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Impact of Cinnamon Ethanolic Extract on Sperm Cryopreservability and Fertility Potential in Buffalo Bulls

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Abstract

THE current research aimed at exploring the action of Cinnamon zeylanicum Bark ethanolic extract on sperm freezability as well as fertility in buffalo bulls. Cinnamon zeylanicum Bark ethanolic extract preparation, assay of its antioxidant status by DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging technique , HPLC (High Performance Liquid Chromatography) analysis to assess the concentrations of its flavonoids and phenolics. Semen samples were harvested from five healthy buffalo bulls. Tris extender with zero cinnamon was kept as a control. The other extenders were Tris containing ethanol extract of cinnamon at concentrations 4, 8, 16 mg/dl as experimental extender. The semen specimens were supplementary and neat sperm cell concentration 60 × 10^6/mL was fulfilled. Diluted semen was filled into 0.25 ml polyvinyl straws and exposed to the freezing process. Diluted semen was evaluated for sperm parameters after refrigeration and freeze-thaw process .The total phenolic content of Cinnamon Ethanolic extract (8256.62 µg/g extract, Cinnamic acid). The total flavonoids content of Cinnamon extract was 334435.79 µg/g extract, Daidzein and Hesperetin). The DPPH of Cinnamon Extract (91.9%) with 1000µg/ml and decreased with decreasing concentration. HPLC chromatograms of cinnamon extract with peaks of Daidzein, Cinnamic acid and Hesperetin. Spermatozoal measures were ameliorated post cooling and thawing especially in TC1. Improvement of conception rate was obvious in TC1 with elevated total antioxidants and decreased Malondialdehyde (MDA). The best concentration of ethanol extract of cinnamon that preserved high quality of spermatozoa, ameliorated post cooling and post freeze-thawing semen quality, and achieved the highest conception rate of buffalo semen extended in Tris dilluent was TC1 containing 4 mg/dL.

Keywords: Cinnamon Ethanolic extract, buffalo, semen, freezing, post-thaw characteristics.

Introduction

Infertility represents the major reproductive harms in the animal life, and roughly thirty percentage of this hazard is attributed to male effects [1].

Sperm freezing plays an important role in assisted reproductive knowledge in humans, and bovines. However, no matter what type of extender and storing conditions are applied, the management and preservation of sperm harmfully affect its value. As well, oxidative effects, which often occur during the freezing process, significantly reduce sperm cell vitality and reduce the spermatozoal ability to fertilize. This oxidative damage causes nucleic acid, lipid and protein compounds to be deteriorated. Recently, plant extracts were explored as a cost-effective natural base of enrichment to maintain and ameliorate spermatozoal survival throughout the preservation process [2].

According to Ros-Santaella and Eliana Pintus [2], the criteria impacting the preservation of semen involve the plant substances used, the extraction technique, the dose, the likely toxic effects, and the antibacterial properties.

Cinnamon herbs are important for tropical medicine and are derived from the family Lauraceae. Cinnamon principally have antioxidant essential oils and other ingredients, mainly cinnamaldehyde, cinnamic acid, and cinnamate [3] causative to the variable metabolic effects exerted by cinnamon [4].

Cinnamon bark has plenty of catechins and procyanidins [5]. Procyanidins ingredient possesses procyanidin A-type and B-type linkages [6-8] which have antioxidant activities [7,9].

The fractions of Cinnamon have multiple benefits including antioxidant and anti-inflammatory effects. Bull fertilization potential is considered one of the

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principal sanitary hazards and that Cinnamon has ameliorating impact on the antioxidant testicular status and sperm value, and its intake has great effects for men having asthenozoospermia [10].

Shalaby and Mouneir [11] observed that Cinnamon zeylanicum Bark orally may be of value for men suffering from diabetes with sexual decline as it improves fertility in male rats.

Hafez [12] recorded that, cinnamon extract orally administred at 0.5 gm/kg to experimentally diabetic rats for sixty five days improved the testes and seminal vesicles weights; enhanced the sperm characteristics with its amount as well as the testosterone levels and fertility . Mahdy [13] decided that daily oral intake of the watery extract of cinnamon for thirty days have positive effects on semen parameters of male mice. Khaki et al. [14] concluded that combination of cinnamon and ginger have marked positive effects on the sperm vitality , testosterone, FSH, LH and serum antioxidants with subsequent improved male reproductive function in rats.

