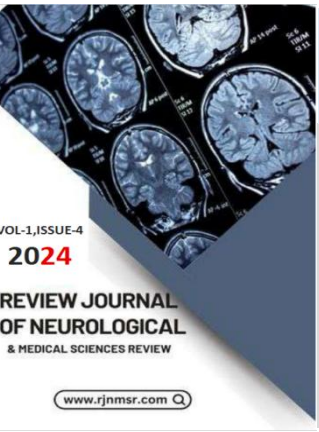

Vol. 3 No. 1 (2025): January - March
https://rjnmsr.com/index.php/rjnmsr/about

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The Antioxidant Effect of Root Bark of Enalai (Zizyphus Oxyphylla) Extract on the Improvement of Bull Semen Quality

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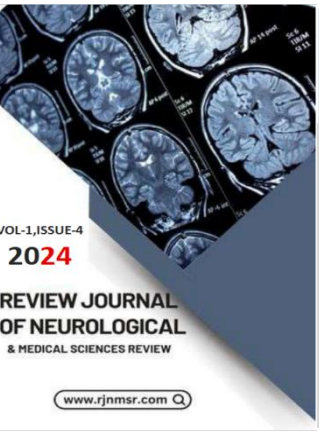
Abstract

Zizyphus oxyphylla is locally called Enalai in Pasthu language. Methanolic extracts of *Zizyphus oxyphylla* (MEZO) root bark possesses antioxidant potential. As reactive oxygen species generate during collection of semen and cryopreservation by thermal and cold shock, causing oxidative stress and thus affecting the quality of semen. The objective of this study was to evaluate the antioxidant effect of MEZO on the improvement of bull semen quality. The research was conducted on Friesian bull semen, collected by artificial vagina at Semen Production Unit (SPU), Harichand, district Charsada, Khyber Pakhtunkhwa, Pakistan. Various inclusions groups (0.5mL, 1mL, 1.5mL, 2mL, 2.5mL and control) of MEZO and semen were extended in egg yolk extenders. Semen quality parameters (spermatozoa motility, spermatozoa viability, spermatozoa acrosomal integrity and spermatozoa plasma membrane integrity) were checked from post-thawed spermatozoa. The semen quality parameters were enhanced significantly ($p < 0.05$) in extended semen containing 0.5 mL extract (mean spermatozoa motility; 68.25, viability; 67.79, acrosomal integrity; 72.17 and plasma membrane integrity; 67.12) compared to control group (the mean spermatozoa motility; 49.21, viability; 50.79, acrosomal integrity; 58.67 and plasma membrane integrity; 50.87) respectively. The extended semen containing 1mL extract were found to be significantly higher in above mentioned parameters compered to control group, however, it was decreased compared to semen containing



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0.5 mL extract. This decline in semen quality parameters was continued in all inclusions above 0.5mL dose dependently. Therefore it can be concluded that Enalai extract (MEZO) in extended semen showed significant antioxidant response that leaded to improve spermatozoa motility, viability, acrosomal integrity and plasma membrane integrity.

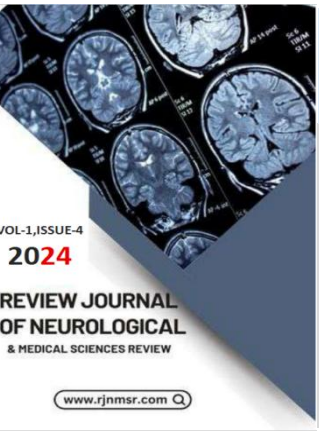
Keywords: *Zizyphus oxyphylla,* antioxidants, Friesian bull semen, oxidative stress.

Introduction

Plants are huge source of drugs. Natural products derived from plants once used by mankind as vast source of all drugs. Clinically 50 % of all drugs derived from natural sources are still in use throughout the world. In which 25 % of drugs are derived from higher plants (Kinghorn and Balandrin, 1993). Four billions of people are using plants and their extracts as drugs in the world (Farnsworth, 1988). Up to 258,650 higher plant species have been reported, out of which only 10% plants are using for medicinal purpose. Plants have diverse bioactive substances which provide foundation of alternative medicine, a common practice in Europe (Rasool Hassan, 2012). These bioactive substances can be used as antioxidant, antimicrobial, anticancer (Altemimi et al., 2017), anti-inflammatory (Rainsford, 2004), anti-aging (Dua and Srivastava, 2016) and anti-fungal agents (Arif et al., 2009). Moreover, these are useful for skin care protection (Ribeiro et al., 2015), enhanced sexual performance (Chauhan et al., 2014), gynaecological disorders, renal ailments, oligospermia (Azu, 2013).

The antioxidant potential of plants is also of great interest and has been tested by a number of researchers in biomedical conditions where oxidative stress was a general phenomenon. Oxidative stress arises, when free radicals are produced in supra physiologic limits in biological medium. Production of free radicals in supra-physiologic limits occurs in cellular damages, cancer, neurodegeneration, disintegration of biological membrane, apoptosis, aging process (Sathisha et al., 2011), inflammatory disease, liver disease, Alzheimer's disease , cataracts (Attanayake and Jayatilaka, 2016).

