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Research Article

Vital Role of Garlic Extract Additive in Cryopreservation and Freezing of Buffalo Bulls Sperm

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Abstract | The preservation of sperm is critical to the effectiveness of assisted reproductive technologies in bovine. However, Plant extracts has lately appeared as a low-cost, it considers a natural source of chemicals for preserving, also enhancing activity of sperm during different period storage. Current paper gives an apprise on the usage of garlic (*Allium sativum*) extract as an alternative additive for sperm preservation in buffalo bulls at animal production research institute, Sakha, Kafr El-Sheikh Governorate, Egypt. The experimental protocol was designed as four groups (Control, G1, G2 and G3). Buffalo-bulls' semen were exposed to different concentrations of garlic extract (0, 3, 6 and 9%, respectively) to evaluate the semen quality parameters after cooling and freezing process. Data obtained showed that, progressive motility, live spermatozoa and acrosome integrity % were higher ($P < 0.05$) in G1, G2 and G3 with reduced mortality percentage than control group after cooling process. In addition, a significantly increased ($P < 0.05$) of progressive motility, acrosome integrity and alive spermatozoa percentage in groups treated with different concentrations of garlic extract than control group. While both the abnormality and chromatin value were significantly lower ($P < 0.05$) in the groups treated with different concentrations of garlic extract than control group. Activity of glutathione peroxidase, activity of superoxide dismutase and alpha glucosidaseLysozyme elevated ($P < 0.05$) in G1, G2, G3 treated groups than control group. Different concentrations of garlic extract reduced the malondialdehyde than control. Finally, it can be concluded that garlic extract additive to extender would be useful to improve semen quality and antioxidant enzymes of buffalo-bulls spermatozoa after different processes (cooling and freezing) at variable time.

Keywords | Plant extract, Preservation, Spermatozoa, Garlic Extender, Antioxidant enzymes.

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INTRODUCTION

There is an urgent need to enhance the efficiency and sustainability of raising animals for food in face of an ever-growing global population; yet, improving the fertility of livestock, throughout the world is critical for solving this challenge (Medeiros et al., 2002). Improved the understanding of reproductive technology mechanisms and problems is critical for increasing the cattle industry's viability. Artificial Insemination (AI) is an important reproductive technique that has been used to promote animal husbandry; also, effective semen cryopreservation and

freezing improves the efficiency and success rate of AI (Walters et al., 2009). Sperm cryopreservation treatments are not always effective since a high proportion of sperm experience physiological damage, resulting in sperm fertility loss after freezing and thawing (Ugur et al., 2019).

The spermatozoa are subjected to osmotic, thermal, and mechanical stressors during cryopreservation, which can be seen throughout the dilution, cooling, equilibration, freezing, and thawing stages (Andrabi et al., 2008). Cold storage of sperm is used to slow metabolism and keep sperm viable for a longer length of time (Perumal et al., 2013).

Semen handling and preservation have a detrimental impact on sperm quality regardless of the extender or storage conditions utilized. Furthermore, oxidative stress, which frequently occurs during sperm storage, greatly lowers sperm function and impairs sperm by causing oxidative damage to proteins, lipids, and nucleic acids, fertilization is possible (Tariq et al., 2015). Plant extracts have recently emerged as a low-cost, natural source of compounds that can help maintain and improve sperm function during storage (Pintus and Ros-Santaella, 2021). Because of enhanced lipid peroxidation, mammalian spermatozoa may be more vulnerable to oxidative stress (Aitken and Drevet, 2020). Although bovine sperm possesses a natural defense system against oxidative stress, when subjected to cryopreservation-induced stress, it is deemed insufficient (Bansal and Bilaspuri, 2010). The extender is a vital component in the cryopreservation process, as it must have an acceptable pH and a buffering capacity, as well as sufficient osmolality and the ability to protect spermatozoa from cryogenic damage (Bustani and Baiee, 2020). To prevent oxidative damage during freeze-thawing of bull and buffalo spermatozoa, semen extender should be reinforced with appropriate components (Patel et al., 2016).

