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RESEARCH ARTICLE

Evaluation of Sesame Seed Oil Efficacy Against Oxidative Stress and Sperm Toxicity Induced by Molybdenum in Swiss Albino Mice

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Abstract

Sesame, one of the oldest oil yielding crops also known as "queen of oilseeds", has unique chemical and physiological properties. It shows high antioxidant properties due to the presence of fatty acids, phenolic compounds, lignans, sesamin, episesamin, sesamol, and sesamolin. In order to evaluate sperm toxic effects of Ammonium Molybdate and protective role of Sesame oil, albino mice were divided into four groups, 8 animals in each group. The animals of Group A were provided with distilled water and considered as control group. Group B and C animals were treated orally with ammonium molybdate (100 mg/ kg/b.wt) and Sesame seed oil (1ml /kg/b.wt) respectively for 30 days. Group D animals were treated orally with Ammonium Molybdate (100mg/kg/b.wt) followed by administration of Sesame seed oil (1ml/kg/b. wt). Treatment of ammonium molybdate resulted in a significant decrease in relative testis weight, sperm count, sperm motility and viability with a significant increase in sperm abnormalities. Ammonium molybdate also induced a significant increase in malondialdehyde (MDA) levels in addition to a significant decrease in reduced glutathione (GSH) content and superoxide dismutase (SOD) activity. Administration of Sesame seed oil alone and along with ammonium molybdate revealed a significant improvement in testicular weight, sperm count, motility, viability, sperm morphology and antioxidant status of testis. The results suggest that Sesame seed oil attenuates the oxidative stress induced toxic effects on sperm parameters in molybdate treated mice.

Keywords: Antioxidant, Mice, Molybdenum, Sesame, Sperm.

Introduction

Metal exposure and toxicity has been emerging as a foremost threat to human beings. Due to their ubiquitous nature, men are more likely to be affected by the metal exposure. Exposure of these metals can occur through inhalation, ingestion and skin absorption. The metals are being used widely in our daily routine life. Various industrial effluents, agricultural products, combustion of fossil fuels, waste water from the industries are a leading cause of metal pollution. Heavy metals have been reported to cause detrimental effects on vital organs in animals and humans at high dose levels (Ali *et al.,* 2019). Molybdenum is an essential trace element which is required as a cofactor for the normal functioning of enzymes related to purine and pyrimidines detoxification in the human body. It has been widely used in metallurgical processing, the manufacture of electronic goods, lubricants, pigments, glass ceramics, and nano materials (Mendel and Bittner, 2006). Food and drinking water are the most important source of Molybdenum exposure. Dietary sources of molybdenum are beans, lentils and organ meats and its excessive intake causes gout, diarrhea, anorexia, seizures, infertility, brain damage and anemia. High level of molybdenum exposure can lead to the copper deficiency or Hypocuprosis. It also has therapeutic approach and being used in the treatment of diabetes, Wilson disease, cancer and delayed amyotrophic lateral sclerosis onset (Brewer, 2005; Waern and Harding, 2004). It has been reported that excessive exposure to molybdenum can cause adverse effects on male reproductive tract by affecting normal sperm quality, spermatogenesis (Pandey and Jain, 2015; Lyubimov *et al.,* 2004) and steroidogenesis (Haywood *et al.,*2004) which in turn can lead to increased incidences of male infertility.

Sesame indicum L. belongs to the family Pedaliaceae. The seeds are used in a variety of ways like production of oil, paste, salads and in food formulations. Sesame seed oil is considered as a rich food due to the presence of high

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content of polyunsaturated fatty acids, tocopherols, phytosterols and lignans, such as sesamin, sesamolin and sesamol, minerals including iron, calcium, magnesium, zinc and copper (Prasad *et al.*, 2012). Kanu *et al.* (2010) reported anticancer effects of Sesame in human colon cancer cells. A number of studies have reported that molybdenum exposure resulted in reduced copper content and increased oxidative stress (Haywood *et al.*, 2004; Pandey and Singh, 2002). As Sesame seeds contain excellent amount of copper and show excellent antioxidant properties. Therefore present study has been undertaken to evaluate the protective effects of Sesame against oxidative stress and sperm toxic effects of molybdenum.

