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Effects of asphaltum (Shilajit) on scrotal circumference and semen quality parameters of adult Lohi rams

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Abstract
The present study was designed to investigate the effects of asphaltum on semen characteristics and scrotal circumference of testes of adult Lohi rams. Twelve clinically normal and healthy rams were divided into three groups A, B and C, with four rams in each group. Group A served as control. After one week of acclimatization period, Asphaltum was administered orally to the animals of the groups B and C @ 800 mg/ram and 1600 mg/ram, respectively daily for a period of seven weeks. Experimental trial was continued for nine weeks. Scrotal circumference was measured at the zero and then 9th week of experiment. Semen was collected at the start and then from the 5th week (twice a week), till the completion of the 9th week and was evaluated for physical characteristics. Data thus obtained was subjected to ANOVA, two factor CRD and LSD at 5% level of significance. It was concluded from the present study that asphaltum may lead to increase significantly (P<0.05) in volume, number of sperms, motility percentage, mass activity and scrotal circumference of the testes and decrease in pH and dead percentage of sperms.

Keywords: Asphaltum, Semen parameters, Lohi ram

Introduction
Asphaltum is a yellowish-brown to blackish gummy secretion obtained from the different mountain ranges of the world. It exudates from the layers of rocks especially in the Himalayas and Hindukush mountains of the subcontinent. In contrast with other herbal extracts and plants, it is mostly found on the steep mountains and its nature is influenced by the plant fossils buried under those rocks from which it is secreted, local temperature, humidity and altitude [1-5]. It is composed of at least 85 minerals which are in the ionic form, includes triterpenes and aromatic carboxylic acid. Other ingredients identified in Asphaltum are moisture, gums, aluminium, calcium, potassium, nitrogen, silica, resin, vegetable matter, magnesium, sulfur, iron, chloride, phosphorous, iodine, glycosides, tannic acid, benzoic acid and a number of vitamins and enzymes [3, 5].
Asphaltum is an adaptogen, a compound that improves strength and enhances stamina, and also relieves stress [2, 6].
Asphaltum has been proved to be very effective and potent and has been used frequently in Europe to improve sexual potency, desire and stamina. It is having a natural quality to increase the sensitivity of nerves in the penis. This improvement in sensitization in turn will increase the testosterone production, which results in boosting the sex drive and desire [5]. Also, it helps to pool the blood towards the genital organ, which in turn can make the erections firmer and longer lasting [10]. Asphaltum has been proved in the market as an important "aphrodisiac" medicine [3, 5]. However, there is scarce information in the literature regarding possible effects of asphaltum on sexual potency in animals. Therefore, the present study was planned to investigate the possible positive effects of asphaltum on scrotal circumference and physical semen characteristics of Lohi rams.

Materials and Methods
This study was conducted at the Livestock Production Research Institute (LPRI) Bahadurnagar, Okara. Twelve clinically normal and healthy adult Lohi rams were used. These rams were kept under naturally prevailing climatic conditions. The study period was of nine weeks. They were offered ample quantity of green fodder and drinking water ad-libitum around the clock.

In addition to fodder, concentrate @ 200g/day was also fed to them for the whole experimental period. After one week of acclimatization, rams were divided into three groups A, B and C with four animals in each group. Rams of group A were kept as control, while rams of groups B and C was given asphaltum (packed in capsules) orally @ 800 and 1600 mg/ram/day, respectively, for a period of seven weeks. Scrotal circumference was recorded at the start and then at the completion of 9th week.

Semen was collected by artificial vagina method at day one of the treatment and then from the 5th week onwards twice a week, till the completion of the trial. Semen samples were evaluated for different parameters like volume, mass activity, percent motility, sperm concentration and dead percentage according to the standard protocols described by [7].

Similarly, scrotal circumference was measured with a measuring tape as described by [8]. Data thus obtained was subjected to analysis of variance (ANOVA) techniques, two factor completely randomized design (CRD) and least significant difference test (LSD) at 5% level of significance through a statistical software SPSS.

