Effect of using *Matricaria Chamomilla* Oil, Vitamin E and Selenium on Reproductive Performance of Male New Zealand White Rabbits

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Abstract

This study was conducted to find out the effect of the use of chamomile flowers, vitamin E and selenium in some reproductive traits of male New Zealand egg rabbits. Twenty males were used in this study at the age of six months; the animals were randomly divided into four groups of five rabbits in each group. The first group was represented by a control without any treatment, the second group was dosed with rabbits (200 mg chamomile flower oil / rabbit/ day), the third group was dosed with rabbits (400 mg chamomile flower oil / rabbit/ day) and the fourth group was dosed with rabbits (vitamin E 250 mg/ kg + selenium 0.3 mg/ day) kg/ per day). The results of the statistical analysis of the reproductive characteristics showed that the third treatment was superior to the rest of the other coefficients for both stages in terms of ejaculate volume, sperm concentration, individual Movement, mass movement of sperm, percentage of deformed sperm and percentage of dead sperm for both physiological before and after mating periods. From other hand, the second and fourth treatment exceeded the control treatment for the same qualities for both periods also.

Key words: Chamomile, Reproductive, Vitamin E, Selenium,Rabbits
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Introduction

In recent years, interest has increased in what figuratively called alternative medicine or herbal medicine, where medicinal herbs often used as an alternative to drugs and chemical preparations. (Furhad and Bokhari, 2021).

Many studies have proven the valuable therapeutic property of many plants because of the active substances they contain, such as alkaloids, glycosides, saponins and volatile essential oils. As there are, 128 plants used to improve the health of animals and as feed additives in their relationships, and the experiments of using these plants and medicinal herbs were transferred from humans to

animals, especially domestic ones, such as rabbit (Tabish, 2008).

Recent studies have begun to try to take advantage of the ability of some medicinal plants to improve the productive, reproductive and physiological qualities in animals. In order to achieve an economic benefit by trying to modify the metabolism of carbohydrates and link this with changes in the metabolism of proteins and fats in order to obtain economic returns in terms of weight gain (Fazili *et al*. 2020; ElMihyaoui*et al*. 2022).The effectiveness of the alcoholic extract of chamomile flowers to reduce the glucose of rabbit blood, improve the coefficient of nutritional conversion, body weight and improve mostof the blood picture, hormonal and reproductiveparameters. So chamomile flowerscontain active basic compound Chamomile as well as compounds of high therapeutic value, namely Bisabolol, Beta-trans Farnesene and Chamaxulene, also contain flavonoids, glycosides, terpenes and coumarins (Saidi*et al*. 2020).As for vitamin E and selenium, they considered essential elements for humans and animals, as they are included in the synthesis of some antioxidant enzymes Antioxidant-peroxidase, including the enzyme glutathion peroxidase glutathione-peroxidase (El-Shobokshy*et al.* 2022). As one study showed a positive relationship between selenium, which is one of the antioxidant nutritional factors found in natural protein-rich foods and the health of testicles responsible for sperm production in male rabbits (Hassan *et al.* 2021). So due to the widespread popular use of medicinal herbs and the scarcity of studies indicating a direct or indirect therapeutic relationship or effect of the use of raw water extract of chamomile flowers, the current study aimed to identify the most important changes in male reproductive traits by examining male sperm.

Material and Methods

This study conducted at the Animal House dedicated to raising small animals at the Technical Institute in Hawija district, located 30 miles southwest of Kirkuk, for the period from April 2021 to January 2021. In this study, 20 adult male rabbits of the White New Zealand breed used at the age of 6 months, the animals randomly divided into four groups of males, five rabbits in each group. The animals were placed in metal cages ranging in size from one cage (2 × 2 m) prepared for the purpose of rabbit breeding and under controlled conditions of a temperature of 20-25 m and a lighting period (12 hours light - 12 hours darkness). The floor was covered with fine sawdust, which was constantly changing, the rabbits were fed on a model barn dedicated to rabbit breeding to meet the needs of the animal for growth and maintenance, as shown in Table (1), as feed and water were provided to the rabbits and open *Add libitum* throughout the duration of the experiment. The first group was represented by a control without any treatment, the second group was dosed with rabbits (200 mg chamomile flower oil / rabbit/ day), the third group was dosed with rabbits (400 mg chamomile flower oil / rabbit/ day) and the fourth group was dosed with rabbits (vitamin E 250 mg/ kg + selenium 0.3 mg/ day) kg/ per day).