Materials and Methods

Ammonium molybdate was purchased from HiMedia Laboratories Pvt. Ltd. Mumbai. Sesame seed oil was purchased from the local market. Healthy male albino mice (*Mus musculus*) weighing about 25-30g was obtained from animal house facility of IIS deemed to be University, Jaipur approved by CPCSEA (Registration No: 1689/PO/a/13/CPCSEA). The animals were kept in plastic cages under standard laboratory conditions with water and food provided *ad libitum*. The experimental protocols were performed in accordance with Guidelines for Ethical Conduct in the Care and use of Animals.

Experimental Protocol

After one week of acclimatization, the animals were divided into four groups (8 albino mice per group) as follow:

Group A (Control group): received distilled water

Group B: 100 mg kg^{-1} bw ammonium molybdate by oral gavage for 30 days

Group C: 1ml/kg bw Sesame oil by oral gavage for 30 days

Group D: 100 mg kg^{-1} bw Ammonium molybdate along with Sesame seed oil (1ml/kg body wt) orally by gavage for 30 days.

Autopsy Schedule

After 30 days treatment, animals were anesthetized via light ether administration. Testes and Cauda epididymides were dissected out. Testes were analyzed for biochemical parameters while sperm parameters were carried out in cauda epididymides tissue.

Parameters of the Study

Following parameters were assessed in each group by standard protocols

Sperm Motility and Density

100 mg of cauda epididymis was taken and was teased gently with the help of needle. Normal saline (2.0 ml) was added to it and was filtered through a nylon mesh. After separation of the tissue from the sperm, one drop of the uniformly mixed sample was put on Neubauer's counting chamber under coverslip and the motility was calculated.

$$\text{Motility (\%)} = \frac{\text{Number of motile sperms}}{\text{Total Number of sperm}} \times 100$$

Sperm count was evaluated by using method of Prasad *et al.* (1972). The sperm suspension was taken in W.B.C. pipette up to 0.5 mark and 5% NaHC03 till the 11 mark. Then a drop of suspension was placed on Neubauer's counting chamber and the number of sperms was counted in 64 small squares. Sperm count was calculated by using formula "Number of spermatozoa = N x 50 x 1000 = X 106/ml".

Sperm Viability

Nigrosin-eosin staining method was used for evaluation of sperm viability. One drop of the Eosin-y (1% aqueous solution) and nigrosin (10% aqueous solution) was taken in a microcentrifuge tube. A thin smear of sperm suspension was prepared and air dried. It was stained with Eosin-nigrosin. After a minute, slide was gently washed with distill water and mounted with glycerin. It was observed under X 400 magnification. The unstained spermatozoa were identified as viable while stained sperms were recognized as dead. Stained and non-stained spermatozoa were counted and percentage of live sperm was determined (Bjorndahl *et al.*, 2003).

Total number of live sperm =

Percentage of live spermatozoa/Total No. of sperm X 100

Morphology of Sperm

A small drop of sperm suspension (5 to 20µl) was taken onto the slide and thin smear was prepared and air-dried. Slides were brought to distilled water after hydration and stained with haematoxylin for 5 minutes. After this it was washed in water and then kept in 1% alcohol for a few seconds and washed in running tap water for bluing. It was then counterstained with 1% Eosin for 1minute and differentiated in 70% alcohol. Then dehydration was done by passing the slides through absolute alcohol followed by clearing in xylene. Lastly the mounting was done in DPX (Bruce *et al.*, 1974). Prepared slides were observed under 100X, oil immersion technique.

Lipid Peroxidation and Antioxidant Defense System Markers

Lipid peroxidation was assessed by quantifying thiobarbituric acid reactive substances (TBARS, Ohkawa *et al.*, 1979). It serves as an indicator of oxidative stress in any tissue. Antioxidant parameters such as Superoxide dismutase (Marklund and Marklund, 1974) and Glutathione (Moron *et al.*, 1979) were analyzed in testes to find out antioxidant capacity.

Statistical Analysis

GraphPad Prism 8.4.0 was used to analyze the data. One-way analysis of variance (ANOVA) was applied to the data set. The data are represented as mean + SEM, and differences were considered as significant when $p < 0.05$; highly significant when $p < 0.01$.

