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Research Article
Ameliorating Potential of Sesame (*Sesamum indicum* L.) On Caffeine Induced Sperm Toxicity in Male Albino Rats

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Abstract

Background and Objective: Globally, men constantly seek medical help for problems associated with fertility. Male infertility has been linked with several factors, therefore, different of approaches are required to resolve this problem. This study evaluated the ameliorating effect of sesame seeds on caffeine-induced sperm toxicity in male albino rats. **Materials and Methods:** Twenty healthy male albino rats of 12 weeks old were used for this study. The 20 male rats were randomly divided into four groups of five rats each using a completely randomized design. The animals were acclimatized for 1 week before the commencement of the treatment. Group A served as control and received only water and pellet feed while Group B received 200 mg kg^{-1} b.wt. of caffeine only. Group C received 1 g kg^{-1} b.wt. of sesame (*S. indicum*) only and group D received 2 g kg^{-1} b.wt. of sesame and 200 mg kg^{-1} b.wt. of caffeine (C+S). The treatments lasted for a period of 65 days. **Results:** There was no significant difference in the weight of testes, sperm viability, semen pH and sperm motility in caffeine treated animals when compared with the control. Results showed statistically significant ($p<0.05$) reduction in weight of epididymis, epididymal sperm count and a concomitant increase in sperm head abnormalities in caffeine treated rats when compared with the control. However, sesame ameliorated the caffeine induced effect in weight of epididymis, sperm count and sperm head abnormalities in albino rat models. **Conclusion:** The results obtained from this study revealed that sesame seeds possess the potential to ameliorating toxicities on weight epididymis, sperm count and sperm head abnormalities induced by caffeine in albino rat as mammalian models.

Key words: Caffeine, sesame, epididymis, ameliorating potential, sperm count, sperm head abnormalities

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Approximately 8% of men across the globe seek medical help for fertility associated problems. Male infertility is caused by a broad variety of etiologies, therefore, different of approaches are needed to resolve this problem[1]. According to the underlying cause, non-surgical or surgical treatments, including hormone therapy with testosterone, human chorionic gonadotropin (hCG), clomiphene citrate and bromocriptine[2] or *in vitro* fertilization (IVF) and intra-cytoplasmic sperm injection (ICSI), may be used to treat an infertile man[3-7]. In addition to the aforementioned treatments, several novel or traditional methods which are different from classic approaches have been used to treat male infertility[8-13].

Caffeine constitutes one of the most constantly consumed psychoactive substances globally and is present in several foods, drugs and beverage products[14-16]. In variance to most other psychoactive substances, it is legalized and unregulated in majority of the countries of the world[17-19] with an estimated 80% of the world's population consuming a caffeine-containing substance daily[14,19]. Caffeine and other methylxanthines are used in clinical medicines as diuretics, analgesics, muscle relaxants and can aid in the treatment of brain disorders such as headache and Parkinson's diseases[20]. High doses cause caffeine dependency with a wide range of unpleasant physical and mental conditions such as nervousness, irritability, restlessness, insomnia, headache and heart palpitations[21]. Consumption of caffeine has also been linked with delayed conception[22], reproductive and developmental toxicities[23-29] and an increase in the frequency of sperm abnormalities[30].

Sesame (*Sesamum indicum* L.) is one of the world's important oil crops. Its primary marketable products are the whole seeds, seed oil and meal. While sesame seeds have been grown in tropical regions throughout the world since prehistoric times, traditional myths hold that their origins go back even further[31]. Sesame oil is mildly laxative, emollient and demulcent. The seeds and fresh leaves are also used as a poultice. Africans use sesame to prepare perfumes and cologne has been made from sesame flowers. Myristic acid from sesame oil is used as an ingredient in cosmetics. Sesame lignans have anti-oxidant and health promoting activities[32].

Sesame is one of the richest sources of lignans including sesamin, episesamin, sesamolin and tocopherol, which are known to have health benefits due to its anti-tumorigenic, estrogenic and/or anti-estrogenic and anti-oxidant properties[33-35]. There are several studies on animal models regarding the effects of sesame on male fertility and sperm quality. Animal studies showed that sesame can improve sperm quality and male fertility[33,34].

Therefore, this study was carried out to determine the ameliorating potential of sesame on the caffeine-induced sperm toxicity in albino rats as a mammalian model.

MATERIALS AND METHODS

Location and duration of study: This study was carried out in the Department of Genetics and Biotechnology, University of Calabar, Calabar, Cross Rivers state of Nigeria. The study lasted for 5 months (February-June, 2018).

Collection and preparation of plant material: Dry and processed sesame seeds were purchased in Akim market, Calabar. The seeds were then blended to powdery form, packed in a container and preserved in a refrigerator throughout the period of the experiment.

