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Effect of Phenolic Extract of Cress Seed on Cations and Osmolality in Rabbit Seminal Plasma

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ABSTRACT

This study aimed to investigated the effect of cress seeds phenolic extract as an antioxidant on cations and osmolality of rabbit seminal plasma. Four groups (24 male and 10 female) of rabbits were taken (N=6). First group was control (Distilled water) and other groups received orally phenol extract (30, 60 and 90 mg/kg), once daily for 60 days. The information of cations concentrations and osmolality of seminal plasma were collected and evaluated. The concentrations of copper, cadmium, lead and potassium were significantly declined (p0.05). While the concentration of iron, sodium, calcium and osmolality increased significantly in the seminal plasma of experimental groups compared to control. Through the results, the appropriate dose of phenolic extract from local plant Rashad can be used as a medicine in improving human fertility through its role in reducing the damage of trace elements and improving semen osmolality, and as results of its antioxidant function.

KEYWORDS: cress seeds, cations, osmolality, seminal plasma, phenolic extract, antioxidant.

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INTRODUCTION

The use of medical plants for treating diseases is a worldwide phenomenon in developing and developed countries. The classical knowledge of medicinal plants has led up to the different medical drugs development, and is as yet the basis of numerous scientific studies (Alalwany *et al.*, 2021)[1]. (Motlagh *et al.*, 2014)[2] confirmed that some medicinal plants have antioxidant potential. The properties of Rashad seeds as total phenolic contents and antioxidative capacity was investigated by Aydemir and Becerik (2011)[3], the activity of seeds extracts to chelate Fe+2 was superior. Cations are a significant class of environmental factors that can interfere with the normal functioning of the male reproductive system, and male rabbits have a relatively low fertility rate compared with other animals. Therefore, male rabbits may be more susceptible of reproductive toxicity (Russell *et al.*, 1990)[4]. A large number of trace elements can exert stimulatory or inhibitory effects on spermatozoa depending on the concentration of each divalent cation (Stegmayer and Ronquist, 1982)[5]. The concentrations of macro mineral in seminal plasma provide the proper environment for sperm survival, and the levels of various ions in the seminal plasma also reflect semen quality (Kalita *et al.*, 2006)[6]. Hamamah and Gatti (1998)[7] the function of sperm strongly depends heavily on the ionic environment, and differences in level of nutrition mineral can have a significant impact on concentrations of SP ions, sodium Na+, potassium K+, and calcium Ca2+in the SP exert an equilibrium of osmotic (Cevik *et al.*, 2007)[8]. Consuming more antioxidants in the diet can enhance the semen's quality, including its motility and sperm count. **(**Eskenazi *et al*., 2005)[9]. An antioxidant activity of phenolic compounds can play an important role in neutralizing and captivating free radicals and decomposing peroxides (Shahidi *et al*., 1992)[10]. The advantages of rashad for health are well known by its antioxidant activity and the capacity to scavenge free radicals. Therefore, the present study aimed to determine the cations concentration and seminal fluid osmolality of male rabbits which affected by cress phenolic extract.

MATERIALS AND METHODS

Preparation of the extract

Standard elements as well as chemicals applied in research were supplied through Sigma-Aldrich, Germany. Rashad seeds of Lepidium sativum plant were gained from the local market in Hilla, Iraq. This seeds were diagnosed by Dr. Nada Adnan in the Faculty of Science at the University of Babylon. The methanolic water extract of plant seeds was prepared with the addition of 1 g of crushed ground seeds with 25 ml of solvent consisting of 80% pure methanol and 20% distilled

water in a 70°C water bath for 40 minutes. The solution was filtered and put in oven of 50°C for 24 hours to obtain the dry extract and then store in a black bottle in the refrigerator until use (Nayak *et.al.,* 2009)[11].

The ethical statement

The provision of animals (rabbits) was approved by the Animal House at the College of Veterinary Medicine, which returns to the University of Baghdad in Iraq. The animals were adapted to the conditions of the laboratory, and the research was completed after the end of the dosage period and the collection of semen samples at the University of Al-Qassim Green after inclusion of the research in the annual education plan with the approval of the Scientific Committee the Department of biotechnology Ethics at Al-Qasim Green University/College of biotechnology sciences.