Results and Discussion

Body weight was highly significantly reduced ($p<0.01$) in mice administered with molybdenum when compared with control mice. Both Group C (SO alone) and D (SO + Mo) revealed a significant ($p<0.05$) improvement in the body weight (Table 1). Ammonium molybdate treatment caused a highly significant decrease ($p<0.01$) in the relative testicular weight as compared to the control mice. A significant improvement was noticed in the relative weight of testes in Group C ($p<0.01$) and Group D ($p<0.05$) mice (Table 1).

A significant diminish ($p<0.05$) in sperm count was noticed in Molybdenum treated animals while treatment with Sesame oil alone and along with molybdenum showed a significant improvement ($p<0.05$) in sperm count. Sperm motility was highly significantly increased ($p<0.01$) in group C and D but Mo group showed significant decrement ($p<0.05$) in sperm motility. Sperm viability was found to be significantly reduced ($p<0.01$) in Molybdenum treated group while significant enhanced sperm viability was noticed in group C ($p<0.05$) and group D ($p<0.01$) (Table 2). Fig. 1a shows normal morphology of sperms in control mice. In ammonium molybdate treated mice, sperms were found to be having some abnormalities like bent tail, headless, tailless and banana like appearance (Fig. 1b). Spermatozoa of mice treated with Sesame oil alone showed normal morphology and presence of healthy spermatozoa. Minor abnormalities like headless and bent tail were also noticed in Group D mice (Fig. 1c and d).

Table 3 showing changes in antioxidant parameters in various treated groups. A significant decrease ($p<0.05$) was found in glutathione level in Group B mice. Glutathione level was found to be increased ($p<0.05$) in group C and D mice. Malondialdehyde level was found to be highly significantly ($p<0.01$) increased in Ammonium treated group. A significant reduction in MDA level ($p<0.05$) was noticed in both group C and D mice. Superoxide dismutase activity was also found to be significantly increased ($p<0.05$) in Sesame treated mice and decreased ($p<0.01$) in Ammonium molybdate treated mice.

Body weight acts as a sensitive indicator of the general health status, the reproductive efficiency and growth. Organ weight changes can also be a sign of chemically-induced alteration in overall body weight (Lazic *et al.*, 2020). Normal body weight gain was observed in control mice throughout the experimental period. A significant reduction in body weight was noticed in ammonium molybdate treated mice which may be a sign of a toxic effect of ammonium molybdate. Similar changes in body weight have been noticed in molybdenum treated animals (Pandey and Jain, 2015 and Johnson and Miller, 1963). Reduction in testicular weight observed in Molybdenum treated mice may be correlated with reduced sperm count. It may be attributed to the degeneration of the germinal epithelium, spermatogenesis discontinuation and decline in

Table 1. Changes in body and testicular weight in control and treated mice

Groups / Parameters	Group A (Control)	Group B (Ammonium Molybdate treated)	Group C (Sesame oil treated)	Group D (Ammonium molybdate+ Sesame oil treated)
Body weight gain (gm)	7.8 ± 0.48	2 ± 0.63[b]	5.6 ± 0.50[a]	4.4 ± 0.50[a]
Testis weight (mg)	0.1136 ± 0.0016	0.0848 ± 0.0064[b]	0.1146 ± 0.0026[b]	0.1176 ± 0.0039[a]

Value represents Mean ± SEM

Levels of significance: represents [a] $p<0.05$ Significant; [b] $p<0.01$ Highly significant

Table 2. Changes in sperm parameters in control and treated mice

Groups / Parameters	Group A (Control)	Group B (Ammonium Molybdate treated)	Group C (Sesame oil treated)	Group D (Ammonium molybdate + Sesame oil treated)
Sperm count (million/ml)	4.9 ± 0.019	1.8 ± 0.017[a]	3.4 ± 0.010 [a]	5.49 ± 0.055 [a]
Sperm motility (%)	64.2 ± 3.23	42 ± 6.18 [a]	69.6 ± 4.17 [b]	69.2 ± 2.55 [b]
Sperm viability (%)	58 .4 ± 1.16	30 ± 0.50 [b]	60.8 ± 2.28 [a]	70.8 ± 0.37 [b]

