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The Effect of Kaempferol on Buffalo Semen Freezability and Redox State

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ABSTRACT

To our knowledge, the roles of kaempferol in semen extension or cryobiology haven't been discovered until now. Furthermore, the intracellular signals triggered by kaempferol in buffalo spermatozoa during semen processing haven't been reported. So, the current study intended to clarify the role of kaempferol during the cryopreservation of buffalo spermatozoa. Six buffalo bulls were adopted for semen collection. Two consecutive ejaculates were collected from each buffalo bull weekly using an artificial vagina. The ejaculates were pooled to eliminate variability. Semen extension was done using the Tris-based diluent supplemented with Kaempferol at concentrations of 5, 10, 15, 25, 50, and 100 µg/ml vs. tris-based diluent only as control. Samples were subjected to freezing then thawing to evaluate the vital sperm characteristics. It was discovered that kaempferol at a concentration of 25 and 50 µg/ml of significantly ($p<0.05$) improved the post thawing motility, acrosomal integrity, viability index, glutathione reductase activity, superoxide dismutase and total antioxidant capacity. Moreover, it significantly ($p<0.05$) decreased the rate of lipid peroxidation, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and DNA fragmentation (%). In conclusion, kaempferol supplementation to buffalo semen extender at 25 and 50 µg/ml significantly enhanced its quality by reducing the cryo-damage rate depending on their enzymatic and non-enzymatic potentials.

1. INTRODUCTION

Cryopreservation of the spermatozoa is a vital tool to preserve the elite genetics to improve the animal population, even though its harmful effects on the structure of mammalian spermatozoa (Kar et al., 2015). The extent of this damage usually differs among the species, but seriously affects the sperm resistance to the freezing-thawing technique and artificial insemination (AI) outcomes (Yeste, 2016). In the recent years, many technical advances were achieved to decrease the rapid loss of sperm quality, which is the main obstacle that indirectly affects the AI results (Mata-Campuzano et al., 2014; Najafi et al., 2014).

Artificial insemination promising outcomes depend chiefly on the competence of the freeze-thawed spermatozoa to achieve the oocyte fertilization (El-Harairy et al., 2011). But the sperm manipulation at room temperature as well as during cooling and freezing in addition to long time of preservation can generate the reactive oxygen species (ROS) in the sperm diluent (Aitken, 1994; Avdatek et al., 2018); which renders the freez-thawed spermatozoa incapable to fertilize the oocyte (Aitken et al., 1993). Sperm structures are extremely vulnerable to the oxidative stressors (Avdatek et al., 2018), due to the high unsaturated fatty acids content in its plasma membrane (Akalin et al., 2015). The generated ROS directly results in energy reduction, lipid peroxidation, DNA damage, apoptosis (Taşdemir et al., 2013), and finally, cell death and fertility loss (Aitken, 1991).

Various policies have been applied to improve the freezing-thawing process by preventing oxidative stress, decreasing the cryo-injury to spermatozoa, and preventing or blocking premature sperm maturation (Ardeshirnia et al., 2017). Supplementing semen freezing media with various antioxidants was one of these policies (Forouzanfar et al., 2013).

Oxidative stress results from the critical imbalance between ROS generation and the antioxidant activity of the sperm cells (Ardeshirnia et al., 2017). The endogenous anti-oxidative capacity of seminal plasma is not sufficient during freezing storage due to reduction of the seminal plasma constituents by their dilution in the diluent media (Daramola et al.,2015). So, supplementing the semen extender with the suitable antioxidants is the main guard against this damage induced by free radicals aggregation (Ardeshirnia et al., 2017). Nowadays, the herbal antioxidants are considered a pioneer point as two-thirds of the world's plants have a medicinal value especially for their antioxidant potential and lower cytotoxicity (Motlagh et al., 2014).

Kaempferol is a natural antioxidant that was discovered in various natural plants (Siddhuraju and Becker, 2003). It possesses phenolic and flavonoid characters (Ampofo-Yeboah, 2013). This flavonoid polyphenol has a strong antioxidant activity more than that of vitamins C and E, as well as it is less toxic. Moreover, the phenolic compounds act as free radical hunters (Ben Abdallah et al., 2013) and metal chelators. So, these compounds act during the generation as well as during the propagation of the oxidative

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process (Silva, 2012). Furthermore, this polyphenol group is functionally similar to some reproductive hormones, so it exerts several biological activities in human and other vertebrates (Turker and Takemura 2011).