Experimental design

Twenty of the white male New Zealand rabbits Oryctolagus cunculus weighing 1.5 ranging from 8 months to 1 year. The male rabbits are placed in plastic cages set up for breeding rabbits under controlled temperature conditions (20 to 25 C°) and light duration (12 h light and 12 h dark). All rabbits were fed according to their standard diet and water ad libitum. Animals were divided into 4 groups of 5 animals after the the adaptation phase. Animals in groups 1 served as a control (Distilled water) were given (2 ml/day) using animal gavages. The rest of the experimental cohorts were treated orally with 2 ml of 30, 60, and 90 mg/kg of the phenolic extract for 60 days.

Semen collection

Two weeks before semen collection, the male was trained to give semen via the artificial vagina (AV) applying a teaser rabbit doe. Animals were placed individually in plastic cages in standard circumstances. In the 50 days following the administration of phenolic extract, the animals under study were placed on semen collection twice a week. One ejaculation of each male rabbit was collected between 07:00 to 14:00 h (local time) on two times a weeks for 4 wk. The semen was centrifuged (Hettiche BH3O) at 1500 rpm for 10 min at 25C° (Xu et al. 2003), and the seminal plasma (sp) was separated from the sperm into tubes and kept at 60°C in the frozen (Ocean).

Measurement of actions (mg/kg) and osmolality (mosm/Kg).

Approximately one ml of sample was digested twice with five ml of acid mixture (1HclO4: 6HNO3) in a glass tube. The remaining material was dissolved in one ml of 1% HNO3. The cations **(Pant *et al.* 2003)[34]** were analyzed using the atomic absorption spectrophotometer (Model AA-7000, Shimadzu Corporation, Kyoto, Japan). The concentrations of macrominerals were analyzed using a flame-photometer (AFP 100, United Kingdom) after diluted samples with deionized distilled water. Osmolality was measured, using a single-sample Micro Osmometer (Model 210, USA).

Statistical analysis

The data from cations and osmolality were analyzed using ANOVA, differences in control and experimental groups of the above parameters were evaluated using F-test, the least significant difference (LSD) was utilized to compare the results, and the descriptive analysis was used to show the mean and the standard error (SE) of the results. P 0.05 was significant, and different characters showed significance in p0.05.

RESULTS

The current study appear in Table 1 that animals supplemented orally with different doses of the phenol extract show a protective role in cations confirmed by the low concentration of Cu2+, Cd2+, Zn2+, Pb2+ and Fe2+ at p0.05 of treated groups compared to control.

Table 1. Concentration of seminal plasma cations in controls and treated rabbits with different concentration of phenol extract.

Phenol extract Mg/kg	Cations Concentration (mg/kg)				
	Cu^{2+}	Cd^{2+}	Pb^{2+}	Zn^{2+}	Fe^{2+}
Control	0.065±0.001a	494.29±0.017a	59.504±0.330a	0.984±0.004a	25.18±0.378a
30	0.045±0.001b	474.09±0.029b	49.349±0.611b	0.863±0.022b	24.69±0.334ab
60	0.026±0.001c	459.23±0.191c	42.366±0.386c	0.711±0.001c	22.72±0.179c
90	0.025±0.001cd	420.61±0.040d	38.427±0.088d	0.705±0.001cd	22.40±0.323cd
LSD	**0.002**	**1.043**	**1.019**	**0.036**	**1.020**

The different letters indicate *significance when the value of p-value 0.05, with the meaning ± Standard Error (SE) and Least significant difference (LSD) is different for treated and control groups.

