Mitigating Effect of Honey on Caffeine Induced Oxidative Stress in Male Albino Rats

U. U. Uno¹,²*, A. J. Umoyen¹ and U. B. Ekaluo¹

¹Department of Genetics and Biotechnology, University of Calabar, Calabar, Nigeria.
²Department of Biology, Cross River State College of Education, Akamkpa, Nigeria.

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. Author AJU managed the analyses of the study. Author UBE managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

Aim: The study was aimed at determining the mitigating effect of honey on oxidative stress induced by caffeine using oxidative stress markers such as superoxide dismutase (SOD), catalase, Glutathione and malondialdehyde, in male albino rats.

Study Design: A completely randomized design (CRD) was used in this study.

Place and Duration of Study: This study was conducted in the Department of Genetics and Biotechnology, University of Calabar and lasted for 65 days.

Methodology: Thirty healthy male albino rats of 12 weeks old were randomly divided into five groups of six rats each. The experimental animals were treated with combinations of caffeine and honey using oral gavage. Group 1 served as the control and was given only water and feed; Group 2 were treated with 10 ml/kgBW of honey (honey group); Group 3 were treated with 200 mg/kgBW of caffeine (caffeine group); Group 4 were treated with 200 mg/kgBW of caffeine and 10 ml/kgBW of honey (C + H₁ group) while Group 5 were treated with 20 ml/kgBW of honey and 200 mg/kgBW of caffeine (C + H₂).
1. INTRODUCTION

Honey is produced by bees from the nectar collected from flowering plants. It is an alkaline forming food with ingredients similar to those found in fruits, which become alkaline in the digestive system [1-2]. Over the years, honey has been used as both a natural sweetener and a healing agent.

Honey has a very complex chemical composition, depending majorly on the composition of nectar where it originates [3-4]. It has more than one hundred and eighty substances which include moisture; sugars such as glucose and fructose; enzymes such as catalase and glutathione reductase; trace essential elements which include iron, copper, zinc and calcium; vitamins such as vitamin A, C and E as well as some flavonoids and phenolic acids [5-9]. It is made primarily of fructose and glucose but also contain 4-5% of fructooligosaccharide which acts as a prebiotic substance [10]. Therefore, the composition of honey differs with different floral components as well as environmental and climatic conditions [9,11].

Caffeine constitute one of the most constantly consumed psychoactive substances globally and is present in several foods, drugs and beverage products such as energy drinks, coffee and tea [12-14]. In variance to most other psychoactive substances, it is legalized and unregulated in majority of the countries of the world [15-17] with an estimated 80% of the world’s population consuming a caffeine-containing substance daily [12,17]. Caffeine and other methylxanthines are used in clinical medicines as diuretics, analgesics, muscle relaxants and can aid in the treatment of brain disorders such as headache, and Parkinson’s diseases [18]. In humans, low and average doses of caffeine produce increase alertness and positive effects on the myocardium, while high doses cause caffeine dependency with a wide range of unpleasant physical and mental conditions such as nervousness, irritability, restlessness, insomnia, headache and heart palpitations [19]. Consumption of caffeine has also been linked with delayed conception [20], reproductive and developmental toxicities [21-27] and an increase in the frequency of sperm abnormalities [28].

Oxidative stress entails the availability of free radicals and reactive oxygen species (ROS) generated under ideal physiological conditions. These become deleterious and harmful when there are not removed by antioxidant systems [29]. In general, Reactive oxygen species are capable to react with diverse cellular components (e.g. DNA, carbohydrates, proteins and lipids) in a destructive manner, therefore, the balance between ROS and antioxidants play an vital role in forestalling oxidative stress [30]. Some of the effects of caffeine may favour the production of free radicals and lead to a subsequent increase in lipid peroxidation thereby increasing oxidative stress [29-34]. 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 [35]. 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 [36-37]. Oxidative stress has also been shown to cause substantial damages to biomolecules including lipid DNA damages, peroxidation, and decline in quality of sperm [38].

Therefore, this study was carried out to determine the potential mitigating effect of honey on the caffeine-induced oxidative stress in albino rats as a mammalian model.
2. MATERIALS AND METHODS

2.1 Treatments and Other Chemicals

Caffeine was obtained from Sigma-Aldrich (St. Louis, MO, USA), while the honey used for this research work was obtained from the Cross River State Ministry of Agriculture, Calabar. All other chemicals used in this study were of analytical grade. Caffeine was adopted considering it widespread and unregulated consumption in various foods and beverages as stated earlier. On the other hand, honey is reported to contain several phytonutrients and antioxidants as stated in the introduction, hence its choice in the study.

