J. Anim. and Poultry Prod., Mansoura Univ., Vol.7 (12): 467- 473 , 2016

Effect of Aqueous Extracts of *Moringa oleifera* leaves or *Arctium lappa* Roots on Lipid Peroxidation and Membrane Integrity of Ram Sperm Preserved at Cool Temperature.

El-Harairy, M. A.[1]; A. E. Abdel-Khalek[1]; W. A. Khalil[1]; E. I. Khalifa[2];A. Y. El-Khateeb[3] and A. M. Abdulrhmn[1].

[1] Animal Production Department, Faculty of Agriculture, Mansoura University, Mansoura, Egypt.

[2] Animal Production Research Institute (APRI), Department of Sheep and Goat Research, Ministry of Agriculture, Dokki, Giza, Egypt.

[3] Agricultural Chemistry Department, Faculty of Agriculture, Mansoura University, Mansoura, Egypt.

ABSTRACT

This study aimed to investigate effect of addition of different levels (0, 100, 500 and 1000 µg/ml) from *Moringa oleifera* leaves (MOL) or *Arctium lappa* L. roots (ALR) extracts, as antioxidants to ram semen extender on sperm characteristics, lipid peroxidation and enzyme activity in seminal plasma of ram semen preserved at cool temperature (5°C)for 48 hours. Four adult Rahmani rams were used. Semen was collected from all rams using an artificial vagina once a week for 8 weeks. Lecithin soybean based-extender was divided into seven portions; control (T1), 100 (T2), 500 (T3) and 1000 (T4) µg MOL extract/ml or 100 (T5), 500 (T6) and 1000 (T7) µg ALR extract/ml extender. In each treatment, percentage of progressive motility (PM), livability (SL), and abnormality (AB) were determined. Also, percentages of spermatozoa having curled tails or chromatin damage were calculated. Polyphenols and flavonoid compounds in MOL or ALR extracts were identified. Concentrations of total antioxidant (TAO) and malondialdehyde (MDA), and activity of acid phosphatase (ACP) and lactic dehydrogenase (LDH) were determined. Results showed that both extracts revealed higher content of most phenolic compounds in ALR than in MOL extract, but Pyrogallol compound showed the highest level of phenolic compounds in both MOL and ALR extracts. ALR extract showed higher contents of Narengenin, Rutin, Quercetin, Apigenin and Luteolin and lower contents of Hisperdin, Romarinic acid, Kampferol and Hesperitin than in MOL extract. Only overall percentages of SL and AB improved ($P<0.05$), while percentage of sperm with chromatin damage increased ($P<0.05$) in T7 as compared to T1. Overall percentage of PM and curled tail of spermatozoa did not differ in T7 compared with T1, while T6 showed poorer ($P<0.05$) results than in T1. All sperm characteristics studied showed deleterious effect ($P<0.05$) by increasing storage period. The effect of interaction between treatment and storage period was not significant on all sperm characteristics. Concentrations of TAO and MDA as well as ACP and LDH activities tended to be higher ($P\geq0.05$) in seminal plasma of semen in all treatments. Effect of storage period or its interaction with treatment was not significant on concentrations of TAO and MDA as well as ALP and LDH activities. The present results showed that adding 1000 µg of *Arctium lappa* roots extract improved livability and abnormality with appropriate progressive motility and response to HOS-t of spermatozoa in Lecithin soybean based-extender of ram semen preserved at cool temperature (5°C) for 48 hours as compared to un-supplemented or those supplemented with 100 or 500 µg of *Moringa oleifera* or *Arctium lappa*.

Keywords: *Ram semen, cool preservation, storage period, Moringa oleifera, Arctium lappa, sperm characteristics.*

INTRODUCTION

Artificial insemination (AI) in sheep is an efficient tool for genetic improvement and also management of reproduction. One of the critical steps of AI is the ram semen preservation, liquid storage or freezing (Allai *et al.* 2015). Oxidative damage (OD) to sperm resulting from reactive oxygen species (ROS) generated by the cellular components of semen during liquid storage is possibly one of the main causes for the decline in motility and fertility during storage, the other detrimental cause is low temperature on the destabilization of sperm membrane structure (Bucak and Tekin 2007).

Antioxidants, in general, are scavengers which restrain the formation of ROS. Enzymatic and non-enzymatic antioxidants have the ability to attack ROS and lipid peroxidation (LPO) to protect sperm cells from membrane damage. A variety of defense mechanisms encompassing antioxidant enzymes, e.g. superoxide dismutase, catalase, glutathione peroxidase and reductase are involved in biological systems. Recent studies described that a balance between the benefits and risks from ROS and antioxidants appears to be essential for the normal functioning of spermatozoa. Seminal plasma also seems to be one of the most powerful antioxidant fluids in the organism, nevertheless supplementation of the extenders with antioxidants is recommended to facilitate the enhancement of sperm cryopreservation techniques (Budai 2014).

