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Effect of Aqueous extract of *Moringa Olivera* leaves(AEMOL) on some characteristics of ram epididymal sperms under cooling conditions

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Abstract

The current study was conducted in the Graduate Studies Laboratory at the College of Agriculture, Al-Muthanna University, for the period from December 3, 2022 to March 9, 2023, to determine the effect of an aqueous extract of Moringa leaves on some characteristics of epididymal sperm of rams under cooling conditions for different periods. The aqueous extract of Moringa leaves was prepared and stored in the refrigerator (5 °C) until the time of use, the testicles were transferred to the laboratory less than an hour after the slaughter of the animal, the caudal epididymis was diluted with a Tris extender containing 0.03 concentration of aqueous extract of moringa olivera leaves.The results of this study showed a significant increase ($P<0.01$) in the percentage of live sperm, individual motility, and the plasma membrane integrity, while the adding of AEMOL leads to a decreasing of the percentage of abnormal sperms. Increasing the cooling hours of sperm leads to a significant decrease in the percentage of live sperm, individual motility, testing the integrity of the plasma membrane with an increase in the percentage of abnormal sperm. Using the aqueous extract of Moringa leaves at different times led to a significant effect on the individual motility, percentage of the plasma membrane integrity represented by the hypo-osmotic swelling test (HOST) , While the treatment did not significantly affect the percentage of live sperm and abnormal sperm. According to the results of this study we conclude that the aqueous extract of Moringa Oleifera leaves improves the characteristics of rams epididymal sperms preserved under cooling conditions for different periods.

Keywords: Moringa water extract, semen characteristics, local ram, cryopreserved periods.

Introduction

Over the last ten years, the global consumption of animal products (milk and meat) has increased by more than 20%, with an expected increase in this percentage in the world (Ahmed et al., 2020). The use of modern agricultural technologies include the assisted reproductive technologies (ART), will definitely stimulate the growing demand for animal products worldwide (Zuidema et al., 2021).

The World Health Organization (WHO) has confirmed that more than 70% of the world's population depends on traditional medicine directly. As for the effect of herbs in general and aromatic and medicinal plants in particular, they have many useful medicinal and therapeutic properties. Biological such as anti-inflammatory, antifungal, antibacterial, insecticidal, antioxidant, analgesic activities and other uses (Joppa et al., 2011).

The medicinal plants have been used to dilute semen instead of industrial diluents, as their extracts have been used by adding this extracts to the semen extenders for animal semen preservation, under cooling or cryopreservation conditions, because it contains an Effective and biologically active substances that act as natural antioxidants with many medical uses and applications (Banana et al., 2020). Plant extracts have also attracted the interest of many researchers to supplement semen diluents in order to preserve the vitality of animals semen (Elsheshtawy & Elnattat , 2020). Moringa leaf extract was used as an antioxidant to improve the results of semen preservation by freezing and cooling (José Maria et al., 2020). Moringa leaves are a rich source of amino acids such as cysteine, methionine, tryptophan, and lysine with a high percentage of proteins. also contains a specific plant dyes that have been proven to It has a strong antioxidant effect (Barakat et al., 2015; Surendra& Roopan, 2016; Young et al., 2019).

The current study aims to clarify the effect of an aqueous extract of Moringa on some characteristics of rams semen preserved under cooling condition for different periods.

Materials and methods:

The current study was conducted to determine the effect of moringa leaves aqueous extract on some characteristics of rams epididymal sperms under cooling conditions preservation for different periods.

Collecting of testicles and obtaining sperm:

The testicles were collected from the rams directly, After slaughtering of animals, and transferred to the laboratory using a container containing ice cubes, to maintain the sperm vitality. Then the scrotum was removed from the testicles, the testicles were dissected, and the sperms war collected from the tail of epididymis according to the method of (Moce et al., 2010).

Preparation of the AEMOL:

Moringa Oleifera leaves were extracted according to (Handa et al., 2008).as the leaves were taken directly from the trees, then washed three times with distilled water to remove impurities and dirt from them, then dried under direct sunlight, with continuous stirring, and after drying, the leaves were ground. plant using an electric mill by adding 300 ml of distilled water to 50 grams of Moringa leaf powder, in sealed plastic containers with stirring and

shaking using an electric vibrator continuously for 72 hours, then the solution is filtered in two stages, the first was drying with layers of medical gauze And the second with filter paper, then it was incubated at 37 ° C in a special incubator, to evaporate the water to obtain a concentrated aqueous extract, then the extract was added to a Tris diluent at a concentration of 0.03.

