



Effect of Soursop (*Annona muricata* L.) Leaf Extract on Sperm Toxicity Induced by Caffeine in Albino Rats

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Abstract Extract of *Annona* spp. have been used for several medicinal purposes such as the management of diabetes and its complications, also as antioxidant and antimutagenic agents. Hence, the mitigating effect of soursop (*A. muricata*) leaf extract on sperm toxicity induced by caffeine was assessed on the weight of testes and epididymes, epididymal sperm count, motility, viability, semen pH and sperm head abnormality in albino rats as a model. The male rats were divided into six groups of six rats each. The rats were administered with treatments of caffeine and Soursop Tea (SST) for 65 days. Results obtained revealed a significant ($p < 0.05$) decrease in the weight of epididymes, sperm motility, sperm viability and sperm count while sperm head abnormalities significantly increased in caffeine – treated animals. No significant differences were observed in semen pH and weight of testes. In conclusion, SST mitigated the caffeine-induced toxicity on weight of epididymes, sperm motility, sperm viability, sperm count and sperm head abnormality in the mammalian models in a dose – dependent manner.

Keywords Soursop, caffeine, toxicity, sperm quality, sperm count, sperm head abnormality.

Introduction

Traditional medicine derived from plant extract has played a vital role in the prevention and cure of various diseases especially in some traditional African localities and among the low socio – economic class. Many medicinal plants with antioxidant properties have been shown to exhibit protective effect against a wide range of toxicants [1-5].

Soursop (*Annona muricata* L.), also known as graviola, belongs to the family Annonaceae which is found throughout the tropics. It has the largest fruits in the genus. The soursop is astringent, cholagogic and promotes digestion [6]. It also has several medicinal uses such as in the management of diabetes and its complications [6-8], also as antioxidant and antimutagenic agent [9]. It is usually recommended in cases of constipation, obesity, hypertension and coronary diseases [6].

All the plant parts of the plants are useful in folk medicine including the bark, leaves, root and fruits. The leaf extract is used in the treatment of cancer and skin infection such as eczema while the white pulp of the fruit is used to make juice, as well as candies, sorbets and ice-cream flavorings. Its flavor is described as a combination of strawberry and pineapple with sour citrus flavor notes contrasting with an underlying creamy flavor reminiscent of coconut or banana. The fruit is rich in B group vitamins, potassium, fructose and vitamin C [6].

Caffeine (1,3,7-trimethylxanthine) is a bitter white crystalline purine and is probably the most frequently ingested psychoactive substance in the world [10-12]. It is found in common beverages like coffee, tea, energy drinks, carbonated beverages, product containing cocoa or chocolate and in medications [13-15]. In human, low and intermediate doses of caffeine produce increase alertness and positive effects on the myocardium, while high doses cause caffeinism usually combine caffeine dependency with a wide range of unpleasant physical and mental



condition including nervousness, irritability, restlessness, insomnia, headache and heart palpitations after caffeine use [16]. It could also cause adverse tachycardia and ventricular arrhythmia [17-18]. Consumption of caffeine has been linked with delayed conception [19], reproductive and developmental risks [20] and increased frequencies of sperm aneuploidy [21].

In view of above finding, this study set out to ascertain mitigating effect of Soursop Tea (SST) on sperm toxicity induced by caffeine in albino rats as mammalian model.

Materials and Methods

Collection of Plant Material

Fresh leaves of soursop (*Annona muricata* L.) were harvested from Ikot Eneobong, 8 miles, Calabar, Cross River State of Nigeria. The fruits were identified and authenticated by Dr. Samuel Udoh, Senior Lecturer, Department of Botany, University of Calabar, Calabar.

Preparation of Plant Extract

The fresh leaves of Soursop were washed, air – dried and then pulverized using an electronic blender. SST was prepared by dissolving 100, 200, and 300mg of powdered leaves in 50ml of hot distilled water. The solution was allowed to cool to room temperature and then filtered. The filtrate was then stored in a refrigerator until when needed.

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

Experimental Animals

Thirty six 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.

Experimental Design and Procedure

The thirty six male rats were randomly divided into five groups of six rats each using a completely randomized design. The animals were acclimatized for one week before the commencement of the study. The treatment lasted for 65 days and the protocol is shown in Table 1. The rats were sacrificed under chloroform anesthesia 24 h 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 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 daily treatment of experimental animals

Treatment groups	Description of treatment
Control	No caffeine and no soursop tea(SST)
C	Caffeine, 200mg kg ⁻¹ BW only via oral gavage
SST ₁	SST 100mg kg ⁻¹ BW only via oral gavage
C+SST ₁	Caffeine, 200mg kg ⁻¹ BW and 10-12 hours SST 100mg kg ⁻¹ BW both orally via oral gavage
C+SST ₂	Caffeine, 200mg kg ⁻¹ BW and 10-12 hours after SST 200mg kg ⁻¹ BW both orally via oral gavage
C+SST ₃	Caffeine, 200mg kg ⁻¹ BW and 10-12 hours after SST 300mg kg ⁻¹ BW both orally via oral gavage



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 [22].