Cinnamon oil and its extract have potent antimicrobial consequence .Therefore, in cinnamon extenders, antibiotics, which may have deleterious effects on sperm cells, might be excluded from these semen diluents [15-17].

Cinnamon oil has preservative function opposite to damages of male reproductive organs [18]. El-Azrak et al. [19] recorded that, cinnamon oil not only enhanced sheep sexual libido, but it also improved the physical characteristics of semen ejaculates.

However, very little literature is available concerning the preservability effect of cinnamon extract on frozen-thawed spermatozoa. So, from this point comes the importance of the current investigation to illustrate the impact of Cinnamon Bark on freezability and fertility of buffalo bulls.

Material and Methods

Processing of Cinnamon extract

To create a fine, dry powder, the cinnamon bark was placed in an Inalsa Mixer Grinder. The powder's weight as determined by an Ohaus electronic weighing balance. 50% of the powder was soaked for eight hours at room temperature with constant stirring, using a 250 ml Erlenmeyer flask and a ratio of 1 g of powder to 5 ml of ethanol. An aluminum foil-wrapped cotton plug was used to seal the flasks. Centrifugation of the mixture for 20 minutes at 3,500 ×g was followed by filtering with Whatmann No. 1. After being collected, the filtrate was placed under lower pressure in a rotary vacuum evaporator (Buchi) to obtain the extract; the filtrate was dried at 45ºC to produce a solid powder [17].

Antioxidant activity measured using the DPPH radical scavenging technique

Huang [20] reported that the free radical scavenging activity of cinnamon extract was determined using 1, 1-diphenyl-2-picryl hydrazyl (DPPH). In short, a 0.1 mM DPPH solution in ethanol was established. Three millilitres of the extract in ethanol were added to one millilitre of this solution (1 ml) at different concentrations (3.9, 7.8, 15.62, 31.25, 62.5, 125, 250, 500, 1000 µg/ml). When those extracts are used, which are soluble in ethanol, and the dilution procedure was used to determine each of their different concentrations, the mixture was well-mixed and allowed to stand at room temperature for half an hour. The experiment was run in triplicate using ascorbic acid as the standard reference chemical. Increased free radical activity can be shown by lower absorbance values of the reaction mixture. The following formula was used to calculate the percentage DPPH scavenging action at 517 nm using a UV-VIS milton roy spectrophotometer. By using a log dosage inhibition curve, the sample concentration needed to inhibit 50% of the DPPH free radical (IC 50 value) was determined. A reaction mixture's lower absorbance values are a reliable indicator of enhanced free radical activity. Using the following formula, the % DPPH scavenging effect was determined: % inhibition or DPPH scavenging action = $A_0 - A_1 / A_0 \times 100$. Where A_1 represented the absorbance while the test or standard sample was present, and A_0 represented the absorbance of the control response.

HPLC parameters

For the HPLC analysis, an Agilent 1260 series was used. Zorbax Eclipse plus C8 column (4.6 mm x 250 mm i.d., 5 µm) was used for the separation. Water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) were combined to form the mobile phase, which was flowing at a rate of 0.9 ml/min. The following was the sequential planning of the mobile phase using a linear gradient: 18–22 min (82% A); 22–24 min (82% A); 0–1 min (82% A); 1–11 min (75% A); 11–18 min (60% A); and 0 min (82% A). At 280 nm, the multi-wavelength detector was observed. For every sample solution, there was one injection volume of five microliters. With few adjustments, the column temperature was maintained at 40°C [21] when a test or reference sample was present

Buffalo bulls

Semen will be collected from five buffalo bulls (3.5 to 5 years old) housed at the Buffalo Semen Freezing Center. The buffalo bulls were kept under standard care and feeding arrangements. With a body weight between 600 and 800 kg, they are in good health and do not suffer from any common or genital ailments. During summer feeding, a few chosen bulls were kept cool and content by being sprayed with water at least three to four times a day; they were also protected from storms, housed in a comfortable

location with little moisture, fed during the cold months, and provided with an abundance of clean drinking water. The animals are fed: 6 kilogram of dry matter + 2 kg roughage plus 2.0 kg dried clover per animal per day in the summer, and 6 kg dry matter plus 2 kg roughage plus 28 kg clover per animal per day in the winter. 72–78 degrees Celsius is indicated by the temperature humidity indicator.