The family of Rhamnacea consists of 58 genra and 900 species all over the world. In Pakistan, there are found only 6 genera and 21 species of this family. Plants of this family grow in tropic region with warm temperature. Genus Zizyphus consists of 100 species at all but in Pakistan 6 species of this genus are found i.e. *Zizyphus jujuba, Zizyphus mauritiana, Zizyphus nummularia, Zizyphus oxyphylla*, *Zizyphus spina christi* and *Zizyphus sativa*. Economically rhamnaceae family is very important because it has some ornamental facts which produce green and yellow dyes. It is a fine source of timber too. Long long ago when propellants were not discovered the wood of rhamnaceae was used to produce charcoal. On pharmacological point of view, genus *Zizyphus* reported in the study of anti-inflamatory effect, analgesic effect, antidiabetic effect, hepatoprotective effect, antipyretic effect, effect on CNS, free radical scavenging effect, antiulcerinic effect, antimicrobial effect, phytotoxic effect, urease inhibitor effect, anticancer effect and antispasmodic effect. Phytochemically this genus comprises many compounds like cyclopeptide alkaloids, polysaccharides and triterpenoids. Up till now 171 cyclopeptide alkaloids are isolated from



Vol. 3 No. 1 (2025): January - March
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Review Journal of Neurological & Medical Sciences Review

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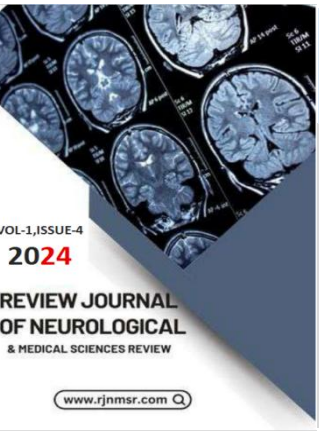
Rhamnaceae family in which 50%, round about 81 cyclopeptide alkaloids are isolated from genus *Zizyphus* (Kaleem et al., 2014).

Zizyphus oxyphylla (ZO) also called Enalai in local Pashtu language (Kaleem et al., 2014). This plant is distributed in Bunir, Swat, Hazara, Dir and Garhi Habibullah in Pakistan. Medicinally it is used in treatment of gastrointestinal disorders, diarrhea, liver disorders, obesity, skin disease, fever, urinary troubles, sleep disorders, jaundice, liver complaints, obesity, diabetes and weakness (Ali *et al*., 2014). Methanolic extract of leaves of *Z. oxyphylla* has marked antinociceptive and antipyretic activities (Nisar et al., 2010). Traditionally it is used in the treatment of inflammatory disease, microbial infections, and allergy and as an antipyretic. The phytochemical study revealed that the extract of this plant is known to contain anthracenedione, alkaloids, flavonoids, tannins, cardiac glycosides, phenolic content, saponins and resins using standard protocols (Kaleem et al., 2012). Almost *Z. oxyphylla* extract was analyzed for cyclopeptide alkaloids as the main constituent (Inayat-Ur-Rahman et al., 2007) & (Kaleem et al., 2012).

Antioxidant and antiglycating activities have also been reported in *Z. Oxyphylla (Ahmad et al., 2013)*. As an antioxidant activity, some phytochemicals i.e. flavonoids like kaempferol and quercetin is isolated which has a DPPH scavenging activity and antioxidant property of the extract mixture of leaves of this plant is shown by antioxidant assay (Ahmad et al., 2016). Prominent antioxidant activity is shown by the ethylacetated leaves extract of *Z. oxyphylla*. *Z. Oxyphylla* mostly contains cyclopeptides alkaloids, the isolated five cyclopeptide alkaloids i.e. oxyphylline D, nummularine C, nummularine R, oxyphylline B and oxyphylline C were tested for antioxidant activity which showed a marked antioxidant activities. Among these five alkaloids the highest antioxidant property was shown by nummularine C and it was clearly reported that the antioxidant effect is dependent on concentration. The 5 isolated cyclopepetide alkaloids have strong antioxidant potential in three in-vitro assays DPPH assay, nitric oxide trapping assay and reducing power assay (Kaleem et al., 2015).

Livestock plays a crucial role in the agro- based economy of Pakistan. It is an integral part of the livelihood of rural population and therefore helpful in poverty alleviation. Almost 8 million families are attached with livestock and earning 35% of income from livestock production activities. It can also play a big role in foreign exchange earnings for the country. Livestock contributed 11.39% in GDP while it contributed 58.33% in agriculture sector during the year 2016-17 and witnessed a growth rate of 3.43% (Ujan et al., 2019). The population growth, urbanization, changed eating patterns, increases in per capita income and export opportunities are fueling the demand of livestock and livestock products in the country. The increase in the total livestock products over the past several years was achieved by increasing the inventory of livestock rather than increase in production per animal. However, the objective is to increase per animal production, explore the livestock sector, its potential for economic growth, food security and rural socioeconomic uplift. Improved and modern animal husbandry practices for production, reproduction and



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Review Journal of Neurological & Medical Sciences Review

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selection of elite class productive animals on the basis of genetic merit can provide opportunity for maximizing livestock production.