Sperm Viability

The sperm viability test was determined using “Eosin-Nigrosin one-step staining technique” [22]. A portion of the sperm suspension was mixed with equal volume of Eosin-Nigrosin stain and five (5) 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 epididymes 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 and was expressed as million/mL of suspension [23].

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 spermatozoa 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.* [24]

Statistical Analysis

Data from weight of testes and epididymes, epididymal semen pH, motility, viability, count and sperm head abnormality were subjected to the Analyses of Variance (ANOVA) test 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 testes between the control and the treatment groups (Table 2). However, the weight of epididymis reduced significantly ($p < 0.05$) in caffeine and C+SST₁ groups (0.30g, respectively) when compared to the control, C+SST₂ and C+SST₃ groups (0.40, 0.40 and 0.41g, respectively), indicating a dose – dependent mitigating effect of SST. The highest weight of epididymis was recorded in SST₁ group as shown in Table 2.

Semen pH and Sperm Motility

There was no significant ($p > 0.05$) effect of caffeine and SST on the semen pH. The motility reduced significantly ($p > 0.05$) in the caffeine group when compared to the control (Table 2). The effect of caffeine was mitigated by SST from 67.34% to 77.41, 80.05 and 81.10%, respectively for C+SST₁, C+SST₂ and C+SST₃.

Sperm Viability

The sperm viability significantly ($p < 0.05$) declined in the caffeine group when compared to other treatment groups (Table 2). The sperm viability in the caffeine group (77.92%) was reduced by 7.86% when compared to the control group (85.78%). However, SST significantly ($p < 0.05$) mitigated the effect of caffeine in a dose – dependent manner (82.71, 85.51 and 86.65% for C+SST₁, C+SST₂ and C+SST₃, respectively).



Table 2: Effect of soursop tea on sperm parameters of rats with caffeine-induced toxicities

Parameters	Treatment Groups					
	Control	Caffeine	C+SST ₁	C+SST ₂	C+SST ₃	SST ₁
Semen pH	7.18±1.59 ^a	7.13±0.03 ^a	7.15±0.03 ^a	7.01±0.03 ^a	7.15±0.03 ^a	7.20±0.00 ^a
Sperm motility (%)	81.71±1.57 ^a	67.34±1.36 ^c	77.41±2.05 ^b	80.05±3.86 ^a	81.10±2.39 ^a	80.43±0.24 ^a
Sperm viability (%)	85.78±2.15 ^a	77.92±1.59 ^b	82.71±1.71 ^a	85.51±1.90 ^a	86.65±1.09 ^a	83.99±2.25 ^a
Sperm head abnormality (%)	6.56±0.38 ^a	9.46±0.32 ^c	8.61±0.40 ^b	8.05±0.31 ^b	7.92±0.41 ^b	6.11±0.24 ^a
Sperm count (x10 ⁶)	7.17±0.30 ^a	6.03±0.20 ^b	6.17±0.13 ^b	6.75±0.11 ^a	6.81±0.08 ^a	6.95±0.14 ^a
Weight of testes (g)	1.00±0.05 ^a	1.30±0.20 ^a	0.90±0.08 ^a	1.10±0.23 ^a	1.10±0.23 ^a	1.10±0.23 ^a
Weight of epididymes (g)	0.40±0.01 ^a	0.30±0.01 ^b	0.30±0.02 ^b	0.40±0.01 ^a	0.41±0.01 ^a	0.42±0.01 ^a

Values are presented as mean ± SEM. Values across the table with similar superscripts are not significantly different at 5% based on ANOVA. C: caffeine at 200mg/kgBW, SST₁: Soursop tea at 100mg/kgBW, SST₂: Soursop tea at 200mg/kgBW, SST₃: Soursop tea at 300mg/kgBW

Sperm Count

The sperm count was significantly ($p < 0.05$) reduced in the caffeine group ($6.03 \times 10^6 \text{ mL}^{-1}$) when compared to the control ($7.17 \times 10^6 \text{ mL}^{-1}$) and C+SST₂, C+SST₃, and SST₁ groups (6.75 , 6.81 and $6.95 \times 10^6 \text{ mL}^{-1}$, respectively) as shown in Table 2. In the same vein, SST also showed mitigating effect on caffeine – induced toxicity of sperm count in a dose – dependent manner.

Sperm Head Abnormality

Results obtained on the effect of SST on caffeine induced sperm head abnormality is presented in Table 2. Animals treated with caffeine alone had the highest percentage of sperm head abnormalities (9.46%) when compared to other treatment groups. SST also had mitigating effect on the caffeine induced sperm head abnormalities reducing the percentage of abnormal sperm heads from 9.46% in the caffeine group to 8.61, 8.05 and 7.92%, respectively in C+SST₁, C+SST₂ and C+SST₃ groups.