Semen Gathering and Initial Assessment

Using a created fake vagina, weekly semen samples were collected for eighteen weeks. Spermatozoal forward motility and abnormalities in the sperm were the main evaluation criteria for semen specimens. Semen samples exhibiting spermatozoal motility of 70% or above and a standard morphological sperm percentage were combined to provide sufficient semen to eradicate the individual bull variation. Before extending, keep the semen in the water bath at 37°C for 10 minutes prior to extension.

Sperm freezing procedures

Tris-citric acid-fructose (TCF), which was constructed along the Foote line, was the basic extender [22]. According to Ijaz et al. [23], the TCFYG diluent was created by dissolving 3.028 g Tris, 1.678 g Citric acid, and 2.000 g Fructose in 100 mL of bi-distilled water. Next, 20% egg yolk and 7% glycerol were added, along with a penicillin-streptomycin mixture as an antibiotic at 0.01 ml/ml of the extender. As a control, Tris extender with no cinnamon was retained. Tris, which contains ethanol extract of cinnamon at dosages of 4, 8, and 16 mg/dl as an experimental extender, is the other extender. After adding the semen samples, a neat sperm concentration of 60×10^6/mL was achieved. The diluted semen was progressively chilled (for around two hours). Semen was poured into 0.25 cc polyvinyl French straws, which were then immediately dipped in liquid nitrogen after being exposed to liquid nitrogen vapor for 10 minutes while positioned horizontally on a specific rack [24].

Assessment of Semen Quality criteria

Sperm motility: Each semen sample (5 µL) diluted with 2.9% Sodium Citrate dehydrate solution will have its progressive forward motility assessed. The drop was found on a cover slip-covered slide, and the warm stage microscope (X400) was used to study it. A minimum of two hundred spermatozoa were counted from various microscopic areas. A percentage of sperm motility was computed [25]. Ratio of living sperm and anomaly: A glass slide was used to create a smear from diluted semen, which was then stained with eosin nigrosin stain and investigated with a 400× under warm stage microscope. From 200 sperms analysed in five microscopical regions, the abnormal sperm percentage was calculated [26].

Integrity of the sperm membrane (Hypo-osmotic swelling test; HOST).

The adding 6.25 grams of sodium citrate dihydrate and 11.25 grams of fructose per liter of pure distilled water to make up the hypo-osmotic solution (125 mosmol/1). A solution containing 1 milliliter and 10µl semen was incubated for 60 minutes at 37°C. Following incubation, a warm stage microscope (400×) was used to measure the percentage of sperm that tested two hundred sperm in various microscopical fields and tested positive for HOST (with a swelled or curled tail) was determined [27].

Acrosome morphology:

The determining acrosome integrity, sperms were stained with Giemsa or Trypan blue. Trypan blue/Giemsa staining was used to evaluate semen samples with a few minor adjustments [28]. Trypan blue was used to stain at a practical concentration of 0.27%. Two smears were inspected for each droplet of semen, and one drop (5 µl) of diluted semen and one drop (5 µl) of Trypan blue were gently combined on a slide, and two smears were looked at for each semen droplet. Slides are placed upright and allowed to air dry before being placed in 10% buffered formol saline (9 gram Nacl, 6.5 gm Na_2HPO_4, 4 grams of NAH_2PO_4) for 30 minutes at 37ºC for fixing. Slides were placed in jars containing the Giemsa solution and allowed to sit for a full day. Giemsa stock solution (Sigma GS-500) was recently added to distilled water at a ratio of 14.3% (v/v) to create the Giemsa staining solution. The slides were cover-slipped, air-dried in an upright position, then gently cleaned once more in distilled water. The frontal portion of the sperm head without an acrosome is light purple, while intact acrosomes are purple.