Favorable genetic changes in non-descript indigenous breed for enhanced productivity can be induced by adapting modern reproductive biotechnologies/breeding practices. Reproductive biotechnologies include, semen collection, semen processing, cryo-preservation, vitrification, sperm and embryo sexing, artificial insemination, super ovulation, embryo collection and transfer, in vitro fertilization, cloning, transgensis, juvenile in vitro embryo transfer, chimera production, aspiration of oocytes from the live animals, zygote intra-fallopian tube transfer and intracytoplasmic sperm injection (Kakar et al., 2012). Artificial insemination (AI) is the less expensive and easily applicable of the reproductive biotechnologies. It has enormous impact on livestock industry and has got world wide acceptance (Foote, 1981). Artificial insemination is the way to enhance the progeny genetics of the low productive animals for sustainable production and reproduction. It is the process of breeding the animals artificially avoiding the physical contact of male and female animals. For this process, semen from elite genetic animals is collected, processed and stored (frozen) in liquid nitrogen at sub-zero temp (-196˚C). The preserving of semen at sub zero temperature is called as cryopreservation.

During the process of cryopreservation, semen is diluted in extender to get multiple doses from a single ejaculate. The process of dilution and cooling expose the sperm cells to osmotic, thermal and pH shocks. All these shocks are responsible for the production of reactive oxygen species (ROS) in supra physiologic limits. Further, the dilution of semen also dilutes the concentration of natural antioxidative enzymes of semen (glutathione peroxidase, catalase, superoxide dismutase, ascorbic acid, α-tocopherol, β-carotene) and hence the balance between ROS production and natural scavenging potential of semen is disrupted (Ijaz et al., 2009). The phenomenon is called as oxidative stress. A small amount of free radicals are produced as a result of spermatozoa normal metabolism which are necessary for spermatozoa functional integrity. However, the disrupted balance between free radicals production and quenching results in excess of free radicals/ROS. These radicals are very unstable and their excess can induce oxidative stress in seminal plasma. The elevated concentration of free radicals can potentially harm spermatozoa cell membrane, acrosomal membrane, mitochondria and affect the spermatozoa motility, viability and functional integrity (Ijaz et al., 2009) and (Gohar et al., 2014). Semen function is impaired by oxidative stress which causes the lipid peroxdation of the semen plasma membrane. Mitochondria generates ROS which damages the organelles of cytoplasm which results in loss of semen motility, plasma membrane integrity, DNA integrity and semen viability (Aitken et al., 2012).

Addition of antioxidants (synthetic or natural) in feed or directly in semen have been reported to reduce or prevent the production of free radicals and can protect the spermatozoa structural and functional integrity. The crude extracts of strawberry and green tea have been studied for their antioxidative potential in the semen of Sahiwal bull. Improvement in the post-thawed semen quality was observed due to reduction

Vol. 3 No. 1 (2025): January - March
https://rjnmsr.com/index.php/rjnmsr/about
Review Journal of Neurological & Medical Sciences Review
E(ISSN) : 3007-3073
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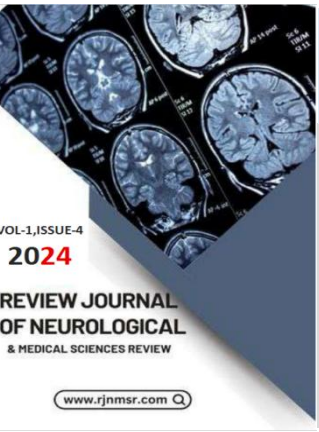
in the concentration of free radicals (Ali et al., 2014). The leaf extract of a tropical plant *Albizia harveyi* has been used in the freezing-thawing process of cryopreserved bull semen. The leaf extract ameliorated the damaging effects of free radicals in a dose-dependent pattern. The antioxidative potential of this extract was due to its polyphenol contents (Sobeh et al., 2017). Vitamin C is a water-soluble antioxidant and its intake prohibits DNA damage. Vitamin E is a fat-soluble antioxidant that neutralizes free radicals and protects cellular membrane against oxygen free radicals. It also prevents lipid peroxidation. (Brigelius-Flohé and Traber, 1999) conducted a study on infertile men. Alpha lipoic acid is a potent antioxidant. Supplementation of extended Nili Ravi bull semen with alpha lipoic acid improved spermatozoa fertility parameters like motility, viability,plasma membrane integrity, acrosomal integrity (Gohar et al., 2014). Similarly the antioxidant butylated hydroxyl toluene can improve the structural and functional integrity of Nili Ravi bull spermatozoa (Ijaz et al., 2009).

Similarly the present study was undertaken to appraise the antioxidant properties of the root bark of *Zizypus oxyphylla* and its effect on the fertility parameters of Friesian bull spermatozoa.

Materials and Methods

The plant Enalai (*Zizyphus oxyphylla*) was collected from Baghcha near Kamrani hill (Dir Lower) in Malakand division. The root barks were separated and washed thoroughly. After shade drying the barks were cut into pieces and grinded with grinder. A total of 100 gm of ground powder was obtained. Previously described protocol (Chan et al., 2007), (Gohar et al., 2014) was followed. 4 grams of powder roots were mixed in 200 ml of methanol and kept at room temperature for 18 hours. The macerates were centrifuged at 6200 rpm for 20 minutes. Total of six inclusions of extract 0.00% (Control) (0.00 ml), 0.25% (0.5 ml), 0.5% (01 ml), 0.75% (1.5 ml), 01% (02 ml) and 1.25% (2.5 ml) were prepared by taking 0.5 ml to 2.5ml from the stock solution.

