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Protective Role of Onion (*Allium cepa* L.) on Caffeine Induced Spermatotoxicity in Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author UBE designed the study, wrote the protocol, interpreted and formatted the final manuscript. Author UUU wrote the first draft of the manuscript. Author NEE performed the statistical analysis and proof reading. Author PBE carried out laboratory experiments under the supervision of author SEE. While author BOV did literature search. All authors read and approved the final manuscript.

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ABSTRACT

Onion is widely consumed and is used for several medicinal and therapeutic purposes such as management of diabetes and also as antimutagenic, antimicrobial and antioxidant agents. Hence, this study investigated the protective role of onion on caffeine induced spermatotoxicity in albino rats as mammalian model. Thirty healthy male albino rats of 12 weeks old were divided into five groups with six rats in each group using a completely randomized design (CRD). The daily caffeine and onion juice treatments were administered orally via oral gavage for sixty five days. Result obtained indicated a significant ($P<0.05$) reduction in the weight of epididymes, sperm viability, sperm motility and sperm count while sperm head abnormality increased in caffeine-treated animals. No significant differences were observed in semen pH and weight of testes. Onion juice protected mammalian model from caffeine induced toxicities on the weight of testes, sperm viability, sperm motility, sperm count and sperm head abnormality. These results show that onion is effective in protecting albino rat models against caffeine induced spermatotoxicity in a dose dependent manner.

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Keywords: Onion; caffeine; toxicity; sperm quality; sperm count; sperm head abnormality.

1. INTRODUCTION

Allium is the most important genus of the family *Lilliaceae* from the dietary standpoint. They are potent in lowering cholesterol and act as anti-carcinogens [1]. It includes members like onion (*Alliun cepa*), shallot (*A. ascalonicum*), leek (*A. porrum*), garlic (*A. sativum*) and Welsh onion (*A. fistulosum*) [1,2].

Onion (*Alliun cepa* L.) is grown primarily for use as food, adding flavor and taste. Young, green leaves with their white bases are also eaten raw in salads. It has several medicinal importances such as; in the management of asthma and diabetes; and their complications [1,3], also as antimicrobial, antiplatelet, antioxidant and antimutagenic agent [1,3-5]. Onion contains plenty of enzymes, vegetal hormone (glycoquinine), trace elements, vitamins (A, B-complex, C, E) and flavonoids [1]. It is also used as aphrodisiac, vermifuge, blood thinner, alkalinizer, hypotensive and diuretic agent [3], as well as decongestant, heart and arterial protector [1].

Caffeine is one of the world's most widely consumed psychoactive substances and is present in several foods, drugs and beverage products such as energy drinks, coffee and tea [6-8]. Unlike most other psychoactive substances, it is legal and unregulated in most part of the world [9-11] with an estimated 80% of the world's population consuming a caffeine-containing substance daily [6,10]. Caffeine dependency has a wide range of unpleasant physical and mental conditions such as nervousness, irritability, restlessness, insomnia, headache and heart palpitations [12].

Consumption of caffeine has also been linked with delayed conception [13], reproductive and developmental toxicities [14-16] and increase in the frequency of sperm abnormalities [9,11,16,17].

Many medicinal plants with antioxidant properties have been shown to confer protective effects from toxicities induced by substances such as caffeine [14-16,18-20]. It is on this premise that this study set out to ascertain the protective role of onion (*Allium cepa* L.) on caffeine induced spermatotoxicity in albino rat as a mammalian model.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Onion Juice

The onion bulbs were obtained from Watt market, Calabar, Cross River State of Nigeria and authenticated at the Department of Botany, University of Calabar, Calabar. The onion bulbs were washed thoroughly, weighed and cut into small pieces and then liquidized into onion juice using mammonlex juice extractor, model: JD 1004. The onion juice was stored in glass bottles and preserved in a refrigerator at 2-4℃ until used for the experiment.

2.2 Chemicals

All chemicals used in the course of the study were of analytical grade.