Table (1): components of the feed used in the experiment

Primary feed material	%	Crude Protein%
Wheat bran	47	7.5
Local barley mash	38	3.6
Soybean meal (44% protein)	10	4.4
Animal protein (50% protein)	2	1
Limestone powder	1	-
Table salt	1.5	-
A mixture of vitamins and minerals	0.5	-
16.5Totals%	100%	16.5%

Semen Collection

Semen collected from rabbits using an electro-Ejaculator type (Electrojac 6) at the end of both physiological before and after mating periods. The rabbit laid on one of its sides on the table and its legs and head tied for easy control, and then the cathode probe inserted into the rectum after cleaning and lubricating it with glycerol. The foreskin opening was cleaned with warm water, the excess hair was cut, and then dried the inserted glass test tube was inserted through the foreskin opening with the tube held to control it during the ejaculation process by electrical stimulation for 2-3 seconds. Then given a rest period after receiving the semen, the nozzle of the tube closed with cotton wool and placed in a water bath at 37Cº for 20 minutes for the purpose of liquefaction for semen evaluation (Hafez, 2000). After the EQ occurs, take down its liquefaction time device picture(1).

Picture (1)Electro Ejaculator 6

Ejaculate Volume

The volume of the ejaculate measured immediately after collection and by means of special inserted glass test tubes for collecting semen and before placing in the water bath. The sample considered hypovolemic if its volume is less than 0.4 ml, while it considered hypervolemic if its volume is more than 6 ml, since the normal volume of sperm flow is 0.4-6 ml (Nizza *et al*., 2003).

Sperm Concentration

Sperm concentration was calculated usingNeubauer Haemocytometer Chamber according to Salisbury*et al.* (1943) by taking 0.1 ml of semen and diluting it with 19.9 ml of a dilution solution consisting of 0.9% NaCl and 0.01 ml of mercuric chloride and 2 g/ L of red eosin dye to color the sperm,so the dilution ratio is 1: 200, as for the counting method. This method carried out by placing a drop of diluted semen in the designated glass slide and after waiting for ten minutes to ensure the stability of the drop. The sperm was counted in the division and the counting process included squares the five median squares in 1 cm3 of the special area after the erythrocytes (WHO, 2010), and then extract the sperm concentration\ cm3 by applying the following equation:

Number of sperm \ cm3= number of sperm in the five squares ×400×200×10/80

Mass Motility Percentage

The mass movement was estimated according to the method of Evan and Maxwell (1987) by taking a drop of semen and placing it on a clean and warm glass slide with a temperature of 37 M to avoid the heat Shock that may occur to the sperm due to the temperature difference, then the sample was examined under a microscope with a magnification of 400 times and was estimated based on the speed and density of moving waves, and then converted into percentages.Table (2) was also used to match the numerical and descriptive values with the moving sperm ratio.

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Table (2) Estimation of mass movement

Numerical value	Descriptive value	Moving sperm ratio	
5	excellent	80%and more	Sperm has a high rotational speed, fast and variable waves
4	very good	70-80%	Sperm has a fast rotational movement
3	good	50-70%	Spermatozoa are mobile with slow rotational waves
2	medium	30-50%	Spermatozoa are mobile without distinct rotational waves
1	Lousy	Less than 30%	Sperm animated slow motion
0	Very lousy	0%	no movement

Individual Motility Percentage

It was estimated by the method of Walton (1933) by placing a drop of semen on a glass slide at a temperature of 37 m with the addition of two drops of sodium citrate sodium citrate by 2.9%, and then put the cover of the slide cover on the sample, examined with an optical microscope with a magnification of 100 times and calculated the percentage of individual movement based on the percentage of moving sperm fast forward progressive movement, as the percentage was estimated as Table (3) was relied on to match the numerical and descriptive values with moving sperm ratio.