2.2 Experimental Animals

Thirty healthy male albino rats of 12 weeks old; with an average body weight of 176.5 g were obtained from the animal house of the Department of Genetics and Biotechnology, University of Calabar, Calabar for this study. The rats were housed in well ventilated wire mesh cages under standard laboratory conditions. They were allowed free access to water and pelleted commercial feed throughout the period of the experiment. Generally, the study was conducted in accordance with the recommendations from the declarations of Helsinki on guiding principles in care and use of animals and the local ethical committee (Approval number: CRS/MH/CGS&EH/ Vol.1/46).

2.3 Experimental Design and Procedure

The thirty rats were 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 daily treatments were given orally via oral gavage which 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. Blood samples were collected through cardiac puncture to get high volume of blood 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).

2.4 Determination of Oxidative Stress Markers

2.4.1 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 nmol mL⁻¹ [39].

2.5 Catalase

Catalase activity was determined according to the method of Aebi [40]. The method is based on the decomposition of H₂O₂ by catalase. Sample containing catalase was incubated in H₂O₂ 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 mL⁻¹.

2.6 Glutathione Peroxidase

Glutathione Peroxidase (GPx) was assessed according to the method of Paglia and Valantine [41], 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 nmol mL⁻¹.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Groups</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control</td>
<td>No honey and no caffeine</td>
</tr>
<tr>
<td>2.</td>
<td>H₁</td>
<td>10 ml/kgBW of honey only via oral gavage</td>
</tr>
<tr>
<td>3.</td>
<td>C</td>
<td>200 mg/kgBW of caffeine only via oral gavage</td>
</tr>
<tr>
<td>4.</td>
<td>C + H₁</td>
<td>10 ml/kgBW of honey and 200 mg/kgBW of caffeine both orally via oral gavage</td>
</tr>
<tr>
<td>5.</td>
<td>C + H₂</td>
<td>20 ml/kgBW of honey and 200 mg/kgBW of caffeine both orally via oral gavage</td>
</tr>
</tbody>
</table>
3. RESULTS

3.1 Superoxide Dismutase (SOD)

Results presented in Table 2 indicate that the serum concentration of SOD was significantly (P<0.05) reduced in caffeine group when compared to the control (Table 2, line 3, column 2, 3). Animals treated with caffeine alone had the lowest value (8.40 nmol mL⁻¹). The concentration of SOD significantly increased in C + H₁ and C + H₂ groups (9.80 and 10.10 nmol mL⁻¹, respectively, Table 2, line 3, column 4, 5) indicating a dose-dependent mitigating effect of honey. The highest concentration of SOD was recorded in the control group (12.30 nmol mL⁻¹) while the honey group had 12.00 nmol mL⁻¹.

3.2 Glutathione Peroxidase (GPx)

Numerical results are detailed in Table 2, line 4. There was a significant (P<0.05) reduction in the serum concentration of GPx in caffeine treated animals when compared with the control: they had the lowest value observed (6.90 nmol mL⁻¹). The effect of caffeine was mitigated in the C + H₁ and C + H₂ groups with mean values of 7.40 and 7.80 nmol mL⁻¹, respectively. The honey group had the highest concentration of GPx (8.40 nmol mL⁻¹), closely followed by the control (8.00 nmol mL⁻¹).

3.3 Catalase (CAT)

Detailed numerical results are given in Table 2, line 2. The concentration of catalase significantly (P<0.05) reduced in the caffeine treated groups when compared to the control. Caffeine group had the lowest value (15.00 nmol mL⁻¹) while C + H₁ and C + H₂ had mean values of 16.90 and 17.20 nmol mL⁻¹, respectively indicating mitigating effect of honey. The control and group had 21.30 and 21.50 nmol mL⁻¹, respectively.

3.4 Malondialdehyde (MDA)

Caffeine caused a significant (P<0.05) increased in the serum concentration of MDA. Caffeine group had the highest concentration of 0.11 nmol mL⁻¹. The value decreased in C + H₁ and C + H₂ (0.08 and 0.09 nmol mL⁻¹, respectively) indicative of mitigating effect of honey. The control and honey groups had 0.04 and 0.05 nmol mL⁻¹, respectively (Table 2).