Moringa oleifera Lam (MO) is a medicinally important plant distributed in many countries of the tropics and subtropics (Mughal *et al.* 1999; Kumar *et al.* 2010). Water extract of MO leaves possesses potent antioxidant activities against free radicals, prevent oxidative damage to major bio-molecules and afford significant safeguard against oxidative stress (Chumark *et al.* 2008; Sreelatha and Padma 2009). Experimental evidence on MO as a natural antioxidant was reported for its capacity to protect organism and cell from oxidative DNA damage associated with aging, cancer and degenerative diseases as well inhibit LPO. Presence of poly-phenolic compounds may therefore be responsible for their overall antioxidant potential (Singh *et al.* 2009; Sreelatha and Padma 2009). Aqueous MO leaf extract was found to contain compounds with wide-spectrum antibacterial activity, capable of inhibiting the growth of gram-positive and negative bacteria (Cristina and Joseí 2011).

Arctium lappa L. (AL) is a traditional Chinese medicinal and an edible perennial plant of the family Compositae. It has also been used therapeutically in Europe, North America and Asia for hundreds of years (JianFeng *et al.* 2012). In this respect, (Duh 1998) cleared that water AL extract (burdock) and hot water extracts of burdock showed significant antioxidant activity, marked reducing power, and a scavenging effect on free-radical and active oxygen. Also, (JianFeng *et al.* 2012) demonstrated that aqueous extract of AL roots enhances

sexual behavior of male rats. The aphrodisiac effects of the plant extract may be related to the presence of flavonoids, saponins, lignans and alkaloids, acting via a multitude of central and peripheral mechanisms.

Therefore, the aim of this study was to investigate effect of addition of different levels (0, 100, 500 and 1000 µg/ml) from *Moringa oleifera* leaves (MOL) or *Arctium lappa* L. roots (ALR) extracts, as antioxidants to ram semen extender (Soybean lecithin-based extender, SBLE), on sperm characteristics, lipid peroxidation and enzyme activity in seminal plasma of ram semen preserved at cool temperature (5°C) for 48 hours.

MATERIALS AND METHODS

This study was carried out at the Laboratory of Physiology and Biotechnology, Animal Production Department, Faculty of Agriculture, Mansoura University, in cooperation with Animal Production Research Institute, during the period from March to November 2016.

Animals:

In this study, four adult healthy Rahmani rams have live body weight (70 -75 kg) and aged 3.0 - 3.5 years were used for semen collection. Rams were healthy and clinically free from external and internal parasites with the sound history of fertility in herd. They were fed a balanced ration to meet the NRC requirements of rams (NRC 2007). Rams were set free in well ventilated semi open pens, so that they were exposed to the natural photo periods. Fresh water was available all over the experimental period.

Semen collection:

Prior to semen collection, the instruments of semen collection were cleaned using distilled water and dried with sterile paper towel. Semen was collected from all rams using an artificial vagina once a week for 8 weeks in existence of an estrous ewe. The collected ejaculates having at least 70% progressive sperm motility, 17% sperm abnormalities and 2.5 x109/ml sperm cells concentration were pooled and used in the experimental work.

Preparation of semen extender:

Semen was extended with SBLE as shown in Table (1).

Table 1. Ingredients of Soybean lecithin-based extender used in semen extension.

Ingredient	Company	Amount
Tris (g/dl distilled water)	AppliChem, Germany	2.442
Citric acid (g/dl distilled water)	AppliChem, Germany	1.340
Sodium citrate (g/dl distilled water)	Sigma-Aldrich, USA	0.145
Fructose (g/dl distilled water)	Oxford,India	0.750
Penicillin (IU/ml)	Misr Co. for Pharm. Egypt	1000
Streptomycin (µg/ml)	AppliChem, Germany	1000
Soybean lecithin (g/dl distilled water)	LabM, UK	1.0
Distilled water (ml)	----	100

Extracts preparation:

Moringa oleifera leaves (MOL) or Arctium lappa L. roots (burdock) were ground into a fine powder; a known quantity of the materials was soxhlted using bi-distilled water. Finally the extracts were lyophilized, weighed and preserved at 4oC and kept for use when required.

Semen extension and experimental treatments:

The prepared semen extender was divided into seven portions; control SBLE (un-supplemented, T1), SBLE supplemented with 100 (T2), 500 (T3) and 1000 (T4) µg MOL extract/ml or 100 (T5), 500 (T6) and 1000 (T7) µg ALR extract/ml extender. Semen on each collection day was extended with SBLE supplemented with different treatment levels at a rate of 1 semen: 5 extender, and the extended semen was kept in water both at 37°C at all times to avoid fluctuations in the temperature of extended semen.

Semen evaluation:

In each treatment, percentage of progressive motility (Rao and Hart 1948), livability (Hackett and Macpherson 1965), and abnormality (Menon *et al.* 2011) were determined. Also, the response of ram spermatozoa to HOS-test was assessed using solution prepared with fructose (0. 675 g) and Na-citrate (0.367 g) in 100 ml distilled water to give osmolarity of 75 mOsmol/l for 30 min at 37oC in water bath. Numbers of spermatozoa with curled tail were determined to calculate the percentage of spermatozoa having curled tails.

