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Protective Role of Aqueous Guava Leaf Extract Against Caffeine Induced Spermatotoxicity in Albino Rats

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ABSTRACT

This study investigated the protecting potential of Aqueous Guava (*Psidium guajava*) Leaf Extract (AGLE) against caffeine induced spermatotoxicity in albino rat models. Thirty healthy and sexually matured albino rats were divided into five groups of six rats each using a completely randomized design. They were treated with caffeine and AGLE combinations orally for 65 days. The result showed that caffeine significantly ($p<0.05$) reduced sperm viability, sperm count and sperm motility, while sperm head abnormality increased in caffeine treated rats when compared to the control. However, AGLE significantly ($p<0.05$) protected the treated albino rat models from caffeine induced spermatotoxicity in a dose-dependent manner. These results show that AGLE is effective in protecting albino rat models against caffeine induced spermatotoxicity in a dose dependent manner.

Key words: Guava, caffeine, toxicity, sperm quality, sperm count, sperm head abnormality

INTRODUCTION

Guava (*Psidium guajava* L.) is an important tropical fruit tree with distinctive greenish layer beneath the bark. The guava berry is an important fruit that is mostly consumed fresh. The fruit contains several small seeds and consist of a fleshy pericarp and seed cavity with pulp (Jimenez-Escrig *et al*., 2001; Marquez and Suarez, 2007). The fruits can also be made into juice, drinks or ice cream (Pamplona-Roger, 2005).

The leaves and bark of the guava tree are widely used for medicinal purposes (Uboh *et al*., 2010). Animal models as well as controlled human studies showed that its fruit, leaf and root are safe and without side effect (Kamath *et al*., 2008; Nwinyi *et al*., 2008). The leaf decoction is taken for its hepatoprotective qualities (Kamath *et al*., 2008), also as a remedy for throat and chest ailments (Gutierrez *et al*., 2008), cough, pulmonary diseases, anti-inflammatory and homeostatic agent (Goncalves *et al*., 2005). It is also taken as an emmenagogue and vermifuge and treatment of leucorrhea (Goncalves *et al*., 2005; Gutierrez *et al*., 2008).

In folk medicine, especially in some traditional African localities, decoctions from guava leaves are used in traditional treatments against diabetes, malaria (Pamplona-Roger, 1999), also as a tonic to treat digestive conditions and gastrointestinal disorder (Garcia *et al*., 2003; Hassan *et al*., 2011), because of it astringency; crushed leaves are applied on wounds, ulcers and rheumatic places. The leaves are also chewed or gargled to relieve oral ulcer, toothache and inflamed gums (Pamplona-Roger, 1999; Goncalves *et al*., 2005).

Guava also possesses antioxidant and free radical scavenging potentials (Jimenez-Escrig *et al*., 2001; Ekaluo *et al*., 2015) and spermatoprotective properties (Akinola *et al*., 2007). In practice, guava leaf extracts are particularly believed to improve erection, treat impotency and sexual dysfunctions in males (Uboh *et al*., 2010), improvement of sperm parameters and boost male fertility (Ekaluo *et al*., 2013a, b).

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 (Best, 1999; Fredholm *et al*., 1999; Smith, 2002). Unlike most other psychoactive substances, it is legal and unregulated in most part of the world (Ekaluo *et al*., 2005, 2009; Craig, 2008) with an estimated 80% of the world's population consuming a caffeine-containing substance daily (Best, 1999; Craig, 2008). Caffeine dependency has a wide range of unpleasant physical and mental conditions such as nervousness, irritability, restlessness, insomnia, headache and heart palpitations (Lanch *et al*., 2007).

Consumption of caffeine has also been linked with delayed conception (Bolumar *et al*., 1997), reproductive and developmental toxicities (Ekaluo *et al*., 2013c, d, 2014) and increase in the frequency of sperm abnormalities (Robbins *et al*., 1997; Ekaluo *et al*., 2005, 2009).

This study set out to determine the protecting potentials of Aqueous Guava Leaf Extract (AGLE) on caffeine induced spermatotoxicity in albino rat models.

MATERIALS AND METHODS

Collection and preparation of test substance: Fresh leaves of guava (*Psidium guajava*) were obtained from a local garden in Calabar, Cross River State. The leaves were authenticated in the herbarium unit of the Department of Botany University of Calabar, Calabar. The leaves were sorted, dried and pulverized using an electric blender and stored in an air-tight container. On weekly basis, the required quantity was measure out and used for the study.

Experimental animals: Thirty healthy male albino rats of 12 weeks old were obtained from the animal house of the department of Zoology and Environmental Biology, University of Calabar, Calabar. The entire animals were housed in well ventilated wire mesh under standard laboratory conditions. They were allowed free access to water and pelleted commercial feed throughout the period of the study. The animals were allowed to acclimatize for two weeks before experimentation.

Experimental design and procedure: There were 30 rats 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 treatment. The treatment lasted for 65 days from May 8-July 11, 2015 and the protocol for treatment is shown in Table 1.

