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Effect of Different Percentage of Dried Aloe Vera Leaf Powder on Quality of Boar Semen

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ABSTRACT

Background: Aloe vera is a perennial herbaceous plant that resembles like a cactus that thrives in arid, warm climates. It has been known and used for centuries for its health, beauty, medicinal and skin care properties. Though it is used for treatment of variety of ailments and improves the body's physiology but continued treatment of fresh extract Aloe vera lowered sperm concentration, motility andpercentage viability, resulting in higher sperm abnormalities. The study was carried out to investigate the effect of different percentage of dried Aloe vera leaf powder on sperm quality of crossbred boar (75% Yorkshire x 25% Zovawk) with GEPS extender.

Methods: The study was conducted on three mature, Large White Yorkshire (LWY) and Zovawk (75% LWY and 25% Zovawk) crossbred boars. A total of 45 ejaculates, 15 from each boar were taken for the study. Each ejaculate was combined 1:1 with extender (GEPS) and divided into three aliquots, each of which was diluted with 0%, 5% and10% Aloe vera powder (PH 7.4) and stored for 48 hours at 17°C in a BOD incubator. At 0, 6, 12, 24 and 48 hours after preservation, the stored semen sample was tested for progressive motile sperm by traditional method, live sperm by Eosin-Nigrosin staining, acrosome integrity by Giemsa staining andsperm membrane integrity by HOST test, total protein, sperm membrane protein, cholesterol, ALT and AST level.

Result: The per cent sperm motility, live sperm, acrosomal integrity and plasma membrane integrity of boar spermatozoa was decreased while preserving the semen sample by using GEPS extenders along with 5% and 10% of dried Aloe vera leaf powder at 17°C for 48 hours. The level of total protein, ALT and AST in seminal plasma was increased while preserving the semen sample by using GEPS extenders along with 5% and 10% of dried Aloe vera leaf powder at 17°C for 48 hours. The level of sperm membrane protein and cholesterol of boar spermatozoa decreased while preserving the semen sample by using GEPS extenders along with 5% and 10% of dried Aloe vera leaf powder at 17°C for 48 hours. The total per cent of sperm abnormalities was increased while preserving the semen sample by using GEPS extenders along with 5% and 10% of dried Aloe vera leaf powder at 17°C for 48 hours. In conclusion, the dried Aloe vera leaf powder @ 5% and 10% level having detrimental effect on semen quality of crossbred (75% Large White Yorkshire and 25% Zovawk) boar while preserving with ZEPS extender at 17°C. The quality of boar semen was deteriorated chronologically at 0, 6, 12, 24 and 48 hours preservation with GEPS extender along with 5% and 10% of dried Aloe vera leaf powder at 17°C.

Key words: Aloe vera, Boar semen, GEPS extender, Preservation, Quality.

INTRODUCTION

Aloe vera is a perennial herbaceous plant that resembles like a cactus that thrives in arid, warm climates in Africa, North America, Europe andAsia. Aloe vera is a medicinal plant used for treatment of variety of ailments and improves the body's physiology (Vinson *et al*., 2005). Aloe vera can support its antioxidant function by boosting vitamin C and E levels (Khooshesh *et al*., 2007). Traditional medicine makes use of this herb (Aloe vera) for a variety of purposes, including as a laxative to alleviate constipation in humans (in patients with anal fissures, anorectal and hemorrhoids surgery) and also minor skin irritation, including burns of second-degree from radiation of thermal, bruises andscratches, as well as for pre-planning GIT diagnostic tests, tuberculosis, fungal infection andlowering blood glucose, mild inflammation of the skin, including burns and burns of second-degree from radiation of thermal, bruises andscratches (Talukdar *et al.,* 2023b). For 20 days, rats were fed 100 mg/kg or 200 mg/kg the quantity of stem cells and primary spermatocytes rose significantly compared to the control group (Modweresi and Khodadadi, 2014). Anti-apoptosis factor in Aloe vera extract can alter spermatogenesis directly by affecting germinal cells and