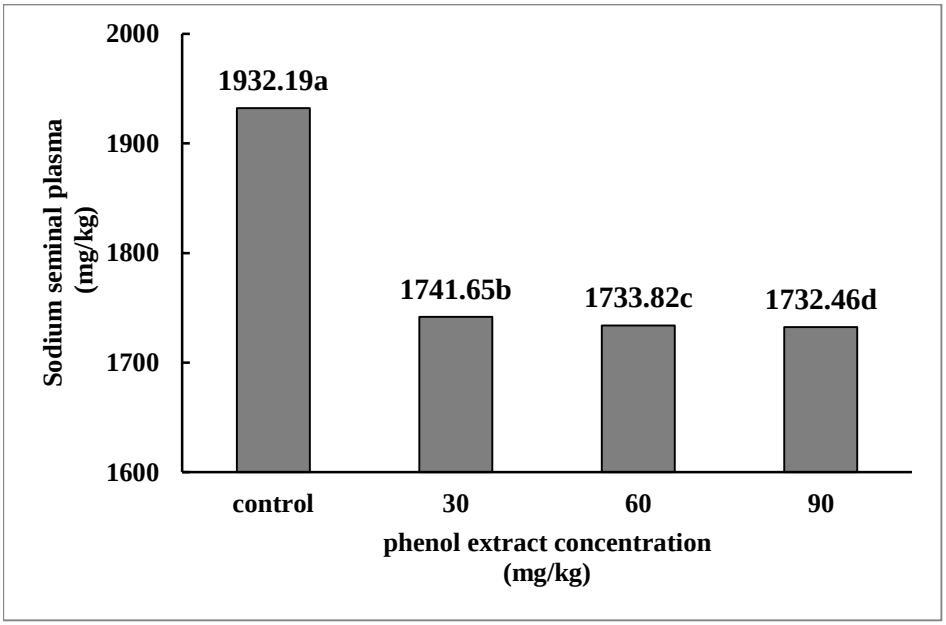

Figure 1. Level of sodium (mg/kg) in seminal plasma of male rabbit in different groups. Group 1 (control group) was compared with treated groups: Group 2 (30 mg/kg phenol extract), Group 3 (60 mg/kg phenol extract) and Group 3 (90 mg/kg phenol extract). Different letters indicate *significance when p0.05. The numbers above the column represent the mean ± Standard error (SE), with least significant difference, LSD = 0.897.

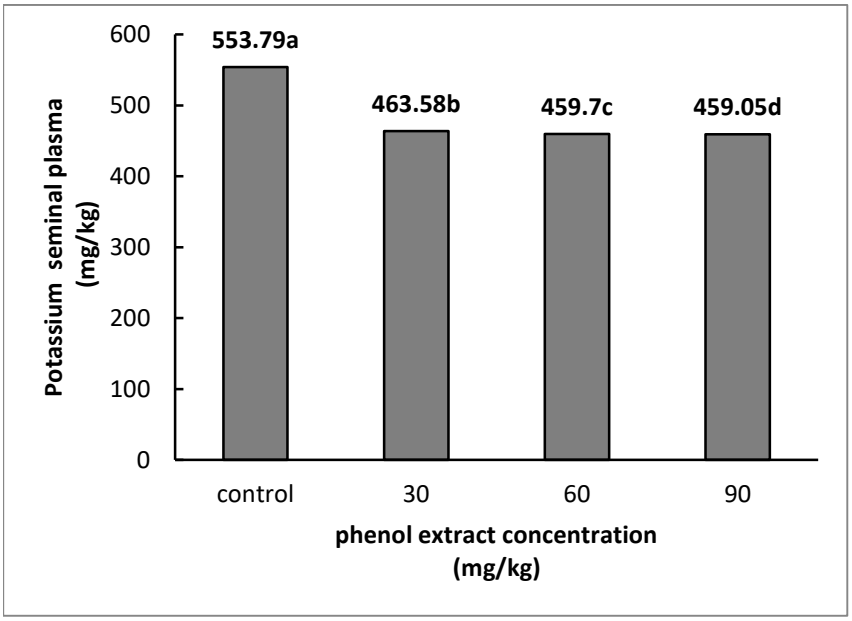

Figure 2. Level of potassium (ppm) in seminal plasma of male rabbit in different groups. Group 1 (control group) was compared with treated groups: Group 2 (30 mg/kg phenol extract), Group 3 (60 mg/kg phenol extract) and Group 3 (90 mg/kg phenol extract). Different letters indicate *significance when p0.05.The numbers above the column represent the mean ± Standard error (SE), with least significant difference, LSD = 2.977.

The concentration of other cations (mg/kg) such as: sodium (Figure 1) and potassium (Figure 2) in the study was significantly lower in treated groups as compared to control. While an increased in calcium concentration (Figure 3) of treated groups, which in turn may lead to significant increase in osmolality (Figure 4) of experimental groups at p0.05.

