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Effect of Soursop (*Annona muricata* L.) Leaf Extract on Oxidative Stress Caused by Caffeine in Albino Rat Models

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Authors' contributions

This work was carried out in collaboration between all authors. Author UUU designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors UBE and PBE managed the analyses of the study. Authors HOO and CMO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was carried out to study the mitigating effect of Soursop (*A. muricata*) Tea (SST) on oxidative stress caused by caffeine in albino rat models.
Study Design: The completely randomized design (CRD) was used.
Place and Duration of the Study: The study was carried out at the animal house of the Department of Genetics and Biotechnology, University of Calabar, Calabar, Nigeria and the duration was for nine weeks.
Methodology: Thirty healthy male albino rats of 12 weeks old were randomly divided into 5 different groups with each containing 6 rats using a CRD. The rats were treated with a mixture of caffeine and SST using oral gavage. The treatment was done for 65 days.
Results: Results obtained on the oxidative stress enzymes indicated a statistically significant (P=.05) decrease in the plasma level of superoxide dismutase (SOD), catalase activity (CAT) and glutathione peroxidase (GPx) in groups treated with caffeine when compared with the control group. There was also an increase in the level of malondialdehyde (MDA) in rats treated with

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caffeine. However, SST mitigated the oxidative stress caused by caffeine in the albino rat models. The effect was in a dose dependent manner.
Conclusion: The findings of study therefore indicate that SST has the propensity to mitigating oxidative stress caused by caffeine in albino rats as mammalian models.

Keywords: Caffeine; soursop; oxidative stress; malondialdehyde.

1. INTRODUCTION

Soursop (*Annona muricata* L.) which is also known as graviola, belongs to the family Annonaceae and is spread throughout the tropics. It has the largest species in the genus. The plant is astringent, cholagogic and increases digestion [1]. It also possesses various medical uses such as in the control of high blood sugar levels and its problems [1-4], also as antioxidant and antimutagenic agent [5]. It is mostly used in cases of indigestion, overweight, hypertension and heart diseases [1]. 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 infections such as eczema while the white pulp of the fruit is used to produce juice, and also candy, sorbets and flavours used in ice-creams. The fruit contains high amount of vitamin B, potassium, fructose and vitamin C [1,4].

Oxidative stress entails the availability of free radicals and ROS produced during normal physiological conditions but is however causes harm and becomes dangerous when there are not destroyed by antioxidant enzymes [6]. In general, reactive oxygen species are able to react with different components of the cell such as DNA, carbohydrates, proteins and lipids in a deleterious way, therefore, the equilibrium between ROS and antioxidant enzymes play a significant role in forestalling oxidative stress [7, 8]. Studies in rodent models suggest mechanism of action such as oxidative stress, DNA damage to sperm, altered hormonal profile, and abnormal progression through spermatogenesis [9].

Caffeine (1,3,7-trimethylxanthine) is a crystalline, white purine and is probably one of the most frequently consumed psychoactive substance globally [10-11]. It is contained in commonly consumed beverage drinks such as coffee, tea, energy drinks and carbonated beverages, products made up of cocoa or chocolate and in drugs [12-14]. In human, low and medium doses of caffeine causes increase alertness and positive impacts on the myocardium, while elevated concentrations can cause caffeinism which implies caffeine dependency with a wide range of adverse physical and mental condition including nervousness, restlessness, irritability, headache, insomnia, and heart palpitations after caffeine use [4,15]. It could also cause adverse tachycardia and ventricular arrhythmia [4,16-17]. Consumption of caffeine has also been linked with delay in conception [4,18], reproductive and developmental abnormalities [4,19] and increased occurrence of sperm mutations [4,16].

Some of the impacts of caffeine may trigger the synthesis of ROS and this can lead to a concomitant increase in lipid peroxidation by enhancing oxidative stress [6,8,20]. Also, pathways in the mitochondrial that breaks down energy substrates and performs respiration, gives rise to significant amounts of free radicals, which leak out of the mitochondria and cause significant damages to various components of the cell. Thus, increased activities in the mitochondrial is expected to enlarge the free radical pool and this in turn, contribute to oxidative stress [8,21-22]. Oxidative stress has also been shown to cause substantial damages to biomolecules including lipid DNA damages, peroxidation, and decline in quality of sperm [8, 23–26].

Considering the increasing intake of caffeine and its abuse which may cause toxic effects, oxidative stress and damage to the cells [8], it is imperative to determine the mitigating effect of SST on oxidative stress caused by caffeine in albino rat models.

2. MATERIALS AND METHODS

2.1 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 plant were identified and authenticated by Dr. Samuel Udoh, Senior Lecturer, Department of Botany, University of Calabar, Calabar.

2.2 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 300 mg of powdered leaves in 50 ml 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.