Kaempferol was stated to have health promoting benefits such as anti-oxidative and anti-inflammatory properties (García-Lafuente et al., 2009), anti-tumor activity (Leung, 2007); it inhibits cancer cell formation by acting as a chemo-preventive agent (Colino et al., 1999). Additionally, Kaempferol exhibit depigmentation activity (Rho et al., 2011).

Kaempferol has strong antioxidant activities by preventing cells 'lipids and DNA oxidative damage (Seifi-Jamadi et al., 2016). It was proposed that flavonoid components prevent lipid peroxidation by inhibiting and neutralizing ROS generation, delay oxidation by interacting with alpha-tocopherol, and stimulating the gene expression of glutathione s-transferase, UDP-glucuronosyltransferase (UGTs), and NAD (P) H-quinone oxidoreductase (Wang et al., 2016). Additionally, they have antimicrobial properties (Nitiema et al., 2012).

Therefore, flavonoid supplements to the semen extender were used to enhance the competence of the cryopreserved ram spermatozoa (Silva et al., 2012), bucks (Silva et al., 2016), bulls (Tvrda et al., 2016), horses (Seifi-Jamadi et al., 2016) and boars (Kim et al., 2014) depending on its antioxidant properties.

To our knowledge, there are no available papers about the use of kaempferol in the cryopreservation of buffalo semen. Therefore, the current study aimed to discover its effects when it was added to the buffalo semen extender. Moreover, the current study tested a wide range of kaempferol levels to determine the suitable concentration that could be used safely during buffalo semen extension and cryopreservation processes.

2. MATERIAL AND METHODS

2.1. Semen collection:

Six fertile buffalo bulls (3-5 years old) were used to collect semen samples using the artificial vagina. Two sequential semen samples were collected from each buffalo bull/weekly. Each ejaculate was evaluated for volume, sperm cell concentration, and motility (%). Semen samples with 800×10^6 sperm cells/ml, >85% normal sperm morphology and had at least 75 % motility were used in the current study. Even though, to eliminate the variability between semen samples, the collected ejaculates were pooled. The experiments were replicated four times for each group.

2.2. Semen Processing:

After semen evaluation and pooling, samples were divided into seven equal portions. Each portion was diluted at 37 °C with tris-based extender (6.6 ml glycerol, 1.48 g citric acid, 1.00 g fructose, 2.42 g Tris, 20 ml egg yolk, 50,000 IU penicillin and 25 mg gentamicin / 100 ml deionized water) that was supplemented with kaempferol (K0133, Pub Chem SID 24896195, Sigma-Aldrich, St. Louis, MO) at concentrations of 0 (Control), 5, 10, 15, 25, 50, 100 µg/ml. 120×10^6 sperm/ml was the final sperm concentration in each group.

The extended semen samples were cooled from 37 °C to 5 °C within an hour (hr) in a cold cabinet then loaded into 0.25 ml polyvinyl chloride straws (IMV, France). Semen straws were placed horizontally in the refrigerator at 4 °C for one hour. After that, the cooled straws were placed about six cm above the liquid nitrogen surface (-120 °C) for 15 min, then were dipped directly into the tank of the liquid nitrogen (− 196 °C) for at least 48 hrs before evaluation.

2.3. Analysis of sperm motility:

Frozen straws (n= 3) were thawed separately in a water bath at 37 °C for 30 sec. Post-thawing sperm viability, motility and acrosomal integrity were assessed for each group. The progressively motile spermatozoa (%) were evaluated visually. The estimated final sperm motility score was recorded as the mean of the three straws in three different microscopic fields per each.

2.4. Analysis of acrosomal membrane integrity:

Acrosomal integrity was examined according to Chinoy et al. (1992) using silver nitrate stain (sigma Aldrich, 209139). At least three hundred spermatozoa were counted per each slide. The percentage of defected acrosome (lack or absence of acrosome, sloughing of the acrosome, vacuolation defect, irregular or ruffled acrosome, disintegrating or incomplete, swelling or thickening especially at the anterior tip) was calculated. Normal acrosome was defined as being closely adherent to the sperm cell head, smooth and entire in shape.