Garlic's exceptional biological qualities include oxidative radical scavenging capabilities and usefulness as an adjuvant in the treatment of number of ailments, as proven by various research studies undertaken in recent decades (Hayat et al., 2018; Alsenosy and Abd El-Aziz, 2019). Garlic has a variety of useful components, including organosulfur chemicals, saponins, and phenolic compounds (Wang et al., 2014).

In this regard, it is critical to enhance sperm preservation techniques by extending sperm life span without affecting sperm function. This might result in higher fertilization rates in AI results. Most plant species have significant levels of antioxidants, which operate as scavengers of reactive oxygen species (ROS) to minimize the negative effects of oxidative stress on sperm function Furthermore, these natural chemicals have been shown to improve the activity of a range of antioxidant enzymes and are antibacterial. The purpose of this study is to examine the efficacy of the usage of different concentrations of garlic extract as a natural factor for sperm preservation in buffalo bulls, as well as the effects on sperm function. Both the proper extraction methods and the most appropriate concentrations for buffalo bulls were also investigated.

MATERIALS AND METHODS

Experimental design

The current study was carried out at the international livestock management training center of the animal production research institution, Sakha Station, Kafr El-Sheikh Governorate, Egypt. All adult buffalo bulls have been kept under the same conditions, feeding and watering throughout the study period.

Preparation of aqueous garlic extract and extenders

A fruit juice extraction machine was used to crush 20 g of garlic in 100 ml of distilled water. The extract mixture was filtered through a cheese cloth as a thin mesh, the clear supernatant was immediately collected and stored in pyrogenic-free bottles until use after centrifugation at 200 g for 10 minutes. 20 mL chicken egg yolk, 3.025 g Tris (hydroxyl methyl amino methane), 1.675 g citric acid, 0.75 g glucose, 7 mL glycerol, 0.25 gm lincomycin, 0.005 g streptomycin, and 100 mL distilled water.

Semen was collected weekly from buffalo bulls (n=6) using an artificial vagina. Ejaculates with high motility (75 percent of mass motility) were collected. The sample was immediately transported to laboratory then placed in water bath with (37-38 ˚C) to perform analysis. Different concentrations (0, 3, 6, and 9 %) of garlic extract were added to extenders. Then, diluted semen (10×10^6 motile spermatozoa/ml) separated into 2 groups corresponding to 2 phases. Phase I (cooling at 5 ˚C for 24, 48 and 72 h; then, semen characteristics progressive motility %, live spermatozoa %, abnormality % and acrosome integrity performed. Phase II (freezing) begin after 4h (equilibration stage), then, examined of individual motility in semen then, positioned to grasses (0.25 ml French straws). Straws were apprehended for ten minutes at surface of nitrogen liquid (vapor, about -120˚C) previously actuality absorbed formerly kept in liquid nitrogen (-196˚C). Additionally, straws thawed for 30 sec. at 37˚C. In contrast the parameters studied were subjective semen characteristics (progressive motility %, live spermatozoa %, abnormality %, acrosome integrity and chromatin integrity).

Table 1: Identified components by GC-MS of *garlic* extract.