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encouraging cell division, which has likely affected and increased primary spermatocytes and stem cells (Talukdar *et al.,* 2021). It can also have an indirect effect by activating leydig cells and raising testosterone levels (Khooshesh *et al.*, 2007). Vitamin E prevents the loss of leydig and sertoli cells and raises testosterone by containing flavonoids, which were natural plant substances with an estrogen-like structure. This vitamin also enhances seminiferous tubule diameter, testis weight andgerminal epithelium thickness (Estakhr and Javdan, 2011). On the other hand, Oyeyemi *et al.* (2011) reported that in the Dwarf buck of West African, continued treatment of fresh extract Aloe vera lowered sperm concentration, motility andpercentage viability, resulting in higher sperm abnormalities. Luwang *et al.* (2021) reported that the use of Aloe vera leaf powder on a regular basis reduced sperm motility, concentration and viability of sperm in boar. The spermiogram of a boar was negatively influenced by Aloe vera leaf powder (Luwang *et al.*, 2021). In light of the foregoing facts, the present study was carried out to investigate the effect of different percentage of dried Aloe vera leaf powder on sperm quality of crossbred boar semen.

MATERIALS AND METHODS

The study was conducted on three mature, healthy with normal reproductive characteristics, Large White Yorkshire (LWY) and Zovawk (75% LWY and 25% Zovawk) crossbred boars, age ranging from 2.5 to 3 years maintained at ICAR-All India coordinated research project on pig, college of veterinary sciences and animal husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram. During the investigation, all of the experimental animals were given a routine check-up to ensure that they were in good reproductive and general health. The boars were raised individually in separate boar pens in a sanitary environment with adequate feed and drinking water (Talukdar *et al.,* 2023a). The semen was collected twice in a week by using gloved hand method (Shipley, 1999; Laskar *et al.*, 2024). A total of 45 ejaculates, 15 from each boar were taken for the study. The semen sample with mass activity 3+ and 70 or more percentage of sperm motility being chosen for further processing. The fresh sperm sample was separated into three portions, each of which was extended using GEPS extender at a 1:1 (v/v) ratio to achieve a concentration of at least 50 million sperm per ml, then each sample was diluted with 0%, 5% and10% Aloe vera powder (PH 7.4) and stored for 48 hours at 17°C in a BOD incubator. At 0, 6, 12, 24 and 48 hours after preservation, the stored semen sample was tested for percentage of progressive motile sperm (Paul *et al.,* 2024), live sperm by Eosin-Nigrosin staining (Blom, 1950), acrosome integrity by Giemsa staining (Watson, 1975) andsperm membrane integrity by HOST test (Jeyendran *et al.*, 1984). The level of total seminal protein, sperm membrane protein, cholesterol, ALT and AST were determined as per standard method described by Talukdar *et al.* (2015). The data collected from the study were subjected to statistical analysis as per methods described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The mean percentage of motile boar sperms were decreased significantly ($P<0.01$) at 5% and 10% of Aloe vera dried leaf powder at 6, 12, 24 and 48 hours of preservation but not at 0 hour in all the boar *i.e.* immediately after dilution as compared to control group (Table 1). The mean percentage of sperm motility was significantly reduced after mixing with different percentages of Aloe vera in GEPS extender as compared to control group during preservation at 17°C. The present finding was in close agreement with the observation reported by Oyeyemi *et al.* (2011). Owoyemi *et al.* (2015) investigated male catfish treated with Aloe vera gel extract. They found that sperm motility was drastically reduced, when they used 2 and 3% Aloe vera gel. According to Fakhrildin and Sodani (2014), five ìL of fresh Aloe vera leaf gel extract can improve sperm parameters, particularly motility. Barbosa *et al.* (2020) investigated the effect of Aloe vera extract at concentrations of 10% and 20% on the cryopreservation of sperm from domestic cats' epididymis. Sperm motility was shown to be reduced. The large disparity in results could be attributable to the effect of phytochemical present in Aloe vera (Talukdar *et al.,* 2023b).

The mean percentage of live sperm in all the boars were decreased significantly ($P<0.01$) at 5% and 10% of Aloe vera dried leaf powder as compared to control group at 6, 12, 24 and 48 hours of preservation but not at 0 hour *i.e.* immediately after dilution (Table 1). It has been observed that the mean percentage of live sperm was significantly reduced after mixing with different percentages of Aloe vera i.e. 5% and 10% as compared to control group in GEPS extender during preservation at 17°C. The present study was in close agreement with the observation reported by Oyeyemi *et al.* (2011). Owoyemi *et al.* (2015) reported that 2 and 3% of Aloe vera gel reduced the per cent viability of sperm. Barbosa *et al.* (2020) investigated the effect of Aloe vera extract at concentrations of 10% and 20% on the cryopreservation of sperm from domestic cats' epididymis. They found that spermatozoa viability has decreased while using Aloe vera extract.

