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Research Paper: The Effects of Plant Extracts Mixture on Sperm Parameters and Damaged Testicular Tissue With Carbon Tetrachloride in Mice

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ABSTRACT

Introduction: Spermatogenesis is a process in which sperm is produced, and its disruption at any stage can lead to infertility. Plant extracts have strong phytochemicals, like s anthocyanin. Applying decoction of this plant's leaves could relieve nausea, and its roots are used to treat dysentery.

Methods: The Naval Medical Research Institute (NMRI) mice were used and grouped into two control and treatment groups. The control group received distilled water and, the treatment group was fed with 250 mg/kg.bw mixture of plants daily after the disruption with Carbon Tetrachloride (CCl_4) for 60 days. After this period, the mice got unconscious, and their testicles were removed from the abdomen. After conducting the morphologic study, including measuring the samples' dimensions and weight, their testicles were transcended and stained with eosin hematoxylin method. All data were analyzed by SPSS V. 22. The significance level was set at $P<0.05$.

Results: The study results revealed significant differences between the testicular size and weight. Moreover, the number of spermatogonia, spermatocytes, spermatozoa, and Leydig cells increased in the experimental group, compared to the controls ($P<0.01$).

Conclusion: The mixture of the plant caused a significant increase in spermatogenesis cells in male mice and increased their fertility.

1. Introduction

Approximately 10%-15% of couples are experiencing infertility. Although the use of health services for their treatment has increased in recent decades, the prevalence of infertility remained steady [1]. Moreover, infertility is a significant problem rising in families and communities. This issue is directly linked to the continuity of generations, and it has long been one of human wishes to raising their children. Thus, various treatment methods have been devised to solve this problem, depend-

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ing on the cause of infertility that might include male or female or both. The number of infertile couples with azoospermia due in case of normal spermatogenesis and a good quality, open biopsy method Testicular Sperm Extraction, Percutaneous Epididymis Sperm Aspiration (TESE.PESA) or aspirate Fine Needle Aspiration (FNA) could be used where sperms from testis or epididymis are obtained, and after washing, injected to ovary. However, there is always the possibility of damage to sperm cells in the lack of special defense mechanism against oxidative agents [2].

Many studies on humans and laboratory animals have reported that substances, such as hypoxia, the alcoholic extract of Riccia fluitans, zinc deficiency, the use of drug colchicine or its injection into the epididymis, phoxim, diltiazem and methylene blue, ifosfamide of selenium deficiency in the diet cypermethrin brown of nicotine, cotinine and marijuana, alcohol, caffeine, and Capparis spinosa leaf extract reduce sperm motility [3-20]. Many studies used various herbal extracts on hormonal axis and testicular tissue. Extracts, such as rosemary, the alcoholic extracts of Centella asiatica, the alcoholic extract of fennel seeds, Yarrow of aqueous extract of aerial parts Mirage, and aqueous extract of fenugreek seeds, decrease testosterone level and sometimes decrease LH level; thus, they could reduce the secondary sex characteristics, and majority of them decrease sperm density and reduce fertility. In addition, saffron extract, carrot seed extract, medicinal plants extract, garlic extract, marjoram, ginger, and velvet bean increase testosterone, LH, and sometimes FSH levels [21-33].

2. Materials and Methods

In this study, 20 male Naval Medical Research Institute (NMRI) mice were used. They were grouped into the control and experimental groups. The control group received distilled water, and the treatment group was fed with 250 mg/kg of mixture of plant extract (Stipa capensis, Crocus sativus, pollen palm tree) after disruption with Carbon Tetrachloride (CCl_4) for 60 days. The sample weight was measured at the onset and end of the experiment. In the treatment period, by anesthesia with ether, about 3 to 4 mL of blood from each mouse was collected from the left ventricle. The collected samples were centrifuged at 3000 rpm for 15 minutes. Then, using the sampler, isolated serums from whole blood were kept at -20°C for measuring the serum concentrations of LH, FSH, and testosterone. Hormonal assays were performed using the hormonal kits.

The testicles were removed and weight after the separation of surrounding connective tissues. Testicular tissue samples were stabilized in 10% buffered formalin solution. Then, testicles washed with normal saline into Catchers were maintained for 24 hours. Next, the specimens were fixed in the basket. For devices, jars with alcohol and paraffin and xylol were filled with a given program catchers devices for 1 hour each and were automatically applied. After dehydration, the samples were maintained in alcohol and xylol for one hour, twice. Then, the samples were stored in paraffin bath for 1 hour twice and got paraffin influenced (Table 1).

Paraffin molds were filled with paraffin melt, and the samples were placed in molds (horizontal or vertical). Paraffin samples were cooled and molded and labeled. Then, paraffin samples were removed from the baskets and kept in the freezer until sectioning. The paraffin samples took place in a special place on the microtome. After adjustment the screw of microtome, the film of paraffin, containing tissue was cut in 4 micron thickness. Then, the slices were lifted with two crossed brushes and placed in water bath (40-45°C) until the loss of paraffin. We transferred the samples in the slides and left them to dry. For staining the testis tissues, hematoxylin and eosin methods were used. Stained tissue sections were studied by optical microscopy camera. The studied spermatogenic cells (spermatogonia, primary spermatocytes, spermatids, spermatozoa, Sertoli) of different views X4, X10, X40, and X100) were used and the number of various experimental and control cells were compared.

