STUDY ON THE ROLE ADMINISTRATION OF CAFFEINE ON SOME BIOCHEMICAL AND HORMONAL ASSAY TO MALE RATS TREATED WITH HYDROGEN PEROXIDE

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Abstract

The present study was aimed to explain the ameliorative effect of the role of caffeine on some physiological parameters in male rats (Rattus norvegicas) treated with hydrogen peroxide (H₂O₂). The study consisted of thirty six of adult male rats randomly divided into six equal group (each of six). Group One (Control): The animals were treated orally by Gavage normal saline, Group Two: The animals treated with H₂O₂ daily by oral garage. Group Three: The animal treated with low dose caffeine 150 mg/kg Bw daily. Group Four: The animal treated with high dose caffeine 250 mg /kg Bw daily, Group Five: Treated animals with H₂O₂ dose after one hour give low dose of caffeine 150 mg/kg Bw. Group Sixth: The animal treated with H₂O₂ dose (5.63 mg/kg Bw) to each rat after one hour give high dose of caffeine 250 mg /Kg Bw. The experiment continue for two month after that rats were sacrificed, blood were collected by cardiac puncture to investigated biochemical parameters which includes Liver enzyme (ALT, AST & ALP), Total serum protein, Blood urea and Creatinine concentration and some hormone like Dopamine, Acetylcholine and Cortisol. The results revealed the following No significant difference in hormones concentration of Dopamine, Acetylcholine, in treated with H₂O₂ and caffeine two doses and significant increase of Serum cortisol in H₂O₂ treated animal and no significant in caffeine treated animals.

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1. Introduction

Caffeine (1.3.7 - trimethylxanthin), is a natural alkaloid found in coffee beans, tea leaves, cocoa beans, cola nuts and other plants. It is probably the most frequently ingested pharmacologically active substance in the world (Wesensten, 2014). The main source of Caffeine includes, decaf coffee, cola sodas, chocolate ice cream, weight loss pills, pain relievers, energy water, alcoholic energy drinks, breath fresheners, sum deeds energized sun flowers seeds, morning spark instant oat, meal and perky jerky (Breda, 2014). The metabolism of Caffeine will remain in the body for up to 6 hours longer than caffeine itself (Breda, 2014). The metabolism of Caffeine will remain in the body for up to 6 hours longer than caffeine itself (Breda, 2014). The metabolism of Caffeine will remain in the body for up to 6 hours longer than caffeine itself (Breda, 2014). The metabolism of Caffeine will remain in the body for up to 6 hours longer than caffeine itself (Breda, 2014). The metabolism of Caffeine will remain in the body for up to 6 hours longer than caffeine itself (Breda, 2014). The metabolism of Caffeine will remain in the body for up to 6 hours longer than caffeine itself (Breda, 2014). The metabolism of Caffeine will remain in the body for up to 6 hours longer than caffeine itself (Breda, 2014). The metabolism of Caffeine will remain in the body for up to 6 hours longer than caffeine itself (Breda, 2014). The metabolism of Caffeine will remain in the body for up to 6 hours longer than caffeine itself (Breda, 2014). The metabolism of Caffeine will remain in the body for up to 6 hours longer than caffeine itself (Breda, 2014). The metabolism of Caffeine will remain in the body for up to 6 hours longer than caffeine itself (Breda, 2014). The metabolism of Caffeine will remain in the body for up to 6 hours longer than caffeine itself (Breda, 2014).
Caffeine is one of few natural substances that been proven to aid fat burning, caffeine increase burning of fat by as much as 10% in obese individuals and 29% in lean people. These effects will decrease when long term caffeine consumption (Kobayashi, 2005).

Hydrogen peroxide is a chemical compound with the formula H₂O₂. In its pure form, it is a pale blue, clear liquid, slightly more viscous than water. The H₂O₂ is the simplest peroxide (a compound with oxygen – oxygen single bond). It is used as an oxidizer (Hill, 2001). Its chemistry was dominated by the nature of its unstable peroxide bond. The H₂O₂ is unstable and slowly decomposes in the presence of base or a catalyst. Because of its instability, hydrogen peroxide was typically stored with a stabilizer in a weakly acidic solution. Hydrogen peroxide is found in biological systems including the human body. Enzymes that use or decompose hydrogen peroxide are classified as Peroxidases. Dougherty et al. (2005), a study published in Nature found that hydrogen peroxide plays a role in the immune system. Scientists found that hydrogen peroxide presence inside cells increased after tissues are damaged in Zebrafish, which is thought to act as a signal to white blood cells to converge on the site and initiate the healing process.

