lumen.In the space between the seminiferous tubules, there was interstitial tissue including Leydig cells and thearrowrepresent thenumber ofsperm (Figure 2),And testes of treatment groups were seen with some changes including: Congestion, tissue regulation disturbance, change and being irregular germinal cells and existence of amyloid bodies in some lumen of seminiferous tubules. Inflammatory view was visible in the testes arrow indicate the destruction of the pipes is seminiferous (Figure 3).

Grouptreatment with formulations of aqueous extract of Melissa officinalis and jujube fruit, there was not congestion and tissue appearance compared toot her groups closer to the control group and arrow indicates the sperm count is increased (Figure 4). In treatment group's testes that treated with lead and formulation, tissue changes were seen in comparison to the normal state while almost the same expressed position was seen in treatment group's testes that treated with lead and the arrow represent the number of sperm (Figure 5).

Epididymis in the control group in the epididymis sections, tubes were seen with simple ciliated cylindrical epithelium.



Figure 1: Plate provided during the test (50 g jujube + 50g Melissa officinal is + 900 g plate)



Figure 2: The testes of control group H& E staining, magnification 100



Figure 3: The testes of treatment group 1 H & E staining, magnification 100

There were many spermatozoids in lumen's tubes, which are produced in the testes and the arrow indicates thesperm count is increased (Figure 6). In epididymis sections at



Figure 4: The testes of treatment group 2 H & E staining, magnification 400



Figure 5: Intervention group's testes, H & E staining, magnification 100

treatment group some tubes were empty (no Spermatozoid) or had amyloid aggregates (Fig. 7).

In epididymis of group which treated with the formulation of aqueous extract of Melissa officinalis and jujube fruit (treatment 2) almost normal tissue appearance was seen, although some of sections had no sperm and arrowindicates thesperm countisincreased(Figure 8). The comparison of animals weight, testes weight and epididymis weight in treatment sham2 was showed meaningful relationship in comparison with all groups (P < 0/05). The comparison of animals weight, testes weight and epididymis weight in treatment sham1 was showed meaningful significantly in comparison with all groups (P < 0/05). And weight of the animals in the control group than in the intervention group, there was a significant relationship (p.vlue= 0/02 < 0/05). But testicles and epididymis weight in the control group than in the intervention group were not statistically meaningful (p.vlue= 0/7 < 0/05).

DISCUSSION

During the past several decades the quality of semen liquid and fertility in human societies have significantly



Figure 6: The epididymis of control group H & E staining, magnification 100



Figure 7: The epididymis of treatment group 1 H & E staining, magnification 400



Figure 8: The epididymis of treatment group 2 E staining, magnification 100

decreased. This indicates that its quality has been changed that related to toxic factors in human environment. Oxygen is one of the compounds that it is essential for the survival of living creatures, while its derivatives such as hydroxyl radicals and superoxide anion have negative effect on the cells biochemically function and structure. Reactive oxygen



Figure 9: The epididymis of intervention group H & H& E staining, magnification

Table 1: Effect of aqueous extract Ziziphuse jujube and Melissa officinalsfrom upon the body testes and epididymis relative weights of male surri mice (laboratory mouse)

Treatment	Body (g)	Testis (g:100g)	Epididymis(g:100g)
control	27/4±1/5	0/082±0/008	0/026±0/005
Sham 1	22/4±1/1	0/072±0/006	0/022±0/003
Sham 2	31/5±1/4	0/108±0/031	0/037±0/004
Test	25/3±1/4	0/081±0/008	0/027±0/003