Discussion

Results obtained in the study revealed that there was no significant effect of caffeine and SST on the weight of testes. However, caffeine caused a significant reduction in the weight of epididymes, sperm motility, sperm viability and sperm count which is similar to the findings of Ekaluo *et al.* [1-4, 25-26], Wilcox [27] and Bassey *et al.* [28].

The significant reduction in the sperm parameters of the caffeine – treated animals could be attributed to disruptions or alterations in the biosynthetic process underlying spermatogenesis in the testes. This assertion is corroborated by Ezzat and El-Gohary [29] who reported that long term consumption of caffeine induces suppression of spermatogenesis. In the same vein, Ikpeme *et al.* [30] showed that distortions in fertility of male mammals are directly correlated with distortion in spermatogenesis. On the other hand, caffeine treatment significantly increased the percentage of sperm head abnormalities suggesting induced mutations during spermatogenesis in the testes as observed by Ekaluo *et al.* [24], Glover and Assinder [31] and Ikpeme *et al.* [30].

SST was observed to mitigate the effect caffeine on the weight of epididymes, sperm motility, sperm count, sperm viability and significantly reduced sperm head abnormalities. The mitigating effect of SST could be due to its rich phytonutrients, antioxidant content and vitamins which provides protective roles against oxidative stress and induced mutations [1, 5, 9, 25].

Conclusion

The present study reveals that SST has the potential to mitigate caffeine induced sperm toxicities in albino rat model in a dose dependent manner.



References

1. Ekaluo, U. B., Ikpeme, E. V., Etta, S. E., Erem, F. A. & Daniel, I. O. Protective role of soursop(*Annona muricata* L.) fruit on testicular toxicity induced by caffeine in albino rats. *Journal of Life Sciences Research and Discovery*, 2014, 1: 26-30.
2. Ekaluo, U. B., Uno, U. U., Ekpo, P. B., Etta, S. E., Odok, T. N. & Daniel, I. O. Mitigating potential of Trevo dietary supplement on caffeine induced sperm toxicities in albino rats. *Asian Journal of Clinical Nutrition*, 2015, 7(3): 84 – 89.
3. Ekaluo, U. B., Uno, U. U., Edu, N. E., Ekpo, P. B., Etta, S. E. & Volunteer, B. O. Protective role of onion (*Allium cepa* L.) on caffeine induces spermatotoxicity in albino rats. *Journal of Applied Life Sciences International*, 2016, 4(4): 1 – 7.
4. Ekaluo, U. B., Ikpeme, E. V., Uno, U. U., Umeh, S. O. & Erem, F. A. Protective role of aqueous guava leaf extract against caffeine induced spermatotoxicity in albino rats. *Research Journal of Medicinal Plants*, 2016, 10(1): 98 – 105.
5. Uno, U. U., Ekaluo, U. B., Okoi, P. E., Ogbe, H. O. & Peter, N. Mitigating role of Trevo dietary supplement on hormonal toxicity induced by caffeine in albino rats. *International Journal of Advance Research*, 2015, 3(11): 586 – 590.
6. Pamplona-Roger, G. D. *Encyclopedia of food and their healing power*. Madrid: Editorial Safeliz, 2005.
7. Shirwaikar, A., K. Rajendran, C.D. Kumar & Bodla, R. Antidiabetic activity of aqueous leaf extract of *Annona squamosa* in streptozotocin-nicotinamide type 2 diabetic rats. *Journal Ethnopharmacology*, 2014, 91: 171-175.
8. Adewole, S. O., Caxton-Martins, E. A. & Ojewole, J. A. O. Histochemical and Biochemical effects of melatonin on pancreatic β -cells on streptozotocin treated diabetic rats. *Pharmacology-Online*, 2006, 2: 1-21.
9. Thakkar, J.H., Solanki, H.K., Tripathi, P., Patel N. J., and Jani, G. K. Evaluation of antimutagenic potential of *Annona squamosa* leaf extract. *Elixir Human Physiology*, 2011, 31: 1960-1965.
10. Best, B. Is caffeine a health hazard? *American Journal of Psychiatry*, 1999, 156: 223-228.
11. Fredholm, B. B., Bahig, K., Holmen, J., Nehlig, A. & Zvartau, E. E. Actions of caffeine in the brain with special references to factors that contribute to its widespread use. *Pharmacological Review*, 1999, 51:83 – 133.
12. Smith, A. Effects of caffeine on human behavior. *Food and Chemical Toxicology*, 2002, 40: 1243-1255.
13. Burkner, M. L. and McWilliams, M. Caffeine content of common beverages. *Journal of American Dietary Association*, 1979, 74: 28-32.
14. Matissek, R. Evaluation of xanthine derivatives in chocolate-nutritional and chemical aspects. *Zeitschrift Lebensmitteluntersuchung-Forschung*, 1997, 205: 175-184.
15. Ekaluo, U. B., Udokpoh, A. E., Udofia, U. U. & Ajang, R. O. Comparative toxicity of five commonly used analgesics on sperm count and sperm head abnormalities. *Global Journal of Pure and Applied Sciences*, 2005, 11: 81-84.
16. Lanch, I., Oimer, A. & Srous, R. D. Caffeinism: History Clinical Features, Diagnosis and Treatment. In: *Caffeine and Activation Theory: Effects in Health and Behaviour*, Smith, B.D., U. Gupta & B.S. Gupta (Eds.). CRS Press, Boca Raton, FL.2007: 331-344.
17. Kaplan, G. B., Greenblatt, D. J., Ehrenberg, B. L., Goddard, J. E., Cotrean, M.M., Harmatz, J. S. & Shader, R. I. Dose-dependent pharmacokinetic and psychomotor effect of caffeine in humans. *Journal of Clinical Pharmacology*, 1993, 37: 693-703.
18. Mosqueda-Garcia, R., Robertson, D. and Robertson, R. M. The Cardiovascular Effects of Caffeine. In: *Caffeine, Coffee and Health*, Garattini, S. (Ed.). Raven Press, New York. 1993: 156-176.
19. Bolumar, F., Olsen, J. Rebagliato, M. & Bisanti, L. Caffeine intake and delayed conception: A European multicenter study on infertility and subfecundity. European study group on infertility subfecundity. *American Journal of Epidemiology*, 1997, 145: 324-334.