Experimental animals: About 20 healthy and sexually mature male albino rats of 12 weeks old were used in this study. The rats were obtained from the Experimental Animal Unit of Department of Genetics and Biotechnology, University of Calabar, Calabar. The rats were housed in conventional wire mesh cages under standard laboratory conditions. They were allowed free access to water and pellet feed throughout the period of the experiment. Generally, the study was conducted in accordance with the recommendation from the declarations of Helsinki on guiding principles in care and use of animals and the local ethical committee (Approval number: CRS/MH/CGS and EH/Vol.1/49).

Experimental design and procedure: About 20 male rats were randomly divided into four groups of five rats each using a completely randomized design. The animals were acclimatized for 1 week before the commencement of the treatment. Group A served as control and received only water and pellet feed while Group B received 200 mg kg^{-1} b.wt. o f caffeine only. Group C received 1 g kg^{-1} b.wt. of sesame (*S. indicum*) only and group D received 2 g kg^{-1} b.wt. of sesame and 200 mg kg^{-1} b.wt. of caffeine (Table 1). The rats were sacrificed under chloroform anaesthesia 24 h after the last treatment which lasted for 65 days. The epididymis and testes were dissected out and weighed using Scout Pro SPU 601 electronic weighing balance. The epididymis were processed for epididymal sperm motility, viability, count and sperm head abnormality, semen pH and sperm motility: Immediately after dissection, a puncture was made in the epididymis with a sterile pin.

Table 1: Protocol for treatment of experimental animals

Groups	Treatments	Description of treatment
A	Control	No caffeine, no sesame
B	C	200 mg kg^{-1} b.wt., of caffeine orally, daily
C	S	1 g kg^{-1} b.wt. of sesame orally, daily
D	S+C	2 g kg^{-1} b.wt. sesame+200 mg kg^{-1} b.wt., caffeine both orally, daily

Table 2: Effect of sesame on male rats exposed to caffeine

Parameters	Control	C	S	S+C
Weight of testes (g)	1.34±0.02[a]	1.26±0.03[a]	1.36±0.04[a]	1.26±0.02[a]
Weight of epididymis (g)	0.54±0.02[b]	0.41±0.02[a]	0.53±0.02[b]	0.51±0.03[b]
Semen pH	7.23±0.03[a]	7.15±0.03[a]	7.26±0.02[a]	7.20±0.04[a]
Sperm count (x10^6 mL^{-1})	7.18±0.31[a]	6.02±0.12[b]	7.55±0.46[a]	7.01±0.35[a]
Sperm motility (%)	80.11±0.68[a]	80.30±1.45[a]	81.41±1.10[a]	85.41±1.62[b]
Sperm viability (%)	88.10±1.12[a]	86.46±0.68[a]	89.93±2.05[a]	88.15±2.26[a]
Sperm head abnormality (%)	6.76±0.20[a]	7.80±0.47[b]	6.84±0.05[a]	6.88±0.20[a]

*Means with different superscript letters along each horizontal array differ significantly ($p<0.05$)

The semen smeared on the pin was rubbed on a pH paper of range 4.0-10.0. The colour change corresponds to the pH and was read from the paper. Two drops of sperm suspension was put on a microscope slide and cover slip was placed. The number of progressively motile cells was divided by the total number of spermatozoa counted under x40 lenses and expressed as a percentage[36].

Sperm viability: The sperm viability test was determined using "Eosin-Nigrosin one-step staining technique"[36]. A portion of the sperm suspension was mixed with equal volume of Eosin-Nigrosin stain and five air-dried smears were prepared on glass slides for each sample. The slides were examined for percentage viability. Normal live sperm cells excluded the stain and appeared whitish, whereas dead sperm cells took up stain and appeared pinkish. Percentage viability was calculated based on the number of live sperm cells out of the total number of sperm cells observed.

Sperm count: The epididymal sperm samples were obtained by macerating known weights of cauda epididymis in physiological saline in the ratio of 1:10 weight by volume. After vigorous pipetting to release the sperm cells. The suspension was filtered using an 80 µm stainless mesh. Epididymal sperm count was obtained by cytometry using the improved Neubauer cytometer (Model: BR723014) and was expressed as m mL^{-1} of suspension[37].

Sperm head abnormality test: A portion of the sperm suspension was mixed with 1% eosin Y solution (10:1) for 30 min and air-dried smears were prepared on glass slides for the sperm head abnormality test. The slides were examined for percentage sperm head abnormalities in every 200 sperm cells observed on each slide and five air-dried smears were prepared on glass slides for each sample. The percentage of sperm head abnormality was calculated according to Ekaluo *et al.*[18].

Statistical analysis: Data from weight of testes and epididymis, epididymal semen pH, motility, viability, count and sperm head abnormality were subjected to one-way Analyses of Variance (ANOVA) test at 5% level of significance while differences in means were separated using Least Significant Difference (LSD) test.