4. DISCUSSION

The present study revealed that caffeine had a significant effect on the oxidative stress markers studied (Table 2). The serum concentration of the antioxidants decreased in caffeine treated animals which agrees with Hatice et al. [43], Uno et al. [28] and Ekaluo et al. [24]. These antioxidants have been reported to convert free radicals or reactive oxygen species (ROS) to non-radical and harmless products [44]. Studies suggest that SOD, CAT and GPx are the major scavengers of harmful ROS in organs [45]. Kermal et al. [46] reported that attack on the DNA by ROS produce base free sites, deletion, frame-shift mutations, DNA cross links and chromosomal rearrangements. It was also observed in a related study that neurological impairment is intrinsically linked to ROS-triggered neuronal apoptosis [30]. The significant reductions in SOD, CAT and GPx concentrations by caffeine treatment implies a decrease in

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Caffeine</th>
<th>Honey</th>
<th>C + H₁</th>
<th>C + H₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malondialdehyde</td>
<td>0.04±0.003a</td>
<td>0.11±0.004c</td>
<td>0.05±0.006a</td>
<td>0.08±0.003b</td>
<td>0.09±0.003b</td>
</tr>
<tr>
<td>Catalase</td>
<td>21.3 ±0.08a</td>
<td>15.0 ±0.05c</td>
<td>21.50 ±0.10c</td>
<td>16.90±0.06b</td>
<td>17.20±0.06b</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>12.30±0.30a</td>
<td>8.40±0.006c</td>
<td>12.00 ±0.43a</td>
<td>9.80±0.06b</td>
<td>10.10±0.40b</td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td>8.00±0.05a</td>
<td>6.90±0.06c</td>
<td>8.40±0.11a</td>
<td>7.49±0.06b</td>
<td>7.80±0.10b</td>
</tr>
</tbody>
</table>

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; Honey: Honey at 10 ml/kgBW; C + H₁: 10 ml/kgBW of honey and 200 mg/kgBW of caffeine; C + H₂: 20 ml/kgBW of honey and 200 mg/kgBW of caffeine.
antioxidant defense system, an increase of free radical activities and consequently, oxidative stress. Honey significantly mitigated the caffeine induced oxidative stress by increasing the concentrations of SOD, CAT and GPx in C + H₁ and C + H₂ groups. This, therefore suggests that oxidative stress has a wide range of effects on different biochemical and physiological processes on the cells, organs and tissues of the body.

Oxidative damage that arises from excess synthesis of ROS has been correlated with abnormal organs and the incapacitation of antioxidant enzymes that play a role in the removal of ROS, thus giving rise to lipid peroxidation (LPO), gene expression alterations and apoptosis. The CAT, SOD and GPx are antioxidants that play an important role in scavenging ROS. The SOD enhances the destruction radicals of superoxide to hydrogen peroxide while CAT and GPx reduce hydrogen peroxide into water and oxygen to prevent oxidative stress and maintain homeostasis in the cell [24,30,47-48].

Our results also showed that the concentration of MDA significantly increased in caffeine treated groups, what indicates an increased lipid peroxidation activity. Lipid peroxidation is an important biological consequence of oxidative cellular damage; therefore, the increased concentration of MDA indicates oxidative stress [6,8] which agrees with the findings of Dianzani et al. [48]. Increase in lipid peroxidation also inhibits the activity of antioxidant enzymes such as SOD, GPx and CAT as well as total antioxidant status [49].

The mitigating capacity of honey was also observed, reducing the concentration of MDA in C + H₁ and C + H₂ groups (Table 2). These mitigating effects can be attributed to the different antioxidants, vitamins and other enzymes present in honey. Moreover, dietary antioxidants have been reported to play a major role in the maintenance of oxidative balance [38,50-51]. Therefore, this implies that the various constituents of the honey must have played a significant role in scavenging free radicals that can accumulate causing lipid peroxidation and oxidative damage [52] as seen in the caffeine treated animals.

5. CONCLUSION

The findings of this study provide evidence that honey has capability of mitigating the effect of caffeine on oxidative stress markers in male albino rat models and the effect is dose dependent.

ETHICAL APPROVAL

As per international standard or university standard written ethical permission has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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9. Gheldof N, Wang XH, Engeseth NJ. Identification and quantification of