Table 1: Protocol for treatment of experimental animals

Treatment groups	Description of treatment
Control	No caffeine and no AGLE
C	Caffeine 200 mg kg^{-1} b.wt. orally only
A_{250}	AGLE 250 mg kg^{-1} b.wt. orally only
$C+A_{250}$	Caffeine 200 mg kg^{-1} b.wt. and AGLE, 250 mg kg^{-1} b.wt. both orally
$C+A_{500}$	Caffeine 200 mg kg^{-1} b.wt. and AGLE, 500 mg kg^{-1} b.wt. both orally

AGLE: Aqueous guava leaf extract

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 count, motility, viability and sperm head abnormality.

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. The colour change corresponding to the pH of the semen was read from the paper.

Sperm motility: The sperm motility was evaluated according to the method of Ekaluo *et al*. (2013c, d), 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 40x lenses and expressed in percentage.

Sperm viability: The sperm viability was determined using Eosin-Nigrosin staining technique (Bjorndahl *et al*., 2003). A portion of the sperm suspension was mixed with equal volume of Eosin-Nigrosin, stain and air-dried smears were prepared on glass slide for each sample. The slides were examined for percentage viability. Normal live sperm cells appeared whitish, while dead sperm cells took up stain and appeared pinkish. The percentage viability was calculated based on the number of live sperm cells out of the total number of cells observed.

Sperm count: Epididymal sperm count was obtained by cytometry using the improved Neubauer Cytometer and will be expressed in million per milliliter of the sperm suspension (Ekaluo *et al*., 2008).

Sperm head abnormality: A portion of the sperm suspension was mixed with 1% eosin Y solution (10:1) for 30 min 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*. (2009).

Statistical analysis: Data obtained from epididymal semen pH, motility, viability, count, sperm head abnormalities and weight of testes and epididymis were subjected to Analysis of Variance (ANOVA) test while Least Significant Difference (LSD) test was used to separate the means.

RESULT

Weight of testes and epididymis: There was no significant ($p>0.05$) difference in the weight of testes and epididymis between the control and the treatment groups. The weight of testes ranged from 1.34 g (Caffeine group) to 1.47 g (A_{250} group) while the weight of epididymis was between 0.35 g (Caffeine group) to 0.39 g (A_{250} group). Although, there were dose-dependent increases as shown in Table 2, with the following trend:

$$C<C+A_{250}<C+A_{500}<control<A_{250}$$

Semen pH and sperm motility: There was no significant ($p>0.05$) effect of caffeine and AGLE treatments on the semen pH and sperm motility. The A_{250} group seem to perform better than the control group for both parameters (Table 2).

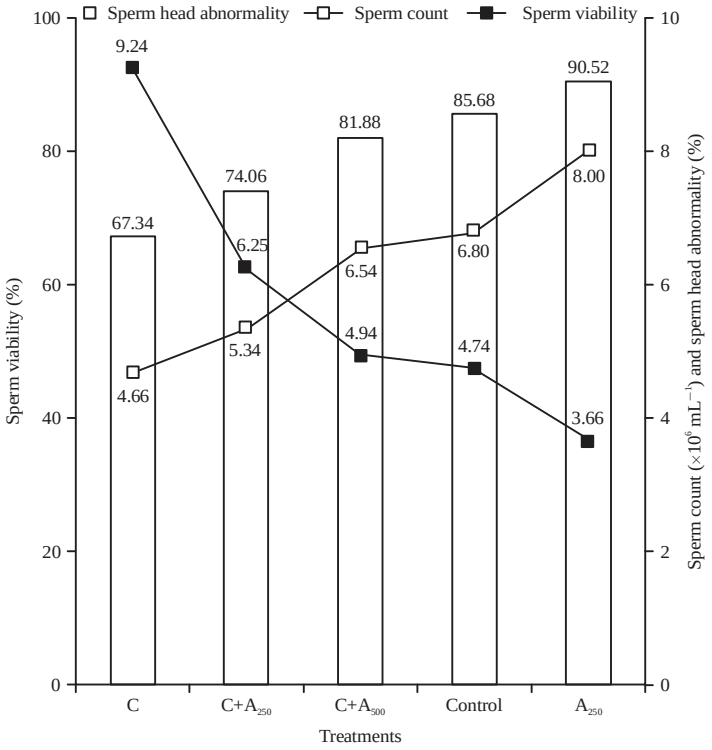

Fig. 1: Effect of aqueous guava leaf extract on caffeine induced sperm toxicities in rats

Table 2: Effect of aqueous guava leaf extract on caffeine induced toxicities in albino rats