Value represents Mean ± SEM
Levels of significance: represents [a] $p<0.05$ Significant; [b] $p<0.01$ Highly significant

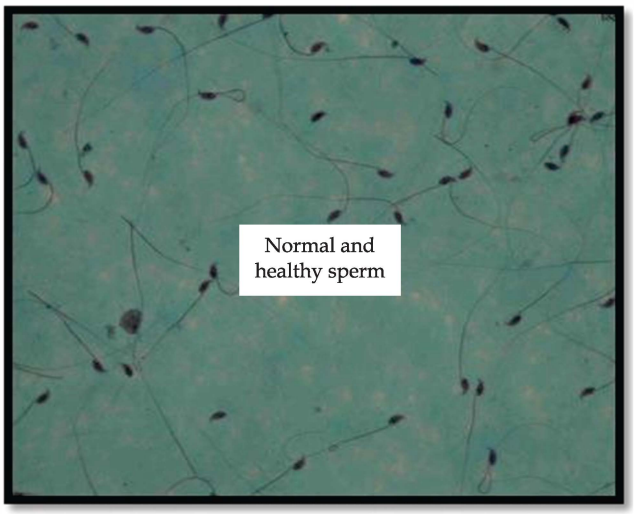

Fig. 1(a). Normal morphology of sperms in control mice (Group A)

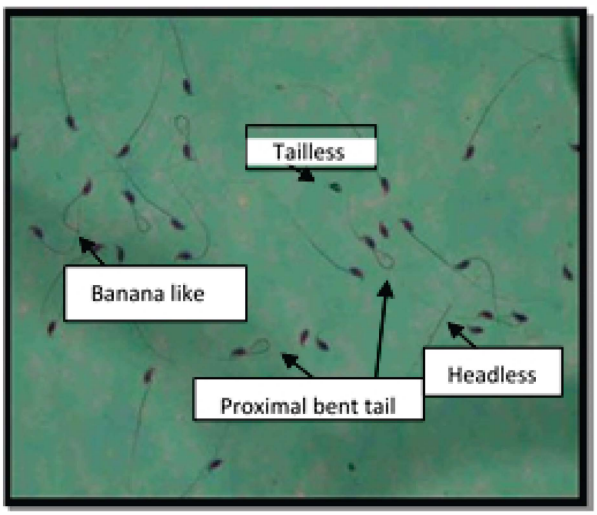

Fig. 1(b). Abnormalities in sperm morphology like tailless, headless, bent tail and banana like sperms in Group B mice treated with ammonium molybdate

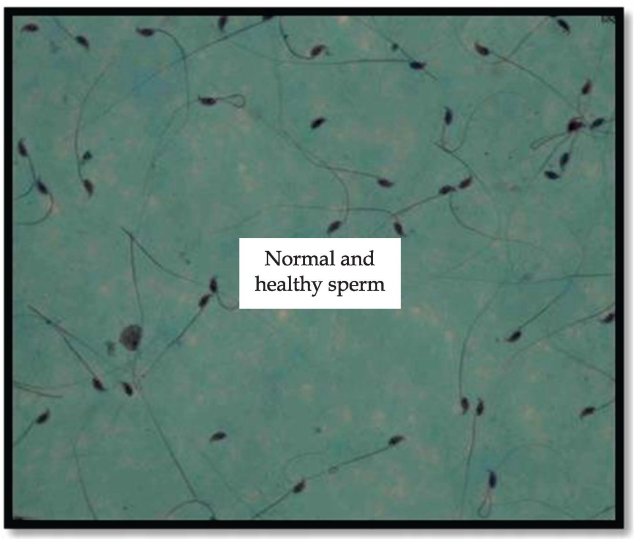

Fig. 1(c). Normal morphology of sperms in Group C mice treated with Sesame oil.