The mean percentage of acrosomal integrity (intact) of boars' spermatozoa in different hours of preservation were decreased significantly ($P<0.01$) in control, 5% and 10% of Aloe vera dried leaf powder at 0, 6, 12, 24 and 48 hours of preservation (Table 1). It has been observed that the mean percentage of acrosomal integrity (intact) was significantly reduced after mixing with different percentages of Aloe vera *i.e.* 5% and 10% as compared to control group in GEPS extender during preservation at 17°C. The present study was in close agreement with the observation reported Fakhrildin and Sodani (2014) and Talukdar *et al.* (2023a). Tatemoto *et al.* (2006) reported that tannic acid present in Aloe vera blocking hyaluronidase activity and reduced boar sperm viability.

The mean percentage of sperm intact plasma membrane (HOST +ve) in all the boars were decreased significantly (P<0.01) at control, 5% and 10% of Aloe vera dried leaf powder at 0, 6, 12, 24 and 48 hours of preservation (Table 1). Barbosa *et al.* (2020) investigated the effect of Aloe vera extract at concentrations of 10% and 20% on the cryopreservation of sperm from domestic cats' epididymis. They found that the mean percentage of sperm intact plasma membrane was decreased (P>0.05) after thawing in all the treated groups.

The total per cent of sperm abnormalities was increased while preserving the semen sample by using GEPS extenders along with 5% and 10% of dried Aloe vera leaf powder at 17°C for 48 hours (Table 1). The findings of this investigation matched those of Oyeyemi *et al.* (2011), who found that continuous treatment of Aloe vera extract increased sperm abnormalities in the West African Dwarf buck by a substantial (P<0.05). The reproductive capacity of male catfish treated with gel extract of Aloe vera plant was investigated by Owoyemi *et al.* (2015). They also reported that that Aloe vera gel was harmful to the catfish's reproductive potential and could be a cause of infertility. Phytochemical constituents of Aloe vera plant *i.e.* Steroid, Alkaloid, Tannins and Terpenoid could be the reason for detrimental effect on semen quality of crossbred boar while preserved upto 48 hours at 17°C (Luwang *et al.*, 2021).

The mean level of total protein in seminal plasma in boar semen was decreased significantly (P<0.01) while preservation period increased at both 5 and 10% of Aloe vera dried leaf powder and highest level of total protein in seminal plasma was released from the boar spermatozoa while using GEPS extenders along with 10% of dried Aloe vera leaf powder at 48 hours of preservation in 17°C (Table 2). The decreased sperm membrane proteins could be due to sublethal damage caused by active ingredients *i.e.* tanins, tarpenoid and alkaloid present in Aloe vera dried leaf powder that occurred during preservation, resulting in sperm surface protein loss (Lessard *et al.*, 2000; Owoyemi *et al.*, 2015), membrane protein segregation (De Leeuw *et al.*, 1990), inactivation of membrane-bound enzymes andreduced lateral protein diffusion within the membrane (Watson, 1995).

The level of sperm membrane protein of boar spermatozoa decreased while preserving the semen sample by using GEPS extenders along with 5% and 10%

Table 1: Per cent sperm motility, live sperm, intact acrosome, intact plasma membrane, sperm abnormalities of crossbred boar (75% LWY × 25% Zovawk) semen after mixing with different percentages of Aloe vera in GEPS extender during preservation at 17°C (Mean± SE).