2.5. Assessment of sperm DNA integrity:

The incidence of DNA fragmentation and DNA integrity were detected according to Boe-Hansen (2005) using the alkaline single cell gel electrophoresis (comet) assay. Briefly, frozen-thawed spermatozoa were diluted in phosphate buffer saline (PPS), embedded in agarose, followed by cell lysis, DNA decondensation, electrophoresis and DNA staining with 50 µL of 20 μgmL^{-1} ethidium bromide (Sigma, St. Louis, MO). The spermatozoa were examined by fluorescent microscopy to differentiate between intact and none-intact sperm DNA. The intact sperm cell DNA showed compact and brightly fluorescent heads, while the damaged sperm cells had strand breaks that show DNA migration in tail shape (comet appearance) behind the head during electrophoresis (Hughes et al., 1996). Sperm nuclei were evaluated by comet IV program (2001).

2.6. Biochemical assays:

Estimating transaminases enzymes and alkaline phosphatase

Aspartate-aminotransferase (AST), alanine-aminotransferase (ALT) and alkaline phosphatase (AKP) enzymes in each group were estimated in the supernatant of the semen straws (n= 5/group) using the spectrophotometer after centrifugation at 800 ×g for 10 min. The test was applied according to Reitman and Frankel (1957) and Tietz (1976) using Span diagnostics AST (55R-1517), ALT (55R-1516) and AKP kits, Ltd, Surat, India.

Superoxide dismutase (SOD)

Superoxide dismutase activity was evaluated according to Flohe and Otting (1984). Briefly, each semen sample was diluted 1:5 with PBS (pH 7.0). The assay solution contained sodium carbonate buffer (50 mM, pH 10.0), 0.1 mM xanthine, 0.025 mM nitroblue tetrazolium, 0.1 mM EDTA, xanthine oxidase and sample were mixed in a small cuvette. The SOD activity was measured spectrophotometrically at 560 nm and expressed as units / milliliter (U/ml).

Glutathione reductase (GSH)

The GSH content of sperm was measured using the method of Sedlak and Lindsay (1968). The semen samples were precipitated with 50% trichloroacetic acid (vol/vol) and then centrifuged at 1,000 ×g at 22°C for 5 min. The reaction mixture contained 0.5 mL supernatant, 2.0 mL Tris-EDTA buffer and 0.1 mL 5, 5-dithiobis-2-nitrobenzoic acid. The solution was incubated at 22°C for 5 min and then read at 412 nm on a spectrophotometer. The concentration of GSH

was recorded as unit / liter (U/L) for the tested sperm samples

Lipid peroxidation

A semen sample (500 µl) from each tested group was centrifuged at 800 ×g for 10 min. The pelleted sperm cells were separated, washed and re-suspended in phosphate buffer saline. The process of centrifugation and re-suspension was repeated three times, and 1 ml of the deionized water was added to the pelleted sperm cells after the final centrifugation. The final mixture was snap-frozen and stored at -70 °C till Malondialdehyde estimation. Before MDA estimation the tested semen samples were thawed and the Malondialdehyde concentration was considered as an index for the level of LPO in the sperm samples according to Placer et al. (1966). The MDA concentrations were expressed in nmol/10^9sperm cells

Total antioxidant capacity

The total antioxidant capacity (TAC) of the frozen-thawed semen was estimated using a commercial kit (Antioxidant Capacity Assay Kit, Cayman Chemical Co. Ann Arbor, MI, USA); according to Cortassa et al. (2004). The reaction was measured spectrophotometrically at 532 nm.

2.7. Statistical analysis:

The results were expressed as means ± SE. Data were statistically analyzed using Costat Computer Program (1986, Version 3.03) Means were compared by Duncan's multiple range test to check the significant differences between groups at 5% levels of probability.

3. RESULTS

Buffalo bull semen treated with the different concentrations of kaempferol showed a significant variance (P<0.05) in its post-thawing motility % (Table 1). Where buffalo semen samples treated with 25 µg/ml of kaempferol showed the highest stability (P<0.001) in motility percentage, viability index and lowest abnormal acrosomal integrity % (69.00 ± 1.35, 131.8 ± 3.11 and 11.50 ± 1.19 %) compared to the control group showed the greatest percentage of motility loss (54.75 ± 1.93) after the freez-thawing process. Additionally, buffalo semen samples treated with high concentration of kaempferol (100 µg/ml) showed adverse reduction in the post-thawing motility percent and viability index (57.25 ± 4.88 and 92.75 ± 2.62), despite it exerts certain degree of protection on the acrosomal integrity (15.50 ± 1.04 %) if compared with semen samples treated with zero concentration of Kaempferol (18.25 ± 0.62).

Table 1 The effects of kaempferol (K) addition to buffalo bull semen extender on sperms characteristics.