Previously described standard procedure for semen collection (Andrabi et al., 2008), (Gohar et al., 2014) by artificial vagina at 42 °C temperature were collected from five bulls of uniform breed (Friesian) and diet from Semen Production Unit (SPU) Harichand, District Charsadda, Khyber Pakhtunkhwa, Pakistan. Bulls were nurtured in healthy and hygienic environment. All the five bulls were in good health and were donating healthy semen twice in a week. The semen were transferred to laboratory immediately after ejaculation and kept in water bath at 37°C for some time. The semen were observed for both macroscopic (colour, volume) and microscopic (percent motility and mass motility) characteristics. Semen having motility greater than 65% were selected for this study. Collected semen having motility above 65% were brought to mix with semen extender. The semen extender was prepared by following standard protocol (Khan and Ijaz, 2007), shortly described as glycerol 70 ml, streptomycin 1g, egg yolk 200ml, citric acid 13.4g, Tris-hydrochloric acid 24.2g, fructose 10g and benzyl penicillin 500,000 IU were added in 1000ml distilled water at 37°C and were kept ready for use.



Vol. 3 No. 1 (2025): January - March
https://rjnmsr.com/index.php/rjnmsr/about

Review Journal of Neurological & Medical Sciences Review

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The six inclusion levels of *Z. oxyphylla* root bark extract (0.00 ml, 0.5 ml, 1 ml, 1.5 ml, 2 ml and 2.5 ml) were added into six test tubes, each test tube was labeled for each inclusion respectively. These inclusions were incubated for 30 minutes at 37°C to evaporate the methanol (Chen *et al.,* 2007; (Gohar et al., 2014). After incubation the extended semen inclusion were added in the extract inclusion and allowed for 5 minutes at 37°C to uptake the extracts by spermatozoa. For each sample (extended semen plus extract), 30ml volumes were prepared and a total of 180 ml of volume was made and cryopreserved(kept in liquid nitrogen -196 c) in AI straw of 0.5 ml.

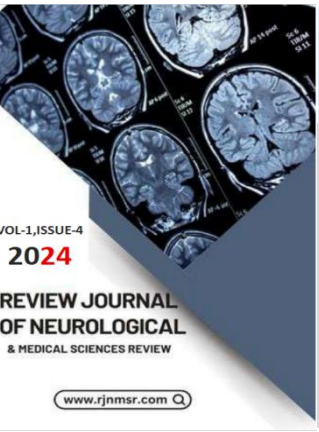
The semen was cryopreserved in 10 liter liquid nitrogen container in lab. Each semen straw (0.5 ml) was adjusted to contain 40 × 10^6 spermatozoa. Each semen straw was thawed in water bath at 37°C for 30 seconds at the time of analysis. 10 semen straws were processed for analysis for each treatment.

Analysis for semen motility was processed just after thawing of semen (Hasan et al., 2001). A small fine drop of each pooled thawed semen was placed on slide and covered with cover slip and observed under phase contrast microscope (40 xs) for percent motility of semen.

Spermatozoa viability was shown by eosin/nigrosin stain. Standard protocol (Mahmood, 2005) was adopted in preparation of this stain. 3g of sodium citrate was dissolved in 100ml of distilled water to prepare 3% Na-citrate solution. Divided it into two equal parts. 1g of eosin was added to one part of the Na-citrate solution and 5g of nigrosin was dissolved in the other half portion of Na-citrate solution. Both portions were incubated at 60°C for 25 minutes. After incubation both portions were mixed with each other and incubated at 37°C and kept them overnight. A small drop of post-thawed semen was placed on slide and mixed with another drop of eosin-nigrosin stain and a thin smear was made by the help of another slide. The slide was air dried and was seen under phase contrast microscope (100 xs). The unstained heads of spermatozoa indicated live while the stained head showed dead spermatozoa.

The spermatozoa plasma membrane integrity was evaluated by hypo-osmotic swelling test (HOST) prescribed by (Adeel et al., 2009). Hypo-osmotic solution was prepared by dissolving 13.51 g of D (-) fructose and 7.35 g of tri-sodium citrate dehydrate in 1 liter of de-ionized water. Thus a 116 mOsm/Kg hypo-osmotic solution was prepared. To bring the osmolarity to 75 mOsm/Kg, distilled water was added and the osmolarity was confirmed by using cryscopic osmometer. Then the test was performed by mixing 0.5 ml hypo-osmotic solution with 500 µL of semen in 1.5 ml Eppendorf tube and kept for 30 minutes in incubator at 37°C. A thin drop was put on slide and covered with cover slip. The slide was seen under phase contrast microscope (100 xs).

For Spermatozoa Acrosomal Integrity, Tryphan Blue Stain was used for intact and damaged acrosome. Procedure established by Jankovicova *et al.,* 2006, a 0.2% solution of tryphan blue was formulated by adding 0.2gm of tryphan blue into 100ml of distilled water. An equal quantity of both tryphan blue and thawed semen were placed on pre-warmed slide and smeared, fixed for 2 minutes with the fixative solution containing 14ml of 37% formaldehyde and 86ml of 1N hydrochloric acid.



Vol. 3 No. 1 (2025): January - March
https://rjnmsr.com/index.php/rjnmsr/about

Review Journal of Neurological & Medical Sciences Review

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P(ISSN) : 3007-3065

Then the slide was air dried and rinsed with tape and distilled water followed by staining with 7.5% of Giemsa stain for 30-35 minutes. Then the slide was rinsed with dist.water and coverd with cover slips. Slide was observed on microscope (100 xs) for intact/damaged apical ridge. The intact acrosomes were light coloured while the damaged acrosmes were violet coloured.