No	Compound Identified	M.wt	Formula	RT (min)	Area (%)
1	Allylthiol	74.14	$C3H_6S$	6.381	97.2
2	Thymoquinone	164.20	$C_{10}H_{12}O_2$	7.056	77.4
3	Methane methyl thiosulfinate	110.20	$C_2H_6OS_2$	7.443	90.6
4	Allyl methyl sulfide	88.17	C_4H_8S	8.338	26.4
5	Butyl 1-propenyl sulfide	130.25	$C_7H_{14}S$	9.449	76.9
6	Diallyl sulfide	114.21	$C_6H_{10}S$	10.556	58.3
7	Methyl allyl thioacetate	90.15	C_3H_6OS	12.808	95.8
8	Methylthiocyclopentane	114.21	$C_6H_{10}S$	14.992	33.3
9	Allyl methyl disulfide	120.2	$C_4H_8S_2$	20.241	9.91
10	Dithymoquinone	328.4	$C_{20}H_{24}O_4$	21.662	55.1
11	Methyl 1-propenyl disulfide	120.2	$C_4H_8S_2$	22.153	30.9
12	Diallyl tetrasulfide	210.4	$C_6H_{10}S_4$	22.713	24.9
13	Cyclopentathiazole	125.19	C_6H_7NS	25.399	16.2
14	2-Ethyl-1,3-dithiane	148.3	$C_6H_{12}S_2$	25.728	21.5
15	Thiazole	85.12	C_3H_3NS	25.809	67
16	2-Hexanol	102.17	$C_6H_{14}O$	28.13	22.6
17	2-Methylfuran	86.13	$C_5H_{10}O$	28.28	12.6
18	Sulfur dioxide	64.07	SO_2	37.082	6.52

(M.wt.) Molecular weight, (RT) Retention time.

Table 2: Effect of garlic extract additive to extender on antioxidant enzymes parameters after post-thaw of buffalo bull spermatozoa (n = 6 bulls; six replicates).

	LSS levels	GPX (U /ml)	SOD (U /ml)	MDA	Alpha glucosidase
Phase I	0	56.3 ± 0.56^{d}	30.4 ± 0.81^{aB}	60.9 ± 0.09^{cB}	5.2 ± 0.42^{aB}
	3	61.1 ± 0.32^{c}	39.1 ± 0.78^{bB}	53.3 ± 1.18^{bB}	5.72 ± 0.21^{bB}
	6	66.4 ± 0.24^{a}	44.1 ± 1.27^{a}	45.1 ± 0.02^{bB}	6.21 ± 0.34^{cB}
	9	63.4 ± 0.62^{b}	40.6 ± 1.65^{b}	50.7 ± 0.09^{aB}	5.84 ± 0.57^{cB}

Data were expressed as mean ±SE. Different superscript letters in the same column are significantly different (ANOVA, $P < 0.05$). Activity of glutathione peroxidase (GPx), activity of superoxide dismutase (SOD) and malondialdehyde (MDA)

Gas Chromatography-Mass Spectrometer (GC-MS) of garlic extract

The composition of the concentrated garlic extract produced was assessed and determined using the Gas Chromatography-Mass Spectrometer Method (GC-MS) (Table 2 and Table 1). An HP-5MS 5 percent phenyl methyl silox-bonded phase column (30 m in length, 250 m in diameter, and 0.25 m in film thickness) and a GC-MS Agilent 7890A Series GC system interfaced to a 5975C inert MSD with Triple-Axis detector and a 7697A auto sampler (Agilent Technologies, USA) were used for the analysis (Agilent Technologies, USA). Whole duration of the GC run was 62 minutes, at a flow rate of 1.22 ml/min and a constant pressure of 22.231 psi using helium as the carrier gas. GC-MS Spectrometer Method (GC-MS) was used to assess and determine the composition of the concentrated garlic extract produced (Table 2 and Table 1). An HP-5MS 5 percent phenyl methyl silox-bonded phase column (30 m in length, 250 m in diameter, and 0.25 m in film thickness) and a GC-MS Agilent 7890A Series GC system interfaced to a 5975C inert MSD with Triple-Axis detector and a 7697A autosampler (Agilent Technologies, USA) were used for the analysis (Agilent Technologies, USA). The GC run lasted 62 minutes, and the carrier gas was helium at a flow rate of 1.22 ml/min with a constant pressure of 22.231 psi (Radwan, 2022).