	Treatment groups				
Parameters	C	$C+A_{250}$	$C+A_{500}$	A_{250}	Control
Weight of testes (g)	1.34±0.44[a]	1.40±0.02[a]	1.42±0.03[a]	1.47±0.44[a]	1.44±0.99[a]
Weight of epididymis (g)	0.35±0.01[a]	0.37±0.04[a]	0.38±0.03[a]	0.39±0.04[a]	0.38±0.04[a]
Semen pH	6.78±0.06[a]	6.86±0.08[a]	6.96±0.09[a]	7.16±0.05[b]	7.12±0.06[b]
Sperm motility (%)	62.60±2.46[a]	67.92±1.96[b]	60.96±3.85[a]	68.40±2.54[b]	54.30±3.09[a]
Sperm viability (%)	67.34±2.01[a]	74.06±2.15[a]	81.88±3.31[b]	90.52±1.76[c]	85.68±1.89[b]
Sperm count ($\times10^6$ mL^{-1})	4.66±0.32[a]	5.34±0.37[a]	6.54±0.34[b]	8.00±0.18[c]	6.80±0.23[b]
Sperm head abnormalities (%)	9.24±0.32[c]	6.25±0.67[b]	4.94±0.55[a]	3.66±0.22[a]	4.74±0.47[a]

Values with similar superscripts are not significantly different at 5% based on ANOVA, C: Caffeine at 200 mg kg^{-1}b.wt., A_{250}: 250 mg kg^{-1} b.wt., of AGLE, A_{500}: 500 mg kg^{-1} b.wt., of AGLE

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 (85.68%). The AGLE significantly protected and increased sperm viability in the groups treated with AGLE in a dose-dependent manner from caffeine induced toxicity as shown in Table 2 and Fig. 1. The sperm viability was increased from 67.34% in caffeine group (C) to 74.06 and 81.88%, respectively for $C+A_{250}$ and $C+A_{500}$ groups.

Sperm count: There was a significant ($p>0.05$) reduction in the sperm count of caffeine treated animals when compared to the control ($6.80\times10^6 mL^{-1}$). The sperm count was significantly protected and increased in the groups treated with AGLE in a dose-dependent manner from caffeine induced toxicity as also shown in Table 2 and Fig. 1, from $4.66\text{-}6.54\times10^6$ mL^{-1}.

Sperm head abnormality: The AGLE also significantly protected and reduced the percentage of sperm head abnormalities in the treated animals in a dose-dependent manner from caffeine induced toxicity as shown in Table 2 and Fig. 1. The AGLE reduced percentage of sperm head abnormalities significantly from 9.24-4.94% in caffeine treated animals.

DISCUSSION

There was no significant effect of caffeine on the weight of testes and epididymis, although there were dose-dependent protection effects of Aqueous Guava Leaf Extract (AGLE) with accompanying increases in weights of testes and epididymis which are similar to earlier findings of Ekaluo *et al*. (2013c, d, 2014). These suggest alterations in spermatogenesis in the tests. According to Ezzat and El-Gohary (1994), long term intake of caffeine induces suppression of spermatogenesis, hence distorts fertility in male animals. On the other hand, caffeine significantly increased the percentage of sperm head abnormalities which suggests induced mutation on the sperm cells during spermatogenesis. This also agrees with the reports of Robbins *et al*. (1997), Harris (2004) and Ekaluo *et al*. (2009).

The significant reduction in the sperm count and viability, in caffeine treated animals is similar to the findings of Wilcox *et al*. (1988), Bassey *et al*. (2011) and Ekaluo *et al*. (2013c, d) and also significantly increased the percentage of sperm head abnormalities which is similar to the findings of Ikpeme *et al*. (2012).

Aqueous Guava Leaf Extract (AGLE) significantly protected and increased sperm count and sperm viability in the rats treated with AGLE in a dose-dependent manner from caffeine induced toxicity as well as reducing the percentage of sperm head abnormalities (Table 2 and Fig. 1). This can be attributed to the high content of vitamin C (Pamplona-Roger, 1999; Begum *et al*., 2002; Suntornsuk *et al*., 2002) and its antioxidant properties (Olajide *et al*., 1999; Jimenez-Escrig *et al*., 2001; Ekaluo *et al*., 2015). This agrees with the reports of Karawya and El-Nahas (2006), Akinola *et al*. (2007), Nashwa and Venes (2008) and Ekaluo *et al*. (2013a, b, 2015).

Epidemiological studies have revealed that consuming fruits and vegetables as well as their extracts reduced free radical oxidative damage (Wang and Su, 2000; Ikpeme *et al*., 2014) and promote fertility (Ikpeme *et al*., 2007; Ekaluo *et al*., 2010, 2011, 2013a, b). Increased Reactive Oxygen Species (ROS) level has been correlated with decreased sperm count and motility (Armstrong *et al*., 1999). Therefore, the protecting effect of AGLE on caffeine induced spermatotoxicity can be attributed to the protective roles of its constituents against oxidative stress and induced mutations.

CONCLUSION

The present study shows that Aqueous Guava Leaf Extract (AGLE) is effective in protecting the albino rat models from caffeine induced spermatotoxicity in a dose dependent manner.

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