Results

Results regarding physical characteristics of semen have been presented as follows:

Volume (ml)

There was no significant difference in volume of semen of all the groups at 0 day of experiment. Weekly analysis of data revealed that the volume of semen increased significantly in groups B and C from 5 to 9th weeks of experiment as shown in table1. This increase in volume is due to aspahlatum feeding.

pH

There was no significant difference in pH of semen of all the groups at 0 day of experiment. Weekly analysis of data revealed that the pH of semen decreased significantly in groups B from 5 to 9th weeks of experiment as shown in table 2. However, there was no significant change in pH of semen in A and C groups.

Mass Activity (0 - 5 scales)

The overall mean of mass activity was significantly different in three groups. The mass activity of semen of all the groups at 0 day of experiment were non-significant with each other. At 5th to 9th week of experiment, there was increased in mass activity in groups B and C as in table 3.

Motility percentage

The mean values of motility percentage were non-significant of three groups at first week of experiment. Weekly analysis of data revealed that the motility of perm increased significantly in groups B and C from 5 to 9th weeks of experiment as shown in table 4. The overall mean values of sperm motility of groups B and C were significantly higher as compared to control group.

Dead percentage

The mean values of dead percentage were non-significant of three groups at 0 day of experiment. Weekly analysis of data revealed that the dead number of sperm decreased significantly in groups B and C from 5 to 9th weeks of experiment as in (Table 5). The overall mean values of sperm motility of groups B and C were significantly higher as compared to control group

Sperm count (billion/ml)

There was no significant difference in count of sperm of all the groups at 0 day of experiment. Weekly analysis of data revealed that the count of sperm increased significantly in groups B and C from 5 to 9th weeks of experiment as in Tab. 6.

Scrotal Circumference

The Mean (± SE) values of scrotal circumference of groups A, B and C were 31.00±0.913, 32.50±0.645 and 28.63±0.554, respectively. After the treatment period, the scrotal circumference of the treated groups increased significantly as compared to the control group (Table 7).

Table 1: Means (± SE) values of volume (ml) of control and experimental rams semen for different weeks of treatment.

Weeks	Groups									Mean		
	A			B			C					
0st	0.73	±	0.075efg	0.93	±	0.131de	0.90	±	0.158def	0.85	±	0.071 C
5th	0.58	±	0.048g	0.63	±	0.048fg	0.90	±	0.129def	0.70	±	0.062 C
6th	0.95	±	0.144de	1.45	±	0.065bc	1.43	±	0.160bc	1.28	±	0.097 AB
7th	0.88	±	0.111ef	1.45	±	0.087bc	1.40	±	0.135bc	1.24	±	0.098 AB
8th	0.58	±	0.048g	1.90	±	0.041a	1.53	±	0.075b	1.33	±	0.171 A
9th	0.90	±	0.147def	1.18	±	0.085cd	1.30	±	0.071bc	1.13	±	0.075 B
Mean	0.77	±	0.049 B	1.25	±	0.090 A	1.24	±	0.069 A			

Means with different letters in a row or in a column statistically non-significant (P<0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table: 2: Means (± SE) values of pH of ram semen during different treatment weeks

Week	Group									Mean		
	A			B			C					
0st	7.68	±	0.125a	7.40	±	0.071a	6.88	±	0.240bc	7.32	±	0.130 A
5th	6.78	±	0.263bc	6.23	±	0.075e	6.35	±	0.185de	6.45	±	0.122 C
6th	6.70	±	0.178bcd	6.95	±	0.050b	6.75	±	0.096bc	6.80	±	0.071 B
7th	6.63	±	0.138bcd	6.75	±	0.050bc	6.63	±	0.075bcd	6.67	±	0.053 B
8th	6.68	±	0.075bcd	6.63	±	0.125bcd	6.88	±	0.240bc	6.63	±	0.046 BC
9th	6.68	±	0.063bcd	6.70	±	0.071bcd	6.83	±	0.063bc	6.73	±	0.040 B
Mean	6.85	±	0.095	6.78	±	0.079	6.67	±	0.061			

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 3: Means (± SE) for Mass activity of semen of three groups during different weeks of treatment