Parameters	Hours	Control	5%	10%	F- Values
Sperm motility (%)	0	86.33±1.14	85.66±1.07	85.83±2.00	0.9^{NS}
	6	$71.20±5.14^{B}$	$17.06±1.76^{A}$	$30.00±1.66^{C}$	45.418**
	12	$47.46±1.29^{B}$	$8.66±0.70^{A}$	$28.33±1.93^{C}$	49.892**
	24	$34.00±0.46^{B}$	$1.40±0.40^{A}$	$16.33±1.24^{C}$	99.513**
	48	$10.04±0.38^{B}$	$0.20±0.10^{A}$	$0.13±0.09^{A}$	16.128**
Live sperm (%)	0	88.00±0.81	85.33±1.24	85.66±1.27	1.649^{NS}
	6	$70.33±1.14^{B}$	$20.66±2.43^{A}$	$26.73±1.95^{A}$	24.202**
	12	$50.26±2.65^{B}$	$16.53±1.24^{B}$	$18.40±0.46^{A}$	12.563**
	24	$34.66±1.33^{B}$	$1.93±0.51^{A}$	$10.44±0.62^{A}$	60.780**
	48	$20.75±0.47^{AB}$	$0.16±0.12^{A}$	$2.07±0.66^{B}$	5.388**
Intact acrosome (%)	0	$88.54±0.63^{B}$	$84.55±0.45^{B}$	$62.56±9.38^{A}$	17.827**
	6	$58.00±2.25^{B}$	$38.46±1.45^{A}$	$39.44±2.26^{C}$	23.946**
	12	$44.06±1.29^{B}$	$23.00±1.02^{A}$	$33.73±1.08^{B}$	30.528**
	24	20.93±0.96	7.93±0.82	9.53±0.63	1.666^{NS}
	48	$10.04±0.46^{B}$	$3.93±0.31^{A}$	$7.26±0.45^{B}$	13.476**
Intact plasma membrane (%)	0	46.73±2.94	41.53±3.30	39.50±5.35	0.674^{NS}
	6	$36.06±2.57^{AB}$	$34.00±2.68^{A}$	$30.44±3.69^{B}$	2.983*
	12	$30.00±1.59^{A}$	$23.86±0.73^{B}$	$22.60±1.57^{B}$	31.59**
	24	$21.40±1.03^{A}$	$15.60±1.90^{A}$	$6.60±0.70^{B}$	56.57**
	48	$11.04±1.26^{B}$	$6.33±0.79^{A}$	$6.80±0.70^{A}$	6.623**
Abnormal sperm (%)	0	8.02±0.00	7.38±0.75	7.57±4.87	0.998^{NS}
	6	$11.33±1.75^{A}$	$18.66±2.41^{B}$	$16.66±2.04^{A}$	3.038*
	12	$15.00±2.58^{A}$	$19.00±1.11^{A}$	$27.66±1.81^{B}$	11.224**
	24	$18.00±1.83^{A}$	$38.33±1.59^{B}$	$50.33±2.78^{C}$	38.199**
	48	$18.33±1.86^{A}$	$39.00±2.61^{B}$	$51.55±3.27^{C}$	38.134**

**P<0.01, *P<0.05, NS: Non significant.

Means bearing different superscript in a row differed significantly.

of dried Aloe vera leaf powder at 17°C for 48 hours (Table 2). The decreased sperm membrane proteins could be due to sublethal damage caused by active ingredients *i.e*. Tanins, Tarpenoid and Alkaloid present in Aloe vera dried leaf powder (Lessard *et al*., 2000; Owoyemi *et al*., 2015), membrane protein segregation (De Leeuw *et al*., 1990), inactivation of membrane-bound enzymes andreduced lateral protein diffusion within the membrane (Watson, 1995). The preservation of diluted liquid boar semen induces damages (Waberski *et al*., 2011). Increased extracellular protein levels in extenders could be related to Aloe vera induced sperm membrane disruption and protein leakage during processing and preservation, according to the current study (Luwang *et al*., 2021).

The mean level of cholesterol in boar semen was decreased significantly (P<0.01) while preservation period increased at both 5 and 10% of Aloe vera dried leaf powder and highest level of cholesterol was released from the boar spermatozoa at 10% of dried Aloe vera leaf powder in 48 hours of preservation at 17°C (Table 2). Cerolini *et al.* (2001) made similar observations. As stated by Srivastava *et al.* (2013) and Talukdar *et al.* (2016b), membrane cholesterol has a stabilizing impact on spermatozoa membrane; thus, any change in its quantity is likely to cause membrane reorganisation or destabilisation. According to Haque *et al.* (2019) there was an increase in phospholipids and cholesterol in the seminal plasma of Hampshire boars with increased preservation hours. The alteration in the cholesterol level might be due to damage to their membrane by active ingredients *i.e*. tanins, tarpenoid and alkaloid present in Aloe vera dried leaf powder (Luwang *et al*., 2021).