Toluidine blue staining was performed as previously described by (Erenpreiss *et al*. 2004). Smears were fixed in ethanol–acetic acid glacial (3:1, v/v) for 1 min and 70% ethanol for 3 min. Smears were hydrolyzed for 20 min in 1 Mm HCl, rinsed in distilled water and air-dried. One droplet of 0.025% Toluidine blue in Mcllvaine buffer (sodium citrate-phosphate) pH 4.0 was placed over each smear and then cover slipped. Smears were evaluated with light microscopy at (X 1000) magnification. The percentage of chromatin damage was estimated by evaluating 300 sperm cells in each smear. Spermatozoa stained as green to light blue were considered to have normal chromatin, while those stained dark blue to violet were considered to have damaged chromatin. All sperm characteristics were evaluated at 0, 24 and 48h.

Analytical procedures:

Polyphenols and flavonoid compounds in MOL or ALR extracts were identified according to the method described by (Goupy *et al.* 1999) and (Mattila *et al.* 2000), respectively, at HPLC laboratory, Food Industries Institute, Agricultural Research Center, Giza, Egypt.

All types of extended semen preserved at cool temperature (5°C) for 0 or after 48 h was centrifuged for 15 minutes at 8000 RCF at 4°C (Sigma 2-16 KL), then seminal plasma was separated and stored at -20oC until the assay of concentration of total antioxidant (Koracevic *et al.* 2001) and malondialdehyde (MDA, (Ohkawa *et al.* 1979), and activity of acid phosphatase (ACP, (Belfield and Goldberg 1971) and lactic dehydrogenase (LDH, (Bais and Philcox 1994) using commercial kit (Biodiagnostic, Egypt) and spectro-photometer (SPECTRO UV-VIS AUTO, UV-2602, Labomed, USA).

Statistical analysis:

Data were statistically analyzed by factorial design (7 treatments x 3 times) using the General Linear Model procedures of (SAS 2004). Duncan multiple

range test was used to test the differences among treatment means (Duncan 1955).

RESULTS AND DISCUSSION

Phenolic and flavonoid compounds in MOL and ALR extracts:

Chemical analysis of both extracts revealed higher content of most phenolic compounds in ALR than in MOL extract, but Pyrogallol compound showed the highest level of phenolic compounds in both MOL and ALR extracts. On the other hand, ALR extract showed higher contents of Narengenin, Rutin, Quercetin, Apigenin and Luteolin and lower contents of Hisperdin, Romarinic acid, Kampferol and Hesperitin than in MOL extract (Table 2). The MO is a medicinally important plant distributed in many countries of the tropics and subtropics (Mughal *et al*. 1999; Kumar *et al*. 2010). Presence of poly-phenols may therefore be responsible for their overall antioxidant potential (Singh *et al*. 2009; Sreelatha and Padma 2009).

Table 2. Phenolic and flavonoid compounds in MOL and ALR extracts.

Item	*Moringa oleifera L.*	*Arctium lappa L.*
Phenolic compounds (ppm):		
Pyrogallol	284.43	163.34
Catechin	1.21	8.93
Catechol	6.35	12.17
Epicatechin	1.53	5.87
Caffeine	2.26	4.15
Coumarin	8.68	24.20
Gallic acid	0.65	5.80
Caffeic acid	1.20	5.78
Chlorogenic acid	2.69	8.57
Protochatchuic acid	2.81	3.31
P-OH-Benzoic acid	2.61	6.39
Vanillic acid	3.65	9.49
P-coumaric acid	1.93	1.74
Ferulic acid	1.89	8.28
Ellagic acid	9.53	10.61
Syringic acid	5.97	10.07
Benzoic acid	26.15	18.95
Salicylic acid	12.18	36.06
Flavonoid compounds (ppm):		
Narengenin	265.87	341.16
Rutin	24.77	93.56
Hisperdin	410.75	106.93
Romarinic acid	69.43	34.49
Quercetin	68.42	431.90
Apigenin	38.85	42.43
Kampferol	76.07	30.01
Luteolin	-	6.44
Hesperitin	5.69	-

However, AL is a traditional Chinese medicinal and an edible perennial plant. It has also been used therapeutically in Europe, North America and Asia for hundreds of years (JianFeng *et al*. 2012). In this respect, (Duh 1998) clearly indicated that water extracts of AL (burdock) and hot water extracts of burdock showed significant antioxidant activity. The aphrodisiac effects of the plant extract may be related to the presence of flavonoids, saponins, lignans and alkaloids, acting via a multitude of central and peripheral mechanisms. Also, MO leaves were reported to contain higher antioxidant contents such as β-carotene, vitamin C, and flavonoids (Amaglo *et al*. 2010; Gowrishankar *et al*. 2010). In this respect, (Luqman *et al*. 2011) indicated presence of polyphenols, tannins, anthocyanin, glycosides, and thiocarbamates in MO leaves. Generally, MO had anti-bacterial properties due to lipophilic compounds (Jabeen *et al*. 2008; Patel *et al*. 2011). (Srinivasan *et al*. 2007; Devaraj *et al*. 2008) observed that in different mammalian cells that were previously exposed to variety of oxidative conditions, improved by Poly-phenolic compounds in MO by enhancing the immune system, scavenge of free radical and reduce the production of DNA mutations.