Viability index

According to Milovanov [29], the post-thawing viability indices are equal to half of the sperm motility after thawing plus the total of the sperm forward motilities after the first, second, and third hours after thawing.

Oxidant/antioxidant parameter determination

After semen was collected, a cooling centrifuge (Sigma 3-18KS, Germany) was used to centrifuge the material at 2773 ×g for 5 minutes at 4°C. After being separated, the seminal plasma was kept at -80°C. Using test kits made by Biodiagnostic Co., Egypt, the amount of total antioxidant capacity (TAC) in the Seminal plasma was measured using test kits made by Biodiagnostic Co., Egypt, in

accordance with the method of Koracevic et al. [30], and lipid peroxidation components as malondialdehyde (MDA) in accordance with the method of Satoh [31]. The Double Beam UV/Visible Spectrophotometer, Model T80, UK, was used for all measurements. Malondialdehyde (MDA) using test kits made by Biodiagnostic Co., Egypt, in accordance with Satoh's procedure [31]. The Double Beam UV/Visible Spectrophotometer, Model T80, UK, was used for all measurements.

Field conception rate (CR)

The buffalo animals (320) will be artificially inseminated with the frozen post- TCFY as a control and the other experimental extenders. Conception rate was implemented by rectal palpation at two months from the artificial insemination. The artificially inseminated animals had been administered through the cooperation with Beni-Suef Governorate. The insemination of animals will be implemented using the insemination gun and semen will be pushed inside the uterus. The CR was estimated following the equation:

CR= [(number of pregnant animals) / (total number of inseminated animals)]×100

Statistical analysis

Statistical analysis data were computed using the SPSS [32] computerized program v. 14.0 to calculate the analysis of variance (ANOVA) for the different criteria between control and experimental groups. Major difference among the mean values was designed by means of Duncan test at $P<0.05$.

Results

Table (1) showed the total phenolic content of Cinnamon Ethanolic extract (8256.62 µg/g extract, Cinnamic acid). Table (2) exhibited the total flavonoids content of cinnamon extract (334435.79 µg/g extract, Daidzein and Hesperetin).

Figure (1A,B) revealed the DPPH of Cinnamon Extract (91.9%) with 1000µg/ml and decreased with decreasing concentration and the standard. Figure (2) demonstrated HPLC chromatograms of cinnamon extract with peaks of Daidzein, Cinnamic acid and Hesperetin. Table (3) revealed that, cinnamon addition to the preservative diluent improved sperm value as evidenced with elevated after cooling spermatozoal motility and the alive sperms, lower sperm abnormalities and sperm premature capacitation. However, sperm cell membrane and acrosome integrities showed no marked variations in all treatments involving the control.

Table (4) revealed that, cinnamon enrichment to the preservative diluent inTC1 exhibited enhanced after- thaw spermatozoal motility, alive sperm per cent ,sperm abnormality per cent , sperm membrane and acrosome status with values nearly similar to the control. Viability index which is considered as indicator of sperm resistance showed significant elevation in TC1 in comparison to other used cinnamon concentrations and the control with significant decrease of per cent of premature capacitation.

Table (5) exhibited eminent lowering in post-thawing sperm motility with the advance of time in all the extenders applied .The spermatozoa forward motility was greater in TC1 in each of the post-thawing times relative to the control and other used cinnamon concentrations.

Table (6) showed significant elevation of the total antioxidant fractions (TAC) with the lower decrease of malondialdehyde (MDA) in TC1 relative to the control and other used cinnamon concentrations.

Discussion

Many agents affecting the sperm cells freezability have been documented mostly osmotic hazard, ice crystals production, the deleterious effect of cryopreservatives and the bull variation [33,34]. Amongst different agents, oxidative damages have been postulated to reduce the fertilizing capacity and function of preserved sperm cells [35-37]. Sperm oxidative deterioration takes place due to imbalanced ratios of oxygen free anions release and the antioxidant potential [38]. Over release of oxygen free radicals are hazardous to the spermatozoa [39]. Reduced levels of these radicals are beneficial for induction of spermatozoal capacitation, a metabolic function that is essential for the spermatozoa fertilizing capacity [40]. Peroxidation of sperm membrane fatty acids and DNA damage are attributed to spermatozoa oxidative stress [41-43], reduced mitochondrial membrane functions [44,45], enzymes inactivation related to sperm motility [46] and Loss of sperm motility due to damage of sperm cell membrane [42,43].