2.3 Experimental Animals

Thirty healthy young adult male albino rats of 12 weeks old with average body weight of 182.5 g ranging from165-215 g were obtained from the animal house of the Department of Zoology and Environmental Biology, University of Calabar, Calabar. The rats were housed in well ventilated 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 recommendations from the declarations of Helsinki on guiding principles in care and use of animals and the local ethical committee.

2.4 Experimental Design and Procedure

The thirty rats were divided into five groups of six rats each using a completely randomized design. The daily treatments were given orally via oral gavage which lasted for 65 days and the protocol is shown in Table 1. The rats were sacrificed under chloroform anaesthesia 24 hours after the last treatment. The epididymes and testes were dissected out and weighed using Scout Pro SPU 601 electronic weighing balance. The epididymes were processed for epididymal sperm count, motility, viability and sperm head abnormality.

Table 1. Protocol for treatment of experimental animals

Treatment group	Description of treatment
Control	No caffeine and no onion juice
C	Caffeine, 200 mg kg^{-1} BW only via oral gavage
OJ_{250}	Onion juice, 250 mg kg^{-1} BW in 2mL via oral gavage
C+ OJ_{250}	Caffeine, 200 mg kg^{-1} BW and onion juice , 250 mg kg^{-1} BW in 2 mL via oral gavage
C+OJ_{500}	Caffeine, 200 mg kg^{-1} BW and onion juice , 500 mg kg^{-1} BW in 2 mL via oral gavage

2.4.1 Semen pH

Immediately after dissection, a puncture was made in the epididymes with a sterile pin. The semen smeared on the pin was rubbed on a pH paper of the range 4.0-10.0. The colour change corresponding to the pH of the semen was read from the paper.

2.4.2 Sperm motility

The sperm motility was evaluated according to the method of Ekaluo et al. [14,15]. Two drops of sperm suspension were put on a microscope slide and cover slip was placed on it. The number of progressively motile cell was recorded and divided by the total number of spermatozoa counted under x40 objective lens and expressed in percentage.

2.4.3 Sperm viability

A portion of the sperm suspension was mixed with 1% eosin Y solution (10:1) for 30 minutes and smeared on glass slides. The air-dried smears were washed with physiological saline and counter stained with 1% Malachite green stain. The slides were examined for percentage viability. Normal live sperm cells appeared colourless, while dead sperm cells took up stain and appeared pinkish in greenish background. The percentage viability was calculated based on the number of live sperm cells out of the total number of cells observed.

2.4.4 Sperm count

Epididymal sperm count was obtained by cytometry using the improved Neubauer Cytometer and will be expressed in million/ml of the sperm suspension [21].

2.4.5 Sperm head abnormality

A portion of the sperm suspension was mixed with 1% eosin Y solution (10:1) for 30 minutes and air-dried smears prepared on glass slides for the sperm head abnormality test. The slides were examined for percentage sperm head abnormalities in every 200 spermatozoa observed on each slide for each sample. The percentage of sperm head abnormality was calculated according to Ekaluo et al. [11].

2.5 Statistical Analysis

Data obtained from weight of testes and epididymes, semen pH, sperm motility, viability, count, and sperm head abnormalities were subjected to analysis of variance (ANOVA) test. Statistical significance were considered if $p<0.05$ while least significant difference (LSD) test was used to separate the means.

3. RESULTS

3.1 Weight of Testes and Epididymes

There was no significant difference ($P>0.05$) in the weight of the testes of the animals. However, caffeine significantly ($P<0.05$) reduced the weight of epiddymes. The caffeine treated group had the lowest weight (0.49 g), closely followed by the C+ OJ_{250} group (0.50 g). The weight of the epididymes significantly increased in the C+OJ_{500} group indicating a dose dependent protective role of onion juice. The highest weight of epididymes was obtained in the OJ_{250} group (0.74 g) while the control group recorded 0.64 g (Table 2).

3.2 Semen pH

The treatments did not significantly affect the pH of the semen of the animals. It was within the range of 6.70 to 7.02 as shown in Table 2.

3.3 Sperm Motility

As shown in Table 2, sperm motility was significantly reduced in the caffeine group (65.06%) compared with the control (72.38%). A significant ($P<0.05$) increase was observed in C+ OJ_{250} and C+OJ_{500} groups (69.78 and 71.53% respectively) indicative of the protective role of

onion juice. The highest value was obtained in the OJ_{250} group (76.53%).