Sperm characteristics:

Effect of treatment was significant on all sperm characteristics in stored ram semen. Only overall percentages of sperm livability and abnormality significantly ($P<0.05$) improved, while percentage of sperm with chromatin damage significantly ($P<0.05$) increased in T7 as compared to T1 (control). However, overall percentage of sperm motility and curled tail of spermatozoa did not differ significantly in T7 compared with T1 and T6 showed significantly ($P<0.05$) poorer results than in T1 (Table 3). These results suggest that supplementation of extender of ram semen with ALR extract may has impact on sperm function during cooling preservation for 48 hours.

Table 3. Sperm characteristics of ram semen as affected by supplementation of extender with different types and levels of MOL and ALR extracts.

Treatment	Sperm characteristics (%)				
	Progressive motility	Livability	Abnormality	Curled tail	Chromatin damage
T1 (control)	73.33^{a}	70.33^{ab}	22.73^{a}	55.00^{a}	2.40^{d}
T2 (100 µg MOL)	67.33^{c}	64.33^{c}	23.00^{a}	48.00^{c}	2.87^{cd}
T3 (500 µg MOL)	68.00^{c}	67.67^{bc}	21.33^{ab}	50.00^{bc}	4.07^{ab}
T4 (1000 µg MOL)	72.33^{ab}	69.00^{abc}	20.87^{b}	49.00^{bc}	3.67^{b}
T5 (100 µg ALR)	74.67^{a}	71.67^{ab}	21.33^{ab}	52.67^{ab}	3.53^{bc}
T6 (500 µg ALR)	69.00^{bc}	65.00^{c}	22.87^{a}	48.33^{c}	4.60^{a}
T7 (1000 µg ALR)	73.33^{a}	73.00^{a}	20.47^{b}	52.67^{ab}	3.93^{ab}
SEM	1.42	1.56	0.57	1.21	0.24
P-Value	0.000***	0.000***	0.010**	0.000**	0.000***

[a, b,c and d]: Means denoted within the same column with different superscripts are significantly different at $P<0.05$.
**** Significant at $P<0.01$. *** Significant at $P<0.001$.**

It is worthy noting that MO leave extract only at 1000 µg had impact on sperm abnormality as compared to control (Table 3). In this line, (Ghodiah 2016) showed marked increase in sperm motility of rabbit semen extended with Tris-extender supplemented with 2 or 4 mg MO extract and stored at cool temperature for 7 days. Also, (Sokunbi *et al*. 2015) found that mean progressive motility significantly increased in bull semen extended with glucose yolk citrate extender containing 12 ml of MO crude extract compared to 0, 8, and 16 ml MO crude extract. The observed improvement on most sperm characteristics (Table 3) for semen extended with 1000 µg ALR extract was indicated by (Duh 1998), who clearly indicated that water extracts of AL

showed significant antioxidant activity, as a scavenging effect on free-radical and active oxygen.

Effect of storage period was significant (P<0.001) on all sperm characteristics in stored semen. All sperm characteristics studied showed significantly deleterious effect by advancing storage period. The most change occurred in sperm motility percentage by advancing storage period (Table 4). Recently, (Ghodiah 2016) reported that rabbit semen extended with tris-extender supplemented with 2 or 4 mg/l of MO extract showed longer preservation time at cool temperature (4-5 d) as compared to un-supplemented semen.

Table 4. Sperm characteristics of ram semen stored for different periods at cool temperature.

Storage period (h)	Sperm characteristics				
	Progressive motility	Livability	Abnormality	Curled tail	Chromatin damage
0	82.29[a]	76.86[a]	17.66[c]	59.14[a]	2.06[a]
24	71.14[b]	68.00[b]	22.29[b]	50.71[b]	3.51[b]
48	60.00[c]	61.29[c]	25.46[a]	42.57[c]	5.17[c]
SEM	0.928	1.019	0.37	0.79	0.16
P-value	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***

a, b and c: Means denoted within the same column with different superscripts are significantly different at P<0.05.
***** Significant at P<0.001.**

The effect of interaction between treatment and storage period was not significant on all sperm characteristics. This effect cleared marked reduction in percentages of motility, livability and curled tail of spermatozoa, which was associated with increase of abnormality and chromatin damage percentages with advancing storage period. Only semen extended with 1000 µg AL showed the best sperm characteristics after 48 hours of storage at cool temperature (Table 5).

Table 5. Sperm characteristics of ram semen extended with different types and levels of MOL and ALR extracts and stored for different periods at cool temperature.