Preparing of tris extender:

A 100 ml of Tris extender was prepared by adding 63.3 g of Tris extender, then 0.5 g of sucrose was added, 1.99 g of citric acid was added, and antibiotics (penicillin - streptomycin) were added, all of the previous materials were added to 80 ml of water distilled, then the medium was filtered through a Melbourne filter twice (22 and 45 µg), with the pH set between 7.2-7.4.according to (Evans &Maxweel,1995).

Sperms assesmeat:-

Rams Epididymal sperm were initially assased according to the following characteristics: the percentage of live and abnormal sperms, individual motility, and HOST, also ware re-evaluated at the periods of cooling 1,24,48,72,96 hrs.

Results and discussion:

The percentage of live sperm:

The results of this study table (1) show that there were no significant differences in the percentage of live sperms between the treatment of AEMOL and the control treatment during the 1hrs. of cooling preservation, while there were a highly significantly superiority ($P<0.01$) to the treatment of AEMOL as compared to the control treatment in the periods 24, 48, 96 and significantly superiority ($P<0.05$) in the period 72hrs. of preservation. The reason for this effect may be due to the content of moringa leaves of antioxidants such as flavonols, flavonoids, phenols, selenium, carotene and many natural compounds that have a role in reducing oxidative stress (Vergara-Jimenez et al., 2019)

Table (1) Effect of cooling on Percentage of Live Sperm (Mean ± Standard Error)

. Sig	Cooling periods					treatments
	96	72	48	24	1	
**	0.78± 71.30 E e	0.62± 76.83 D b	2 79.8 0.48± C b	0.26± 85.14 B b	0.27± 91.09 A a	T1
**	0.85± 74.42 E a	0.60± 77.79 D b	83.69 0.39± C a	0.52± 88.59 B a	0.26± 91.31 A a	T2
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Averages with different uppercase letters within the same column and lowercase letters within the same row are significantly different among themselves. * ($P<0.05$), ** ($P<0.01$).

Percentage of abnormal sperm:-

The results of table (2) shows a significantly (P<0.05) decreasing during the period 24hrs. and a highly significantly (P<0.01) decreasing during the periods 48 and 72 hrs in the percentage of abnormal spermatozoa in the treatment of AEMOL. As compared to the control treatment, while no significant differences occurred during the periods 1 and 96hrs. of preservation.

Table 2: Effect of cooling on the Percentage of Abnormal Sperms (Mean ± Standard Error)

. Sig	Cooling periods					treatments
	96	72	48	24	1	
**	0.42±9.49 A a	0.29±8.32 B a	0.28±7.21 C a	0.26±4.92 D a	0.15±4.51 D a	T1
**	0.27±7.92 A a	0.29±6.22 B c	0.23±6.26 B b	0.18±4.81 C a	1.15±3.68 D b	T2
	N.S	**	**	*	N.S	. Sig

Averages with different uppercase letters within the same column and lowercase letters within the same row are significantly different among themselves. * (P<0.05), ** (P<0.01).

individual sperm motility:-

The results of table (3) shows that non-significant differences in the percentage of individual motility between the different periods of cooling preservation except the last period (96hrs.). The cell wall and the inner tissue of the moringa leaves contains a percentage of Carbohydrates includes a sugars as a source of energy that affect increasing of sperm motility during the long times of preservation (joshi, 1997; Yeh et al., 2003).

Table 3 Effect of cooling preservation on the Percentage of Individual Sperm motility (Mean ± Standard Error)

. Sig	Cooling periods					treatments
	96	72	48	24	1	
**	1.26±22.58 E c	1.46±34.48 D b	1.79±47.47 C b	1.56±60.57 B b	1.30±81.03 A a	T1
**	1.63±28.50 E b	1.50±38.10 D b	1.49±51.20 C b	1.71±60.34 B b	1.19±81.14 A a	T2
	**	N.S	N.S	N.S	N.S	. Sig

Averages with different uppercase letters within the same column and lowercase letters within the same row are significantly different among themselves. * (P<0.05), ** (P<0.01).

Hypo Osmosis Swelling test (HOST):-

Table (4) shows that a significantly (P<0.05) increasing in the percentage of the plasma membrane integrity during the last period of preservation in the treatment of AEMOL as compared with the control treatment .