Weeks	Groups									Mean		
	A			B			C					
0st	3.00	±	0.000	3.00	±	0.000	3.00	±	0.000	3.00	±	0.000
5th	3.00	±	0.000	3.00	±	0.000	3.00	±	0.000	3.00	±	0.000
6th	3.00	±	0.000	4.00	±	0.000	4.00	±	0.000	3.67	±	0.142
7th	3.00	±	0.000	4.00	±	0.000	4.00	±	0.000	3.67	±	0.142
8th	3.00	±	0.000	4.00	±	0.000	4.00	±	0.000	3.67	±	0.142
9th	3.00	±	0.000	4.00	±	0.000	4.00	±	0.000	3.67	±	0.142
Mean	3.00	±	0.000	3.67	±	0.098	3.67	±	0.098			

Means with different letters in a row or in a column statistically non-significant ($P<0.05$). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 4: Means (± SE) values of motility %age of all the groups during different weeks of treatment

Week	Group									Mean		
	A			B			C					
0st	68.75	±	1.250cd	67.50	±	1.443d	68.75	±	1.250cd	68.33	±	0.711 C
5th	65.00	±	0.000e	70.00	±	0.000c	70.00	±	0.000c	68.33	±	0.711 C
6th	70.00	±	0.000c	80.00	±	0.000b	80.00	±	0.000b	76.67	±	1.421 B
7th	70.00	±	0.000c	80.00	±	0.000b	80.00	±	0.000b	76.67	±	1.421 B
8th	70.00	±	0.000c	85.00	±	0.000a	80.00	±	0.000b	78.33	±	1.880 A
9th	70.00	±	0.000c	80.00	±	0.000b	80.00	±	0.000b	76.67	±	1.421 B
Mean	68.96	±	0.423 C	77.08	±	1.309 A	76.46	±	1.063 B			

Means sharing similar letter in a row or in a column are statistically non-significant ($P>0.05$). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 5: Means (± SE) values of dead sperm %age for rams of three groups during six treatment weeks

Weeks	Groups									Mean		
	A			B			C					
0st	4.83	±	0.118 b	5.38	±	0.239 a	5.00	±	0.204 b	5.07	±	0.123 A
5th	5.00	±	0.000 b	5.00	±	0.000 b	5.00	±	0.000 b	5.00	±	0.000 A
6th	5.00	±	0.000 b	4.08	±	0.111 c	3.88	±	0.095 cd	4.32	±	0.154 B
7th	5.00	±	0.000 b	3.73	±	0.229 de	3.53	±	0.138 ef	4.08	±	0.213 C
8th	5.00	±	0.000 b	2.95	±	0.065 g	3.43	±	0.085 ef	3.79	±	0.266 D
9th	5.00	±	0.000 b	3.08	±	0.085 g	3.25	±	0.096 fg	3.78	±	0.265 D
Mean	4.97	±	0.022 A	4.03	±	0.196 B	4.01	±	0.157 B			

Means sharing similar letter in a row or in a column are statistically non-significant ($P>0.05$). Small letters represent comparison among interaction means and capital letters are used for overall m

Table 6: Means (± SE) values of sperm count during six treatment weeks

Week	Group									Mean		
	A			B			C					
0st	1.21	±	0.053 d	1.25	±	0.033 d	1.24	±	0.017 d	1.23	±	0.020 C
5th	1.20	±	0.072 d	2.21	±	0.020 c	2.20	±	0.030 c	1.87	±	0.145 B
6th	1.21	±	0.073 d	2.26	±	0.024 c	2.30	±	0.034 c	1.92	±	0.155 B
7th	1.22	±	0.077 d	2.30	±	0.020 c	2.34	±	0.033 bc	1.95	±	0.158 B
8th	1.21	±	0.065 d	2.46	±	0.055 ab	2.51	±	0.052 a	2.06	±	0.183 A
9th	1.21	±	0.065 d	2.48	±	0.061 a	2.54	±	0.049 a	2.08	±	0.187 A
Mean	1.21	±	0.025 B	2.16	±	0.089 A	2.19	±	0.092 A			

Means sharing similar letter in a row or in a column are statistically non-significant ($P>0.05$). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 7: Means (± SE) values of scrotal circumference of experimental rams during six weeks.

