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Attenuating Effect of Some Antioxidants on Caffeine Induced Spermatotoxicity and Mutagenicity in Male Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background and Objective: Infertility is one of the major health problems in life and has been linked to several factors; therefore different approaches are required to address the problem. This study investigated the attenuating potential of some antioxidants: Cellgevity, Max one, purslane and Vitamin C on caffeine induced spermatotoxicity in male albino rats.
Location and duration of Study: This study was carried in the Department of genetics and Biotechnology, University of Calabar, Calabar and lasted for sixty five days.
Methodology: Sixty sexually matured male albino rats were randomly divided into ten groups consisting of two rats in three replicates using completely randomized design (CRD). Group one served as control and received water and feed only. Group two were given 200 mg/kgBw of Cellgevity, group three received 200 mg/kgBW of Max one, group four received 100 mg/kgBW of

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Vitamin C, group five received 200 mg/kgBW of caffeine, group six received 200mg/kgBW of purslane, group seven received 200 mg/kgBW of caffeine and 200 mg/kgBW of Cellgevity, group eight received 200 mg/kgBW of caffeine and 200 mg/kgBW of Max one, group nine received 200mg/kgBW of caffeine and 200 mg/kgBW of purslane, group ten received 200 mg/kgBW of caffeine and 100 mg/kgBW of vitamin C. Administration was done orally and lasted for 65days. The rats were sacrificed after administration using chloroform anaesthesia. Testes and epididymes were processed for testes and epididymal weights as well as sperm profile.

Results: The results showed that caffeine significantly ($p<0.05$) negatively affected all the parameters studied. The sperm profile significantly reduced in caffeine treated animals. However, Cellgevity, Max one, purslane and Vitamin C attenuated the effect of caffeine in all the parameters evaluated by increased the sperm viability, sperm motility, sperm count and reduced sperm head abnormalities and mutation index in the combination groups.

Conclusion: Results show that Cellgevity, Max one, purslane and Vitamin C have the potential to attenuate spermatotoxicity caused by caffeine in albino rats.

Keywords: Caffeine, cellgevity; max one; purslane; vitamin C; attenuating potential; spermatotoxicity; mutagenicity.

1. INTRODUCTION

Infertility is one of the major health problems in life, and is defined as the inability of a couple to achieve pregnancy after a year of unprotected sexual intercourse. This issue reportedly affects 8–12 percent of couples of reproductive age. More so, male factors contribute to approximately 50percent of all cases of infertility and affects one in twenty men of reproductive age [1]. A plethora of evidences suggest that reactive oxygen species (ROS)-mediated damage to reproductive processes and spermatogenesis is a major contributor to the pathology of infertility in 30–80percent of infertile men [2].

Free radicals manufactured by external and internal agents are very high reactive substance of oxygen origin with half-life that is within the ranges of nano to milliseconds range. The biomolecules have been reported to be significant in altering reproductive parameters. Modifications in styles of living, advancement in technology, increase in pollution, intake of alcohol, smoking, exposure to stress and toxins are part of the major external factors that contributes to the production of reactive oxygen species. In addition, various processes which involve the metabolisms that take place in the membranes of cells, peroxisomes, mitochondria and endoplasmic reticulum give rise to internal reactive oxygen species [3-4].

Glutathione is an endogenous antioxidant present in almost every cell in the body, playing a role in detoxification of drugs and xenobiotics. It is a coenzyme that mediates the protection of the cell against free radical generated during cellular oxidative mechanisms. The intake of whole glutathione does not have effect due to the fact that it would be denatured along the digestive tract before it can be utilized by the cells. The potent components in the riboceine supplement which include Cellgevity and Max one is the D-Ribose-L-Cysteine. The ribose constituent in the riboceine supplement addresses the problem by ensuring the fragile cysteine molecules are protected and delivered, which enables the cells to manufacture the glutathione as the need arises in the cells [5].

