Effect of adding aqueous extract of Kalgan seeds to Tris diluent on some semen traits of Holstein bulls after cooling.

Alaa Fadel Abdul Wahid1 Makki Khalaf Al-Dulaimi2
Al-Mussaib Technical College , Al-Furat Al-Awsat Technical University
Email: dr.makkikhalaf@atu.edu.iq , alaafadil8585@gmail.com

Abstract

The study was conducted in the artificial insemination department from September 2024 to December 2024 with the aim of demonstrating the effect of adding different concentrations of the aqueous extract of the seeds of the Kalgan plant to Tris diluent on some traits of the semen of Holstein bulls after cooling. Four Holstein bulls were used in this experiment. Semen was collected from the four bulls every week at a rate of one ejaculation per bull. The semen of four bulls was mixed and mixed together (Pooled Semen) to eliminate individual differences between bulls. The experiment was divided into four treatments: The first treatment was the control treatment (T1) Tris diluted without adding antioxidant, T2: (Tris diluted + 501 micrograms Kalgan seed extract / 10 ml diluted), T3: (Tris diluted + 250 micrograms Kalgan seed extract / 10 ml diluted), (T4: Tris diluted + 350 micrograms Kalgan seed extract / 10 ml diluted). The results showed that adding 250 micrograms of Kalgan seed aqueous extract / 10 ml / Tris diluted in treatment (T3) led to a highly significant increase (P>0.01) in the percentage of individual sperm motility after cooling the semen at a temperature of 5 ºC. The results also showed a highly significant increase (P>0.01) in the percentage of live sperm for treatment T3) when adding 250 µg of aqueous extract of Kalgan seeds/10 ml/Tris diluent) after cooling compared to the rest of the treatments. The results also showed a highly significant increase (P>0.01) for treatment T3) when adding 250 µg of aqueous extract of Kalgan seeds/10 ml/Tris diluent) in the percentage of plasma membrane integrity and acrosome integrity after semen cooling compared to the rest of the treatments.

Introduction

The global consumption of animal products (milk and meat) has increased by more than 20% over the past ten years, with an expected increase in this percentage all over the world, especially in developing countries [2]. The use of modern agricultural technologies will certainly meet the increasing demand for animal products globally, and perhaps the most prominent of these technologies is artificial insemination [27]. The process of cryopreservation of semen has contributed to reducing the obstacles and factors that hinder the process of obtaining semen from genetically distinct bulls, such as geographical location and time factor [3].The process of cryopreservation of semen in bulls stimulates an additional source of reactive oxygen species; ROS) which harm sperm and lead to a decrease in the activity of antioxidant enzymes and the sperm membrane becomes more susceptible to lipid oxidation [9]. Adding some natural plant extracts to semen diluents protected the sperm of agricultural animals from reactive oxygen species by improving their qualitative properties after cryopreservation and freezing in Holstein bulls [4] due to their containing large amounts of antioxidants. The aqueous extract of the seeds of the Kalgan plant (Silybum marianum) is rich in Silymarin, a powerful antioxidant that has the ability to inhibit free radicals and

ISSN 2072-3857

provides protection from lipid oxidation in the cell membrane [17]. Therefore, the current study aims to demonstrate the effect of adding different concentrations of the aqueous extract of the seeds of the Kalgan plant to Tris diluent on some traits of the semen of Holstein bulls after cooling, including individual motility, percentage of live sperm, percentage of integrity of the plasma membrane of sperm, and percentage of integrity of the acrosome of sperm.

