

Original Research Article

EFFECT OF SOURSOP (*Annona muricata* L.) LEAF EXTRACT ON CAFFEINE INDUCED OXIDATIVE STRESS IN ALBINO RAT MODELS

ABSTRACT

Aim: This study was aimed at investigating the mitigating effect of Soursop (*A. muricata*) Tea (SST) in caffeine induced oxidative stress in albino rat models.

Study design: The study employed a completely randomized design (CRD).

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 lasted for nine weeks.

Methodology: Thirty healthy male albino rats of 12 weeks old were divided into five groups with six rats in each group using a completely randomized design (CRD). The rats were treated with combinations of caffeine and SST orally via oral gavage. The treatment lasted for 65 days.

Results: Results of the oxidative stress markers showed a statistically significant ($P=.05$) reduction in the plasma level of superoxide dismutase (SOD), catalase activity (CAT) and glutathione peroxidase (GPx) in caffeine treated groups when compared with the control group; also with an increase in the level of malondialdehyde (MDA) in caffeine treated rats. However, SST mitigated the caffeine-induced oxidative stress in the albino rat models in a dose dependent manner.

Conclusion: This study therefore, reveals that SST has the potential to mitigating caffeine-induced oxidative stress in albino rat models.

Keywords: Caffeine, soursop, oxidative stress, malondialdehyde.

1. INTRODUCTION

29 Soursop (*Annona muricata* L.), also known as graviola, belongs to the family Annonaceae
 30 which is found throughout the tropics. It has the largest fruits in the genus. The soursop is astringent,
 31 cholagogic and promotes digestion [1]. It also has several medicinal uses such as in the management
 32 of diabetes and its complications [1-3], also as antioxidant and antimutagenic agent [4]. It is usually
 33 recommended in cases of constipation, obesity, hypertension and coronary diseases [1]. All the plant
 34 parts of the plants are useful in folk medicine including the bark, leaves, root and fruits. The leaf
 35 extract is used in the treatment of cancer and skin infection such as eczema while the white pulp of
 36 the fruit is used to make juice, as well as candies, sorbets and ice-cream flavorings. Its flavor is
 37 described as a combination of strawberry and pineapple with sour citrus flavor notes contrasting with
 38 an underlying creamy flavor reminiscent of coconut or banana. The fruit is rich in B group vitamins,
 39 potassium, fructose and vitamin C [1].

40 Oxidative stress depicts the presence of free radicals and reactive oxygen species (ROS)
 41 formed under normal physiological conditions but become harmful and deleterious when there are not
 42 eliminated by antioxidant systems [5]. In general, ROS are able to react with diverse cellular
 43 components (e.g. DNA, carbohydrates, proteins and lipids) in a destructive way, therefore, the
 44 balance between ROS and antioxidant species play an important role in preventing oxidative stress
 45 [6].

46 Some of the effects of caffeine may favour the production of free radicals and lead to a
 47 subsequent increase in lipid peroxidation by increasing oxidative stress [5, 7]. Also, mitochondrial
 48 pathways that oxidize energy substrates and carry out respiration, produces significant amounts of
 49 ROS, which leak out of the mitochondria and cause substantial damage to various cellular
 50 components. Thus, enhanced mitochondrial activity is expected to enlarge the ROS pool and, in turn,
 51 contribute to oxidative stress [8, 9]. Oxidative stress has also been reported to cause significant
 52 damage to biological molecules such as lipid peroxidation, DNA damage and decline in sperm quality
 53 [10 – 13].

54 Caffeine (1,3,7-trimethylxanthine) is a bitter white crystalline purine and is probably the most
 55 frequently ingested psychoactive substance in the world [14-16]. It is found in common beverages like
 56 coffee, tea, energy drinks, carbonated beverages, product containing cocoa or chocolate and in
 57 medications [17-19]. In human, low and intermediate doses of caffeine produce increase alertness
 58 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 [20]. It could also cause adverse tachycardia and ventricular arrhythmia [21-22]. Consumption of caffeine has been linked with delayed conception [23], reproductive and developmental risks [24] and increased frequencies of sperm aneuploidy [21].

In view of the increasing intake of caffeine and its abuse which is arbitrary and unregulated that may cause toxicities, oxidative stress and cellular damage. This study is designed to ascertain the mitigating effect of SST on caffeine induced oxidative stress in albino rats as mammalian model.

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 fruits 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 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.

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).

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
SST₁	SST 100mg kg ⁻¹ BW only via oral gavage
C+SST₁	Caffeine, 200mg kg ⁻¹ BW and 10-12 hours after SST 100mg kg ⁻¹ BW both orally via oral gavage
C+SST₂	Caffeine, 200mg kg ⁻¹ BW and 10-12 hours after SST 200mg kg ⁻¹ BW both orally via oral gavage
C+SST₃	Caffeine, 200mg kg ⁻¹ BW and 10-12 hours after SST 300mg kg ⁻¹ BW both orally via oral gavage

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 [25].

2.6.2. Catalase

Catalase activity was determined according to the method of Aebi [26]. 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 [27], 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 [28]. 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.

3. RESULTS AND DISCUSSION

3.1. Superoxide dismutase (SOD)

Table 1 shows that the concentration of SOD was significantly ($P=.05$) reduced in caffeine group when compared to the control. Treated animals in the caffeine group had the lowest value of $89.037\mu mol^{-1}$. The values were significantly increased in C+SST₃ groups ($117.16\mu mol^{-1}$) indicating a

dose-dependent mitigating effect. The highest concentration of SOD was recorded in the SST₁ group (131.56 μmol^{-1}) while the control had 124.4 μmol^{-1} .