Statistical analysis was conducted with the Statistical Package for Social Science (Windows version 15, SPSS Inc., Chicago, IL, USA). The data was implanted as mean±SD. The data was analyzed using one-way analysis of variance (one-way ANOVA). The group differences were compared by Duncan test. The differences were considered significant at $P < 0.05$.

Results

	Post thawed semen characteristics			
con	**SM**	**SV**	**SAI**	**SPMI**
0.0	49.20±5.90	50.79±14.72	58.67±13.80	50.87±11.35
0.5	68.25±7.13***	67.79±13.33***	72.17±6.86***	67.12±8.80***
1.0	55.62±5.54**	55.91±9.67**	65.25±12.00**	56.04±8.01**
1.5	48.83±6.34	48.91±9.44	54.25±14.50	51.71±7.77
2.0	46.50±6.94	49.41±6.70	47.75±10.30	48.67±10.08
2.5	37.54±9.17	43.41±11.65	43.12±14.32	46.67±11.21

The effect of different concentrations of root bark extracts on semen motility, semen viability, semen acrosomal integrity and semen plasma membrane integrity was determined in table-1. As results indicated that all post thawed semen characteristics (SM, SV, SAI and SPMI) were significantly higher ($p < 0.5$) at low doses of 0.5 ml and 1ml as compare to control group. Significant decreasing orders were observed while the dose was increased from 0.5 ml.

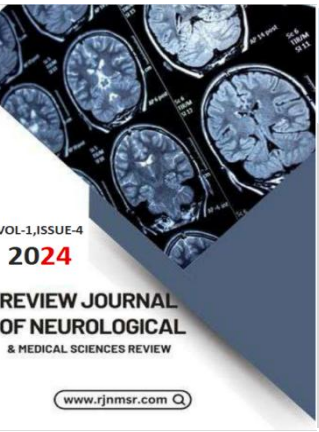
Table-1 Post thawed semen characteristics under various inclusions of root bark extracts of *Zizyphus oxyphylla* in semen extender.

(con, concentration in ml of root bark extract of *Zizyphus oxyphylla*; SM, spermatozoa motility; SV, spermatozoa viability; SAI, spermatozoa acrosomal integrity; SPMI, spermatozoa plasma membrane integrity) values are represented as Mean ± S.E. While stars (***) represent the significant differences ($p < 0.5$) among the groups.

Discussion

Plants contain a broad spectrum of phytochemical substances. These phytochemical substances and their metabolites have anti-inflammatory, anticancer, antibacterial, antifungal and antioxidant properties. These properties can be used for prevention and treatment of various diseases in human and animals and for counteracting or reversing deleterious reactions occurring in biological medium.

The plant *Zizyphus oxyphylla* is known to contain natural antioxidants (Kaleem et al., 2015). If extracted properly, these antioxidants can be used for alleviating oxidative stress exerted by reactive oxygen species in in-vivo and in-vitro. To test this hypothesis, we selected an in vitro oxidative stress phenomenon faced during bovine



Vol. 3 No. 1 (2025): January - March
https://rjnmsr.com/index.php/rjnmsr/about

Review Journal of Neurological & Medical Sciences Review

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semen cryopreservation. During bovine semen cryopreservation, reactive oxygen species are generated by oxidation of unsaturated fatty acids in sperm membrane, dead or abnormal sperms and by activation of an enzyme amino oxidase. These reactive oxygen species when produced in supra physiologic limits can harm the morphologic and physiologic fertility characteristics of sperm resulting in low fertility (Gohar et al., 2014) and (Khan and Ijaz, 2007). So it is assumed that addition of natural antioxidants to semen can decrease the intensity of oxidative stress by decreasing the production, scavenging or detoxifying reactive oxygen species.

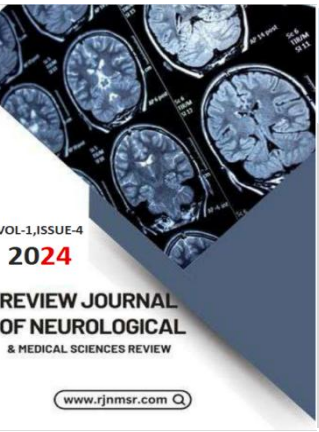
In this research study, the various inclusion levels of root bark methanolic extract of *Zizyphus oxyphyla* (MEZO) were used as a source of antioxidants to protect the sperms from damaging effects of reactive oxygen species and hence improved the fertility parameters (SM, SV, SAI & SPMI) of spermatozoa. Semen samples from five Holstein Friesian (HF) bulls were collected with artificial vagina, processed and filled in 0.5 ml straws according to the standard operating procedure maintained at semen production unit Harichand, Cattle Breeding & Diary Farm (C.B. & D.F) district Charsadda, Khyber Pakhtunkhwa, Pakistan. The straws were then brought to laboratory of physiology, College of Veterinary Sciences and Animal Husbandry, Abdul Wali Khan University Mardan and were assessed for spermatozoa motility, viability, plasma membrane and acrosomal integrity.

