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

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Extracts 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. There have been attempts to attenuate toxic effects using medicinal plants. Hence, the mitigating effect of soursop (*A. muricata*) fruit extract on sperm toxicity induced by caffeine was accessed 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 five groups of six rats each. The rats were administered with treatments of caffeine and Soursop Fruit Extract (SFE) for 65 days. In conclusion, SFE mitigated the caffeine-induced toxicity on weight of testes and epididymes, sperm motility, sperm count and sperm head abnormality in the mammalian model.

Key words: Soursop, toxicity, caffeine, sperm abnormality, sperm count, testes, albino rats

INTRODUCTION

Soursop (*Annona muricata* L.) 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 (Pamplona-Roger, 2005). It also has several medicinal uses such as in the management of diabetes and its complications (Shirwaikar *et al.*, 2004; Pamplona-Roger, 2005; Adewole *et al.*, 2006), also as antioxidant and antimutagenic agent (Thakkar *et al.*, 2011). It is usually recommended in cases of constipation, obesity, hypertension and coronary diseases (Pamplona-Roger, 2005).

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 (Pamplona-Roger, 2005).

Caffeine (1,3,7-trimethylxanthine) is probably the most frequently ingested psychoactive substance in the world (Best, 1999; Fredholm *et al.*, 1999; Smith, 2002). It is found in common beverages like coffee, tea, energy drinks, carbonated beverages, product containing cocoa or chocolate and in medications (Burker and McWilliams, 1979; Matissek, 1997; Ekaluo *et al.*, 2005).

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 (Lanch *et al.*, 2007). It could also cause adverse tachycardia and ventricular arrhythmia (Kaplan *et al.*, 1993; Mosqueda-Garcia *et al.*, 1993). Consumption of caffeine has been linked with delayed conception (Bolumar *et al.*, 1997), reproductive and developmental risks (Christian and Brent, 2001) and increased frequencies of sperm aneuploidy (Robbins *et al.*, 1997).

In view of above finding, this study set out to ascertain mitigating effect of Soursop Fruit Extract (SFE) on sperm toxicity induced by caffeine in albino rats as mammalian model; using short-term *in vivo* assays.

MATERIALS AND METHODS

Collection of plant material: The ripe fruits of soursop (*Annona muricata* L.) purchased from Marian market, Calabar, Cross River State of Nigeria. Calabar. The fruits were identified and authenticated by Dr Samuel Udoh, Senior Lecturer, Department of Botany, University of Calabar, Calabar.

Preparation of plant extract: The fruits of Soursop (*Annona muricata* L.) were washed, peeled and cut into tiny pieces and then liquidized into the Soursop Fruit Extract (SFE) using Mammonlex juice extractor; model: JD 1004. The SFE (or juice) was stored in glass bottles and preserved in a refrigerator at 2-4°C until used for experiment.

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

Experimental animals: Thirty 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 male rats were randomly divided into five groups of six rats each. 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 anaesthesia 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

Table 1: Protocol for treatment of experimental animals

Group	Treatment	Description of treatment
A	Control	No SFE and no caffeine
B	S ₁	SFE, 5 mL kg ⁻¹ body weight (BW) orally only
C	C	Caffeine, 200 mg kg ⁻¹ BW orally only
D	C + S ₁	Caffeine, 200 mg kg ⁻¹ BW and SFE, 5 mL kg ⁻¹ BW both orally
E	C + S ₂	Caffeine, 200 mg kg ⁻¹ BW and SFE, 10 mL kg ⁻¹ BW both orally

sterile pin. 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 was expressed as a percentage.

Sperm viability: The sperm viability test was determined using “Eosin-Nigrosin one-step staining technique” (Bjorndahl *et al.*, 2003). 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 (Ekaluo *et al.*, 2008).

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.* (2009).

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 epididymes: The weight of the testes and epididymes of the rats were significantly ($p < 0.05$) reduced in groups C, D and D by caffeine treatments, compared to the control group; with 1.46 and 0.48 g, respectively. Table 2 shows that SFE had a mitigating effect on the caffeine-induced toxicity on weight of testes and epididymes. The mitigating effect of SFE on the testes and epididymes was in a dose-dependent manner; from 1.01 to 1.32 g and 0.25 to 0.36 g, respectively for testes and epididymes.