Determination of Semen parameters

Progressive sperm motility was estimated according to (Melrose and Laing, 1970), live sperm and abnormal sperm was evaluated according to (Salisbury et al.,1978). Acrosome integrity detected with Giemsa stain procedure which was labeled by (Watson, 1975). Briefly, one drop of diluted semen was smeared on warmed slide which dehydrated in existing warm air, moreover, smears were fixed with engagement in 10% buffered saline of formal for 15

min, then washed consecutively in tap water. Regarding to smears were air-dried then absorbed in buffered Giemsa solution in a Coplin jar for 90 min, after which they cleaned temporarily in refined water and dried, then examined under light microscope at magnification 100x by using oil immersion lens. Calculated percentage of normal acrosome (at least 200 spermatozoa were aimlessly nominated from at least four microscopic fields). Finally, acrosome considered normal when stain was clearly and consistently dispersed ended spermatozoa anterior to equatorial segment. Furthermore, for determined chromatin after air drying of smears, ten were fixed at a concentration of fresh 96 percent ethanol-acetone (1:1) at 4°C for 30 minutes, then hydrolyzed in 0.1 NHCl at 4°C for 5 minutes continuously, slides washed thrice in distilled water for 2 minutes, and finally stained with 0.05 percent Toluidine blue (TB) in 50 percent McIlvaine buffer (pH=3.5) for 10 minutes at room temperature. The metachromatic staining of sperm heads was measured using light microscopy at 1000 magnification using a stock solution of 1 percent TB in distilled water maintained at 48C to determine the chromatin quality of spermatozoa (Erenpreisa et al., 2003).

Antioxidant enzymes

The activity of superoxide dismutase (T-SOD), glutathione peroxidase (GPx), and malondialdehyde (MDA) in seminal plasma was tested using a commercially available kit and the manufacturer's instructions (Bio diagnostic, Cairo, Egypt). The spermatozoa were homogenized in phosphate buffered saline PBS solution using an ultrasonic disintegrator before being centrifuged at 1000 g for 10 minutes at 4 °C. The supernatant was used for the analysis. One unit of T-SOD activity was defined as the amount of sample protein capable of inhibiting NBT reduction by 50% of maximum inhibition, whereas one unit of GSH-Px activity was defined as the amount of enzyme necessary to oxidize 1 mol/NADPH/min at pH 7.0 at 25 °C. The thiobarbituric acid approach was used to determine MDA concentrations, which is based on the interaction of MDA with thiobarbituric acid to form a pink chromogen (Fan et al., 2011) and MDA concentrations were expressed as nmol/mg protein.

Assay of alpha glucosidase in seminal plasma

Seminal plasma alpha-glucosidase were determined with by glucose oxidase method by a commercially available kit EpiScreen Plus-α-glucosidase assay (FertiPro, Beernem, Belgium) giving to the instructions assumed with the manufacturer, whereby absorbance stayed quantified at 505 nm wave length and 1 unit of activity was defined as per ml sample producing 1 µmol of D-glucose /min at 37 °C, pH 6.8 (Mahmoud et al., 1998).

Data analysis

The obtained data were examined for normality and homogeneity of distribution using Levene's test. With 95 percent confidence, one-way ANOVA was conducted in the SPSS software to investigate the differences between LSSD levels ($P < 0.05$). When significant differences ($P < 0.05$) between data were detected, Tukey's HSD post-test was utilised. All data was presented as mean ± (standard mean of error) SEM except for the antibacterial and antifungal data, which were shown as mean ± (standard deviation) SD.

RESULTS

Evaluation of Semen Quality Parameters after cooling period

The effects of garlic extract additive to extender on progressive motility quality in spermatozoa of buffalo bull at different time are illustrated (Figure 1). The results showed that addition of different concentrations of garlic extract (G1, G2, and G3) significantly enhanced the levels of progressive motility compared with the control group (Figure 1). Live spermatozoa declared significant decrease in control group at different period than garlic extract additive concentration (Figure 2). Moreover, Figure 3 declared that, garlic extract-treated groups resulted in lower level of sperm abnormality than that recoded in control group. Increased in the average value of acrosome integrity (92.25 ±1.03%) with G2 at zero times and the lowest (38.90 ±0.31%) after 72h at control group (Figure 4).