3.4 Sperm Viability

There was a significant ($P<0.05$) reduction in the percentage of viable sperm cells in caffeine treated animals when compared to the control (89.12%). The onion juice significantly protected and increased sperm viability in the groups treated with onion juice in a dose-dependent manner from caffeine induced toxicity as shown in Fig. 1 and Table 2. The sperm viability was increased from 74.18% in caffeine group to 93.49% in OJ_{250} group with the following trend: C < C+ OJ_{250} < C+OJ_{500} < control < OJ_{250}.

3.5 Sperm Count

The sperm count was significantly ($P<0.05$) reduced by the caffeine treatment when compared with the control. Fig. 1 and Table 2 show that the caffeine group has the lowest value of 5.80 x10^6/ml and the onion juice protected the rats from the effect of caffeine in C+ OJ_{250} and C+OJ_{500} groups (6.99 and 7.330x10^6/ml) respectively in a dose-dependent manner with the following trend: C < C+A_{250} < C+A_{500} < control < A_{250}.

3.6 Sperm Head Abnormality

The sperm head abnormality was also significantly ($P<0.05$) increased by the caffeine treatments as shown in Table 2. The percentage of sperm head abnormalities were significantly reduced from 9.24 to 3.66%; indicative of the protective role of onion juice in a dose-dependent manner with the following trend: C < C+A_{250} < C+A_{500} < control < A_{250} (Fig. 1 and Table 2).