Materials and methods

Semen was collected from four bulls per week, with one ejaculation per bull. The semen of four bulls was mixed and mixed together (Pooled Semen) in each replicate to eliminate individual differences between bulls. The necessary tests were performed to evaluate the semen and then diluted using Tris diluent, which was prepared in advance according to the method of [20]. The experiment was divided into four groups and the diluent was added to the semen samples gradually. The distribution of treatments was as follows: (T1) Tris diluent without adding antioxidant, T2: (Tris diluent + 501 micrograms of Kalgan seed extract / 10 ml diluent), T3: (Tris diluent + 250 micrograms of Kalgan seed extract / 10 ml diluent), (T4: Tris diluent + 350 micrograms of Kalgan seed extract / 10 ml diluent). The tests were performed after the semen was cooled to 5°C.

Studied Traits

:1Individual Motility Percentage

The individual motility percentage of sperm was estimated based on what was stated by [6.[

.2Live Sperms Percentage

The percentage of live sperm was calculated based on what was indicated by [23[

:3The percentage of integrity of the sperm plasma membrane

The percentage of sperm with an intact plasma membrane was estimated according to the method of [14.[

:4Sperm Acrosome Integrity Percentage

The percentage of integrity of the acrosome was estimated based on the method of [12.[

Statistical analysis

The statistical program Statistical Analysis System -SAS [21] was used to analyze the data to study the effect of different treatments on the studied traits according to the design Complete randomization (CRD), and significant differences between means were compared by [7] multinomial test.

Results and discussion

Effect of adding different levels of aqueous extract of Kalgan seeds on the percentage of individual motility of sperm after cooling, freezing and liquefaction for 48 hours and after one month

The results of Table 1 showed that there were highly significant differences ($P<0.01$) in the percentage of individual motility after cooling the semen at a temperature of 5 °C, as treatment T3, which recorded (49.00±1.00), outperformed treatments T1, T2 and T4, which recorded (1.22±43.00), (1.00±44.00) and (1.22±38.00) respectively. The excelled of individual motility can be due to the addition of aqueous extract of Kalgan seeds to the semen diluent compared to the control treatment without addition. [10] indicated that adding silymarin to the semen diluent of rams can maintain the individual motility of sperm and reduce the percentage of non-motile sperm compared to the control treatment, [19] reported that using ram semen diluent containing silymarin and storing it at 5 °C for 72 hours gave significant differences ($p<0.05$) in individual sperm motility compared to the control treatment .

ISSN 2072-3857

Table 1: Effect of adding different levels of aqueous extract of Kalgan seeds on the percentage of individual sperm motility after cooling

treatment	Mean ± Standard Error(%)
	After cooling
T1	43.00 ±1.22 B
T2	44.00 ±1.00 B
T3	49.00 ±1.00 A
T4	38.00 ±1.22 C
Significance Level	**

T1 = control treatment diluted Tris without antioxidant addition, T2 = 150 µg Kalgan seed extract/10 ml diluted, T3 = 250 µg Kalgan seed extract/10 ml diluted, T4 = 350 µg Kalgan seed extract/10 ml diluted

Means with different uppercase letters within the same column and lowercase letters within the same row are significantly different from each other. * (P≤0.05), ** (P≤0.01).

Effect of adding different levels of aqueous extract of Kalgan seeds on the percentage of live sperm after cooling

The results of Table 2 showed that there were highly significant differences (P<0.01) in the percentage of live sperm after cooling the semen at a temperature of 5°C, as treatment T3, which recorded (81.80±0.80)), outperformed treatments T1), T2 and T4, which recorded (1.03 ± 73.40), (±0.86 72.80) and (± 1.06 71.20) respectively.

The improvement in the percentage of live sperm may be due to the fact that silymarin works to inhibit free radicals such as hydroxyl radicals in different cellular systems such as platelets, fibroblasts and mitochondria and inhibits oxidative stress [15] just as silymarin has an effective role in improving the vitality and reducing sperm abnormalities caused by alloxan [13] and many studies have reported that silymarin is a strong antioxidant whose activity is attributed to its work to inhibit the formation of free radicals and scavenge them and enhance the mechanisms of enzymatic and non-enzymatic antioxidants. Its strong antioxidant activity makes it a herbal drug that inhibits oxidative stress in cellular systems [22.[