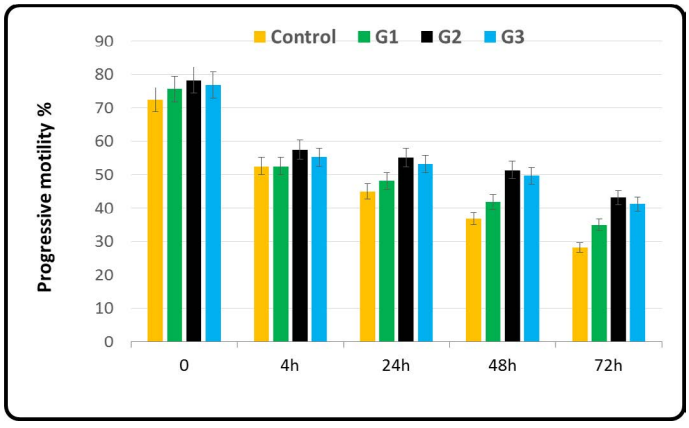

Figure 1: Effect of garlic extract additive to extender after cooling at 5 °C for 24, 48 and 72 h on progressive motility % quality of buffalo bull spermatozoa (n = 6 bulls; six replicates). (Control: 0; G1: 3; G2: 6 and G3: 9% of concentrations).

Evaluation of Semen Quality Parameters after freezing processes

The effects of thawing rates on frozen buffalo bull spermatozoa post-thaw quality measurements examined in this research were presented in Figure 5. The elevated av-

erage value of progressive motility and live spermatozoa were recorded in G2 and lowest in control group being 38.13 ±0.98, 65.63±0.91 and 27.62 ±1.27, 52.78±0.94 % for both progressive motility and live spermatozoa, respectively. The minimum average value of acrosome integrity (50.00±0.74%) was detected in control group. It increased gradually in G1 group (53.50±0.70%), through G3 (63.41±0.71%) and reached maximum value (68.63±0.64%) in G2 group. However, data revealed significant decreases in abnormality and chromatin percentage in G2 than other groups.

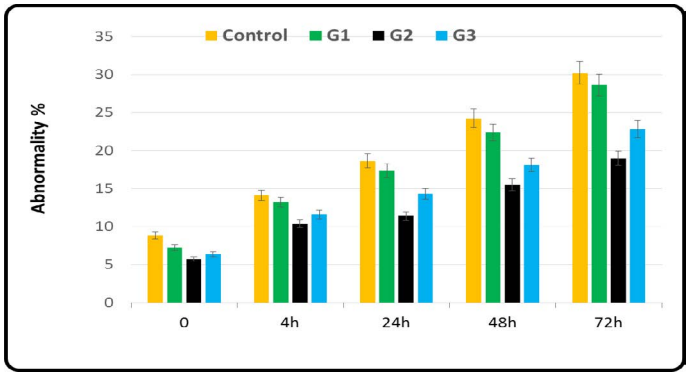

Figure 2: Effect of garlic extract additive to extender after cooling at 5 °C for 24, 48 and 72 h on abnormality % quality of buffalo bull spermatozoa (n = 6 bulls; six replicates). (Control: 0; G1: 3; G2: 6 and G3: 9% of concentrations).