Treatment	Pre-cooling motility (%)	Cooling motility (%)	Post-thawing motility (%)	Viability index*	Abnormal acrosomal integrity (%)
Control	74.25 ± 2.17 ^{a}	68.75 ± 2.39^{b}	54.75 ± 1.93^{c}	95.50 ± 2.10^{de}	18.25 ± 0.62^{a}
K 5 µg/ml	74.75 ± 0.62 ^{a}	69.50 ± 0.86^{b}	56.25 ± 3.11^{bc}	97.00 ± 2.48^{de}	15.25 ± 0.85^{b}
K 10 µg/ml	78.50 ± 1.93 ^{a}	72.75 ± 1.10^{ab}	64.00 ± 2.08^{abc}	103.3 ± 2.68^{cd}	15.00 ± 0.70 ^{b}
K 15 µg/ml	76.75 ± 1.18 ^{a}	73.50 ± 1.19^{ab}	64.25 ± 2.78^{ab}	117.5 ± 3.22^{b}	13.00 ± 0.91^{bc}
K 25 µg/ml	77.75 ± 1.79 ^{a}	75.25 ± 1.70^{a}	69.00 ± 1.35^{a}	131.8 ± 3.11^{a}	11.50 ± 1.19^{c}
K 50 µg/ml	74.50 ± 1.04 ^{a}	71.00 ± 0.91^{ab}	60.75 ± 2.52^{abc}	106.5 ± 3.61^{c}	14.25 ± 0.62 ^{b}
K100 µg/ml	73.50 ± 2.53 ^{a}	69.25 ± 1.65^{b}	57.25 ± 4.88^{bc}	92.75 ± 2.62^{e}	15.50 ± 1.04 ^{b}

Results were presented as mean ± SEM (n= 4 replicates/group)

Values with different superscript letters (a,b,c,..) in the same column were significantly different (P<0.05).

Viability index = $\frac{\text{post-thawing motility}}{2}$ + [Motility after (1h + 2h + 3h)].

Table (2) showed that there were significant increases (P< 0.0001) in TAC (0.49 ± 0.02 and 0.46 ± 0.01), SOD (69.34 ± 1.50 and 71.70 ± 1.94) and GSH (59.62 ± 5.62 and 58.70 ± 2.39) activities in semen sample treated with 25 and 50 µg/ml of kaempferol, respectively. Additionally, there was a significant decrease (P< 0.001) in the level of lipids peroxidation in buffalo spermatozoa plasma membrane especially samples treated with 25 µg/ml (12.57 ± 0.58), 25 (12.05 ± 0.76), and 50 µg/ml (11.07 ± 1.01) of kaempferol. Where, the lowest degree of the oxidative damage in buffalo spermatozoa plasma membrane (high level of un-saturated fatty acids) was observed with (50 µg/ml.

Table 2 The effects of kaempferol (K) addition to buffalo bull semen extender on its antioxidant potentials.

Treatment	TAC (mµ/mL)	SOD (U/mL)	GSH (U/L)	MDA (nmol/mL)
Control	0.18 ± 0.01^{d}	28.80 ± 1.69^{e}	29.94 ± 3.60^{d}	20.46 ± 2.32^{a}
K 5 µg/ml	0.19 ± 0.01 ^{d}	34.81 ± 2.14d^{e}	37.11 ± 1.47^{cd}	17.36 ± 1.96^{ab}
K 10 µg/ml	0.24 ± 0.02^{cd}	40.74 ± 1.87^{cd}	41.29 ± 1.55^{bc}	13.98 ± 0.68^{bc}
K 15 µg/ml	0.37 ± 0.01^{b}	55.39 ± 3.78^{b}	46.72 ± 1.52^{b}	12.57 ± 0.58^{c}
K 25 µg/ml	0.49 ± 0.02^{a}	69.34 ± 1.50^{a}	59.62 ± 5.62^{a}	12.05 ± 0.76^{c}
K 50 µg/ml	0.46 ± 0.01^{a}	71.70 ± 1.94^{a}	58.70 ± 2.39^{a}	11.07 ± 1.01^{c}
K 100µg/ml	0.28 ± 0.04^{c}	42.89 ± 2.80^{c}	36.92 ± 1.59^{cd}	13.97 ± 1.65^{bc}

Results are presented as mean ± SEM (n= 4 replicates/group). Values with different superscript letters (a,b,c,..) in the same column are significantly different (*P<0.05*). TAC: Total antioxidant capacity, SOD: Superoxide dismutase, GSH: Glutathione Reductase, and MDA: Malondialdehyde.