ISSN 2072-3857

Table 2: Effect of adding different levels of aqueous extract of Kalgan seeds on the percentage of live sperm after cooling

Mean ± Standard Error(%)	treatment
After cooling	
73.40 ±1.03 B	T1
72.80 ±0.86 B	T2
81.80 ±0.80 A	T3
71.20 ±1.06 B	T4
**	Significance Level

T1 = control treatment diluted Tris without antioxidant addition, T2 = 150 µg Kalgan seed extract/10 ml diluted, T3 = 250 µg Kalgan seed extract/10 ml diluted, T4 = 350 µg Kalgan seed extract/10 ml diluted

Means with different uppercase letters within the same column and lowercase letters within the same row are significantly different from each other. * ($P \leq 0.05$), ** ($P \leq 0.01$).

The effect of adding different levels of aqueous extract of Kalgan seeds on the percentage of integrity of the plasma membrane of sperm after cooling

The results of Table (7) showed highly significant differences ($P<0.01$) in the percentage of integrity of the plasma membrane after cooling the semen at a temperature of 5°C, as treatment (T3) eas excelled, which recorded (±0.20 79.80) compared to treatments T1), T2 and T4, which recorded (±0.37 78.20), (±0.37 78.80) and (±0.31 77.00) respectively.

The excelled of T3 treatment over the control treatment may be due to the fact that this superiority may be evidence that silymarin has antioxidant properties, as it improved the antioxidant capacity of the system and may have increased the stability of membranes[1,11] expressed that silymarin improves the antioxidant defense system in human semen, as it increased the activity of antioxidant defense enzymes of sperm, such as superoxide dismutase (SOD), glutathione peroxidase, and catalase (CAT). Cryopreservation leads to physical, chemical, and mechanical changes in sperm membranes of all mammalian species[8] and El-Nattat), as these changes are attributed to differences in temperature, lipid oxidation, production of reactive oxygen species, osmotic pressure, and others [5] and since sperm are highly susceptible to oxidative damage due to the presence of large quantities of unsaturated fatty acids in the membrane Plasma [16] as silymarin works to maintain the redox balance in the cell by activating a group of non-enzymatic antioxidants such as vitamins C and E and enzymatic antioxidants such as GSH-Px, SOD, CAT [26 .[

ISSN 2072-3857

Table 3: Effect of adding different levels of aqueous extract of Kalgan seeds on the percentage of integrity of the plasma membrane of sperm after cooling

Mean ± Standard Error(%)	treatment
After cooling	
78.20 ±0.37 B	T1
78.80 ±0.37 B	T2
79.80 ±0.20 A	T3
77.00 ±0.31 C	T4
**	Significance Level

T1 = control treatment diluted Tris without antioxidant addition, T2 = 150 µg Kalgan seed extract/10 ml diluted, T3 = 250 µg Kalgan seed extract/10 ml diluted, T4 = 350 µg Kalgan seed extract/10 ml diluted

Means with different uppercase letters within the same column and lowercase letters within the same row are significantly different from each other. * ($P \leq 0.05$), ** ($P \leq 0.01$).

Effect of adding different levels of aqueous extract of Kalgan seeds on the percentage of acrosome integrity of sperm after cooling

The results of Table (4) showed highly significant differences ($P<0.01$) in the percentage of acrosome integrity after cooling the semen at a temperature of 5 °C, as treatment (T3) eas excelled, which recorded (±0.24 90.40) compared to treatments T1, T2 and T4, which recorded (±0.37 88.20) and (±0.37 88.20) and (±0.37 87.80) respectively.