Treatment	Period (h)	Progressive motility	Livability	Abnormality	Curled tail	Chromatin damage
T1 (control)	0	86	79	19.4	62	1.4
	24	72	71	23.0	55	2.4
	48	62	61	25.8	48	3.4
T2 (100 µg MOL)	0	80	75	17.4	58	1.4
	24	67	62	24.0	48	2.8
	48	55	56	27.6	38	4.4
T3 (500 µg MOL)	0	78	76	16.4	59	2.6
	24	67	67	22.4	50	3.8
	48	59	60	25.2	41	5.8
T4 (1000 µg MOL)	0	83	76	16.6	57	2.4
	24	72	69	20.8	48	3.4
	48	62	62	25.2	42	5.2
T5 (100 µg ALR)	0	85	78	17.6	60	2.0
	24	76	71	21.6	53	3.6
	48	63	66	24.8	45	5.0
T6 (500 µg ALR)	0	80	73	19.0	58	2.4
	24	70	64	23.8	49	4.6
	48	57	58	25.8	38	6.8
T7 (1000 µg ALR)	0	84	81	17.2	60	2.2
	24	74	72	20.4	52	4.0
	48	62	66	23.8	46	5.6
SEM		2.45	2.69	0.99	2.09	0.42
P-Value		0.99	0.99	0.89	0.93	0.64

Antioxidant capacity and enzyme activity in seminal plasma:

Although the effect of treatment was not significant, concentration of total antioxidants (TAO) and malondialdehyde (MDA) as well as enzyme activity of ACP and LDH tended to be higher in seminal plasma of semen extended with all types and sources of MOL or ALR extracts (Table 6). Such trend was associated with the observed insignificant effect of all treatments on sperm motility percentage, but not with other sperm characteristics studied (Table 3).

Table 6. Antioxidant capacity and enzyme activity in seminal plasma of ram semen as affected by supplementation of extender with different types and levels of MOL and ALR extracts.

Treatment	Total antioxidants (Mm/l)	Malon-dialdehyde (nmol/ml)	Acid phosphatase (IU/l)	Lactic dehydrogenase (U/ml)
T1 (control)	1.24	17.97	39.11	333.25
T2 (100 µg MOL)	1.61	19.63	43.10	393.51
T3 (500 µg MOL)	1.81	20.99	45.68	407.00
T4 (1000 µg MOL)	2.14	20.01	44.37	397.55
T5 (100 µg ALR)	1.99	19.98	53.08	459.17
T6 (500 µg ALR)	2.00	19.23	43.36	406.10
T7 (1000 µg ALR)	1.83	18.94	41.97	334.14
SEM	0.27	2.32	3.77	69.30
P-value	0.32	0.98	0.28	0.87

Effect of storage period was also not significant on concentration of TAO and MDA as well as ACP and LDH activities, but there was a tendency of increasing TAO and MDA concentrations and decreasing ACP and LDH activities in seminal plasma of stored semen after 48 h storage period (Table 7).

Table 7. Antioxidant capacity and enzyme activity in seminal plasma of ram semen stored for different periods at cool temperature.

Storage period (day)	Total antioxidants (Mm/L)	Malon-dialdehyde (nmol/ml)	Acid phosphatase (IU/L)	Lactic dehydrogenase (U/ml)
0	1.68	19.49	46.88	399.61
48	1.93	19.58	41.89	380.59
SEM	0.15	1.24	2.01	37.04
P-Value	0.23	0.96	0.09	0.72

Treatment insignificantly interacted with storage period for TAO and MDA concentration and ACP and LDH activities, reflecting an increase in TAO and LDH activity and decrease in MDA concentration and ACP activity in most treatments after 48 h of semen storage (Table 8). It is of interest to note that T6 showed an opposite trend of most treatments in term of slight increase in MDA concentration and ACP activity as well as decreasing LDH activity by advancing storage period.

Recently, (Ghodiah 2016) reported that TAO concentration increased in blood of rabbit bucks orally treated with 60 mg (P<0.05) and 120 mg (P≥0.05) of

MO extract for 21 days. This may indicate effect of MO extract depending on dose of administration.

Also, (Sofidiya *et al.* 2006; Ogbunugafor *et al.* 2011) mentioned that the medicinal effects of MO were ascribed to their possession of anti-oxidants, which are known to suppress ROS formation and free radicals. Also, (Luqman *et al.* 2011) found that MO extract remove free radicals, activate antioxidant enzymes, and inhibit oxidases.

Table 8. Antioxidant capacity and enzyme activity in seminal plasma of ram semen extended with different types and levels of MOL and ALR extracts and stored for different periods at cool temperature.