Various antioxidant enzymes are existing inside the sperm cells and as ingredients of the seminal plasma expressed by the antioxidant enzymes (GSH, CAT and SOD). Their antioxidant potential is not enough and stepwise reduced on prolongation of the preservation protocol, therefore, antioxidant additives ought to be supplementary in the semen diluent [47].

There is an important worldwide focus on the vital synergistic action of herbal additives and their different ingredients in comparison to their single metabolically functional fractions [48]. Sperm freezing induces spermatozoal hazard with consequent reduction in sperm value [49], despite it is indispensable for conserving the superior genetic

properties of local buffalo breeds. Sperm preservation is accompanied with cryoinjury due to excessive release of free oxygen anions [50]. Therefore, the natural supplement to the semen extender enhances the antioxidant action with consequent improving the fertilizing potential of frozen-thawed spermatozoa [51]. Undesirable premature capacitation of sperm used in AI is a main agent of bull subfertility and a potential indicator for detection of low fertile males [52].

The current investigation exhibited that, cinnamon enrichment in the preservative extender inTC1 improved the post- thaw sperm motility, alive sperms, sperm abnormalities, viability index which is considered as indicator of sperm resistance with significant decrease of per cent of premature capacity potential. These findings could be attributed to the potent antioxidant capacity, metal chelating and free radical scavenging property of phenolic compounds [53-55] and oil [56-58] compounds derived from Cinnamon zeylanicum . These results could be confirmed by the significant elevation of the total antioxidant compounds (TAC) accompanied with the lower decrease of malondialdehyde (MDA) in TC1 and are compatible to the the findings demonstrated by Jayaprakasha et al. [53,58].

Cinnamomum cassia consists of a broad range of compounds such as cinnamaldehyde, cinnamic acid, cinnamyl alcohol, cinnamyl acetate, eugenol and to a large extent of coumarin [59]. Great Performance Liquid Chromatography (HPLC) however is better technique to separate the more polar compounds present in Cinnamomum cassia which dissolves in water. In the current research the total phenolic content of Ethanolic cinnamon extract is (8256.62 µg/g extract, Cinnamic acid) and the total flavonoids content is (334435.79 µg/g extract, Daidzein and Hesperetin).

In this investigation, the high total phenolic content in cinnamon extract is confirmed by the results of Shan et al. [60] and the high total flavonoids is in agreement with Ramadan et al. [61]. The potential antioxidant character of cinnamon extract is referred to its high phenolic and flavonoids content [61,62]. In this regard, Shahid et al. [63] recorded that, Cinnamic acid derivatives in cinnamon are implicated in its strong antioxidant activity. Raeeszadeh et al. [64] documented that the metabolic effects of broccoli extract containing Cinnamic acid improved spermatogenesis and improved sperm function, forward motility, and decreased DNA damage of rat sperm with ameliorating effect on the increase of antioxidant enzymes concentration among the sperm freeze-thaw procedures. Toutou et al. [65] recorded the biological activities of propolis extract rich in Cinnamic acid including antibacterial, antifungal, antioxidant, anti-inflammatory effects, and sperm cells cryopreservation effect involving sperm cells cryopreservation preservation in rams.

Li et al. [66] recommended that, daidzein spplementation could enhance sow reproductive capacity via alteration of serum hormones, increasing anti-oxidant potential and up-regulating critical functional genes in the placenta. Daidzein at the dose of 5 mg/kg ration markedly increased serum testosterone intensities, high testis gain and improved spermatozoal value [67].

Cinnamon extract is rich in Hesperetin. This bioflavonoid compound can increase the quality of human sperm and also protect human sperm against reactive oxygen species, LPO, and apoptosis among the freeze-thaw protocol [68]. Addition of 1 µM Hesperetin in the bull semen extenders can ameliorate the value of the bull sperms after thawing through its effect as an excellent antioxidant. It can also reduce oxidative damage and significantly preserve spermatozoa membranes, as indicated by the evaluation of the sperm characteristics. Therefore, Hesperetin can be applied as semen additive due to its positive actions on the frozen-thawed semen [69].