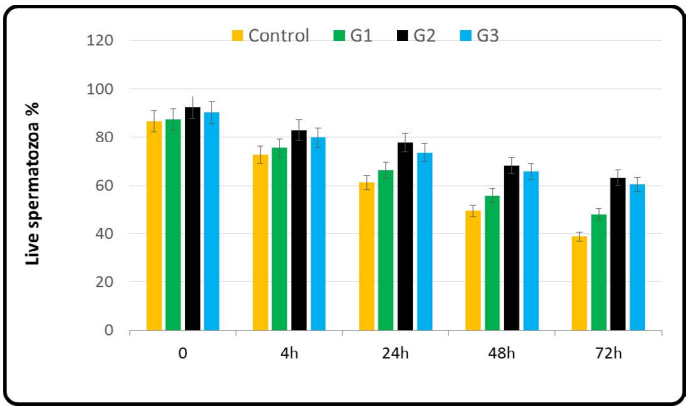

Figure 3: Effect of garlic extract additive to extender after cooling at 5 °C for 24, 48 and 72 h on live spermatozoa % quality of buffalo bull (n = 6 bulls; six replicates). (Control: 0; G1: 3; G2: 6 and G3: 9% of concentrations).

Evaluation of Garlic extract additive on antioxidants parameter

The activity of glutathione peroxidase (GPx) significantly differed (P < 0.05) in different concentration groups, with the G2-6 percent group having the more activity and the control group having the lowest. Similarly, the activity of the SOD showed a similar pattern, with the G2 (6% garlic extract) group reporting the most activity and the control group reporting the lowest. Moreover, the lowest value of MDA was obtained in G2, and the maximum values were recorded in the control group. The alpha glucosidase activity significantly differed (P < 0.05) between the groups, with the G2 group having the more activity and the control group having the lowest (Table 2).

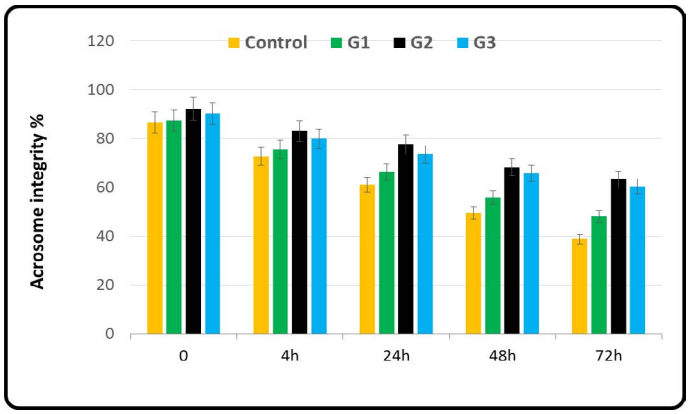

Figure 4: Effect of garlic extract additive to extender after cooling at 5 °C for 24, 48, and 72 h on live acrosome integrity % quality of buffalo bull (n = 6 bulls; six replicates). (Control: 0; G1: 3; G2: 6 and G3: 9% of concentrations).

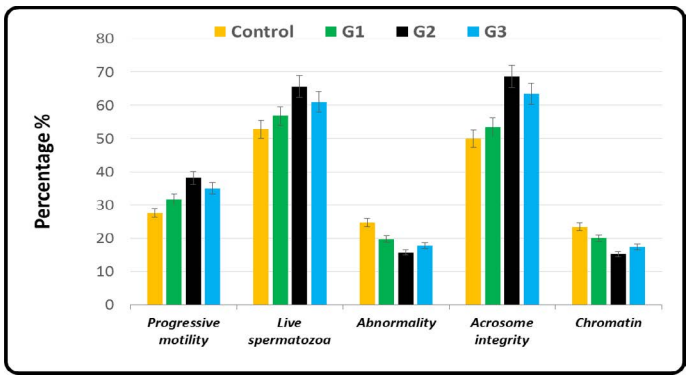

Figure 5: Effect of freezing rates post-thaw quality on parameters of buffalo bull spermatozoa (n = 6 bulls; six replicates).