3. Results

The effects of the mixture of plants in mice body and testis weights: The extract combined therapy increased the animal's body and their testis weights in the treatment group, compared to the control group. The effects of the mixture of plants in sperm count: The count of sperm significantly increased in the treatment group compared with the controls. The effects of the mixture of plants in hormone levels: The level of LH had no significant

Table 1. The studied spermatogenic cells

1	2	3	4	5	6	7	8	9	10	11
Ethanol 30	Ethanol 50	Ethanol 70	Ethanol 80	Ethanol 90	Ethanol 100	Ethanol 100	Xylol	Xylol	Paraffin	Paraffin

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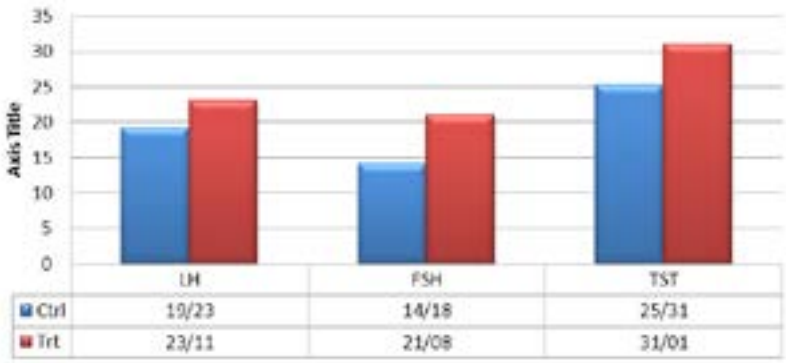

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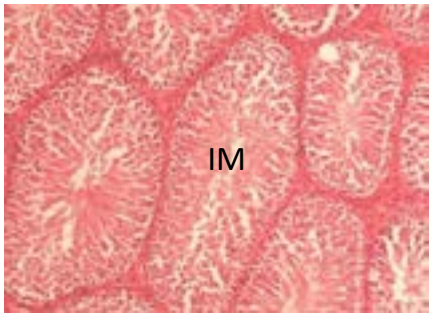
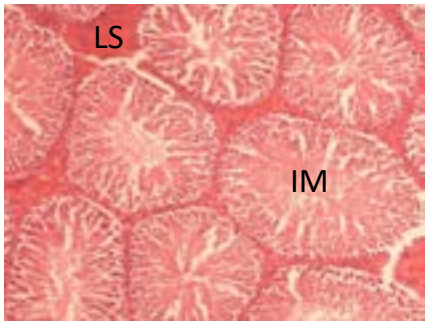
Figure 1. The effects of the mixture of plants in hormone levels



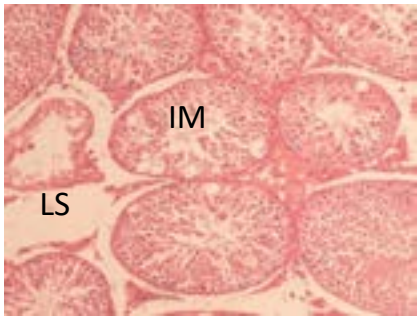
Microscopic image of testis x10

(The control group)



Microscopic image of testis x10

(The treatment group receving the mixture of plants)



Microscopic image of testis x10

(The disrupted group with CCl_4)

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Figure 2. The histology analysis results

A. The control group; B. The treatment group receving the mixture of plants; C.The disrupted group with CCl4. Microscopic image of testis x10

IM: Immature sperm, LS: Interstitial cell

differences between the control and treatment groups; however, the FSH and testosterone levels significantly increased in the treatment group (Figure 2). The effects of the mixture of plants in testis tissue: The histology analysis results suggested that both groups were normal in terms of appearance, shape, and distribution of seminiferous tubules (Figure 1).

However, the average number of spermatogonia, primary spermatocytes, spermatids, and sperm increased significantly in the experimental group.

4. Discussion

The present study investigated the effect of mixed plant extracts on the hormonal axis and testes of adult male mice. Mixed plants, despite having many different compounds, can have numerous effects. The study results indicated that the mixed extract usage could lead to changes in testicular and pituitary-gonadal function and structure in mice. Bodyweight and testes were affected by testosterone [28, 32]. Previous studies have reported that testosterone directly affects Sertoli cells, liquid discharge tube, and several proteins, including growth factors and transferrin.

Moreover, a special role in sex cells was dividing power sexual cell division, and finally, their sperm production [34]. Furthermore, tubular atrophy and reduced sperm are signs of impaired spermatogenesis [35]. Many medicinal plants used in concentration can have an essential role in its effects. According to nurser, rosemary, at low doses, has no significant influence on spermatogenesis [21, 36]. We used the extracts of mixed herbs in this research. However, different combinations require accurate determination of regulatory pathways as well as combinations that could increase some hormones and sperm. Finally, applying the mixed extract dose was effective in the improvement of spermatogenesis. However, more investigations are required to identify the active ingredient and mechanism of action and efficacy of different doses of the extract.

The plant extracts had androgenic properties and increased androgen-dependent parameters. Moreover, it caused polyspermy and increased the weight of reproductive organs. The obtained results and further investigations could be beneficial for infertile patients and prevent or reduce the symptoms of menopause in men.

Ethical Considerations

Compliance with ethical guidelines

All ethical principles were considered in this article.

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Authors contributions

Initial idea and costs: Mehdi panahian; Responsible for the tests: Mehdi Ahmadifar; Data analysis: Nazila Vahidi.

Conflict of interest

The authors declared no conflict of interest.

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