Week	Group									Mean		
	A			B			C					
0st	31.00	±	0.913 cd	32.50	±	0.645 bc	28.63	±	0.554 e	30.71	±	0.611 B
5th	22.50	±	0.402 f	23.55	±	0.247 f	23.50	±	0.248 f	23.18	±	0.217 C
6th	31.00	±	0.707 cd	33.38	±	0.625 ab	29.38	±	0.625 de	31.25	±	0.601 AB
7th	31.00	±	0.577 cd	33.85	±	0.630 ab	29.63	±	0.718 de	31.49	±	0.628 AB
8th	31.00	±	0.577 cd	34.50	±	0.645 a	30.00	±	0.816 de	31.83	±	0.683 A
9th	31.00	±	0.913 cd	34.50	±	0.645 a	30.00	±	0.816 de	31.83	±	0.716 A
Mean	29.58	±	0.708 B	32.05	±	0.833 A	28.52	±	0.534 C			

Means sharing similar letter in a row or in a column are statistically non-significant ($P>0.05$). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Discussion

Asphaltum is used extensively in Europe to augment the sexual activity. It improves the pooling of blood to the genital area [9, 10]. In this study, there was noticeable increase in the volume of the treated groups animals after the treatment when compared to the control group. The results of present were in accordance to the findings of [11]. This increase in volume may be due to higher amount of testosterone, as the testosterone controls the secretion and synthesis of accessory sex glands [12].

The normal pH of semen varies from 7.2 to 8.0 [13]. The pH of semen is an important factor for the explanation of motility and count of sperm [14]. Graves (1978) [15] reported that good-quality semen is usually on the acid side of neutrality than semen with lower sperm concentration, and semen containing many dead spermatozoa may evolve ammonia, which will increase the pH value. Accordingly, in the present investigation, the significant decrease in pH value in asphaltum -treated group may be due to the decrease of dead sperm and increase sperm concentration.

The mass activity is dark movement of sperms, which was increased significantly in treated animals as compared to control. This improvement in mass activity may be due to more count and motile sperms.

The individual motility of sperm plays an important role in transport of the sperm to site of fertilization and its penetration into zona pelleucida surrounding the egg. The importance of sperm motility has achieved a central role in the routine diagnosis of male fertility [16]. Sperm motility was significantly (P<0.05) higher in treated rams when compared to control and it was maximum at 7th week of experiment. [11] Biswas *et al*. used processed shilajit (PS) in oligospermic patients and examined 12.4 to 17% increase in sperm motility.

The dead sperm percentage in treated groups was significantly decreased from control group. At the end of 9th of experiment dead sperm percentage started to increase in group B while not in group C which may be due to dose difference. This decrease of dead sperm percentage may be due to elimination of free radical as asphaltum plays an important role in the elimination of free radicals [17].

Similarly, mean sperm count was also increased after the treatment which is in accordance to the previously reported results by [9] and [18] who stated that the mean sperm count was increased after the asphaltum treatment. These results are in accordance to the [11], who used shilajit in the oligospermic patients and found that there was increase in sperm concentration up to 37%. As compared to that study these results were quite better because in this experiment 50% increase in sperm concentration was noticed after seven weeks of treatment. In that study, initial sperm concentration was less than 20 million/ml and increase after 90 days of treatment was 37% but in contrast, sperm count was 1.12 billion/ml which increased up to 2.46 billion/ml after the treatment. This difference in the results may be due to species variation, geographical variation or dosage variation of the treatment.

After the treatment, the scrotal circumference of the treated groups increased considerably as compared to the control group. This increase in scrotal circumference is yet be clear. Howevr this due to antioxidant properties of the asphaltum.

Conclusion

It was concluded from the present study that Asphaltum was a quite safe drug to Lohi rams at the given doses of 800mg and 1600mg. At this dose rate it was not only spermatogenic but also increases semen volume, mass activity, sperm motility and sperm count. Its administration also increased the scrotal circumference of the treated rams. It was also concluded that better results are drawn in group C in which it is used @1600 mg/ram. The mechanism of improvement of these semen quality parameters is not yet clear, which demands its clarification in the terms of hormonal profile in future studies.