Table (3) Individual motion estimation

lue	alue	n ratio
5	very good	
	good	
	medium	
	Lousy	
	Very lousy	

Dead Sperm Percentage

The percentage of dead sperm was calculated by the method of Campbell *et al.* (1956) by taking a drop of fresh semen and placing it on a clean glass slide at a temperature of (37 M) and then one drop was added every two dyes eosin (5%) and necrosin Nigrosine (10%). Then a swab made on another glass slide at an angle of 45 degrees and examined under a microscope with a magnification of 400. The dead sperm appeared pink in order to color the Cellular membrane (Swanson and Beardon, 1951), and 200 spermatozoa calculated in different fields of the slide after which the percentage of dead sperm in the ejaculate estimated as shown in the picture. The percentage estimated according to the following equation:Percentage = (number of dead sperm) \ (total sperm count)× 100

Abnormal Sperm Morphology Percentage

The milovanov (1960) method adopted to calculate the percentage of abnormal or deformed sperm, as the same slides used previously to calculate the percentage of healthy and dead sperm, as 200 sperm randomly examined within each slide under an optical microscope with a magnification of 400. Bearden *et al.* (2004) classified abnormal or deformed spermatozoa into primary abnormalities of the head, which are abnormalities that occur in the sperm head Sperm Head, microcephaly head, asymmetric head, Piriform pyriform head, giant head, tapered head tapered head, biceps double head, round-headed round head, irregular-headed amorphous head. As for the presence of cytoplasmic droplets Cytoplasmic droplets in the sperm neck, they are classified as secondary abnormalities secondary abnormalities, and tertiary abnormalities Tertiary abnormalities are abnormalities in the sperm tail sperm tail include double tail, twisted tail twisted tail, curved tail Bent tail, arched tail, truncated tail truncated tail and enlarged tail enclosed tail (zemjanis, 1970).

The percentage estimated according to the following equation:

Percentage = (number of abnormal sperm) \ (total sperm count)× 100

Statistical analysis

Statistical analysis of the experiment data was carried out according to a complete random CRD design using the ready-made statistical program (SAS, 2001), and Duncan Multiple Range Test Duncan (1955) used to compare averages at a significant level (p≤0.05).

Results and discussion

The results of the statistical analysis of the table (4) showed the superiority of the fourth and fifth treatment over the first, second, third, fifth and seventh treatment significantly at the level of (p≤0.05) for the pre-mating stage. The fourth and sixth treatment were significantly higher at the level of (p≤0.05) than the first, second, third and seventh treatment of the post-mating stage

Table (4) the effect of chamomile flower oil, vitamin E and selenium on some reproductive qualities in male rabbits(Standard Error ± Mean)

Trait	Treatments / Period	T1 Control	T2 Chamomile 200 mg	T3 Chamomile 400mg	T4 Vit E + Selenium
Ejaculate volume	Before mating	.416±0.0690 c	0.550±0.831 b	0.840±0.133 a	0.866±0.134 a
	After mating	0.602±0.075 cd	1.300±0.197 ab	1.300±0.197 ab	1.308±0.175 ab
Sperm concentration	Before mating	±1.49235.24 c	452.20±1.007 b	582.06±1.20 a	438.60±0.57 b
	After mating	376.30±1.68 g	587.40±1.53 e	912.70±0.8 a	658.22±0.84 d
Individual Motility Percentage	Before mating	32.86±0.73 c	57.96±1.01 b	69.44±0.74 a	57.16±0.83 b
	After mating	43.40±1.08 c	62.64±1.03 b	88.86±0.94 a	67.72±1.02 b
Mass Motility Percentage	Before mating	32.86±0.87 f	57.96±0.73 b	57.96±0.73 b	57.16±0.79 b
	After mating	43.40±0.97 d	62.64±0.76 C	62.64±0.76 C	67.72±1.01 b
Abnormal Sperm Morphology Percentage	Before mating	53.30±0.9753 c	33.36±1.26 b	15.56±0.77 a	38.24±0.84 b
	After mating	62.68±0.96 d	19.76±0.58 b	9.62±0.71 a	25.90±0.94 bc
Dead Sperm Percentage	Before mating	64.92±1.30 d	38.24±0.91 bc	25.32±0.62 a	35.80±0.86 b
	After mating	52.82±1.37 c	29.34±0.82 b	11.66±0.67 a	29.34±1.18 b