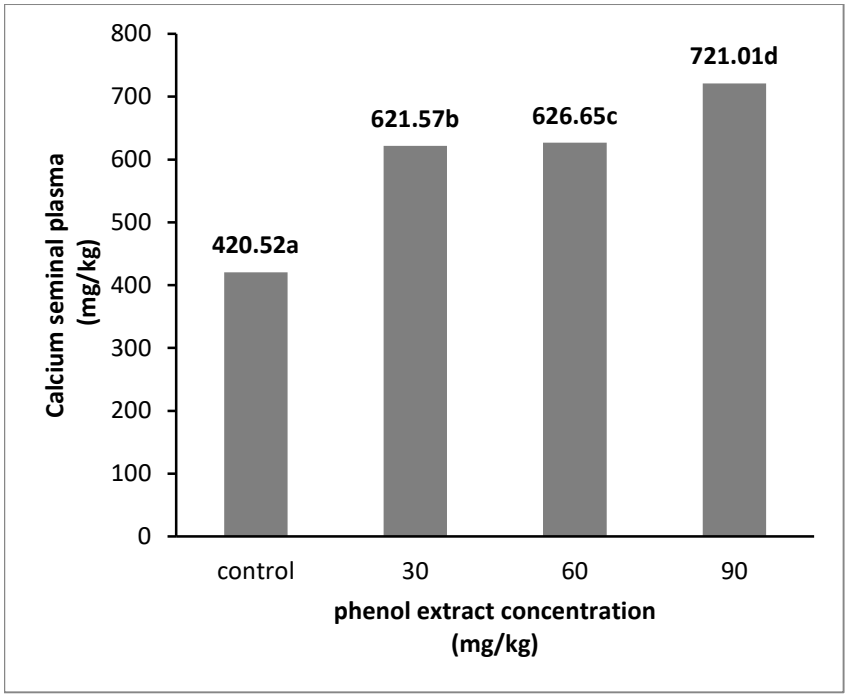

Figure 3. Level of calcium (mg/kg) in seminal plasma of male rabbit in different groups. Group 1 (control group) was compared with treated groups: Group 2 (30 mg/kg phenol extract), Group 3 (60 mg/kg phenol extract) and Group 3 (90 mg/kg BW phenol extract). Different letters indicate *significance when p0.05. The numbers above the column represent the mean ± Standard error (SE), with least significant difference, LSD = 3.394.

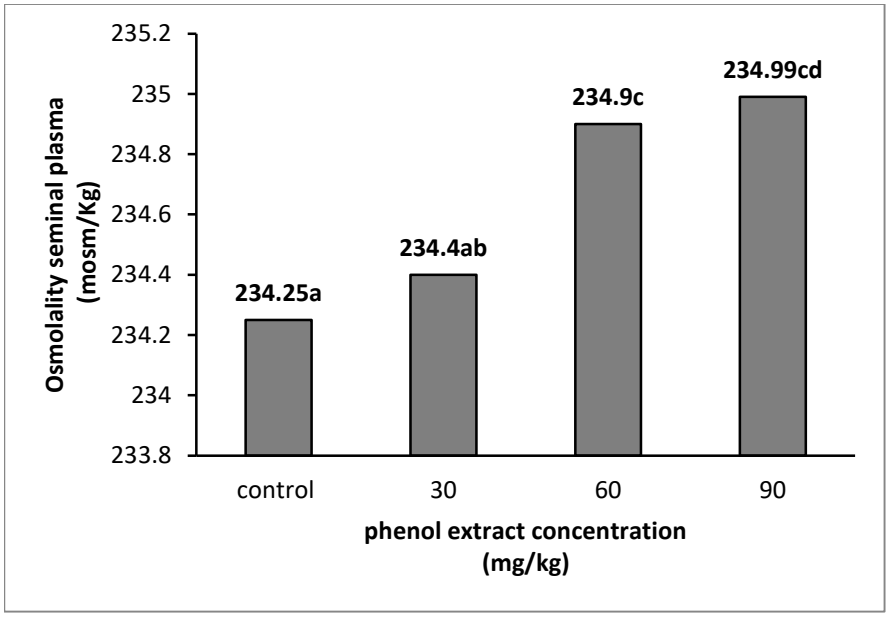

Figure 4. Level of osmolality (mosm/Kg) in seminal plasma of male rabbit in different groups. Group 1 (control group) was compared with treated groups: Group 2 (30 mg/kg phenol extract), Group 3 (60 mg/kg phenol extract) and Group 3 (90 mg/kg phenol extract). Different letters indicate *significance when p0.05. The numbers above the column represent the mean ± Standard error (SE), with least significant difference, LSD = 1.052.

DISCUSSION

Male reproductive potential may be significantly modified by diet. Therefore, it is important to emphasize the impact of daily food intake in preserving male fertility or preventing male infertility (Ferramosca and Zara, 2022)[12]. Natural antioxidants, which operate as reducing agents and free radical scavengers against reactive oxygen species and free radicals, are mostly found in plants. (Maury *et al.*, 2020)[13], because of the negative health effects of some artificial antioxidants. Additionally, plant-derived diets contain a variety of secondary plant metabolites such as polyphenols which the oxidation of low density lipoprotein (Frei, 1995), and due to their antioxidant activity and ability to reduce tissue damage by oxidative stress, which is linked to a number