Treatment	Period (h)	Total antioxidants (Mm/L)	Malon-dialdehyde (nmol/ml)	Acid phosphatase (IU/L)	Lactic dehydrogenase (U/ml)
T1 (control)	0	1.16	18.05	39.95	322.00
	48	1.31	17.90	38.27	344.49
T2 (100 µg MOL)	0	1.37	19.70	45.27	465.91
	48	1.85	19.57	40.92	321.10
T3 (500 µg MOL)	0	1.57	21.50	47.57	423.64
	48	2.05	20.48	43.79	390.36
T4 (1000 µg MOL)	0	2.22	20.22	49.17	396.65
	48	2.07	19.79	39.57	398.45
T5 (100 µg ALR)	0	1.70	18.91	60.12	440.73
	48	2.27	21.05	46.04	477.61
T6 (500 µg ALR)	0	1.93	19.00	43.32	423.64
	48	2.07	19.46	43.41	388.56
T7 (1000 µg ALR)	0	1.79	19.07	42.72	324.70
	48	1.87	18.81	41.21	343.59
SEM		0.39	0.39	3.27	5.33
P-Value		0.96	0.100	0.84	0.97

CONCLUSION

The present results showed that adding 1000 µg of *Arctium lappa* roots extract improved livability and abnormality with appropriate progressive motility and response to HOS-t of spermatozoa in Lecithin soybean based-extender of ram semen preserved at cool temperature (5°C) for 48 hours as compared to un-supplemented or those supplemented with 100 or 500 µg of *Moringa oleifera* or *Arctium lappa*.

REFERENCES

Allai, L., Druart, X., Contell, J., Louanjli, N., Moula, A.B., Badi, A., Essamadi, A., Nasser, B. and El Amiri, B. (2015). Effect of argan oil on liquid storage of ram semen in Tris or skim milk based extenders. Animal Reproduction Science, 160: 57-67.

Amaglo, N.K., Bennett, R.N., Curto, R.B.L., Rosa, E.A., Turco, V.L., Giuffrida, A., Curto, A.L., Crea, F. and Timpo, G.M. (2010). Profiling selected phytochemicals and nutrients in different tissues of the multipurpose tree *Moringa oleifera* L. grown in Ghana. Food Chemistry, 122(4): 1047-1054.

Bais, R. and Philcox, M. Approved recommendation on IFCC methods for the measurement of catalytic concentration of enzymes. Part 8. IFCC Method for Lactate Dehydrogenase (l-Lactate: NAD+ Oxidoreductase, EC 1.1. 1.27). International Federation of Clinical Chemistry (IFCC). In 'European journal of clinical chemistry and clinical biochemistry: journal of the Forum of European Clinical Chemistry Societies, 1994, p. 639.

Belfield, A. and Goldberg, D. (1971). Colorimetric determination of alkaline phosphatase activity. Enzyme, 12(5): 561-568.

Bucak, M.N. and Tekin, N. (2007). Protective effect of taurine, glutathione and trehalose on the liquid storage of ram semen. Small Ruminant Research, 73(1): 103-108.

Budai, C. (2014) The protective effect of antioxidants on liquid and frozen stored ram semen. Scientific Papers Animal Science and Biotechnologies, 47(1): 46-52.

Chumark, P., Khunawat, P., Sanvarinda, Y., Phornchirasilp, S., Morales, N.P., Phivthong-ngam, L., Ratanachamnong, P., Srisawat, S. and Klai-upsorn, S.P. (2008). The *in vitro* and *ex vivo* antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of *Moringa oleifera* Lam. leaves. Journal of ethnopharmacology, 116(3): 439-446.

Cristina, J.R.O.P.G. and Joseí, S.R.A.C. (2011). *In vitro* antibacterial effect of aqueous and ethanolic Moringa leaf extracts. Asian Pacific Journal of Tropical Medicine, 4(3): 201-204.

Devaraj, S., Mathur, S., Basu, A., Aung, H.H., Vasu, V.T., Meyers, S. and Jialal, I. (2008). A dose-response study on the effects of purified lycopene supplementation on biomarkers of oxidative stress. Journal of the American College of Nutrition, 27(2):, 267-273.

Duh, P.D. (1998). Antioxidant activity of burdock (*Arctium lappa* Linne): its scavenging effect on free-radical and active oxygen. Journal of the American Oil Chemists' Society, 75(4): 455-461.

Duncan, B.D. (1955). Multiple range and multiple F-test. Biometrics. 11: 1-42.

Erenpreiss, J., Jepson, K., Giwercman, A., Tsarev, I., Erenpreisa, J. and Spano, M. (2004) Toluidine blue cytometry test for sperm DNA conformation: comparison with the flow cytometric sperm chromatin structure and TUNEL assays. Hum. Reprod., 19(10): 2277-2282.

Ghodiah, A.E.B. (2016). Physiological studies on some factors affecting semen quality and preservation of rabbit bucks fed moringa. Ph.D. Thesis, Faculty of Agric., Mansoura Univ., Egypt.

Goupy, P., Hugues, M., Boivin, P. and Amiot, M.J. (1999) Antioxidant composition and activity of barley (*Hordeum vulgare*) and malt extracts and of isolated phenolic compounds. Journal of the Science of Food and Agriculture, 79(12): 1625-1634.