In this investigation, Cinnamon Extract as a free radical scavenger (91.9%) with 1000µg/ml and decreased with decreasing concentration (DPPH assay). The improvement in fertility potential caused by Cinnamon zeylanicum extract is related to its reported antioxidant property [70,71]. Yang et al. [72] confirmed that the herbal antioxidant compounds can preserve DNA and other structures from cell injuiry created by oxidative stress and can ameliorate sperm quality and enhance the reproductive potency in human.

Excessive levels of deteriorate sperm metabolic functions among the freezing protocol [73]. It was also documented that flavonoids, as strong antioxidants, could lower the oxygen free radicals levels [74]. Phenolic compounds are the principal antioxidants widely present in plants, and capable of scavenging the free radicals directly so that their antioxidative power is more than that of synthetic antioxidants and of vitamins C and E [75,76]. This character of phenolic compounds can be related to their potentiality to donate hydrogen ions (H+) [77]. Furthermore, many research categorized Cinnamomum zeylanicum phenyl propanoids as free radical scavengers. researchs show also that Cinnamomum zeylanicum is a good source of polyphenolic compounds, predicting antioxidative, metal chelating and free radical scavenging activities for these ingredients [78,79].

Lipid peroxidation results in inferior sperm motility and deteriorated membrane flexibility by damage of the lipid components of the sperm cell membranes.

Cinnamon extract in this study revealed strong antiradical scavenging activities. A strong correlation between the total phenolics and antioxidant activity is documented confirming the fact that, the total phenolic is responsible for free radical scavenging activity [80].

Cinnamomum ethanolic extracts only and with trehalose enhanced the spermatozoa quality and could be applied for freezing of goat epididymal spermatozoa. [81].

Varalakshmi et al. [82] documented the antioxidant effect of cinnamon on sperm DNA.

Conclusion

The best concentration of ethanol extract of cinnamon that preserved high quality of spermatozoa, ameliorated post cooling and post freeze-thawing semen quality, and achieved the highest conception rate of buffalo semen extended in Tris dilluent was TC1 containing 4 mg/dL.

Aacknowledgments

Not applicable.

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study follows the ethics guidelines of the Faculty of Veterinary Medicine, Benha University, Egypt (ethics approval number; 49/11/2023).

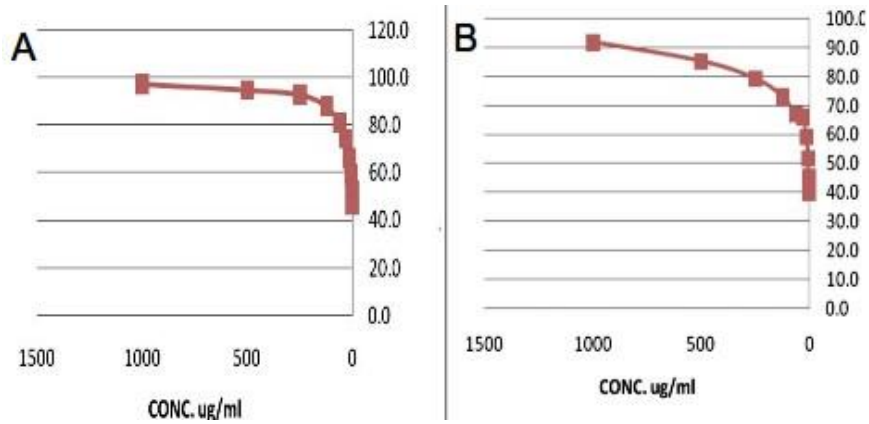

Fig. 1. DPPH scavenging % of ascorbic acid in standard (A) and Sample (B).