20. Christian, M.S. & Brent, R. L. Teratoga update: Evaluation of the reproductive and developmental risks of caffeine. *Teratology*, 2001, 64: 51-78.
21. Robbins, W. A., Vine, M. F., Truong, K. Y. & Everson, R. B. Use of Fluorescence *In situ* Hybridization (FISH) to assess effects of smoking, caffeine and alcohol on aneuploidy load in sperm of healthy men. *Environmental and Molecular Mutagenicity*, 1997, 30: 175-183.
22. Bjorndahl, L., Soderlund, I. & Kvist, U. Evaluation of the one-step eosin-nigrosin staining technique for human sperm vitality assessment. *Human Reproduction*, 2003, 18: 813-816.
23. Ekaluo, U. B., Udokpoh, A. E., Ikpeme E. V. & Peter, E. U. Effect of chloroquine treatment on sperm count and weight of testes in male rats. *Global Journal of Pure and Applied Sciences*, 2008, 14: 175-177.
24. Ekaluo, U. B., Ikpeme, E. V. & Udokpoh, A. E. Sperm head abnormality and mutagenic effects of aspirin, paracetamol and Caffeine containing analgesics in rats. *The Internet Journal of Toxicology*, 2009, 7: 1-9.
25. Ekaluo, U. B., Ikpeme, E. V., Ibaing, Y. B. & Omordia, F. O. Effect of soursop (*Annona muricata* L.) fruit extract on sperm toxicity induced by caffeine in albino rats. *Journal of Medical Science*, 2013a, 13: 67-71.
26. Ekaluo, U. B., Ikpeme, E. V., Ibiang, Y. B. & Amaechina, O. S. Mitigating role of vitamin C on sperm toxicity induced by monosodium glutamate in albino rats. *Journal of Biological Sciences*, 2013b, 13: 298-301.
27. Wilcox, A., Weinberg, C. & Baird, D. Caffeinated beverages and decreased fertility. *Lancet*, 1988, 2:1453-1456.
28. Basseyy, R. B., Yama, O. E., Osinubi, A. A., Noronha, C. C. & Okanlawon, A. Effects of Tahitian Noni dietary supplement on caffeine- induced testicular histo – pathology alterations in adult Sprague – Dawley rats. *Middle East Fertility Society Journal*, 2011, 16: 61 – 66.
29. Ezzat, A. R., & El-Gohary, Z. M. Hormonal and histological effects of chronic caffeine administration on the pituitary-gonadal and pituitary-adrenocortical axes in male rabbits. *Functional Development and Morphology*, 1994, 4: 45-50.
30. Ikpeme, E V., Udensi, O., Ekaluo, U. B. & Efieneokwu, N. Biological response of male wistar rats to crude extracts of *Ficus exasperate* (VAHL). *International Journal of Current Research*, 2010, 7:9-13.
31. Glover, A. & Assinder, S. J. Acute exposure of adult male rats to dietary phytoestrogens reduces fecundity and alters epididymal steroid hormone receptor expression. *Journal of Endocrinology*, 2006, 189:565-573.