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Conflict of Interest: The authors have no conflict for the publication of this manuscript in this journal.

Authors' Contribution: First two authors played an active role in designing and conducting this trial. Similarly, data analysis and manuscript preparation was performed by last two authors.

References

1. Ali M, Sahrawat I, O Singh. Phytochemical investigation of Shilajit. Indian J Chem. 2004; 43:2217-2222.
2. Agarwal SP, Khanna R, Karmarkar R, Anwer MK, Khar RK. Shilajit: A review. Phytother Res, 2007; 21:401-405.
3. Khanna R, Witt M, Anwer MK, Agarwal SP, Koch BP. Spectroscopic characterization of fulvic acid extracted from the rock exudate shilajit. Organic Geochem, 2008; 39:1719-1724.
4. Meena H, Pandey HK, Arya MC, Ahmed Z. *Shilajit:* A panacea for high-altitude problems. Int J Ayurveda Res. 2010; 1(1):37-40. doi: 10.4103/0974-7788.59942.
5. Wilson E, Rajamanickamb GV, Dubeyc GP, Klosea P, Musiala F, Sahaa FJ *et al.* Review on shilajit used in traditional Indian medicine. J Ethnopharmacol. 2011; 136:1-9.
6. Carrasco-Gallardo C, Guzmán L, Maccioni RB. *Shilajit*: A Natural Phytocomplex with Potential Procognitive Activity. Int J Alzheimers Dis. 2012. doi: 10.1155/2012/674142.
7. Salisbury GW, Van Demark NL, Lodge JR. Physiology of Reproduction and Artificial Insemination of Cattle. 2nd Ed, Freeman, San Francisco, USA, 1978.
8. Akpa GN, Suleiman IO, Alphonsus C. Relationships between body and scrotal measurements, and semen characteristics in Yankasa ram. Contit J Anim Vet Res. 2012; 4:7-10.
9. Ahmed KA, Venkataraman BV, Mitra SK. Assessment of a polyherbal ayurvedic medicine for sexual activity in rats. Indian Drugs, 1999; 9:576-582.
10. Mirza MA, Alam MN, Faiyazuddin M, Mahmood M, Bairwa R, Mustafa G. Shilajit: An ancient panacea. Intern J Current Pharmaceutical Rev and Res. 2010; 1:2-11.
11. Biswas TK, Pandit S, Mondal S, Biswas SK, Jana U, Ghosh T *et al.* Clinical evaluation of spermatogenic activity of processed shilajit in oligospermia. Andrologia, 2010; 42:48-56.
12. Mann T. Fructose, polylos, and organic acids. In The Biochemistry of Semen and the Male Reproductive Tract; John Wiley and Sons Inc.: New York, U.S.A, 1964, 237-264.
13. Harraway C, Berger NG, Dubin NH. Semen pH in-patients with normal versus abnormal sperm characteristics. Am J Obstet Gynecol. 2000; 182:1045-

1047.
14. Kuo HW, Wang CS, Lai JS. Semen quality in workers with long-term lead exposure: a preliminary study in Taiwan. Sci Total Environ. 1997; 204:289-292.
15. Graves CN. Semen and its components. In Physiology of Reproduction and Artificial Insemination of Cattle, 2nd Ed. Salisbury GW, Van Demark NL, Lodhe JR, Eds. W.H. Freeman and Company: San Francisco, 1978, 247.
16. Aitken R J. Development of *in vitro* tests of human sperm function: a diagnostic tool and model system for toxicological analyses. Toxicol *in Vitro*, 1990; 4:560-569.
17. Bhattacharya SK, Sen AP. Effects of Shilajit on biogenic free radicals. Phytotherapy Research, 1995; 9:56-59.
18. Park JS, Kim GY, Han K. The spermatogenic and ovogenic effects of chronically administered shilajit to rats. J Ethnopharmacol. 2006; 107:349-353.