Hydrogen peroxide has important roles as a signaling molecule in the regulation of a wide variety of biological processes. The compound is a major factor implicated in the free-radical theory of aging, based on how readily hydrogen peroxide can decompose into a hydroxyl radical and how superoxide radical byproducts of cellular metabolism can react with ambient water to form hydrogen peroxide. These hydroxyl radicals in turn readily react with and damage vital cellular components, especially those of the mitochondria, at least one study has also tried to link hydrogen peroxide production to cancer (Niethammer et al., 2009).

2. Materials and Methods

Thirty-six adult male rats were used, the body weight average between 250 ± 25 g, the animals were housed in individual cages measuring (50 × 50 cm), in house of Veterinary Medicine College, University of Basra, Iraq. All animals were exposed to the same environmental including climate of management feeding and acclimatize, adaptive on place for two weeks before treatment. The animals were divided randomly into 6 equal groups (6 rats in each group) as follow:

a) Group One (Control group): In which rats were given normal saline orally by oral gavages.

b) Group Two: In which rats were given of H₂O₂ doses (5.63 mg/kg BW) orally by gavages.

c) Group Three: In which rats were given low doses Caffeine (150 mg/kg/BW) by oral gavages daily.

d) Group Four: In which rats were given high doses Caffeine (250 mg/kg/BW) by oral gavages daily.

e) Group Five: Male rats given H₂O₂ doses (5.63 mg/kg BW) and after one hour given low dose Caffeine (150 mg/kg/BW) by oral gavage.

f) Group Six: Male rats given H₂O₂ doses (5.63 mg/kg BW) and after one hour given high dose Caffeine (250 mg/kg/BW) by oral gavage.

The experiment was continued for two months and after that we measures the following.

Collection of blood samples

Blood sample (5 ml) was collected from heart puncher, after anaesthetized the rats with chlorophorme. Three ml of blood collected from each animal were stored in a tube without anticoagulant and allowed to clot at room temperature. Then, the blood samples were centrifuged at 5000 rpm for 30 minutes and serum sample were stored in polyethylene tubes at -20 °C) until used for biochemical analysis.
Biochemical test

The biochemical tests were done in the laboratory by using Chemistry Autoanalyzer made in Germany by Human Star Company, Serial Number – 20628. The machine has 54 wells which numbered from 1 to 54. The serum samples deposited in each specific wells. The reagent was put in a special container beside the wells. The serum biochemical parameters estimated by this instrument were AST, ALT, ALP, Total serum protein, Blood urea and Serum creatinine concentration.

Hormonal assay
Measurement of Acetylcholine

Ach hormone concentration Ach Elis A was intended for the quantitative determination of Ach (Acetylcholine hormone) in blood plasma, saliva, urine, human serum and related biological liquid, kit was used (Human Ach [Acetylcholine]), YHB0068 HU/China.

Measurement of Dopamine Concentration

Dopamine molecule consists of a catechol structure (abenzenering with two hydroxyside groups). Dopamine was broken down into inactive metabolites by a set of enzymes- monoamine oxidase (MAO).

Cortisol hormone concentration

Intended Use: The quantities determination of Total Cortisol concentration in serum by Microplate enzyme immunoassay, kit was used (Monobind Inc. Lake Forest CA 92630, USA).