With regard to the highly significant increase in the percentage of acrosome integrity for treatment T3 when adding 250 micrograms of Kalgan seed extract/10 ml diluted. The result of the current study can be interpreted that the integrity of the acrosome is linked to the integrity of the sperm plasma membrane, as the sperm plasma membrane in turn protects the healthy cells in the acrosome vesicle at the tip in the head so that the acrosome vesicle remains intact and the protection of the acrosome protective cap is increased [18]. The superiority is also attributed to the ability of the aqueous extract of the seeds of the Kalgan plant to affect cellular metabolism by removing the toxicity resulting from sperm metabolism processes or perhaps its role in protecting lipid membranes, proteins and nucleic acids from oxidative stress damage in sperm [24,25] indicated, silymarin is effectively used to increase fertility due to its antioxidant properties as it inhibits the formation of free radicals and lipid oxidation in membranes and increases the content of antioxidants inside cells as the sperm membrane in mammals is rich in polyunsaturated fatty acids, which makes it very sensitive to lipid oxidation and by oxidizing agents, silymarin acts as an antioxidant as it can reduce the high percentage of reactive oxygen species produced by metabolic processes that occur in mitochondria for the purpose of energy production .

ISSN 2072-3857

Table 8: Effect of adding different levels of the aqueous extract of Kalgan seeds on the percentage of acrosome integrity of sperm after cooling

Mean ± Standard Error(%)	treatment
After cooling	
88.20 ±0.37 B	T1
88.20 ±0.37 B	T2
90.40 ±0.24 A	T3
87.80 ±0.37 B	T4
**	Significance Level
T1 = control treatment diluted Tris without antioxidant addition, T2 = 150 µg Kalgan seed extract/10 ml diluted, T3 = 250 µg Kalgan seed extract/10 ml diluted, T4 = 350 µg Kalgan seed extract/10 ml diluted Means with different uppercase letters within the same column and lowercase letters within the same row are significantly different from each other. * (P≤0.05), ** (P≤0.01).	

References

1. Aghashahi, M., Momeni, H.R. and Darbandi, N. (2020). Impact of aluminium toxicity on vital human sperm parameters—Protective effects of silymarin. Andrologia, 52(10):1-10.
2. 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.
3. Al-Jashmi, Atheer Saad Mohsen. (2017). The effect of adding garden sage and melatonin hormone to Tris and Soybean diluents on the traits of Holstein bulls' semen preserved for different periods of refrigeration and freezing. PhD thesis/ College of Agricultural Engineering Sciences/ University of Baghdad.
4. Al-Mufarji, Ayser Hamid Salman. (2018). The effect of adding alcoholic extract of Ferula hermonis and some antioxidants to Tris diluent on the quality of chilled and frozen semen in Holstein bulls. Master's thesis. College of Agriculture/ University of Diyala.
5. Camara, D.R., Mello-Pinto, M.M.C., Pinto, L.C., Brasil, O.O., Nunes, J.F. and Guerra, M.M.P. (2011). Effects of reduced glutathione and catalase on the kinematics and membrane functionality of sperm during liquid storage of ram semen. Small. Rumin. Res., 100(1): 44-49.
6. Chemineau,P.,Caginie,Y.,Guerin,Y.,Arguer,P.and.Vallet,J.C.(1991). Training Manual on Artificial Insemination in Sheep and Goat. F. A. O. Anim. Prod. and Health, 83(2): 122-128.
7. Duncan, D.B.(1955). Multiple Rang and Multiple F-test. Biometrics. 11: 4-42.
8. El-Sheshtawy, R.I., El-Nattat, W.S., Sabra, H.A. and Ali, A.H. (2014). Effect of honey solution on semen preservability of local breeds of cattle bulls. World. Appl. Sci. J., 32(10): 2076-2078.

ISSN 2072-3857

9. El-Sisy, G.A., El-Nattat, W.S. and El-Sheshtawy, R.I. (2007). Buffalo semen quality, antioxidants and peroxidation during chilling and cryopreservation. Online J. Vet. Res., 11(2): 55-61.