Table 4 Effect of cooling preservation on the percentage of HOST (Mean ± Standard Error)

. Sig	Cooling periods		treatments
	96	1	
**	1.15±70.75 B b	0.52±92.37 A a	T1
**	1.22±76.54 B a	0.49±92.69 A a	T2
	*	N.S	. Sig

Averages with different uppercase letters within the same column and lowercase letters within the same row are significantly different among themselves. * (P<0.05), ** (P<0.01).

Refrence:-

Ahmed, J., J. Lorch, L. Ong and J. Wolfgram .2020. How the global supply landscape for meat protein will evolve? McKinsey & Company, USA. pp. 1-8.

Banana, H.J.H., Alkhazreji, H.T.A. and Mahdi, Z.A. (2020). Adding different concentrations of pomegranate peels alcoholic extract.

Barakat, A.H., Wagdy, K.B., Himaidia, A.R. 2015. Moringa oleifera extract modulates the expression of fertility related genes and elevation of calcium ions in sheep oocytes. Small Ruminant Research 130: 67–75.

Elsheshtawy, R., & Elnattat, W. (2020). Assessment of Buffalo Semen Preservability Using Tris Extender Enriched With Moringa Oleifera Extract. *Egyptian Journal of Veterinary Sciences*, *51*(2), 235-239. doi: 10.21608/ejvs.2020.23502.1150.

Evans G, Maxwell C. 1987. Salamon's artificial insemination of sheep and goats. Sidney: Butterworth. pp.66-76, 158-161.

Handa, S.S., Khanuja, S.P.S., Longa, G., & Rakesh, D.D. (2008). Extraction Technologies for Medicinal and Aromatic Plants (1st Edition) P. 66, Italy: United Nations industrial development organization and international centre for science and high technology.

Joppa, L.N.; Roberts, D.L.; Myers, N.; Pimm, S.L. 2011. Biodiversity hotspots house most undiscovered plant species. Proc. Natl. Acad. Sci. USA.

José Maria Carrera-Chávez, Edson Eduardo Jiménez-Aguilar, Theisy Patricia Acosta-Pérez, José Alberto Núñez-Gastélum, Andrés Quezada-Casasola, Angélica María Escárcega-Ávila, Mateo Fabián Itza-Ortiz & Ernesto Orozco-Lucero (2020). Effect of *Moringa oleifera* seed extract on antioxidant activity and sperm characteristics in cryopreserved ram semen, Journal of Applied Animal Research, 48:1, 114-120.

joshi SP. (1997). Chemical Constituents and Biological Activity of Aloe barbadensis—A Review. Journal of Medicinal and Aromatic Plant Science; 20:768-773.

Moce, E., Blanch, E., Tomas, C., Graham, J.K. (2010). Use of cholesterol in sperm cryopreservation: Present moment and perspectives to future. Reprod. Dom. Anim. Sci., 45(3):67-75.

Surendra, T.V., Roopan, S.M. 2016. Photocatalytic and antibacterial properties of phytosynthesized CeO2 NPs using Moringa oleifera peel extract. Journal of Photochemistry & Photobiology, B: Biology 161: 122–128.

Vergara-Jimenez, M., et al., 2019. Bioactive Components in Moringa Oleifera Leaves Protect against Chronic Disease. Antioxidants (Basel, Switzerland), 6,4 91. 16

Yassen, S.G. 2021. Molecular Diagnosis of Some Medicinal Plants and Studying the Effect of Their Extracts on Some Dermatophytes Compared with Nano-zinc. Thesis, Education College for Pure Science, University of Kerbala, Iraq.

Yeh GY, Eisenberg DM, Kaptchuk TJ, Phillips RS. (2003). Systematic Review of Herbs and Dietary Supplements for Glycemic Control in Diabetes. Diabetes Care; 26:1277-1294.

Young, Q.W., G., Yujie, D. Hui, D., Shejiang, L., Xu, H., Jianzhou, G., y Dan, L. 2017. Subcritical ethanol extraction of flavonoids from Moringa oleifera leaf and evaluation of antioxidant activity. Food Chemistry. 218: 152–158.

Zuidema, D., K. Kern, and P. Sutovsky. 2021. An exploration of current and perspective semen analysis and sperm selection for livestock artificial insemination. Animals, 11: Article # 3563.