The results of our study indicated that methanolic extract of *Zizyphus oxyphyla* has positive effect on sperm fertility parameters at the inclusion levels of 0.5 ml and 1.00 ml. The positive results of the MEZO may be due to the flavonoids content and more specifically may be due to the glycoside of quercetin and kaempferol. As quercetin and kaempferol is isolated from the flavonoid content from the extract of the *Z. oxyhylla* (Ahmad et al., 2016*)*. The extract of this plant contains cyclopeptide alkaloids like nummularine C & R and oxyphylline B & D which were tested for antioxidant activities. Among these nummularine C was proved to have a potent antioxidant activity (Kaleem et al., 2015). Thus providing a rationale for our study that MEZO in low inclusion levels in semen have improved semen quality parameters like motility, viability and integrity.

Other *Zizyphus* species, including *Zizyphus jujube* and *Zizyphus mauritiana* are also reported with potential antioxidants phytochemicals (Rasool et al., 2011) and (Krishna and Parashar, 2013). Being from the same genus, The *Z. oxyphylla* might share some of the constituents with above mentioned species which account for its antioxidant potential.

Previously reported studies on use of natural antioxidants against oxidative stress induced sperm degradation were reviewed. Our research study is in coordination with previously reported study conducted on bull semen to evaluate the antioxidant effect of alpha lipoic acid and strawberry respectively. The inclusion level containing 0.5 mM of alpha lipoic acid and 0.5% strawberry improved semen quality parameters while on increasing the inclusion levels, the decreasing order was seen in the results (Ali et al., 2014, Gohar et al., 2014).

However, the pattern of decrease in fertility parameters was dose dependent as the concentration of the extract of *Zizyphus oxyphylla* exceeded beyond 1.0 ml. The



Vol. 3 No. 1 (2025): January - March
https://rjnmsr.com/index.php/rjnmsr/about

Review Journal of Neurological & Medical Sciences Review

E(ISSN) : 3007-3073
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reason of this damaging effect may be due to the fact that the plants of the genus *Zizyphus* have alkaloids like betulinic acid. These alkaloids posses cytotoxic potential (Jafarian et al., 2014), (Hoshyar et al., 2015). It has also been reported by (Wang et al., 2007) that saponin causes mitochondrial dysfunction thereby inducing oxidative stress through generation of reactive oxygen species. Therefore, the decline in sperm fertility parameters at increasing concentration of MEZO may be attributed to increasing concentration of saponin in the extract.

The extract of *Zizyphus oxyphylla* was prepared in methanol. It has been documented by (Parthasarathy et al., 2006) that methanol generates superoxide anions. The superoxides anions have oxidant nature and may induce lipid peroxidation. It has been mentioned earlier that lipid peroxidation lead to oxidative stress thereby damaging sperm quality. It is therefore evident that at higher concentration of MEZO, methanol will be higher in sperm medium (extender) and will provoke oxidative stress through lipid peroxidation.

Other *Zizyphus* species, including *Zizyphus jujube* and *Zizyphus mauritiana* are also reported with phytochemicals having cytotoxic potential like colubrinic acid, oleanolic acid, coumaroylalphitolic acid, ziziberenalic acid, betulenic acid, anthrquinone glycosides, tannins, saponins, cardiac glycosides, alkaloids and flavonoids (Rasool et al., 2011), (Krishna and Parashar, 2013) and (Kanbargi et al., 2016). Being from the same genus, The *Z. oxyphylla* might share some of the constituents with above mentioned species which account for its antioxidant potential.

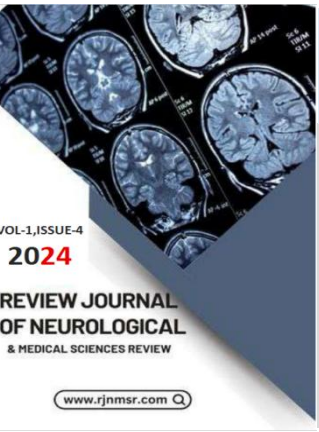
In previously reported study (Ahmad et al., 2016) the cytotoxic effect of some of cyclopeptide alkaloids (oxyphylline A, nammularine R & hemesine) on bacterial and plasmodium cell were checked and the cytotoxic effect was confirmed on both of the cells. The same effect may be applied on the spermatozoa cell and it may be possible that it can cause damage at higher doses and affect the SM, SV, SAI and SPMI.

The present study demonstrated that extract of Zizyphus *oxyphylla* improved post-thawed quality parameters of Friesian bull spermatozoa. This study provides information for improving semen quality by addition of natural antioxidant compounds. However, the results also indicated that the higher concentration of MEZO intoxicated the extending media of sperm leading to the production of reactive oxygen species in supra physiologic limits and hence decreasing the sperm motility, viability, plasma membrane and acrosomal integrity. It is therefore recommended to use advanced biochemical techniques for isolation and purification of active antioxidant ingredients in the plant of *Zizyphus oxyphylla* and the same could be used for enhancement of sperm quality.

References

ADEEL, M., IJAZ, A., ALEEM, M., REHMAN, H., YOUSAF, M. & JABBAR, M. 2009. Improvement of liquid and frozen-thawed semen quality of Nili-Ravi buffalo bulls (Bubalus bubalis) through supplementation of fat. *Theriogenology,* 71, 1220-1225.

AHMAD, R., AHMAD, N., NAQVI, A. A., EXARCHOU, V., UPADHYAY, A., TUENTER, E., FOUBERT, K., APERS, S., HERMANS, N. & PIETERS, L. 2016.



Vol. 3 No. 1 (2025): January - March
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Review Journal of Neurological & Medical Sciences Review

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Antioxidant and Antiglycating Constituents from Leaves of Ziziphus oxyphylla and Cedrela serrata. *Antioxidants,* 5, 9.