Gowrishankar, R., Kumar, M., Menon, V., Divi, S.M., Saravanan, M., Magudapathy, P., Panigrahi, B., Nair, K. and Venkataramaniah, K. (2010) Trace element studies on *Tinospora cordifolia* (Menispermaceae), *Ocimum sanctum* (Lamiaceae), *Moringa oleifera* (Moringaceae), and *Phyllanthus niruri* (Euphorbiaceae) using PIXE. Biological Trace Element Research, 133(3): 357-363.

Hackett, A. and Macpherson, J. (1965) Some staining procedures for spermatozoa. The Canadian Veterinary Journal, 6(3): 55.

Jabeen, R., Shahid, M., Jamil, A. and Ashraf, M. (2008). Microscopic evaluation of the antimicrobial activity of seed extracts of *Moringa oleifera*. Pak. J. Bot., 40(4): 1349-1358.

JianFeng, C., PengYing, Z., ChengWei, X., TaoTao, H., YunGui, B. and KaoShan, C. (2012). Effect of aqueous extract of *Arctium lappa* L.(burdock) roots on the sexual behavior of male rats. BMC Complementary and Alternative Medicine, 12: 1-8.

Koracevic, D., Koracevic, G., Djordjevic, V., Andrejevic, S., and Cosic, V. (2001) Method for the measurement of antioxidant activity in human fluids. *Journal of clinical pathology* 54(5), 356-361

Kumar, P.S., Mishra, D., Ghosh, G. and Panda, C.S. (2010). Medicinal uses and pharmacological properties of *Moringa oleifera*. International Journal of Phytomedicine, 2: 210-216.

Luqman, S., Srivastava, S., Kumar, R., Maurya, A.K. and Chanda, D. (2011). Experimental assessment of *Moringa oleifera* leaf and fruit for its antistress, antioxidant, and scavenging potential using *in vitro* and *in vivo* assays. Evidence-Based Complementary and Alternative Medicine, 2012: 1-12.

Determination of flavonoids in plant material by HPLC with diode-array and electro-array detections. Journal of Agricultural and Food Chemistry, 48(12): 5834-5841.

Menon, A.G., Thundathil, J.C., Wilde, R., Kastelic, J.P. and Barkema, H.W. (2011). Validating the assessment of bull sperm morphology by veterinary practitioners. The Canadian Veterinary Journal, 52(4): 407-408.

Mughal, M.H., Ali, G., Srivastava, P. and Iqbal, M. (1999). Improvement of drumstick (*Moringa pterygosperma* Gaertn.) a unique source of food and medicine through tissue culture. Hamdard Med., 42(1): 37-42.

NRC (2007). Nutrient requirements of small ruminants: Sheep, goats, cervids, and new world camelids. National Academies Press: Washington, D.C., U.S.A.

Ogbunugafor, H., Eneh, F., Ozumba, A., Igwo-Ezikpe, M., Okpuzor, J., Igwilo, I., Adenekan, S. and Onyekwelu, O. (2011). Physico-chemical and antioxidant properties of *Moringa oleifera* seed oil. Pakistan Journal of Nutrition, 10(5): 409-414.

Ohkawa, H., Ohishi, N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical biochemistry, 95(2): 351-358.

Patel, J., Gami, B., Patel, K. and Solanki, R. (2011). Antibacterial activity of methanolic and acetone extract of some medicinal plants used in indian folklore. International Journal of Phytomedicine, 3:261-269.

Rao, C. and Hart, G. (1948). Morphology of bovine spermatozoa. Am. J. Vet. Res., 9: 117-124.

SAS (2004). SAS/Stat. User's Guide Static's, Ver., 6.06 4th Ed. SAS Institute Inc. Cary, NC.

Singh, B.N., Singh, B., Singh, R., Prakash, D., Dhakarey, R., Upadhyay, G. and Singh, H. (2009). Oxidative DNA damage protective activity, antioxidant and anti-quorum sensing potentials of *Moringa oleifera*. Food and Chemical Toxicology, 47(6): 1109-1116.

Sofidiya, M., Odukoya, O., Familoni, O. and Inya-Agha, S. (2006). Free radical scavenging activity of some Nigerian medicinal plant extracts. Pak. J. Biol. Sci., 9(8): 1438-1441.

Sokunbi, O., Ajani, O., Lawanson, A. and Amao, E. (2015). Antibiotic potential of moringa leaf (*Moringa oleifera* Lam.) crude extract in bull semen extender. European Journal of Medicinal Plants, 9(2):1-8.

Sreelatha, S. and Padma, P. (2009). Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. Plant foods for human nutrition, 64(4): 303-311.

Srinivasan, M., Sudheer, A.R. and Menon, V.P. (2007). Ferulic acid: therapeutic potential through its antioxidant property. *J*ournal of Clinical Biochemistry and Nutrition, 40(2): 92-100.