3. Results

The mean values of ALT, AST and ALP concentration were presented in Table – 1 and appeared a significant (p≤0.05) increase in ALT concentration in animals treated \( \text{H}_2\text{O}_2 \) compared with Control group, also a significant (p≤0.05) increase in ALT concentration in animals treated caffeine two doses 150 - 250 mg /kg BW, while no significant difference in AST concentration in animals treated with caffeine two doses compared with Control group. Also the periods of treatment caused a significant (p≤0.05) increase in ALT concentration in animals treated \( \text{H}_2\text{O}_2 \), while no significant difference in ALT concentration in animals treated with caffeine two doses 150 - 250 mg/kg BW and \( \text{H}_2\text{O}_2 \) one hour give Caffeine two doses compared with control group. The Table - 2 showed the mean values of Urea, Creatinin and Protein concentration. The table showed a significant (p≤0.05) increase in Serum urea concentration in animals treated with \( \text{H}_2\text{O}_2 \), while no significant difference in serum urea concentration in animals treated with caffeine two doses 150 - 250 mg/kg BW, also a significant (p≤0.05) increase in Serum urea concentration in animals treated \( \text{H}_2\text{O}_2 \) one hour give caffeine two doses compared with control group. A significant (p≤0.05) increase in Serum creatinin concentration in \( \text{H}_2\text{O}_2 \) treated groups while no significant difference in Serum creatinin concentration in animals treated caffeine two doses 150 - 250 mg/kg BW, the same table showed a significant (p≤0.05) increase in Serum creatinin concentration in animals treated \( \text{H}_2\text{O}_2 \) after one hour give caffeine two doses. The table showed a significant (p≤0.05) increase in serum total protein concentration in animals treated \( \text{H}_2\text{O}_2 \) compared control group, while no significant difference in serum creatinin concentration in animals treated with caffeine two doses 150 - 250 mg/kg BW and \( \text{H}_2\text{O}_2 \) after one hour give caffeine two doses compared with control group. The Table - 3 show that the mean values of dopamine concentration were a significant (p≤0.05) increase in \( \text{H}_2\text{O}_2 \) treated groups, also a significant (p≤0.05) increase in dopamine concentration in animals treated with caffeine two doses 150 - 250 mg/kg BW, the periods of treatment show significant (p≤0.05) increase in dopamine concentration in animals.
Table - 1: The effects of caffeine and H\textsubscript{2}O\textsubscript{2} on ALT, AST and ALP (Mean ± SD) n = 6

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>32.15±2.21</td>
<td>112.97± 6.25</td>
<td>49.28±4.46</td>
</tr>
<tr>
<td>H\textsubscript{2}O\textsubscript{2}+</td>
<td>43.89± 4.21</td>
<td>172.59±11.41</td>
<td>141.86±7.95</td>
</tr>
<tr>
<td>Caffeine 150 mg/kg</td>
<td>36.85±1.93</td>
<td>107.55±8.83</td>
<td>54.05±5.81</td>
</tr>
<tr>
<td>Caffeine 250 mg/kg</td>
<td>32.57±2.43</td>
<td>110.31±7.27</td>
<td>60.43±5.95</td>
</tr>
<tr>
<td>Caffeine 150 mg/kg + H\textsubscript{2}O\textsubscript{2}</td>
<td>38.62±5.32</td>
<td>132.74±10.55</td>
<td>57.22±4.99</td>
</tr>
<tr>
<td>Caffeine 250 mg/kg + H\textsubscript{2}O\textsubscript{2}</td>
<td>35.64±2.33</td>
<td>153.29±13.11</td>
<td>63.09±6.6162</td>
</tr>
</tbody>
</table>

The different small letters refer to significant differences at (p≤0.05) among day

Table - 2: The effects of caffeine and H\textsubscript{2}O\textsubscript{2} on urea, creatinin and total protein (Mean ± SD) n = 6

<table>
<thead>
<tr>
<th>Group</th>
<th>Urea (mg/dL)</th>
<th>Creatinin (mg/dL)</th>
<th>Total Protein (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24.40±2.41</td>
<td>0.28±0.01</td>
<td>5.75±0.39</td>
</tr>
<tr>
<td>H\textsubscript{2}O\textsubscript{2}</td>
<td>32.07±4.22</td>
<td>0.93±0.02</td>
<td>8.07±1.14</td>
</tr>
<tr>
<td>Caffeine 150 mg/kg</td>
<td>22.19±4.05</td>
<td>0.24±0.02</td>
<td>5.99±0.89</td>
</tr>
<tr>
<td>Caffeine 250 mg/kg</td>
<td>23.89±2.88</td>
<td>0.28±0.03</td>
<td>5.35±0.88</td>
</tr>
<tr>
<td>Caffeine 150 mg/kg + H\textsubscript{2}O\textsubscript{2}</td>
<td>25.32±3.32</td>
<td>0.25±0.02</td>
<td>5.98±0.97</td>
</tr>
<tr>
<td>Caffeine 250 mg/kg + H\textsubscript{2}O\textsubscript{2}</td>
<td>26.11±4.25</td>
<td>0.28±0.02</td>
<td>5.88±1.00</td>
</tr>
</tbody>
</table>