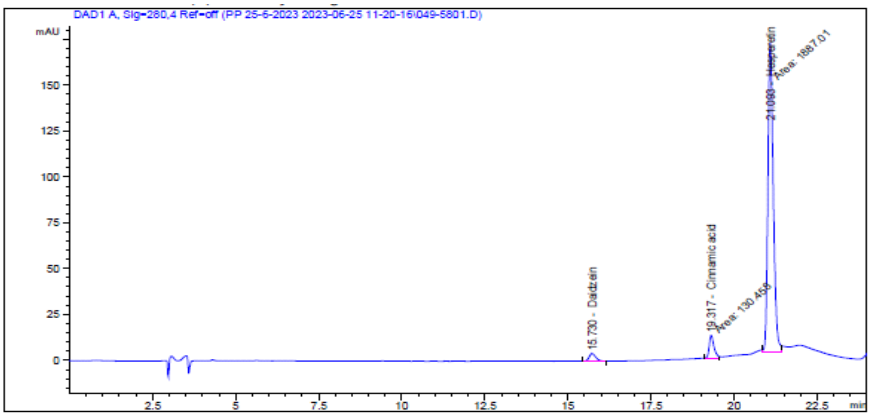

Fig. 2. HPLC Chromatograms of standard metabolites revealing signal from diode array detector at 280 nm wavelength . Peak: Daidzein- Cinnamic acid- Hesperetin.

TABLE 1. HPLC analysis of polyphenolic ingredients concentration in cinnamon extract

Compound	Concentration of compounds (µg / g extract) in cinnamon
Gallic acid	0.00
Chlorogenic acid	0.00
Methyl gallate	0.00
Syringic acid	0.00
Pyrocatechol	0.00
Ellagic acid	0.00
Coumaric acid	0.00
Vanillin	0.00
Ferulic acid	0.00
Rosmarenic acid	0.00
Cinnamic acid	8256.6
Total	8256.62

TABLE 2. HPLC analysis of flavonoid compounds concentration in cinnamon extract

Compound	Concentration of compounds (µg / g extract) in cinnamon
Catechin	0.00
Caffeic acid	0.00
Rutin	0.00
Naringenin	0.00
Daidzein	10877.01
Querectin	0.00
Kaempferol	0.00
Hesperetin	323558.78
Total	334435.79

TABLE 3. Impact of Tris extender augmented with cinnamon extract on the post-cooled sperm characteristics of diluted buffalo bull semen (Mean±SE).

Diluent	Motility %	Alive %	Abnormality%	HOST	Acrosome %	Capacitation%
Control(TCFYG)	68.33±1.66^{ab}	73.66±1.33^{a}	19.66±0.33^{b}	55.00±2.88^{a}	92.00±2.00^{a}	35.00±2.88^{b}
TC1(4 mg /dL)	81.66±1.66^{c}	86.00±1.00^{c}	15.00±1.15^{a}	58.36±0.36^{a}	89.00±0.57^{a}	26.00±1.00^{a}
TC2(8 mg /dL)	75.00±2.88^{bc}	80.33±0.33^{b}	25.66±0.66^{c}	57.60±0.60a^{a}	89.33±2.33^{a}	33.00±3.00^{ab}
TC3(16 mg /dL)	63.33±3.33^{a}	79.00±2.08^{b}	30.33±0.33^{d}	56.66±0.88^{a}	87.66±2.66^{a}	42.00±2.00^{c}
pP-value	0.004	.001	.000	.493	.537	.0001

Means bearing different superscripts between different extenders and differ at 5% levels of probability. Control Tris-citrate-fructose-egg yolk-glycerol (TCFYG); TC1(TrisC1); TC2(TrisC2); TC3(TrisC3)

TABLE 4. Impact of Tris diluent augmented with cinnamon extract on the post-thawed sperm characteristics of diluted buffalo bull semen (Mean±SE).