3.2. Glutathione peroxidase (GPx)

Caffeine caused a significant ($P=.05$) decrease in the concentration of GPx in the treated animals with the lowest value observed in the caffeine group (9.91 μgHb^{-1}). The effect of caffeine was significantly mitigated in the C+SST₂ and C+SST₃ groups with mean values of 14.64 and 16.86 μgHb^{-1} , respectively. SST₁ group had mean value of 22.19 μgHb^{-1} while the highest concentration of GPx was recorded in the control (31.65 μgHb^{-1}) as shown in Table 1.

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 $\pm$ 1.51 ^c	89.03 $\pm$ 1.57 ^a	98.69 $\pm$ 0.03 ^a	102.89 $\pm$ 1.71 ^a	117 $\pm$ 1.29 ^b	131.56 $\pm$ 1.01 ^c
GPx (μgHb^{-1})	31.65 $\pm$ 0.39 ^a	9.91 $\pm$ 0.08 ^a	10.94 $\pm$ 0.28 ^a	14.64 $\pm$ 0.46 ^b	16.86 $\pm$ 0.17 ^b	22.19 $\pm$ 1.57 ^a
CAT (nmol)	0.64 $\pm$ 0.02 ^b	0.30 $\pm$ 0.03 ^a	0.45 $\pm$ 0.01 ^a	0.48 $\pm$ 0.01 ^a	0.57 $\pm$ 0.01 ^b	0.71 $\pm$ 0.01 ^c
MDA (nmol)	1.47 $\pm$ 0.02 ^a	2.46 $\pm$ 0.01 ^c	2.11 $\pm$ 0.03 ^b	1.99 $\pm$ 0.02 ^b	1.37 $\pm$ 0.02 ^a	1.74 $\pm$ 0.03 ^b

Values are presented as mean $\pm$ 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

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+SST₁ and C+SST₂ had mean values of 0.30, 0.45 and 0.48nmol, respectively while the control group had 0.64nmol. The highest concentration of catalase was obtained in the SST₁ group (0.71nmol). The effect was also mitigated by SST in the C+SST₃ group in a dose dependent manner.

3.4. Malondialdehyde (MDA)

MDA was significantly increased ($P=.05$) by caffeine treatment. Caffeine group had the highest concentration of 2.64nmol/ml, followed by C+SST₁ (2.11nmol/ml), C+SST₂ (1.99nmol/ml) and C+SST₃ (1.37nmol/ml) indication of dose-dependent mitigating effect of SST. The control and SST₁ groups had 1.47 and 1.74nmol/ml, respectively (Table 2).

3.5. DISCUSSION

Result obtained in this study indicated that caffeine significantly affected the oxidative stress markers assayed. The concentration of the antioxidants (SOD, GPx and CAT) significantly ($P=.05$) reduced in caffeine treated animals which agrees with the findings of Hatice *et al.* [27] and Ekaluo *et al.* [30]. These antioxidants convert free radicals or reactive oxygen intermediates to non-radical and harmless products [28]. Previous studies have shown that SOD, CAT and GPx are the major scavengers of harmful ROS in organs [29]. Kermal *et al.* [30] showed that attack on the DNA by ROS produce base free sites, deletion, frame-shift mutations, DNA cross links and chromosomal rearrangements. Oxidative damage arising from excessive production of Reactive Oxygen Species (ROS) has been associated with defective organs and the inhibition of enzymes involved in the removal of free radicals, thus leading to lipid peroxidation, alteration in gene expression and cell death [34 – 35]. The SOD, CAT and GPx are known to play a vital role in scavenging ROS. The SOD catalyzes the destruction of superoxide radicals to H_2O_2 while CAT and GPx reduce H_2O_2 into water and oxygen to prevent oxidative stress and maintain cell homeostasis.

In a related study, it was observed that neurological impairment is intrinsically linked to ROS-triggered neuronal apoptosis [6]. The significant dose-dependent reductions in SOD, CAT and GPx concentrations by caffeine treatment indicate a decrease in antioxidant defense system, increased free radical activities and consequently resulting in oxidative stress. However, SST mitigated the caffeine-induced oxidative stress by increasing the concentrations of SOD, CAT and GPx (Table 2).

On the other hand, the concentration of MDA significantly increased in caffeine-treated groups which indicate an increased lipid peroxidation activity. Lipid peroxidation is an important biological consequence of oxidative cellular damage; hence the increased concentration of MDA reflects oxidative stress [5, 8]. The increase in the concentration of MDA in caffeine-treated animals is similar to the findings of Dianzani *et al.* [36]. Increase in lipid peroxidation also inhibits the activity of antioxidative enzymes such as SOD, GPx and CAT as well as total antioxidant status [37].

The mitigating potential of SST was also observed as it reduced the concentration of MDA from 2.64 nmol/ml in caffeine group to 1.37 nmol/ml in C+SST₃ group (Table 2). These mitigating effects can be attributed to the different antioxidants and the phytochemicals in the extract. This therefore implies that the various constituents of the SST must have played a major role in

scavenging free radicals that can accumulate to cause lipid peroxidation and oxidative damage to proteins and DNA [38] as seen in the caffeine treated animals.

4. CONCLUSION

The results of the present study suggest that Soursop Tea (SST) is effective in mitigating caffeine induced oxidative stress in albino rat models; and also in a dose dependent manner.

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