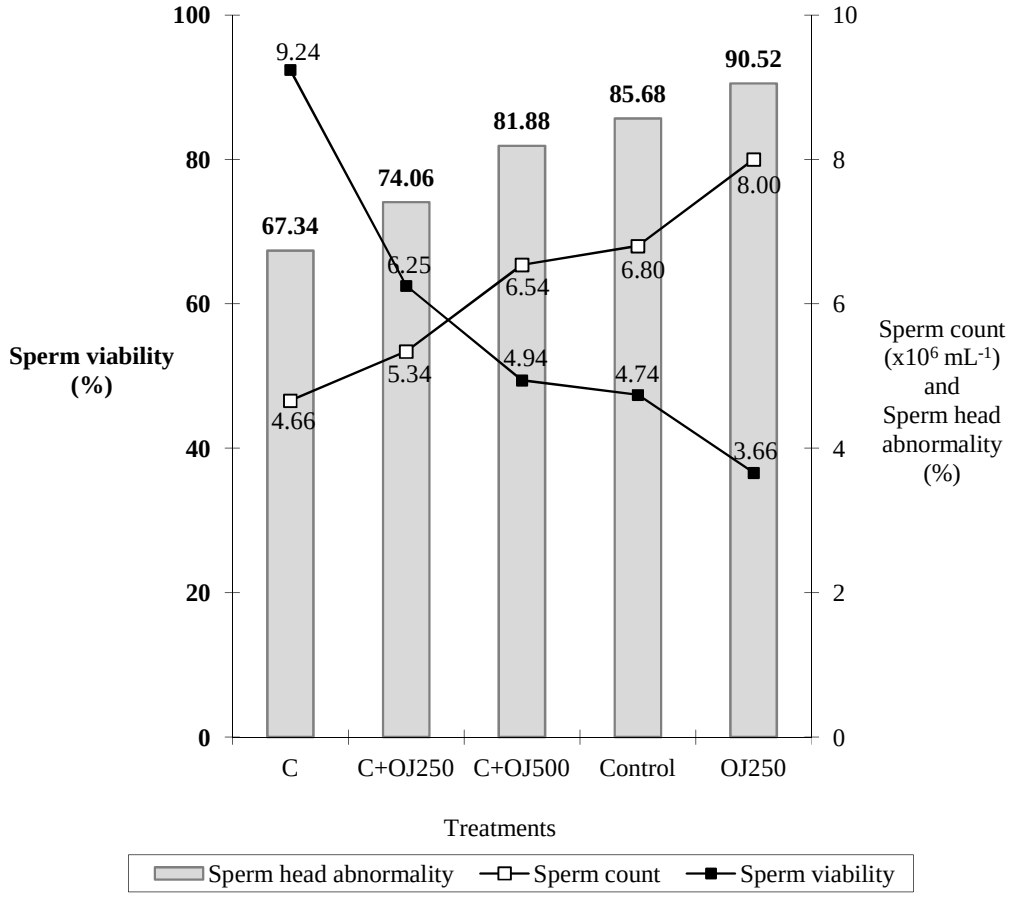

Fig. 1. Protective effect of onion juice on sperm parameters in albino rats

Table 2. Effect of onion juice on caffeine induced toxicities in albino rats

Parameters	Treatment groups				
	C	C+ OJ_{250}	C+ OJ_{500}	OJ_{250}	Control
Weight of testes (g)	1.28±0.08[a]	1.29±0.03[a]	1.41±0.80[a]	1.33±0.04[a]	1.35±0.10[a]
Weight of epididymes (g)	0.49±0.06[c]	0.50±0.04[c]	0.63±0.04[b]	0.74±0.11[a]	0.64±0.43[b]
Semen pH	6.70±0.95[a]	6.98±0.10[a]	6.92±0.10[a]	7.02±0.09[a]	6.76±0.11[a]
Sperm motility (%)	65.06±3.03[c]	69.78±3.00[b]	71.53±2.89[b]	76.53±2.01[a]	72.38±3.95[b]
Sperm viability (%)	74.18±2.00[d]	83.73±1.27[c]	85.54±1.27[c]	93.49±0.50[a]	89.12±0.58[b]
Sperm count ($x10^6$ mL^{-1})	5.80±0.18[c]	6.99±0.34[b]	7.30±0.36[b]	8.10±0.42[a]	7.14±0.27[b]
Sperm head abnormalities(%)	10.60±0.79[a]	7.70±0.57[b]	5.60±0.29[c]	3.50±0.45[e]	4.19±0.31[d]

Values across the table with similar superscripts are not significantly different at 5% based on ANOVA.
C = Caffeine at 200 mg kg^{-1}BW; OJ_{250} = 250 mg kg^{-1}BW of onion; OJ_{500}= 500 mg kg^{-1}BW of onion

4. DISCUSSION

In this study, caffeine significantly ($P<0.05$) reduced the weight of epididymes, sperm viability, sperm motility and sperm count of the treated rats. These findings agree with the reports of Wilcox et al. [22]; Bassey et al. [23]; Ekaluo et al. [14-16,24], and suggest a distortion in the biosynthetic processes underlying spermatogenesis, hence distorts in the fertility indices of the male animals [25]. The significant ($P<0.05$) decrease in the weight of the epididymes is also corroborated by the reduction in the sperm count of the caffeine-treated animals. The caffeine treatment also significantly increased the percentage of sperm head abnormalities which suggests induced mutations on the sperm cells during spermatogenesis. This also agrees with the reports of Harris [26] and Ekaluo et al. [11].

Onion juice protected the rats from the caffeine induced toxicities on the weight of epidiymes, sperm viability, sperm count, sperm motility and sperm head abnormality. The protective role of onion juice could be attributed to its rich vitamin C [27], which agrees with the protective role of vitamin C reported by Nashwa and Venes [28], Karawya and El-Nahas [29]; Ekaluo et al. [14,15]. The protective role of onion might also be due to the antioxidant properties of the onion juice against oxidative stress, which has been implicated as one factor that affects fertility [30]. Epidemiological studies have revealed that consuming fruits and vegetables as well as their extracts reduced free radical oxidative damage [20,31] and promote fertility [18,19,32]. Increased reactive oxygen species (ROS) level has been correlated with decreased sperm count and motility [33]. Therefore, the protecting effect of onion juice on caffeine induced spermatotoxicity can be attributed to the protective roles of its constituents against oxidative stress and induced mutations.

5. CONCLUSION

The present study shows that onion juice is effective in protecting the albino rat models from caffeine induced spermatotoxicity in a dose dependent manner.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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