L-cysteine is called semi-essential amino acid because humans can synthesize it from the amino acid, methionine along with a host of proteins. It is a precursor of glutathione which is considered very important for the detoxification of cellular oxidative stress. Elevated levels of oxidative stress can potentially impair cellular glucose metabolism through a variety of mechanism including redox imbalance, insulin resistance and reproductive dysfunction [5].

Purslane (*Portulaca oleracea*, William Darlington, 1859: Portulacaceae: Caryophylales) also commonly called *mmong mmong ikong mbakara* in Efik. Purslane has the potential to relax muscles, treat convulsion, reduces pains and has anti-inflammatory capabilities, and possesses anti-anxiety abilities. Furthermore, studies have revealed that purslane exhibits liver protective properties in rats with liver diseases [6-7]. Studies have indicated that the plant provides richer nourishment as compared to other vegetables that are widely cultivated due to their shoots which are rich sources of omega-3-fatty acids, ascorbic acid, b-carotene, a-

tocopherols, and glutathione. The seeds of the plant also have a high amount of a-linolenic acid. These compounds play a vital part of its anti-oxidative potentials [8].

The total phenolic content (TPC) in extracts of *P. oleracea* ranged from 127 ± 13 to 478 ± 45 mg/100 g fresh weight of plant. The IC_{50} ranged from 0.89 ± 0.07 to 3.41 ± 0.41 mg/mL, the ascorbic acid equivalent antioxidant capacity (AEAC) values ranged from 110 ± 14 to 430 ± 32 mg AA/100 g, and the FRAP values ranged from 0.93 ± 0.22 to 5.10 ± 0.56 mg/g [6]. The 2,2-diphenyl-1-picrydrazyl (DPPH) scavenging (IC_{50}) capacity ranged from 1.30 ± 0.04 to 1.71 ± 0.04 mg/mL, while AEACvalues were from 229.5 ± 7.9 to 319.3 ± 8.7 mg AA/100 g, the TPC varied from 174.5 ± 8.5 to 348.5 ± 7.9 mg/100 g, ascorbic acid content (AAC) varied from 60.5 ± 2.1 to 86.5 ± 3.9 mg/100 g, and ferric reducing antioxidant power (FRAP) ranged from 1.8 ± 0.1 to 4.3 ± 0.1 mg GAE/g [7].

Antioxidants decrease stress by ROS by removing free radicals and because most of the antioxidants are compounds of phenol, they have been shown to be contributory factors for the antioxidant properties of many plants [9]. Evidences from researches have revealed that purslane is very effective in antioxidant properties, and also nourishes the kidneys, liver, heart tissues and testes [10].

Vitamin C is needed for many physiological functions. It is a natural antioxidant that prevents increased production of free radicals due to oxidative damage against to lipids and lipoproteins in cellular compartments and tissues. The protective role of vitamin C against oxidative stress has been well reported [11].

Caffeine constitute one of the most constantly consumed psychoactive substances globally and is present in several foods, drugs and beverage products such as energy drinks, coffee and tea. In variance to most other psychoactive substances, it is legalized and unregulated in majority of the countries of the world with an estimated 80percent of the world's population consuming a caffeine-containing substance daily [12]. It is also used in the clinics for various medical purposes such as analgesics, muscle relaxants, diuretics, and has the potential to treat brain disorders such as Parkinson's diseases and headache [13]. In man, minute and medium dosages of caffeine exert alertness and positively impact the myocardium, whereas the high dosages give rise dependency on caffeine with various unwanted mental and physical effects which give rise to irritability, nervousness, lack of rest, headache, loss of sleep, and palpitations of the heart [14]. Frequent intake of caffeine has been reportedly correlated with delay in conception, toxic effects on reproduction and development and advancement in the rate at which sperm abnormalities occur [15-19].

2. MATERIALS AND METHODS

2.1 Location of the Study

This research was conducted in the Animal House of the Department of Genetics and Biotechnology, University of Calabar, Calabar, Cross River State. The study lasted for 6 months (September 2020-February, 2021).