In table (3) there was a significant decrease (P< 0.0001) in AST (69.11 ± 2.71 and 67.35 ± 1.03), ALT (18.49 ± 0.80 and 16.44 ± 0.78) and AKP (16.48 ± 0.84 and 14.63 ± 1.10) levels in the kaempferol treated semen samples mainly those supplemented with 25 and 50 µg/ml, respectively if compared with control group (106.0 ± 2.17, 28.09±1.11 and 25.86 ± 0.52, respectively).

Table 3 The effect of Kaempferol addition to buffalo bull semen extender on its enzymatic activity.

Treatment	AST (U/L)	ALT (U/L)	AKP (U/L)
Control	106.0 ± 2.17^{a}	28.09 ± 1.11^{a}	25.86 ± 0.52^{a}
K5 µg/ml	101.6 ± 3.26^{ab}	24.25 ± 1.65^{b}	24.13 ± 1.03^{a}
K 10 µg/ml	96.53 ± 2.54^{b}	22.80 ± 0.99^{bc}	21.03 ± 0.78^{b}
K 15 µg/ml	87.15 ± 2.80^{c}	20.53 ± 0.91^{cd}	18.99 ± 0.71^{b}
K 25 µg/ml	69.11 ± 2.71^{d}	18.49 ± 0.80^{de}	16.48 ± 0.84^{c}
K 50 µg/ml	67.35 ± 1.03^{d}	16.44 ± 0.78^{e}	14.63 ± 1.10^{c}
K 100 µg/ml	100.5 ± 1.72^{ab}	25.33 ± 1.05^{ab}	25.90 ± 0.77^{a}

Results are presented as mean ± SEM (n= 4 replicates/groups)

Values with different superscript letters (a,b,c,..) in the same column are significantly different (P<0.05). AST: Aspartate amino transferase, ALT: Alanine aminotransferase, and AKP: Alkaline phosphatase.

There was a significant decrease (P< 0.0001) in tail moment of buffalo spermatozoa treated with 25 µg/ml (5.68 ± 0.55), and 50 µg/ml (4.69 ± 0.69) of kaempferol compared with control (4.69 ± 0.69) and other treatment groups of Kaempferol (Table, 4 and Fig. 1). Moreover, supplementation of buffalo semen extender with kaempferol at concentration of 25 and 50 µg/ml could provide an efficient DNA protection (2.51 ± 0.15 and 2.31 ± 0.17%, respectively) especially if compared with control, 5 and 10 µg/ml of kaempferol (5.68 ± 0.36, 5.49 ± 0.21 and 4.27 ± 0.22%, respectively).

Table 4 The effect of kaempferol addition to buffalo bull semen extender on its DNA fragmentation percentage.

Treatment	DNA Fragmentation (%)	Tail length (µm)	Tail moment*
Control	5.68 ± 0.36^{a}	4.93 ± 0.17^{a}	28.24 ± 2.61
K5 µg/ml	5.49 ± 0.21^{a}	4.62 ± 0.06^{a}	25.42 ± 1.30^{a}
K 10 µg/ml	4.27 ± 0.22^{b}	3.46 ± 0.16^{b}	14.86 ± 1.21^{b}
K 15 µg/ml	3.25 ± 0.31^{cd}	2.64 ± 0.21^{c}	8.76 ± 1.38^{cd}
K 25 µg/ml	2.51 ± 0.15^{de}	2.25 ± 0.16^{cd}	5.68 ± 0.55^{d}
K 50 µg/ml	2.31 ± 0.17^{e}	2.02 ± 0.20^{d}	4.69 ± 0.69^{d}
K 100 µg/ml	3.92 ± 0.42^{bc}	3.30 ± 0.19^{b}	13.22 ± 2.18^{bc}

Results are presented as mean ± SEM (n= 4 replicates/group)
Values with different superscript letters (a,b,c,..) in the same column are significantly different (P<0.05). Tail moment = DNA fragmentation (%) ×Tail length*

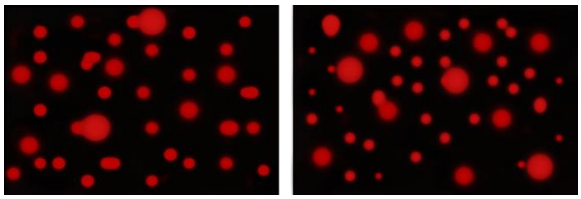

Figure 1 Single cell gel electrophoresis.
A: Low DNA fragmentation (%) that was illustrated by limited amount of DNA in the comet tail of buffalo bull spermatozoa cryopreserved in tris extender supplemented with 25 or 50 µg/ml of kaempferol .
B: High DNA fragmentation (%) that was illustrated by huge amount of DNA in the comet tail of buffalo bull spermatozoa cryopreserved in tris extender without kaempferol.