AHMAD, R., UPADHYAY, A., AHMAD, M. & PIETERS, L. 2013. Antioxidant, antliglycation and antimicrobial activities of Ziziphus oxyphylla and Cedrela serrata extracts. *European journal of medicinal plants*, 520-529.

AITKEN, R. J., JONES, K. T. & ROBERTSON, S. A. 2012. Reactive oxygen species and sperm function—in sickness and in health. *Journal of andrology,* 33, 1096-1106.

ALI, H., RIAZ, A., GHAFOOR, A., JAVEED, A., ASHRAF, M. & SATTER, A. 2014. Antioxidative protection by Strawberry and green tea extracts during cryopreservation of Sahiwal bull semen. *Pak J Life Soc Sci,* 12, 97-100.

ALTEMIMI, A., LAKHSSASSI, N., BAHARLOUEI, A., WATSON, D. G. & LIGHTFOOT, D. A. 2017. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants,* 6, 42.

ANDRABI, S., ANSARI, M., ULLAH, N. & AFZAL, M. 2008. Effect of non-enzymatic antioxidants in extender on post-thaw quality of buffalo (Bubalus bubalis) bull spermatozoa. *Pakistan veterinary journal,* 28.

ARIF, T., BHOSALE, J., KUMAR, N., MANDAL, T., BENDRE, R., LAVEKAR, G. & DABUR, R. 2009. Natural products–antifungal agents derived from plants. *Journal of Asian natural products research,* 11, 621-638.

ATTANAYAKE, A. & JAYATILAKA, K. 2016. Evaluation of antioxidant properties of 20 medicinal plant extracts traditionally used in Ayurvedic medicine in Sri Lanka.

AZU, O. O. 2013. The sausage plant (Kigelia africana): Have we finally discovered a male sperm booster? *Journal of Medicinal Plants Research,* 7, 903-910.

BRIGELIUS-FLOHÉ, R. & TRABER, M. G. 1999. Vitamin E: function and metabolism. *The FASEB Journal,* 13, 1145-1155.

CHAN, E. W. C., LIM, Y. Y. & CHEW, Y. L. 2007. Antioxidant activity of Camellia sinensis leaves and tea from a lowland plantation in Malaysia. *Food chemistry,* 102, 1214-1222.

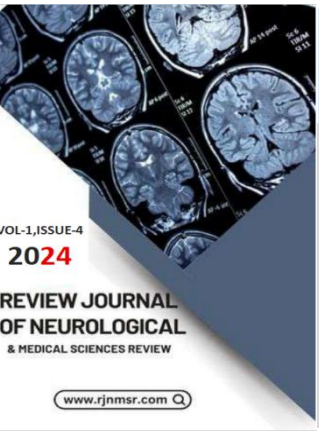
CHAUHAN, N. S., SHARMA, V., DIXIT, V. & THAKUR, M. 2014. A review on plants used for improvement of sexual performance and virility. *BioMed research international,* 2014.

DUA, D. & SRIVASTAVA, N. S. 2016. A study on antioxidant and anti-aging properties of few medicinal plants. *International Journal of Pharmacy and Pharmaceutical Sciences,* 8, 344-347.

FARNSWORTH, N. R. 1988. Screening plants for new medicines. *Biodiversity,* 15, 81-99.

FOOTE, R. H. 1981. *The Artificial Insemination Industry*, New Technologies in Animal Breeding, B.]. Brackett, GE Seidel Ir. and SM ….

GOHAR, A., KHAN, H., YOUSAF, M., AHMAD, J., ALI, Q., KHAN, M., KHAN, D., HAYAT, Y., ALI, F. & AHMAD, I. 2014. Assessment of alpha lipoic acid inclusion



Vol. 3 No. 1 (2025): January - March
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Review Journal of Neurological & Medical Sciences Review

E(ISSN) : 3007-3073
P(ISSN) : 3007-3065

in semen extender on cryopreservation of Nili-Ravi buffalo bull spermatozoa. *Life Sci J,* 11, 45-50.

HASAN, S., ANDRABI, S., MUNEER, R., ANZAR, M. & AHMAD, N. 2001. Effects of a new antibiotic combination on post-thaw motion characteristics and membrane integrity of buffalo and sahiwal bull spermatozoa and on the bacteriological quality of their semen. *Pakistan Veterinary Journal,* 21, 6-12.

HOSHYAR, R., MOHAGHEGH, Z., TORABI, N. & ABOLGHASEMI, A. 2015. Antitumor activity of aqueous extract of Ziziphus jujube fruit in breast cancer: an in vitro and in vivo study. *Asian Pacific Journal of Reproduction,* 4, 116-122.

IJAZ, A., HUSSAIN, A., ALEEM, M., YOUSAF, M. & REHMAN, H. 2009. Butylated hydroxytoluene inclusion in semen extender improves the post-thawed semen quality of Nili-Ravi buffalo (Bubalus bubalis). *Theriogenology,* 71, 1326-1329.

INAYAT-UR-RAHMAN, KHAN, M. A., ARFAN, M., AKHTAR, G., KHAN, L. & AHMAD, V. U. 2007. A new 14-membered cyclopeptide alkaloid from Zizyphus oxyphylla. *Natural product research,* 21, 243-253.