10. Eskandari, F. and Momeni, H.R.(2016b). Protective effect of silymarin on viability, motility and mitochondrial membrane potential of ram sperm treated with sodium arsenite. Int. J. Reprod. Biomed., 14(6): 397.

11. Feitosa, W.B., Rocha, A.M.D., Mendes, C.M., Milazzotto, M.P., Delboni, C.C., Visintin, J.A. and Assumpcao, M.E.(2008). Kinetics of changes in plasma membrane related to apoptosis and necrosis in bovine sperm cells at different incubation times. Braz J. Vet. Res. Anim. Sci., 45: 398-404.

12. Hancock, J.L. (1951). A staining technique for the study of temperature-shock in semen. Nature, 167(2): 323-324.

13. Ikpeme, E.V., Okonko, L.E. and Udensi, O.U. (2016). Detrimental effects of chlorpyrifos and cypermethrin on reproductive physiology of male albino rats. Res. J. Environ. Toxicol., 10(1): 68.

14. Jeyendran, R.S., Vander Van, H.H., Perez-Pelaez, M., Crabo, B.G. and Zaneveld,L.J.D.(1984). Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen traits. J. Reprod. Fertile., 70(1): 219- 228.

15. Kamel, R.O. (2016). Interactions between mefloquine and the anti-fibrotic drug silymarin on Schistosoma mansoni infections in mice. J. Helminthol., 90(6): 760.

16. Laudat, A., Lecourbe, K., Guéchot, J. and Palluel, A.M. (2002). Values of sperm thiobarbituric acid-reactive substance in fertile men. Clin. Chim. Acta., 325(1-2): 113-115.

17. Luangpirom, A., Junaimuang, T., Kourchampa, W., Somsapt, P. and Sritragoo, O. (2013). Protective effect of pomegranate (Punica granatum Linn.) juice against hepatotoxicity and testicular toxicity induced by ethanol in mice. Biol. Anim. Husb., 5(1): 87-93.

18. Rizal ,M., Teoliher, M. R., Yusuf, T.L., Purwantara, B .and Situmorang, P. (2003). Media Kedokteran Hewan 1979.

19. Roostaei-Ali Mehr, M. and Parisoush, P. (2016). Effect of different levels of silymarin and caproic acid on storage of ram semen in liquid form. Reprod. Domest. Anim., 51(4): 569-574.

20. Salamon, S. and Maxwell ,W.M . (2000) . Storage of ram semen . Anim Reprod Sci, 62:77-111.

21. SAS. (2018). Statistical Analysis System, User's Guide. Statistical. Version 9.6th ed. SAS. Inst. Inc. Cary. N.C. USA.

22. Sun, J., Li, X. and Yu, X. (2017). Polysaccharides, total flavonoids content and antioxidant activities in different parts of Silybum marianum L. plants. In AIP Conference Proceedings (Vol. 1794, No. 1, p. 050004). AIP Publishing LLC.

23. Swanson,E.W.and Beardon,H.J. (1951). An eosin nigrosin stain differentiating live and dead bovine spermatozoa. J. Anim. Sci., 10(4): 981-987.

24. Verschoyle, R.D., Greaves, P., Patel, K., Marsden, D.A., Brown, K., Steward, W.P. and Gescher, A.J. (2008). Evaluation of the cancer chemopreventive efficacy of silibinin in genetic mouse models of prostate and intestinal carcinogenesis: relationship with silibinin levels. Eur. J. Cancer, 44(6): 898-906.

25. Wellington, K. and Jarvis, B. (2001). Silymarin: A review of its clinical properties in the management of hepatic disorders. Bio. Drugs, 15(7): 465-489.

ISSN 2072-3857

26. Zhao, F., Shi, D., Li, T., Li, L. and Zhao, M. (2015). Silymarin attenuates paraquat- induced lung injury via Nrf2-mediated pathway in vivo and in itro. Clin. Exp. Pharmacol. Physiol., 42(9): 988-998.

27. 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.

ISSN 2072-3857