DISCUSSION

The purpose of this study was to examine how adding different concentrations of garlic extract to the extender during the cooling and freezing stages affected the spermatozoa quality of buffalo bull and antioxidant enzymes. The cryopreservation is known to harm spermatozoa during the freezing-thawing process (Watson, 2000) because to its pronounced influence on mitochondrial and plasma membranes, also in worst-case, nucleus (Blesbois, 2007). It is better to state that Garlic extract (3, 6, and 9 %) was significantly higher than control (0%) at cooling and post-thaw sperm quality (via, progressive motility, abnormality, live spermatozoa, acrosome integrity ect.) compared to another group. This enhancement in semen quality may be attributed to the presence of some phenolic compounds in garlic as, gallic acid, flavonoid aglycones and quercetin that have antioxidant activities through reduction of oxidative stress, lipid peroxidation and free radical generation (Bo-

zin et al., 2008; Türk et al.,2013). On the other side, comparable to (Kumar et al., 2015) and cattle, several studies have found enhanced post-thaw sperm quality in buffalo (Celeghini et al., 2008; Leite et al., 2010; Andrabi et al., 2014). However, the presence of iron, calcium, potassium, sodium, copper, and zinc in garlic may be responsible for sperm maturation and many enzymes that preserve sperm (Borek, 2001; Fernandez-Gago et al., 2013). Buffalo bull spermatozoa not only exhibited good resistance to garlic extract, but also behaved as kept characteristics, signaling that the cryo-protocol utilized in this work may assisted to minimize overproduction of ROS. This finding agrees with (Shahverdi et al., 2014; Shah et al., 2016).

The metabolic environment and physical situations that spermatozoa are subjected to are reflected in the pattern of sperm motility. The acrosome ridge, plasma membrane integrity, and the motion properties of buffalo spermatozoa (visual or computed motility and curvilinear velocity) are all considerably degraded by freezing and thawing (Rasul et al., 2001).

The decrease in curvilinear velocity after thawing might be attributable to cryoinjuries to spermatozoa's mitochondrial machinery and axoneme (Mortimer, 2000; Larson-Cook et al., 2003). Furthermore, spermatozoa are exposed to unfavorable conditions for a shorter period of time when frozen at optimal rates (Woelders, 1997).

In current study, buffalo bull spermatozoa showed high tolerance to garlic extract; it also increased the quality, indicating a promising future for its usage as an extender. Our findings revealed a varying pattern of sperm motility at different doses of garlic extract (3–9%), whereas at concentrations (6%), sperm motility is practically stable to the control group. This difference in sperm motility could be attributed to thymoquinone, which possesses antioxidant qualities at low concentrations that protect spermatozoa from ROS and pro-oxidant properties at high concentrations that increase ROS production, resulting in decreased sperm motility (Burits and Bucar, 2000; Alenzi et al., 2010).

Sperm chromatin structure indicates sperm's capacity to fertilise the egg and is a measure of sperm quality, according to (Ugur et al., 2019). Data reported, considerably lowered at different groups as compared to control group; this might be attributable to a variety of biological activities, as anti-inflammatory, anti-carcinogenic, anti-oxidative, and several other biological actions. Garlic has potential antioxidant capabilities due to the presence of phenolic and flavonoids components (Miller et al., 2000). Moreover, Sokunbi et al. (2020) stated the, Allium sativum ethanolic extract has cytoprotective effect on semen quality and spermatozoa fertilizing capacity in prolonged porcine semen.