تأثير المستخلصات المائية لكل من أوراق المورينجا أو جذور الأرقطيون على أكسدة الدهون وسلامة الغشاء البلازمي للحيوان المنوي للكباش المحفوظ على درجة حرارة التبريد

مصطفي عبد الحليم الحرايري[1] ، عبد الخالق السيد عبد الخالق[1] ، وائل أحمد خليل[1] ، عز الدين إبراهيم خليفه[2] ، أيمن يحي الخطيب[3] و أحمد محمد عبد الرحمن.[1]

[1] قسم إنتاج الحيوان - كلية الزراعة - جامعة المنصورة.

[2] معهد بحوث الإنتاج الحيوانى – قسم بحوث الأغنام والماعز- وزارة الزراعة - الدقى – الجيزة - مصر

[3] قسم الكيمياء الزراعية - كلية الزراعة - جامعة المنصورة.

إستهدفت هذه الدراسة بحث تأثير إضافة مستويات مختلفة (صفر ، ١٠٠ ، ٥٠٠ ، ١٠٠٠ ميكرو جرام/مل مخفف) من مستخلص أوراق المورينجا أو جذور الأرقطيون كمضاد أكسدة إلى مخفف السائل المنوي للكباش على خصائص الحيوانات المنوية، وأكسدة الدهون، ونشاط الإنزيمات فى بلازما السائل المنوي المحفوظ على درجة حرارة التبريد (٥°م) لمدة ٤٨ ساعة. استخدم فى هذه الدراسة أربع كباش رحمانى ناضجة لجمع السائل المنوي بإستخدام المهبل الصناعي مره واحدة أسبوعيا ولمدة ٨ أسابيع. تم تقسيم مخفف ليسثين فول الصويا إلى ٧ معاملات هى: المعاملة القياسية (م١)، ١٠٠(م٢)، ٥٠٠ (م٣) و ١٠٠٠(م٤) ميكروجرام من مستخلص أوراق المورينجا لكل مل مخفف أو ١٠٠(م٥)، ٥٠٠(م٦) و ١٠٠٠(م٧) ميكروجرام من مستخلص جذور الأرقطيون لكل مل مخفف. تم تقدير الحركة التقدمية والحيوية والشواذ، وكذلك نسبة الحيوانات المنوية ذات الذيل الملفوف وتم حساب ضرر الأكرسوم. تم تحديد مركبات البوليفينول والفلافونويد فى كل من مستخلص المورينجا والأرقطيون. تم قياس تركيز مضادات الأكسدة الكلى ومالون داي الدهيد ونشاط كل من إنزيم الفوسفات الحامضى واللاكتيك ديهيدروجينز. اظهرت النتائج أن كلا المستخلصين يحتوي على محتوى عالى من معظم المركبات الفينولية فى مستخلص الأرقطيون عن مستخلص المورينجا ، لكن Pyrogallol أظهر أعلى تركيز فى المركبات الفينولية فى كلا المستخلصين. مستخلص الأرقطيون أظهر مستوى عالى من (Narengenin, Rutin, Quercetin, Apigenin and Luteolin) ومستوى منخفض من (Hisperdin, Romarinic acid, Kampferol and Hesperitin) مقارنة بـ مستخلص المورينجا. تحسن كل من من حيوية الحيوانات المنوية وإنخفضت نسبة الشواذ بينما زادت نسبة الحيوانات المنوية ذات الأكرسوم غير السليم فى المعاملة (م٧) بالمقارنة بالمعاملة القياسية (م١). متوسط الحركة الفردية والحيوانات المنوية ذات الذيل الملفوف لم تختلف فى (م٧) بالمقارنة مع (م١). بينما أظهرت النتائج إنخفاضاً فى الصفات المدروسه للحيوانات المنوية فى (م٦) بالمقارنة مع (م١). بزيادة مدة الحفظ تضررت كل الصفات المدروسه للحيوانات المنوية. لم يكن هناك تأثيرا معنوياً للتداخل مابين المعاملات ومدة التخزين على كل خصائص الحيوانات المنوية المدروسة. كان تركيز مضادات الأكسدة الكلى والمالون داى ألدهيد، كذلك نشاط إنزيم الفوسفات الحامضي واللاكتيك ديهيدروجينيز أعلى فى بلازما السائل المنوي لكل المعاملات ولم يكن هناك تأثيراً معنوياً لمدة التخزين أو التداخل بين المعاملات ومدة التخزين على كل من تركيز مضادات الأكسدة الكلى، والمالون داى ألدهيد، ونشاط إنزيم الفوسفات الحامضي واللاكتيك ديهيدروجينيز. أظهرت هذه الدراسة أن إضافة ١٠٠٠ميكروجرام من مستخلص جذور الأرقطيون حسنت من حيوية الحيوانات المنوية وقللت نسبة الشواذ مع حركة تقدمية واستجابة مناسبة لإختبار انخفاض الإسموزية للحيوانات المنوية المخففه والمحفوظة على درجة حرارة التبريد (٥°م) لمدة ٤٨ ساعة بالمقارنة بالمعاملة القياسية أو التى أضيف إليها ١٠٠ أو ٥٠٠ ميكروجرام من مستخلص أوراق المورينجا أو جذور الأرقطيون.