4. DISCUSSION

The current results illustrated promising roles of kaempferol in enhancing the post thawing motility and viability index especially at 25 µg/ml (69.00 ± 1.35 and 131.8 ± 3.11, respectively), while the high dose (100 µg/ml) exerts a deleterious effect on the same parameters (57.25 ± 4.88 and 92.75 ± 2.62, respectively). These results came in harmony with Ďuračka et al. (2019), who reported the positive effects of kaempferol on boar sperm motility in a dose-dependent manner. The enhancing role of kaempferol on buffalo sperm cell motility might be due to its effects on the sperm mitochondria. Where, Montero et al. (2004) reported that flavonoids, including kaempferol, could modulate the mitochondrial Ca^{2+} uniporter in the sperm plasma membrane without requiring ATP, thus phosphorylation. This mechanism offers an alternative way for flavonoid compounds to stimulate mitochondrial activities which play an essential role in the sperm motion (Montero et al., 2004). Furthermore, it was observed that flavonoid polyphenol compounds can increase sperm longevity in fresh semen by inhibiting glycolysis (Seddiki et al., 2017). Moreover, Castañeda-Arriaga et al. (2018) stated that the bioactive flavonoid substances could act as a double-edged sword where, at low concentrations improve the structural and functional characteristics of spermatozoa, while at high concentrations have cytotoxicity and cell death effects. Additionally, kaempferol (25 µg/ml) enhancing role on the acrosomal integrity of buffalo sperms (11.50 ± 1.19 %, Table 1) might be due to its powerful ROS scavenging effect that directly and/or indirectly affects the sperm viability where high ROS generation and aggregation trigger sperm apoptosis (Seifi-Jamadi et al., 2016, Wang et al., 2016 and Zhao et al., 2020). According to the table (2) it has been found that kaempferol at 25 and 50 µg/ml increase the total antioxidant capacity (0.49 ± 0.02 and 0.46 ± 0.01, respectively). Furthermore, kaempferol supplementation to buffalo semen extender potentiates the activities of antioxidants like superoxide dismutase (69.34 ± 1.50 and 71.70 ± 1.94, respectively).

GSH is a very potent intracellular ROS scavenger. Till the current study, there was no report stated the relevance between kaempferol and glutathione level during buffalo semen processing. Though, this relationship with other compounds of the same flavonoid group to which Kaempferol belongs had been estimated (Wang et al., 2016; Avdatek et al., 2018). In harmony with these previous reports, the results of the current study showed that kaempferol (as a member of flavonoid groups) also enhance GSH level at 25 and 50 µg/ml (59.62 ± 5.62 and 58.70 ± 2.39 U/L, respectively). According to the current results kaempferol was observed to decrease the rate of lipid peroxidation (12.05 ± 0.76 and 11.07 ± 1.01nmol/ml, respectively). These results came in agreement with Wang et al. (2016), who reported that the flavonoid components could prevent lipid peroxidation by inhibiting and/or neutralizing ROS generation, delay oxidation by interacting with alpha-tocopherol as well as promoting the gene expression of detoxifying enzymes such as glutathione s-transferase, UDP-glucuronosyltransferase (UGTs) and NAD (P) H-quinone oxidoreductase. Similarly, kaempferol additions to the extender of buffalo semen improved its quality by decreasing the rate of its destruction during cryopreservation, especially at 25 and 50 µg/ml (Table 3 and 4). These results run parallel with Seddiki et al. (2017) and Zhao et al. (2020) who reported that flavonoid group to which kaempferol belongs regulates gene expression, inhibits protein kinases, inhibits DNA topoisomerases and regulate the mitochondrial function. Taken together, buffalo spermatozoa that characterized by the high content of the unsaturated fatty acids are essentially in need for high protection from the cryo-damage by supplementing the extension medium with a potent bioactive broad spectrum cheap anti-oxidant like kaempferol.

5. CONCLUSION

The current study revealed that the addition of kaempferol to buffalo semen extender at concentration of 25 or 50µg/ml is promising as an efficient bioactive broad-spectrum polyphenol antioxidant that should be used in buffalo semen processing.

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