2.3 Treatments and Other Chemicals

Caffeine was obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used in this study were of analytical grade.

2.4 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.

2.5 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 anaesthesia 24 hours after the last treatment. Blood samples were collected through cardiac puncture and the serum was used for the analyses of the following oxidative stress markers: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA).

2.6 Determination of Oxidative Stress Markers

2.6.1 Superoxide dismutase

Packed erythrocytes were obtained from blood sample and washed four times with 5 mL of 0.9% saline solution and centrifuged at 3500 rpm for 10 min. The cells were lysed with ice cold distilled water and centrifuged twice to obtain erythrocyte membrane and hemolysate. The cells were further treated with chloroform and ethanol and used to determine SOD enzyme activity which was expressed in µmol [27].

2.6.2 Catalase

Catalase activity was determined according to the method of Aebi [28]. The method is based on the decomposition of H_2O_2 by catalase. Sample containing catalase was incubated in H_2O_2 and then mixed with 4-aminophenozone and 3, 5-dichloro-2-hydrobenzenesulfonic acid and catalysed by horseradish peroxidase. The resulting quinoneimine dye was measured at 510 nm and expressed in nmol.

2.6.3 Glutathione peroxidase

Glutathione Peroxidase (GPx) was assessed according to the method of Paglia and Valantine [29], using the Fortress diagnostic kit. GPx catalyses the oxidation of glutathione and then the oxidized glutathione is converted to the reduced form in the presence of glutathione reductase and NADPH. The NADPH is oxidized to NADP and decrease in absorbance at 340 nm is measured and expressed in micro per gram Hb.

2.6.4 Malondialdehyde

Malondialdehyde (MDA) is the end product of lipid peroxidation. MDA was determined by the method of Draper and Hadley [30]. The principle of this method is based on fluorometric measurement of the colour produced during the reaction of Thiobarbituric Acid (TBA) with MDA. Absorbance was measured using a spectrophotometer at 532 nm. The concentration of MDA was calculated and expressed in nmol mL^{-1}.

2.7 Statistical Analysis

Data from the oxidative stress markers (superoxide dismutase, catalase, glutathione peroxidase and malondialdehyde) were subjected to analyses of variance (ANOVA) test for significant difference. Statistical significance were considered if p=.05 while least significant difference (LSD) test was used to separate the means.

Table 1. Protocol for daily treatment of experimental animals

Treatment groups	Description of treatment
Control	Caffeine and soursop tea (SST) not administered
C	Caffeine administered at 200 mg kg^{-1} bw using oral gavage tube
SST_1	SST administered at 100 mg kg^{-1} bw using oral gavage
$C+SST_1$	Caffeine administered at 200 mg kg^{-1} bw and after 10-12 hours, SST was administered at 100 mg kg^{-1} BW both orally via oral gavage
$C+SST_2$	Caffeine, 200 mg kg^{-1} bw and 10-12 hours after SST 200 mg kg^{-1} BW both orally via oral gavage
$C+SST_3$	Caffeine, 200 mg kg^{-1} bw and 10-12 hours after SST 300 mg kg^{-1} BW both orally via oral gavage

3. RESULTS AND DISCUSSION

3.1 Superoxide Dismutase (SOD)

Table 1 indicated that the serum level of SOD reduced significantly (P=.05) in group treated caffeine in comparison to the control group. Animals treated in the caffeine group possessed the least value (89.037 μmol^{-1}). The levels were increased significantly in $C+SST_3$ group being 117.16 μmol^{-1} revealing a dose-dependent mitigating effect. The highest serum level of SOD was found in the SST_1 group being 131.56 μmol^{-1} while the control had 124.4 μmol^{-1}.

3.2 Glutathione Peroxidase (GPx)

Caffeine resulted to a significant (P=.05) reduction in the serum level of GPx in the treated animals. The lowest level was observed in the caffeine group being 9.91 μgHb^{-1}. The effect of caffeine was mitigated significantly in the $C+SST_2$ and $C+SST_3$ groups with both having mean levels of 14.64 and 16.86 μgHb^{-1}, respectively. SST_1 group had mean level of 22.19 μgHb^{-1} while the highest level of GPx was observed in the control animals being 31.65 μgHb^{-1} as shown in Table 1.

3.3 Catalase (CAT)

The concentration of catalase significantly (P=.05) reduced in the caffeine group when compared to the control (Table 2). Caffeine, $C+SST_1$ and $C+SST_2$ had mean values of 0.30, 0.45 and 0.48 nmol, respectively while the control had mean level of 0.64 nmol. The highest serum level of catalase was obtained in the SST_1 group (0.71 nmol). The effect was as well significantly mitigated by SST in the $C+SST_3$ group depending on the dose.