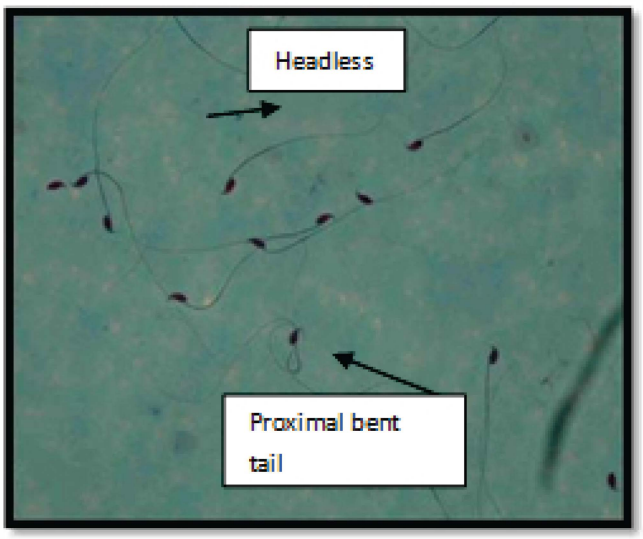

Fig. 1(d). Few abnormalities like headless and proximal bent tail in Group D mice treated with Sesame oil along with ammonium molybdate.

Fig. 1(a-d). Sperm morphology of mice in control and treated groups

Table 3. Changes in antioxidant parameters in various treated groups

Groups / Parameters	Group A (Control)	Group B (Ammonium Molybdate treated)	Group C (Sesame oil treated)	Group D (Ammonium molybdate+ Sesame oil treated)
Glutathione (nmole/mg protein)	2.15 ± 0.0083	0.05 ± 0.0012[a]	2.64 ± 0.1904 [a]	1.61 ± 0.0615 [a]
Lipid peroxidation (n moles MDA/mg protein)	0.15 ± 0.0152	0.43 ± 0.0056 [b]	0.22 ± 0.0134 [a]	0.20 ± 0.0246 [a]
Superoxide dismutase (unit/mg protein)	673 ± 26.62	219.2 ± 11.74 [b]	612.8 ± 40.99[a]	283 ± 19.02 [b]

Value represents Mean ± SEM
Levels of significance: represents [a] $p<0.05$ Significant; [b] $p<0.01$ highly significant

testosterone level (Pandey and Singh, 2002; Zhai *et al.*, 2013). Seasame oil along with ammonium molybdate induced significant increase in testicular weight which may be due to antioxidant potential of Sesame.

Decrease in the level of glutathione and superoxide dismutase activity along with concomitant increased MDA level in testis indicates that Molybdenum induces oxidative stress in testes. A number of studies have been reported similar increased oxidative stress in testis of animals treated with molybdenum (Bersényi *et al.*, 2008, Sharma *et al.*, 2004). Increased oxidative stress by molybdenum might be responsible for deterioration in sperm count, mobility and viability. Zhai *et al.* (2013) suggested that molybdenum reduce the capacity of the testes to eliminate free radicals which may cause damage to the spermatozoa and lead to decline in sperm count or viability. Reduced sperm count, viability and motility may also be correlated with low testosterone concentration for spermatogenesis and supporting normal sperm morphology (Guardo *et al.*, 2020). Diminution in the sperm concentration may possibly due to lowered testosterone level as androgens control secretion of epididymal secretory products which are required to maintain sperm quality (Robaire *et al.*, 2006).

Sesame oil shows an ameliorative effect on sperm parameters in Mice treated with Sesame oil along with molybdate which reflect anti-inflammatory and antioxidant properties of Sesame oil. Sesame lignans inhibit the cytochrome P450 3A-dependent n-hydroxylase pathway of tocopherol catabolism or regeneration of oxidized tocopherol and raise the level of tocopherol which may work against the oxidative damage caused by reactive oxygen species (Parker *et al.* 2000). Such harmonization between Vitamin E and Sesame lignans might be responsible for improvement in the sperm quality. Shittu *et al.* (2008) reported that Sesame improves the sperm density by influencing the hypothalamic pituitary –testicular pathways. Sesame lignans may bind to the estrogen receptors and can also modulate the activity of the androgen receptors in the testis, thereby ultimately stimulate hypothalamo-pituitary-gonadal axis.

Conclusions

Ammonium molybdate treatment can induce significant adverse effects on male reproductive system by deteriorating sperm quality and disturbing antioxidants level. Administration of Sesame seed oil revealed a significant improvement in testicular weight, sperm count, motility, viability, sperm morphology and antioxidant status of testis. The results indicate that Sesame seed oil can improve fertility potential of the male by modulating the sperm parameters due to its antioxidant properties.

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