Semen pH and sperm viability: There was no significant effect of SFE and caffeine treatments on the semen pH. The sperm viability was significantly ($p < 0.05$) affected by SFE with a mitigating effect, which was also in a dose-dependent manner as shown in Table 2.

Sperm motility: The sperm motility was significantly ($p < 0.05$) reduced in groups C, D and D by caffeine treatments when compared with the control group (89.15%). The mitigating effect of SFE on the sperm motility was in a dose-dependent manner; from 54.30 to 64.12%.

Sperm count: The sperm count was significantly ($p < 0.05$) reduced in groups C, D and D by caffeine treatments when compared with the control group; with 7.63×10^6 mL. Table 2 shows that the mitigating effect of SFE on the sperm count in a dose-dependent manner; from 3.61 to 4.94×10^6 mL⁻¹.

Sperm head abnormality: The sperm head abnormality was significantly ($p < 0.05$) reduced in groups C, D and D compared to the control group; with 4.26%. Table 2 shows that SFE had a mitigating effect on the caffeine-induced toxicity on sperm head abnormality; which was in a dose-dependent manner; from 8.47-5.50%.

Table 2: Effect soursop fruit extract and caffeine on sperm parameters in rats

Parameter	Group A (control)	Group B(S ₁)	Group C(C)	Group D(C+S ₁)	Group E(C+S ₂)
Semen pH	7.05±0.12 ^a	6.89±0.09 ^a	7.06±0.13 ^a	7.05±0.16 ^a	6.99±0.12 ^a
Sperm count (x10 ⁶ /ml)	7.35±0.02 ^a	6.98±0.06 ^b	3.61±0.11 ^c	4.29±0.08 ^d	4.94±0.10 ^e
Sperm motility (%)	89.15±1.95 ^a	93.13±1.14 ^a	54.30±2.71 ^d	56.52±2.70 ^e	64.12±1.57 ^b
Sperm viability (%)	90.01±1.95 ^a	83.32±1.69 ^b	42.36±1.19 ^e	59.16±0.49 ^d	68.88±0.50 ^c
Sperm head abnormality (%)	4.26±0.09 ^a	4.78±0.10 ^b	8.47±0.29 ^e	7.29±0.18 ^d	5.50±0.08 ^c
Weight of testes (g)	1.46±0.02 ^a	1.43±0.02 ^a	1.01±0.05 ^e	1.23±0.04 ^b	1.32±0.01 ^d
Weight of epididymes (g)	0.48±0.02 ^a	0.45±0.02 ^a	0.25±0.01 ^d	0.03±0.01 ^e	0.36±0.07 ^c

Values with similar superscripts are not significantly different at 5% based on ANOVA

DISCUSSION

The rats treated with caffeine showed significant ($p < 0.05$) reduction in weight of the testes and epididymes, sperm count and sperm motility that agree with Ekaluo *et al.* (2005, 2009) and Ikpeme *et al.* (2012), as well as significant ($p < 0.05$) increase in sperm head abnormalities that agree with Robbins *et al.* (1997), Christian and Brent (2001), Ekaluo *et al.* (2005, 2009) and Ikpeme *et al.* (2012).

SFE mitigated the caffeine-induced toxicity on weight of testes and epididymes, sperm motility, sperm count and sperm head abnormality. The mitigating effect of SFE could be attributed to its rich vitamin C content (Pamplona-Roger, 2005), which agrees with the protective role of vitamin C (Karawya and El-Nahas, 2006; Nashwa and Venes, 2008; El-Sokkary and Awadalla, 2011; Al-Amoudi, 2012). It could also be attributed to the antioxidant properties of soursop fruit extract (Pamplona-Roger, 2005; Thakkar *et al.*, 2011).

In conclusion, Soursop Fruit Extract (SFE) mitigated the caffeine-induced toxicity on weight of testes and epididymes, sperm motility, sperm count and sperm head abnormality in the mammalian model.

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