species ROS are one of the factors that are considered to human infertility or sterility. Production of reactive oxygen species in healthy and active sperm cells is known as a physiological process that causes the sperm acrosome reaction. The positive effects of reactive oxygen species are dependent on presence of antioxidants. In sperm any kind imbalance between production and elimination of reactive oxygen species causes oxidative stress processes in cell. Reactive oxygen species by disrupting in DNA's morphology and structure causes to reduce sperm motility and finally sperm can't connect to the oocyte's surface.^[13] In natural conditions antioxidants take into action in order to collect and then neutralizing reactive oxygen species in inside and outside of cell. In recent years it has been demonstrated that in semen liquid plasma of infertile men exists less amount of antioxidants compared with fertile men.^[14] For example, the presence of reactive oxygen species in semen liquid of smokers reduces their fertility chances. Such men have less antioxidant capacity in seminal plasma in comparison with normal males.^[15] In this present study it was shown that aqueous extracts of Melissa officinals and jujube fruit increased weight of animals, testis and epididymis at dosage of 200 mg/kg and has not created morphological changes in comparison with control group.Considering to the antioxidant property and androgenic activity of aqueous extracts of jujube fruit and Melissa officinals these results may explain its role in reproductive activities.^[16] Studies have shown that

lead causes cell damage and genetic alterations and also causes oxidative damage. This increases free radicals and decreases available antioxidants which are stored in the cell for response to injury.^[17] Lead connects to Sulfhydryl and causes changes in calcium metabolism and also decreases available antioxidants in body.^[9] Clinical and experimental studies on laboratory animals have shown that intense exposure with lead causes abnormal sperm production and weight loss of testes and epididymis, and reduces the weight of pituitary gland and changes the natural structure of the testes. This also makes epithelial structure disruption, reduces sperm quality, and causes structural changein spermand reduces androgen levels.^[18-19] That confirms the reason of lose weight in animals, testis and epididymis in treatment group 1 compared with all of groups. It was also noted that the formulation of aqueous extract of Melissa officinals and jujube fruit has a significant effect in reducing lead poisoning that this could be due to existence of effective materials such as phenol, flavonoid, saponin, vitamin A, B and C and minerals such as calcium, phosphorus, magnesium, sodium and potassium in the jujube and Linoleic acid, Carnosic and Ursonic in Melissa officinals.^[20] Although each of these substances lonely cannot have a strong performance against lead, the combination of these materials together can greatly reduce gastrointestinal absorption of lead.^[21] The simultaneous presence of these materials in the formulation can be one reason for the reduction histopathological lesions in the intervention group and Jujube also contains thiamin and folic acid vitamins.^[20] Studies have shown that minerals such as calcium which exist in this formulation protect from destructive effects in the body with the mechanisms that calcium can be replaced with lead in the tissues.^[8-9] In this experiment, testes and epididymis in the intervention group has less morphological changes and lesions which are seen in this group had less intensity in comparison with treatment group 1 that this could be due to the presence of vitamin A and B12 in the formulation.^[22] Because previous studies have shown that vitamin A prevents from atrophy, testicular size reduction and abnormal sperm forming^[23] And alsoVitamin B12 prevents from testis loss weigh weight, seminiferous atrophy and prevents from failure or defect in spermatogenesis that is the reason for increasing body weight, testis and epididymis in treatment sham1 compared with all of groups.^[24]

CONCLUSION

Lead consider to the toxic effects can causes impairment in fertility in male. But with prescription of antioxidants and regard to the results of the present studyit seems that consume the aqueous extract *Melissa officinals* and jujube fruit with proper prescribed period can be have positive effect on protection of lead poisoning and spermatogenesis and causes strengthens the sexual power and reduction the testis and epididymis tissue destruction in men.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Vice Chancellor for research and technology, ministry of health and medical education Kermanshah University of Medical Science, Kermanshah, Iran.

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How to cite this article: Alimoradi M, Alimoradi Z, Ghasemi M, Tajehmiri A, Jahanmir S. Protective Effect of Aqueous Extract of *Melissa* officinals and Ziziphus jujube against Mice's Testis and Epididymis Lead Poisoning. Int J Sci Stud 2017;5(5):229-234.

Source of Support: Nil, Conflict of Interest: None declared.