RESULTS

Weight of testes and epididymis: There was no significant difference ($p>0.05$) in the weight of the testes of animals treated with both caffeine and sesame seeds when compared with the control. However, significant difference was observed in the weight of epididymis. A significant decrease ($p = 0.05$) in the weight of epididymis was obtained in animals treated with caffeine when compared to the control (0.54 g) as shown in Table 2. The epididymal weight significantly increased ($p<0.05$) in group of animals treated with combination of caffeine and sesame indicating ameliorating potential of sesame seeds.

Semen pH and sperm count: Results presented in Table 2 showed that there was no significant effect of the treatments on the semen Ph. A significant reduction ($p<0.05$) was observed in the sperm count of animals treated with caffeine alone when compared to the control. The sperm count was

increased in C+S group indicative of the ameliorating potential of sesame seed.

Sperm motility and viability: There was no significant difference in sperm motility of rats treated with caffeine when compared to the control and sesame group as presented in Table 2. Results obtained showed that sperm motility significantly increased in C+S group. The results also indicated no significant difference in the percentage of viable sperm cells in all the treatment groups when compared with the control (Table 2).

Sperm head abnormality: Result obtained on the effect of the treatments on sperm head abnormalities is presented in Table 2. Rats treated with caffeine alone had the highest percentage of sperm head abnormalities when compared to the control and those treated with sesame only. The percentage of sperm head abnormalities significantly decreased ($p<0.05$) in C+S group implying that sesame seeds possess ameliorating potential against caffeine induced sperm head abnormalities.

DISCUSSION

Results of this study showed that caffeine had a significant effect ($p<0.05$) on some of the sperm parameters studied. The weight of epididymis and sperm count significantly ($p<0.05$) reduced in caffeine treated animals when compared with the control. This agrees with the findings of Uno *et al.*[38], Ekaluo *et al.*[24,26] and Smith[16], they observed spermatotoxic effect of caffeine on sperm quality.

The reduction in the sperm parameters observed as a result of caffeine treatment might be due to its effect on spermatogenesis in the animals which is supported by Ezzat and El-Gohary[39], who posited that long term intake of caffeine impedes spermatogenesis. In the same vein, Ikpeme *et al.*[40] reported that disruptions in fertility in male mammals has direct correlation with disruptions in spermatogenesis. Therefore, this suggested that caffeine treatment might have distorted spermatogenic processes and pathways with a resultant reduction in sperm count and weight of epididymis. The reduction in sperm count corroborates the decrease in weight of epididymis observed in group of rats treated with caffeine alone which could also imply disruptions in sperm maturation in the epididymis.

Also, degenerative changes in testicular histological of rats have been reported to cause a decline in the testosterone levels and consequently distorts spermatogenesis[41,42]. This might be the underlying cause of the significant reduction in the sperm count and weight of epididymis observed in caffeine treated animals.

Results obtained also revealed a significant ($p<0.05$) increased in sperm head abnormalities in caffeine treated animals which is indicative of induced mutation on the sperm cells during the spermatogenic processes in line with the findings of Smith[16], Glover and Assinder[43], Uno *et al.*[38] and Ikpeme *et al.*[40]. However, sesame seeds significantly ameliorated the effect of caffeine in rats treated with a combination of caffeine and sesame (C+S group). The ameliorating potential of sesame could be due to its rich phytonutrients such as lignans including sesamin, episesamin, sesamolin and tocopherol, which have been reported to have health benefits due to its anti-tumorigenic, estrogenic and anti-oxidant properties[33-35]. Anti-oxidants prevent oxidative stress and testicular degeneration with its resultant effect on spermatogenesis and sperm profile. Caffeine has been reported to cause significant decline in anti-oxidant defense system, increased free radical activities and consequently resulting in oxidative stress[24,28]. Increase in active oxygen species (ROS) level and oxidative stress have been shown to correlate with decreased sperm count and motility[44]. Epidemiological studies have revealed that consuming vitamins and mineral containing foods, fruit and products such as sesame as well as their extracts reduced free radical oxidative damage[45] and its effect on sperm profile.

CONCLUSION

The findings of this study indicated that sesame seedshave the potential to ameliorating caffeine induced toxicities on weight epididymis and sperm count and sperm head abnormalities in albino rat models.

SIGNIFICANCE STATEMENT

This study discovered the sesame seeds that can be beneficial for ameliorating the harmful effect of caffeine consumption on sperm profile. Findings of this study will help the researchers to uncover the critical areas of malefertility enhancement that many researchers were not able to explore.

Thus a new theory on spermatotoxicity and pro-fertility as well as amelioration potential of many commonly used plants may be arrived at.

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