of chronic diseases (Rio *et al.,* 2013[15]; Doke and Guha, 2015 [16]), also due to Quercetin and other antioxidants work together synergistically to enhance the freezing-thawing of rams. (Ardeshirnia *et al.,* 2017)[17]. However, In vivo antioxidant activity (Malara *et al.,* 2014) [18] of phenol extract on the chemical compositions of seminal plasma from minerals, and its osmolality may have some impact on the regular activity of rabbit spermatozoa. As demonstrated in Naji and Abood (2013)[19] study, MED50 (36.1 mg/kg) of phenol extract of garden cress seeds has improved the sperm parameters of rabbits. The reducing the concentrations of positive ions in seminal plasma matched with several studies including: study of Sakhaee *et al.,* (2016)[20], After long-term treatment of copper sulfate, the flavonol glycosides produced protective effects on mouse testes since copper sulfate is a harsh emetic and strong oxidizing agent that damages mucosal membranes (Babaei and Abshenas, 2013)[21]. In the male rabbit reproductive system, It is well known that cadmium damages the reproductive system and lowers sperm quality (Al-Taee, 2005)[22], and phenolic compounds that found in grape juice protects male rats reproductive parameters against cadmium-induced damage (Pires *et al,* 2013)[23] through modulation of the antioxidant system. When addition, lead another cation that causes a wide range of physiological, biochemical, and behavioral dysfunctions by producing free radicals. Bhattacharjee *et al.,* (2018)[24], reported that natural polyphenolic compounds can reverse the harmful effects of lead by detoxifying it from the major organs. In another word, flavonoids compound decreased rats serum and tissues concentrations of the trace elements (zinc, copper and iron) compared with control (Hussain and Jaccob, 2013)[25]. Numerous investigations have shown comparable findings, and they suggest that chelation or the complexation of phenols with trace elements as possible mechanism and the beneficial effects of phenol extract in the concentration of harmful cations in the present study were observed.

The seminal plasma's cation concentrations, which create a favorable environment for the survival of sperm, also reflect the quality of the semen and the physiological condition of the reproductive accessory gland. (Kalita et al., 2006) 6. A major constituent of the seminal plasma, potassium can inhibiting sperm motility in the sperm duct, and Sperm motility can be maintained by sodium and chloride (Morisawa 1983)26. The two ions have the responsibility in maintaining osmolarity and metabolic activities of the spermatozoa (Ambali et al., 2018)27. Zamiri and Khodaei, (2005)28 found that a high percentage of motile sperm was related with low levels of Na+ and K+ ions. However, (Akpa et al., 2013)29 described positive correlation between sperm motility and concentration of seminal potassium ion in study on Red Sokoto goats. So another beneficial effects of phenol extract in cation concentration have been shown in current study. Quercetin is an antioxidant that scavenges ROS by inhibiting their production by both enzymatic and non-enzymatic systems, including NADPH oxidase and NADH-dependent oxidoreductase, which are found in the mitochondria and plasma membrane of sperm. This antioxidant promote the cell to release calcium, Controlling the intracellular free calcium concentration is essential for the preservation of appropriate cell activity, depending on the period and polyphenol concentrations (Silva et al., 2012)30, and this may explain the increase in calcium concentration of sperm plasma in the current study, and in turn the only harmful effect of the phenol extract, that supports the results of the previous study by Naji and Abood (2013)19 an increased in abnormal sperm morphology percent of epididymis caudal at (36.1 mg/kg) of phenol extract, because a rise in seminal Ca2+ concentration would coincide with an increase in the incidence of acrosome defects in spermatozoa, highlighting the importance of Ca2+ in semen quality. In media with the same osmolality as semen or blood, mammalian spermatozoa may sustain their motility for the greatest time. Additionally, hypertonic solutions are generally safer than hypotonic ones (White, 1962 ; Mann, 1964). Thus phenol extract of Rashad seeds increased the fertilizing capacity of rabbit by increasing the osmolality of rabbit seminal plasma. This result is consistent with the findings of researcher Chang and Thorsteinsson (1958)33, Additionally, rabbit spermatozoa's ability to fertilize is demonstrated to be superior in hypertonic than hypotonic solutions. However, a number of variables, including the way phenolic compounds are administered, their dosage, and their structural makeup may affect their activities (Silva et al., 2012)30. In conclusion, the current results show that regular orally doses of phenolic extract were able to decrease harmful concentration of cations in rabbit seminal plasma, through antioxidant modulation. Thus, the using of foods and supplements which high in phenols may enhance health and protect against a variety of diseases , but the conditions It's unclear whether consuming large amounts of phenols poses any hazards.

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Conflict of interest

The author declares no conflict of interest and this study was self-funded.

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