The different small letters refer to significant differences at (p≤0.05) among day
Table - 3: The effects of Caffeine and H₂O₂ on Hormone Dopamine, Acetylcholine and Cortisol (Mean±SD)  

<table>
<thead>
<tr>
<th>Group</th>
<th>DOP</th>
<th>Ach</th>
<th>CORTI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.22±0.00</td>
<td>0.04±0.00</td>
<td>0.42±0.01</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>0.31±0.01</td>
<td>0.22±0.01</td>
<td>1.23±0.08</td>
</tr>
<tr>
<td>Caffeine 150 mg/kg</td>
<td>0.26±0.01</td>
<td>0.07±0.00</td>
<td>0.44±0.02</td>
</tr>
<tr>
<td>Caffeine 250 mg/kg</td>
<td>0.28±0.02</td>
<td>0.07±0.00</td>
<td>0.46±0.02</td>
</tr>
<tr>
<td>Caffeine 150 mg/kg + H₂O₂</td>
<td>0.27±0.03</td>
<td>0.06±0.00</td>
<td>0.69±0.05</td>
</tr>
<tr>
<td>Caffeine 250 mg/kg + H₂O₂</td>
<td>0.29±0.03</td>
<td>0.05±0.00</td>
<td>0.56±0.03</td>
</tr>
</tbody>
</table>

The different small letters refer to significant differences at (p≤0.05) among day

treated H₂O₂ after one hour give Caffeine two doses compared with control group. The mean values of Serum cortisol concentration show a significant (p≤0.05) increase in H₂O₂ treated groups, while no significant difference in Serum cortisol concentration in animals treated caffeine two doses 150 - 250 mg/kg BW, also a significant (p≤0.05) increase in Serum cortisol concentration in animals treated H₂O₂ after one hour give caffeine two doses compared with Control group.

4. Discussion

The liver is a large, complex organ that is well designed for its central role in carbohydrate, protein and fat metabolism. It is the site where waste products of metabolism are detoxified (Dufour et al., 2000). Serum liver enzyme significantly increased in blood serum AST, ALT and ALP. The Table - 1 in animal group treated H₂O₂ and caffeine the results were agreements with study done by Durate et al. (2012) who found that caffeine consumption significant increase the liver enzymes it has been that caffeine caused tissue damage is usually associated with the release of these enzymes, also treated with H₂O₂ significant increase the levels of ALT, AST and ALP (Jeroh et al., 2012). This was in agreement with present study, Sallie et al. (1991) show that H₂O₂ induced hepatotoxicity in treated rats this result was evidenced by the marked increase in serum AST, ALP and ALP.

The result in the Table - 2 showed that the animal treated with H₂O₂ caused a significant increased in the levels of serum urea, creatinin and total protein concentration. The result was in agreement with study done by study Salahudeen et al. (1991) and found that the H₂O₂ induced increased the level of serum urea, creatinin and total protein that may be due to decreased in GFR rather than interference with serum creatinine assay or blocking tuloularcreatinine secretion.

Caffeine treated animals show no significant difference in the levels of serum urea, creatinin and total protein, the present study differ from result done by Tofovic et al. (2002) showed that caffeine treated group in rats associated with decrease the serum urea, creatinin and total protein but with advanced dose of caffeine with H₂O₂ significant increase the serum urea, creatinin and protein due to renal vascular resistance.

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The Table - 3 showed that a significant increased in the level of Dopamine, Ach and Cortisol level concentration in group of animals treated H₂O₂. This result was coordinated with study done by Jerem et al. (2015) they show that the H₂O₂ induced significant increase in Cortisol hormone level due to H₂O₂ activation of the Hypothalamus – pituitary adrenal (HPA) Axis induce the activity in the cellular reduction – oxidation system. The same table showed that the animals treated caffeine two doses 150 – 250 mg/kg BW lead to increased significantly in hormone Dopamine and Acetylcholine while the changes in hormone cortisol do not reach a significant. The result was in agreement with study done by Carter et al. (1995). They show that the caffeine showed significant increase Ach, that caffeine significant increase Ach due to enhanced action potential dependent of acetylcholine in release by antagonism of local receptors.

5. References


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