Antioxidants are chemicals that prevent the development of ROS and lipid peroxidation. The well-known antioxidants that are important for sperm function include SOD, GPx, and catalase, which protect sperm cells from oxidative stress (Ansari et al., 2011). Plant-derived extracts, on the other hand, are sources of natural antioxidants with reduced cytotoxicity when compared to chemical antioxidants (Khan et al., 2007). Garlic extract's antioxidant capabilities are ascribed to key components found in its seeds, such as thymol, thymoquinone, and dithymoquinone. It also contains a lot of vitamin E, which contributes to its antioxidant properties (Desai et al., 2015). Some studies have found that a 4 h equilibration interval improves post-thaw sperm quality of buffalo (Kumar et al., 2015; Celeghini et al.,2008) . Due to its major components, the addition of garlic extract improved all sperm quality measurements, including progressive motility, plasma membrane integrity, viability, and DNA integrity (Leite et al., 2010). Furthermore, (Türk et al., 2013) observed that oral ingestion of Allium Sativum herbal extract results in a considerable reduction in MDA levels as well as significant increases in GSH levels, GPx, and catalase activities. Furthermore, garlic contains antioxidants such as organosulfur and flavonoids such as quercetin, which may lead to healthier animals and enhanced sperm characteristics (Schepetkin et al., 2019). In contrast, depending on the quantity and kind of antioxidant, garlic extender can change the activity of endogenous antioxidants (Hammami et al., 2010). Due to the presence of phenolic chemicals and flavonoids, which are considered natural antioxidants, studies have shown that using garlic at modest levels can greatly enhance antioxidant enzymes (Banerjee et al., 2001).

Depending on the amounts of antioxidants used, addition of garlic extract can modify the activity of antioxidant enzymes (Hammami et al., 2010). Furthermore, because alpha-glucosidase is generated by the epididymis, a low level of glucosidase implies epididymal blockage, according to (Alsenosy and Abd El-Aziz, 2019). When compared to the control group, it is better to state that Garlic extract (3, 6, and 9 %) was significantly higher than control (0%) concentration significantly boosted alpha-glucosidase. These findings can also be explained by the beneficial chemicals found in garlic (Borek, 2001). Phytochemicals from plant-rich diets, such as garlic, provide important extra protection against oxidant damage, and the relevance of -glucosidase is reflected in our discovery of a high activity in garlic extended group (Roaiah et al., 2007; Nepomuceno, 2011). Many studies have indicated that garlic has substantial antioxidant qualities, as well as anti-inflammatory and immune-protective characteristics (Percival, 2016; Hayat et al., 2018).

CONCLUSION

Addition of garlic extracts to a semen extender successfully restored majority of the harmful effects of oxidative stress on semen properties during preservation in the current investigation. Garlic extract addition in sperm extender increased -glucosidase activity considerably. Garlic has antioxidant qualities, as evidenced by lower MDA levels and increased SOD and GPx activities and may be regarded one of the most effective antioxidant agents, shielding the cell from ROS destructive damage. Finally, the mechanism of sperm quality impediment during storage is related to the creation of oxidative stress and inflammatory reactions, which leads to cell membrane damage and may diminish sperm energy sources. Garlic extract-containing extender may protect sperm from the detrimental effects of ROS, enhance sperm quality and energy source during sperm storage, and may be effective in enhancing sperm quality and fertility rates.

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CONFLICT OF INTEREST

The authors declare that we did not have any conflict of interest regarding publishing or funding of this article

NOVELTY STATEMENT

According to results the garlic extract addition in sperm extender at different concentrations, it may be effective in enhancing sperm quality, protected of damage and fertility rates. Positive role in restored majority of the harmful effects of oxidative stress on semen properties during preservation. It is consider alternative safety sours in cryopreservation of Buffalo Bulls Sperm.

AUTHORS CONTRIBUTION

Conceptualization of this study was performed by Entesar Z Eliraqy: Writing – original draft, Formal analysis, Methodology, Investigation, Conceptualization, review and editing. Basant M Shafik: Formal analysis, Statistical analysis, Methodology, Editing. Yasser S Hussein: Formal analysis, Statistical analysis, Methodology, Editing. Abdelwahab A Alsenosy: Formal analysis, Methodology, Conceptualization, Editing. Emad A Abd allah: Formal analysis, Methodology, Visualization, Editing and Mohamed F Saad: Writing – original draft, Methodology, Investigation, Conceptualization. All authors read and approved the final manuscript.

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