2.2 Collection of Materials

Caffeine was acquired from Sigma-Aldrich (St. Loius, USA). The antioxidant agents: Max one and Cellgevity were purchased from Max International, LLC, (Salt Lake city, USA).Vitamin C was purchased from Emzor Pharmaceutical Industries Limited, Lagos. Purslane leaves were obtained from the University of Calabar Botanical Garden and its environs. The leaves were authenticated by Mr. Effa Anobeja of the Herbarium Unit, Department of Plant and Ecological Studies, University of Calabar. Voucher specimen was deposited with the Resource Room of the Department of Genetics and Biotechnology, University of Calabar, Calabar with the number GBT/PLT/20/098. The leaves were processed into crude extracts. The leaves were air-dried, pulverized and aqueous extracts obtained using distil water.

2.3 Experimental Animals

Sixty (60) sexually matured male albino rats, twelve weeks old weighing between 160 – 200g was purchased from the Department of Zoology and Environmental Biology, University of Calabar, Nigeria. The animals were kept in steel cages covered with wire mesh under standard laboratory environment. They were given water and commercial feed from Top Feed Limited (crude protein: 18 percent; metabolizable energy: 2800kcal/kg) *ad libitum* during the study. Animals were allowed to adapt to their environment for one week before treatment.

2.4 Experimental Design and Procedure

The 60 albino rats were randomly divided into ten groups consisting of two rats in three replicates using the completely randomized design (CRD). Treatment protocol was as shown in Table 1 and lasted for 65 days [12]. Chloroform fume was used to anesthetize the rats twenty-four hours after administering the last dose. Epididymes of male rats were surgically removed and processed for epididymal sperm estimation.

2.5 Weight of Some Organs

Testes and epididymes were weighed with an electronic weighing balance (Scout Pro SPU 601).

Sperm parameters: The epididymal sperm specimen was obtained by maceration of a known weight of epididymes in physiological saline (1:10 w/v ratio). An 80 micrometer mesh was used to sieve the suspension to remove tissues. Sperm analysis was carried using the methods of Ekaluo et al. [15].

Semen pH: A sterile pin was used to puncture the epididymes after dissection, and then the collected semen was smeared on a pH paper ranging from 4.0 to 10.0. Semen pH was read based on the colour change on the pH paper.

Sperm viability: The Eosin-Nigrosin staining method was used to determine sperm viability. A drop of sperm suspension was mixed with equivalent quantity of stain, smeared on clean slides and allowed to dry. Normal live cells were white in colour, while dead sperm cells were pink in colour. Percentage sperm viability was computed as number of live cells to the entire cells counted using the formula

$$\text{Percentage sperm viability} = \frac{\text{No. of live sperm cells}}{\text{Total no. of sperm cells}} \times \frac{100}{1}$$

Sperm motility: Sperm motility was evaluated based on the percentage of progressive and non-progressive movements of the sperm cells seen with the help of a compound microscope using the formula:

$$\text{Percentage sperm motility} = \frac{\text{No. of sperm cells with progressive movements}}{\text{Total no. of sperm cells}} \times \frac{100}{1}$$

Sperm count: Sperm cells were counted using the improved Neubauer Cytometer (Model: BR723014) and presented in million/ml of sperm suspension.

Sperm head abnormality: A drop of sperm suspension was mixed with a drop of 1 percent Eosin, spread on a sterile slide and left to dry for thirty minutes. For each sample, two hundred spermatozoa were examined for sperm head abnormality. The result was expressed in percentage and calculated using the formula:

$$\text{Sperm Viability} = \frac{\text{No. of abnormal sperm heads}}{\text{Total no. of sperm cells}} \times \frac{100}{0}$$

Mutation index: Mutation index were calculated according to Ekaluo et al. [16] using the following formula:

$$\text{Mutation index} = \frac{\text{Frequency of abnormal sperm heads (treatment + control)}}{\text{Frequency of abnormal sperm heads (control)}}$$

2.6 Statistical analysis

Data obtained were analyzed using analysis of variance (ANOVA) on SPSS version 20. Least significant difference was utilized to compare means at $p<0.05$.