The reason for the improvement of semen properties such as the increase in sperm volume, sperm concentration, the percentage of individual and group motility, the decrease in the percentage of deformed sperm and dead sperm may be due to the protective effect of chamomile flowers on the testis and epididymis (Lim, 2017). As well as the biological function of chamomile flowers as an antioxidant and inhibitor of free radicals in the seminal plasma, which are harmful products of many metabolic processes(McKayandBlumberg,2009).

It can also have natural antioxidants found in chamomile flowers such as vitamin E and alkaloids, which work to reduce the effect of effective oxygen varieties on the cells and tissues of the body, including testicular tissues, subsequent glands and sperm cells (Malviya*et al*., 2016). As well as also that among the protection carried out by these antioxidant compounds is to prevent lipid peroxidation in the cellular membranes of sperm and prevent or reduce the effect of effective oxygen varieties on the respiratory chain of

mitochondria in sperm-generating cells, which facilitates the production of mature and natural sperm(Khaki, *et al.* 2009).In addition, they have an important role in the stability of cellular membranes, including the membranes of energy houses (mitochondria), and thus prevents changes in the construction of proteins and the electron transport chain (Alahmadi*et al*., 2020). The reason for this improvement of the male reproductive system may also be that chamomile plants contain flavonoid compounds that have a structure and action similar to android hormone (Harborne*et al*., 1975). Bisabolol and Chamazulene oil, as the two active substances in chamomile, also contribute to providing energy available to the sperm, which positively affects the movement, maturation and number of sperm, and has an important role in the metabolism of sperm where it found in the epididymis fluid (Soltani *et al*., 2018). As well as its antioxidant role reduces the harmful effects of free radicals on germ cells.

As for the treatment of selenium and vitamin E, Underwood(1981) pointed out that there is a contribution of some minerals, including selenium, to increasing the effectiveness of the subsequent sex glands of the male reproductive system, including the prostate gland and the vesicular gland. Smith *et al.* (1979) also pointed out that the highest concentration of selenium in the subsequent sex glands, in the prostate, the vesicular gland and the Cooper gland. Its effect on increasing the effectiveness of the enzyme GSH-px and its ability to get rid of free radicals produced internally by metabolic and thus increase the secretory efficacy of these glands. Selenium is a trace element in humans and animals (Hawkes and Turek, 2001) and selenium is essential for sperm function and male fertility reproductive problems of cows, cattle, mice, rats and chickens have linked to selenium deficiency (Combs and Combs, 1986). Where improved productive performance has reported because of the use of selenium (Tang *et al*., 1991). Selenium and glutathione are essential for the formation of phospholipid hydroxyperoxidase GSH-Px, which is an enzyme, is present in the primary sperm, which is a structural protein. Therefore, it is 50% of the middle part of the mitochondrial capsule in mature sperm, and any imbalance in these two substances causes instability in the middle piece hence the imbalance of sperm motility (Ursinif *et al*., 1999). As for vitamin E, it also mentioned that with the use of vitamin E orally, the concentration of malondialdehyde (MDA) decreases significantly. As well as sperm, motility improves.The improvement of semen qualities may also be due to the improvement in testicular function as a result of the direct effect of selenium on testicular intercellular and its active effect in stimulating pituitary anterior lobe hormones affecting testicular function (Yousef *et al*., 1990).

The Zubair Study (2017), which matched the results of this experiment, indicated the role of vitamin E and selenium in increasing collective and individual mobility and reducing the percentage of dead and deformed sperm because of the role of vitamin E in protecting the plasma membrane from peroxides of unsaturated fatty acids. As well as the role of selenium in reducing the occurrence of abnormalities in the sperm tail and maintaining sperm straightness and its motor and metabolic functions (Qazi*et al.,* 2019).

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