Diluent	Motility	Alive	Abnormality	Host	Acrosome	Viability index	capacitation
Control (Tris extender)	35.00±5.00^{b}	78.33±1.66^{c}	24.33±0.66^{b}	50.66±0.66^{b}	89.66±2.60^{b}	68.60±1.14^{c}	59.00±3.78^{ab}
TC1 (4 mg/dL)	36.66±3.33^{b}	81.66±1.66^{c}	22.66±2.66^{b}	51.00±2.08^{b}	86.33±1.33^{ab}	81.99±1.15^{d}	34.33±2.33^{a}
TC2 (8 mg/dL)	28.33±1.66^{b}	41.66±1.66^{b}	24.66±0.33^{b}	48.33±1.66^{b}	81.00±1.00^{a}	50.83±0.83^{b}	49.66±2.60^{ab}
TC3 (16 mg/dL)	11.66±1.66^{a}	34.00±1.00^{a}	29.33±0.66^{a}	36.66±3.33^{a}	79.00±4.00^{a}	14.00±0.01^{a}	61.00±1.00^{c}
P-value	0.002	0.0001	0.05	0.005	.060	0.0001	0.003

Means different superscripts (a, b, c, d) within column differ at P<0.05. Control Tris-citrate-fructose-egg yolk-glycerol (TCFYG).

TABLE 5. Effect of Tris extender enriched with cinnamon extract on post-thaw total motility % of frozen-thawed bull spermatozoa.

Hours	Control (Tris extender)	TC1 (4 mg /dL)	TC2 (8 mg /dL)	TC3 (16 mg /dL)	P-value
0	35.00±5.00[b]	36.66±3.33[b]	28.33±1.66[b]	11.66±1.66[a]	0.001
1	26.66±1.66[c]	30.66±0.66[c]	18.33±1.66[b]	10.00±0.01[a]	.0001
2	18.33±1.66[b]	21.66±1.66[b]	11.66±1.66[a]	7.50±2.50[a]	.004
3	8.33±1.66	13.33±1.66	8.33±1.66	6.50±1.50	0.105

Means different superscripts (a, b, c) within rows differ at P<0.05. Control Tris-citrate-fructose-egg yolk-glycerol (TCFYG).

TABLE 6. Effect of Tris extender enriched with cinnamon extract on Antioxidant concentration-TAC (mM) and MDA concentration (µM).

Diluent	TAC	MDA
Control (Tris extender)	0.20±0.06[a]	7.11±0.05[b]
TC1 (4 mg /dL)	0.39±0.05[b]	4.34±0.24[a]
TC2 (8 mg /dL)	0.20±0.01[a]	7.87±0.18[b]
TC3 (16 mg /dL)	0.08±0.01[a]	9.63±0.57[c]
P-value	0.014	.0001

Means with different superscripts (a, b, c) within column differ at P<0.05. Control : Tris-citrate-fructose-egg yolk-glycerol (TCFYG).

TABLE 7. Effect of Tris extender enriched with cinnamon extract on pregnancy rate in buffalo.

Treatment	No of inseminated females	No of conceived females	In vivo fertility rate (CR, %)
Control (Tris extender)	100	52	52%
TC1 (4 mg /dL)	90	60	66.7%
TC2 (8 mg /dL)	80	36	45%
TC3 (16 mg /dL)	90	5	5.6%

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كفاءة حفظ الحيوانات المنوية والخصوبة لطلائق الجاموس باستخدام المستخلص الايثانولى للقرفة

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الملخص

استهدفت الدراسة الحالية تقييم كفاءة حفظ الحيوانات المنوية والخصوبة لطلائق الجاموس باستخدام المستخلص الايثانولى للقرفة اثناء التبريد والتجميد.تم قياس مستوى الأنتى أوكسيدانت والتحليل الكروماتوجرفى لقياس تركيزات الفينولات والفلافونويد فى المستخلص الايثانولى للقرفة .تم تخفيف السائل المنوى باستخدام مخفف التريس المحتوى على تركيزات مختلفة المستخلص الايثانولى للقرفة (4, 8, 16 mg/dl)واحتوت أنابيب الكنترول على (0)من المستخلص الايثانولى للقرفة. وقد تم تبريد السائل المنوى فى قصيبات وترك 4 ساعات عند درجة5 مئوية ثم تعريضه 10 دقائق وغمره فى النيتروجين السائل للتجميد .تم التقييم أظهرت النتائج تحسن فى صفات السائل المنوى بعد التبريد والتجميد وكانت أحسن النتائج فى التركيز الأول (4 mg/dL).

الكلمات الدالة:المستخلص الايثانولى للقرفة، الجاموس، السائل المنوى، تجميد، خصائص السائل المنوى.