3. RESULTS

Results obtained on sperm parameters are presented in Table 2. Results indicated that the treatment administered had no significant effect on Semen pH which ranged from 7.00 ± 0.63 (C+Mx) to 7.10 ± 0.68 (Control). Sperm viability was significantly reduced ($p<0.05$) in caffeine group (67.05 ± 4.48 %) when compared with the control (92.01 ± 1.78 %). The percentage of viable sperm cells increased in C + Cg, C + Mx, C + Pu and C + Vc groups indicating alternating effect of the antioxidants containing treatments. Groups of rats treated with Cg, Max one, Vc and Purslane only recorded 84.28 ± 1.72, 80.09 ± 0.42, 83.84 ± 0.75 and 89.27 ± 0.64 percent, respectively. Vc had the best result on sperm viability.

A significant difference was observed in the sperm motility of animals treated with caffeine alone (71.20 ± 3.26 percent) when compared with the control (77.34 ± 4.16 percent). However, sperm motility significantly ($P< 0.05$) increased in

Table 1. Protocol for treatment of experimental animal

Treatment groups	Description of treatment
Control	1ml of physiological saline, No Caffeine, purslane, Vit. C, Max one and Cellgevity
C	Caffeine in 1ml of physiological saline, 200mg/kgBW orally by gavage [12]
P	Purslane, 200 mg/kgBW orally
Vc	Vitamin C, 100mg/kgBW, orally
Mx	Max One, 200mg/kgBW orally
Cg	Cellgevity, 200mg/kgBW orally
C+P	Caffeine, 200mg/kgBW and purslane, 200mg/kgBW both orally
C+Vc	Caffeine, 200mg/kgBW and Vit. C, 100mg/kgBW both orally.
C+Mx	Caffeine, 200mg/kgBW and Max One, 200mg/kgBW both orally
C+CG	Caffeine, 200mg/kgBW and cellgevity, 200mg/kgBW both orally

C + Cg, C + Pu and C + Vc groups (83.34 ± 1.51, 83.16 ± 2.14 and 89.21 ± 2.82 percent, respectively) with Cg having the best attenuating effect. Animals in Cg, Mx, Vc and Pu groups recorded 80.74 ± 2.93, 79.22 ± 2.82, 76.14 ± 4.68 and 84.69 ± 2.48 percent, respectively. Sperm count significantly declined (P< 0.05) in caffeine group treated with caffeine only when compared with the Control animals. The effect of caffeine on sperm count was attenuated in C+Pu, C+Vc, C+Mx and C+Cg with sperm counts of 4.43 ± 0.13, 4.88 ± 0.14, 5.76 ± 0.23 and 7.30 ± 0.13x10^6/ml, respectively. The Cg, Mx, Vc and Pu groups had 4.94 ± 0.83, 6.81 ± 0.83, 6.87 ± 0.10 and 5.49 ± 0.86x10^6/ml. Cg had the best result in attenuating sperm count.

On the other hand, sperm head abnormalities was significantly high in animals treated with caffeine only (13.61 ± 0.73 percent) when compared with the control (8.32 ± 0.27 percent) and other treatment groups. Animals treated with only CG, Mx, Vc and Purslane had 8.37 ± 0.20, 9.16 ± 0.21, 6.92 ± 0.23 and 8.10 ± 0.25 percent, respectively. The percentage of abnormal sperm cells significantly decrease in C + Pu, C + Mx, C + Cg and C + Vc groups (11.65 ± 0.37, 10.55 ± 0.46, 10.10 ± 0.27, 9.49 ± 0.25 percent, respectively) with the least recorded in C + Vc group indicating attenuating potentials. Mutation index was highest in animals treated with only caffeine (0.64) when compared with the control (0.00) as shown on Table 2. Vc had the best result on reducing mutation index (0.14).