3.4 Malondialdehyde (MDA)

The serum level of MDA was increased significantly (P=.05) by caffeine treatment. Caffeine group had the highest concentration of 2.64 nmol/ml, followed by $C+SST_1$ (2.11 nmol/ml), $C+SST_2$ (1.99 nmol/ml) and $C+SS_3$ (1.37 nmol/ml) indication of dose-dependent mitigating effect of SST. The control and SST_1 groups had 1.47 and 1.74 nmol/ml, respectively (Table 2).

3.5 Discussion

Result gotten in this study revealed that caffeine had significant effect on the oxidative stress enzymes assessed. The serum levels of the antioxidants (SOD, GPx and CAT) declined significantly (P=.05) in animals treated with caffeine which is in line with the observations of Hatice et al. [29] and Ekaluo et al. [8]. These antioxidants transforms ROS or reactive oxygen intermediates to products that are not radical or harmful [8,30]. Earlier researches have revealed that CAT, SOD and GPx are the main garners of ROS that can cause havoc in organs [31]. Kermal et al. [32] and Ekaluo et al. [8] reported that attack on the DNA by free radicals gives rise base free sites, frame-shift mutations, deletion, chromosomal rearrangement and DNA cross links. Oxidative damage that arises from excess synthesis of ROS has been correlated with abnormal organs and the reluctance of catalyst that play a role in the removal of ROS, thus producing LPO, gene expression alterations and apoptosis [8,33–34]. The CAT, SOD and GPx play an important role in garnering ROS. The SOD enhances the destruction radicals of superoxide to hydrogen peroxide while CAT and GPx reduce hydrogen peroxide into water and oxygen to forestall oxidative stress and maintain homeostasis in the cell.

In a study similar to the present study, it was seen that neurological dysfunction has inward linkage to neuronal cell death triggered by reactive oxygen species [8-7]. The significant dose-dependent decrease in CAT, SOD and GPx level by treatment with caffeine shows a decrease in antioxidant defense system,

Table 2. Effect of SST on oxidative markers in rats

Parameters	Treatment groups					
	Control	Caffeine	C+SST_1	C+SST_2	C+SST_3	SST_1
SOD (μmol^{-1})	124.41 ± 1.51^{c}	89.03 ± 1.57^{a}	98.69 ± 0.03^{a}	102.89 ± 1.71^{a}	117 ± 1.29^{b}	131.56 ± 1.01^{c}
GPx (μgHb^{-1})	31.65 ± 0.39^{a}	9.91 ± 0.08^{a}	10.94 ± 0.28^{a}	14.64 ± 0.46^{b}	16.86 ± 0.17^{b}	22.19 ± 1.57^{a}
CAT (nmol)	0.64 ± 002^{b}	0.30 ± 0.03^{a}	0.45 ± 0.01^{a}	0.48 ± 0.01^{a}	0.57 ± 0.01^{b}	0.71 ± 0.01^{c}
MDA (nmol)	1.47 ± 0.02^{a}	2.46 ± 0.01^{c}	2.11 ± 0.03^{b}	1.99 ± 0.02^{b}	1.37 ± 0.02^{a}	1.74 ± 0.03^{b}

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 200 mg/kgBW, SST_1: Soursop tea at 100 mg/kgBW, SST_2: Soursop tea at 200 mg/kgBW, SST_3: Soursop tea at 300 mg/kgBW

increased ROS activities and as a result, oxidative stress. On the other hand, SST mitigated the oxidative stress caused by caffeine by raising the serum levels of CAT, SOD and GPx based on the dose of the treatment administered (Table 2).

More so, the serum level of MDA improved in a significant manner in animals treated with caffeine which reveals an elevated LPO activity. LPO is an essential biological impact of oxidative destruction to the cell; hence the elevated levels of MDA depicts oxidative stress [6,10]. The increase in the serum level of MDA in animals given caffeine treatment agrees with Ekaluo et al. [8] and Dianzani et al. [35]. An increase in LPO also impede the actions of enzymes with antioxidant properties such as GPx, SOD and CAT including the total antioxidant status [8,36].

The mitigating effect of SST was also seen as it cause a decline in the concentration of MDA from 2.64 nmol/ml in group of animals treated with caffeine to 1.37 nmol/ml in C+SST_3 group suggesting a mitigating effect based on the dose of the treatment (Table 2). These mitigating effects might be due to the different antioxidants and the phytochemicals in the extract. Therefore, it shows that the different components of the SST might have performed a vital part in garnering ROS that can build up to cause LPO and oxidative damage to proteins and DNA [8,37] as observed in the groups that were given caffeine treatment.

4. CONCLUSION

The findings of this study reveals that SST has the propensity to effectively mitigate oxidative stress caused by caffeine in albino rats as mammalian models and the effect is dose dependent.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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