The mean level of ALT and AST in boar semen was increased significantly (P<0.01) while preservation period increased at both 5 and 10% of Aloe vera dried leaf powder and highest level of ALT and AST was released from the boar spermatozoa at 48 hours of preservation in 17°C (Table 2). The mean activity of ALT and AST in the present study was lower than the values reported by Nath *et al.* (1996) in bull semen and higher than the values reported by Pratap *et al.* (1999); Laskar *et al.* (2024). Transaminase activity (ALT and AST) is a good indication of semen quality (Talukdar *et al.,* 2016a). After cell injury, the mean activity of ALT and AST changed considerably (Talukdar *et al.,* 2017). It might

Table 2: Level of total protein present in seminal plasma, sperm membrane protein, cholesterol, ALT, AST (Mean±SE) of crossbred boars' (75 % LWY × 25% Zovawk) semen after mixing with different percentages of Aloe vera in GEPS extender during preservation at 17°C

Parameters	Hours	Control	5%	10%	F- Values
Total protein (mg/dl)	0	1.43±0.04	1.35±0.01	1.40±0.01	2.312^{NS}
	6	1.71±0.15^{a}	1.75±0.00^{b}	1.75±0.00^{b}	4.092*
	12	1.81±0.11	1.78±0.01^{SY}	1.83±0.17	2.2168^{NS}
	24	2.02±0.11^{ab}	2.28±0.12^{b}	1.87±0.42^{a}	4.273*
	48	2.71±0.04	2.74±0.26	2.77±0.25	0.232^{NS}
Sperm membrane protein (mg/10^{9} spermatozoa)	0	12.57±0.33	13.15±0.37^{ab}	13.36±0.15^{b}	1.357^{NS}
	6	8.97±0.30^{a}	9.55±0.13^{b}	9.91±0.17^{b}	4.732*
	12	7.82±0.25^{a}	9.18±0.20^{b}	8.78±0.20^{b}	9.635**
	24	6.65±0.10^{a}	7.08±0.05^{b}	7.01±0.92^{b}	7.302**
	48	5.65±0.25	6.25±0.05	5.93±0.27	1.967^{NS}
Cholesterol (mg/10^{8} spermatozoa)	0	30.38±1.07	32.11±0.41	51.74±20.11	1.357^{NS}
	6	27.57±0.32	28.46±0.75	27.83±0.26	0.834^{NS}
	12	23.07±0.13	23.05±0.28	23.54±0.74	2.234^{NS}
	24	19.28±0.10	19.25±0.11	19.37±0.09	0.354^{NS}
	48	16.18±0.14	16.22±0.16	16.59±.12	2.416^{NS}
ALT (IU/L)	0	0.81±0.02	0.82±0.03	0.86±0.02	0.758^{NS}
	6	0.73±0.01^{a}	0.85±0.00^{b}	0.86±0.00^{b}	34.641**
	12	5.13±0.15	5.01±0.10	5.30±0.07	1.657^{NS}
	24	10.03±0.23^{a}	10.78±0.16^{b}	10.61±0.13^{b}	4.563*
	48	11.73±0.33^{a}	12.63±0.23^{b}	12.64±0.29^{b}	3.224*
AST (IU/L)	0	12.37±0.30	13.00±0.98	12.75±0.12	2.526^{NS}
	6	43.10±1.16	44.89±1.06	45.52±1.07	1.293^{NS}
	12	57.54±0.28^{a}	57.06±0.26^{ab}	56.22±0.34^{a}	5.01*
	24	87.32±0.51	87.24±1.47	85.83±1.40	0.479^{NS}
	48	95.86±0.44	96.29±0.38	95.81±0.42	0.392^{NS}

**P<0.01, *P<0.05, NS: non significant.

Means bearing different superscript in a row differed significantly.

be due to alterations in mitochondrial sheath with protein loss from the mid piece and an increase in cell membrane permeability with or without rupture (Haque *et al.*, 2018).

CONCLUSION

The dried Aloe vera leaf powder @ 5% and 10% level having detrimental effect on semen quality of crossbred (75% large white yorkshire × 25% zovawk) boar while preserving with GEPS extender at 17°C. The quality of boar semen was deteriorated chronologically at 0, 6, 12, 24 and 48 hours preservation with GEPS extender along with 5% and 10% of dried Aloe vera leaf powder at 17°C.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Institution Animal Ethics Committee of College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram, India.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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