The weight of testes decreased significantly (P< 0.05) in animals treated with caffeine only (1.02 ± 0.17 g) when compared with the control (1.48 ± 0.1 g). Testes weight increased significantly in C + Pu, C + Cg and C + Vc groups (1.13 ± 0.21, 1.17 ± 0.33 and 1.46 ± 0.76 g, respectively) when compared with animals administered with caffeine alone depicting attenuating effect with the highest attenuating potential observed in C + Vc group. Groups Cg, Mx, Vc and Pu recorded 1.17 ± 0.33, 0.97 ± 0.21, 0.97 ± 0.33 and 1.10 ± 0.12 g, respectively as shown in Table 3. In the same vein, weight of epididymes reduced significantly (P < 0.05) in caffeine group (0.42 ± 0.02g) when compared with the control (0.57g ± 0.03). The Cg, Mx, Vc and Pu groups had 0.48 ± 0.01, 0.38 ± 0.04, 0.45 ± 0.05 and 0.57 ± 0.03, respectively. This significantly increased in C + Cg, C + Mx and C + Vc groups to 0.48 ± 0.02, 0.48 ± 0.02 and 0.52 ± 0.03g, respectively depicting attenuating effect of the antioxidants with Vc having the best result (Table 3).

4. DISCUSSION

Results obtained showed that the treatments did not have any significant effect on semen pH. However, caffeine caused a significant (p<0.05) reduction in sperm motility, sperm viability and sperm count when compared with the control and other treatment groups. The effect of caffeine on the sperm parameters suggested spermatotoxic effect of caffeine on sperm quality and quantity was in line with the findings of Smith [20], Ekaluo et al. [16] and Uno et al. [21]. The significant reduction in sperm count, viability and motility could be due to deleterious effect of caffeine on spermatogenic processes in the animals. This also agreed with the reports of Ekaluo et al. [22] and Ikpeme et al. [23] who noted that disruptions in spermatogenesis is positively correlated with disruptions in fertility in male animals.

More so, oxidative stress has been shown to be a major contributor to male infertility and correlates positively with decrease in sperm count and motility [24]. This maybe an underlying factor in the reduction in the sperm count and motility observed in groups of animals treated

Table 2. Effect of some antioxidantants on caffeine induced toxicity on sperm parameters of male albino rats

Parameters	Control	CG	Mx	Vc	Caffeine	Purslane	C+CG	C + Mx	C+Pu	C+Vc	LSD
Sperm motility	77.34 ± 4.16	80.74 ± 2.93	79.22 ± 2.82	76.14 ± 4.68	71.20 ± 3.26	84.69 ± 2.48	83.34± 1.51	76.12 ± 2.48	83.16 ± 2.14	89.21 ± 2.82	2.45
Sperm viability	92.01 ± 1.78	84.28 ± 1.72	80.09 ± 0.42	83.84 ± 0.75	67.05 ± 4.48	89.27 ± 0.64	81.35 ± 2.87	81.89 ± 0.19	84.84 ± 1.14	89.27 ± 0.65	2.87
Sperm count	7.64 ± 0.11	4.94 ± 0.83	6.81 ± 0.67	6.87 ± 0.10	4.30 ± 0.15	5.49 ± 0.86	7.30 ± 0.13	5.76 ± 0.23	4.43 ± 0.21	4.88 ± 0.14	0.83
SHAT	8.32 ± 0.27	8.37 ± 0.20	9.16 ± 0.21	6.92 ± 0.23	13.61 ± 0.73	8.10 ± 0.25	10.10 ± 0.27	10.55 ± 0.46	11.65 ± 0.37	9.49 ± 0.25	1.02
pH	7.10 ± 0.68	7.05 ± 0.22	7.05 ± 0.34	7.03 ± 0.33	7.08 ± 0.31	7.05 ± 0.34	7.07 ± 0.42	7.00 ± 0.63	7.05 ± 0.22	7.08 ± 0.40	NS
Mutation index	0.00	0.006	0.10	-0.16	0.64	-0.03	0.21	0.27	0.40	0.14	0.11