JAFARIAN, A., GHANNADI, A. & MOHEBI, B. 2014. Cytotoxic effects of chloroform and hydroalcoholic extracts of aerial parts of Cuscuta chinensis and Cuscuta epithymum on Hela, HT29 and MDA-MB-468 tumor cells. *Research in pharmaceutical sciences,* 9, 115.

KAKAR, M. A., KAKAR, E., SHAHWANI, M., JAN, M., RAZA, A., HASSAN, J., SAEED, M., BABAR, S. & BALOCH, M. 2012. Reproductive biotechnologies in dairy industry in Pakistan. *J. Anim. Plant Sci,* 22, 84-86.

KALEEM, W. A., MUHAMMAD, N., KHAN, H. & RAUF, A. 2014. Pharmacological and phytochemical studies of genus Zizyphus. *Middle-East J Sci Res,* 21, 1243-63.

KALEEM, W. A., MUHAMMAD, N., KHAN, H., RAUF, A., ZIA-UL-HAQ, M., QAYUM, M., KHAN, A. Z. & NISAR, M. 2015. Antioxidant Potential of Cyclopeptide Alkaloids Isolated from Zizyphus oxyphylla. *Journal of the Chemical Society of Pakistan,* 37.

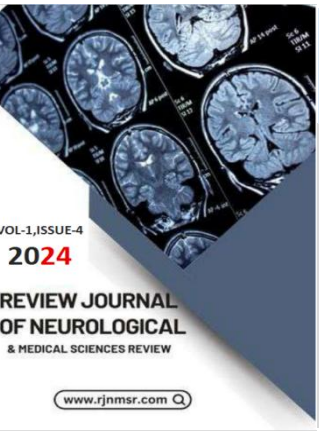
KALEEM, W. A., NISAR, M., QAYUM, M., KHAN, S., ZIA-UL-HAQ, M. & CHOUDHARY, M. 2012. Biologica l screening of oils from Zizyphus oxyphylla edgew. *Pak J Bot,* 44, 1973-1976.

KANBARGI, K. D., SONAWANE, S. K. & ARYA, S. S. 2016. Functional and antioxidant activity of Ziziphus jujube seed protein hydrolysates. *Journal of food measurement and characterization,* 10, 226-235.

KHAN, M. & IJAZ, A. 2007. Assessing undiluted, diluted and frozen-thawed Nili-Ravi buffalo bull sperm by using standard semen assays. *Italian Journal of Animal Science,* 6, 784-787.

KINGHORN, A. D. & BALANDRIN, M. F. 1993. *Human medicinal agents from plants*, ACS Publications.

KRISHNA, H. & PARASHAR, A. 2013. Phytochemical Constituents and Antioxidant Activities of Some I ndian Jujube (Z iziphus mauritiana Lamk.) Cultivars. *Journal of Food Biochemistry,* 37, 571-577.



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https://rjnmsr.com/index.php/rjnmsr/about

Review Journal of Neurological & Medical Sciences Review

E(ISSN) : 3007-3073
P(ISSN) : 3007-3065

MAHMOOD, S. 2005. Effect of cold shock on frozen-thawed spermatozoa of buffalo and cow bulls.

NISAR, M., KALEEM, W. A., QAYUM, M., HUSSAIN, A., ZIA-UL-HAQ, M., ALI, I. & CHOUDHARY, M. 2010. Biological screening of Zizyphus oxyphylla Edgew leaves. *Pak. J. Bot,* 42, 4063-4069.

PARTHASARATHY, N. J., KUMAR, R. S., MANIKANDAN, S. & DEVI, R. S. 2006. Methanol-induced oxidative stress in rat lymphoid organs. *Journal of occupational health,* 48, 20-27.

RAINSFORD, K. 2004. History and Development of the. *Aspirin and related drugs*, 11.

RASOOL HASSAN, B. 2012. Medicinal plants (importance and uses). *Pharmaceut Anal Acta,* 3, 2153-2435.

RASOOL, N., RIZWAN, K., ZUBAIR, M., GULZAR, T., BUKHARI, I. H., GILANI, M. A. & AMJAD, A. 2011. Antioxidant activity of various extracts and organic fractions of Ziziphus jujuba. *International Journal of Phytomedicine,* 3, 346.

RIBEIRO, A. S., ESTANQUEIRO, M., OLIVEIRA, M. B. & SOUSA LOBO, J. M. 2015. Main benefits and applicability of plant extracts in skin care products. *Cosmetics,* 2, 48-65.

SATHISHA, A., LINGARAJU, H. & PRASAD, K. S. 2011. Evaluation of antioxidant activity of medicinal plant extracts produced for commercial purpose. *E-Journal of Chemistry,* 8.

SOBEH, M., HASSAN, S. A., EL RAEY, M. A., KHALIL, W. A., HASSAN, M. A. & WINK, M. 2017. Polyphenolics from Albizia harveyi exhibit antioxidant activities and counteract oxidative damage and ultra-structural changes of cryopreserved bull semen. *Molecules,* 22, 1993.

UJAN, I. A., BHUTTO, A. & ISMAILI, I. A. 2019. SURVEY OF PAKISTAN HEALTH SECTOR. *17th*, 121.

WANG, Y., CHE, C.-M., CHIU, J.-F. & HE, Q.-Y. 2007. Dioscin (saponin)-induced generation of reactive oxygen species through mitochondria dysfunction: a proteomic-based study. *Journal of proteome research,* 6, 4703-4710.