Key: CG: Cellgevity; Mx: Max one; Vc: Vitamin C; C+CG: Caffeine and Cellgevity; C+Mx: Caffeine and Max one; C+Pu: Caffeine and Purslane; C+Vc: Caffeine and Vitamin C

Table 3. Effect of some antioxidants on caffeine induced toxicity on weight of some organs of albino rats

Parameters	Control	Cg	Mx	Vc	Caffeine	Pu	C+Cg	C+Mx	C+Pu	C+Vc	LSD
Weight of testes (g)	1.48 ± 0.17	1.17 ± 0.33	0.97 ± 0.21	0.97 ± 0.33	1.02 ± 0.17	1.10 ± 0.12	1.17 ± 0.33	1.07 ± 0.21	1.13 ± 0.21	1.46 ± 0.76	0.67
Weight of epididymes (g)	0.57 ± 0.03	0.48 ± 0.01	0.38 ± 0.04	0.45 ± 0.05	0.42 ± 0.02	0.57 ± 0.03	0.48 ± 0.02	0.48 ± 0.02	0.45 ± 0.02	0.52 ± 0.03	0.04

Key: CG: Cellgevity; Mx: Max one; Vc: Vitamin C; C+CG: Caffeine and Cellgevity; C+Mx: Caffeine and Max one; C+Pu: Caffeine and Purslane; C+Vc: Caffeine and Vitamin C

with caffeine in line with the findings of Kemal et al. [25], Misro et al. [26] and Singh et al. [27]. Sperm cells have high vulnerability to free radicals because they are made up of huge amounts fatty acids that are polyunsaturated in their membrane as well as cytoplasm. More so,oxidative stress can give rise to a fast loss of intracellular adenosine triphosphate resulting in axonemal damage with resultant decrease in sperm viability and motility and increased mid-piece structural defects as well as harmful effects on sperm capacitation and the acrosome reaction. Lipid peroxidation of the sperm membrane is a key mediator of ROS-induced sperm damage leading to infertility [25-26].

Results obtained also revealed that abnormalities of the sperm heads escalated significantly ($p<0.05$) in the group of animals that were administered caffeine which indicates the occurrence of mutation induced on the spermatozoa during sperm synthesis. These findings were in agreement with reports of Harris [28], Glover and Assinder [29] and Ekaluo et al. [15]. The result on sperm head abnormality is also in consonance with result obtained on mutation index with the highest values obtained in caffeine treated animals. Increase in mutation index might be an indication of DNA damage and DNA damage and mutation have been shown to contribute to apoptosis, poor fertilization of sperm cells, high frequency of miscarriage and morbidity in offsprings [30].

However, the effect of caffeine was attenuated in C+Cg, C+Mx, C+Pu and C+Vc groups. The attenuating effect of Cellgevity, Max one, purslane and vitamin C can be attributed to their antioxidant properties [31]. Antioxidants prevent oxidative stress with its resultant impact on spermatogenesis and sperm profile. Caffeine has been reported to cause significant decline in antioxidant defense system, increased free radical activities and consequently resulting in oxidative stress [32] and the increase in antioxidants level have positive correlation with increase in fertility in men [33-34].

5. CONCLUSION

The present study revealed the significant spermatotoxic effect of caffeine. The findings of this study provided substantial evidence that Cellgevity, Max one, Purslane and Vitamin C can attenuate caffeine-induced spermatotoxicity in male rats.

ETHICAL APPROVAL

Animals were handled in line with the Helsinki protocol for care of experimental animals and with approval from the local ethical committee (Approval number: CRS/MH/CGS and EH/Vol.1/102).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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