Possible Impact of Antioxidant Properties of Cocoa (Theobroma Cacao L.) Against Irradiation-Induced Some Biochemical Disorders in Rats

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ABSTRACT

Man is exposed to natural radiations either from cosmic or terrestrial origins. Furthermore, it is well known that the gamma irradiation of animals induce biochemical alterations which depend Mostly on oxidative stress. This work aimed at evaluating the radio protective efficiency of Cocoa (Theobroma cacao L.) against whole body γ- irradiation of rats. The virtue of cocoa aqueous extract (CAE) was given to rats at a dose of 1 g/ kg for 6 weeks to determine changes in hepatic marker enzymes, lipid profile and antioxidant status. The animals exposed to γ-rays exhibited a pronounced increment in serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transpeptidase (γGT), total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C) and liver thiobarbituric acid reactive substance (TBARS). On the other hand, a significant decline was demonstrated in high density lipoprotein cholesterol (HDL-C). A decrease of hepatic reduced glutathione (GSH) content, superoxides dismutase (SOD) and catalase (CAT) activities was sustained. The CAE administered orally to rats has significantly modulated all the radiation-induced biochemical alterations. These findings revealed that cocoa would exert radio-protective properties.

Key Words: Gamma Irradiation / Cocoa / Liver Enzymes / Lipid Profiles / Oxidative Stress.

INTRODUCTION

Human exposure to ionizing radiation has become inevitable due to its vast application in diagnosis and industry. Everyone on earth is continuously exposed to radiation either as a result of natural radiation background or through the occasional medical or dental X-ray treatments ¹. Radiation damage is, to a large extent, caused by over production of reactive oxygen species (ROS) which cause disruption of lipid cell membranes leading to subsequent formation of peroxide radicals ². Oxygen free radicals are highly reactive and may cause cell and tissue damage by interacting with cell membranes and organelles, lipid peroxidation is a ubiquitous phenomenon in the body under the influence of oxidative stress ³.

Evidences have shown that over production of ROS results in an imbalance between pro-oxidants and antioxidants that cause ROS production exceed the activity of endogenous antioxidants which increase oxidative stress ⁴,⁵. Once this imbalance takes place, cellular molecules such as nucleic acids, proteins, structural carbohydrates, and lipids may be damaged by oxidative modifications ⁶. Also, ROS play a causative role in numerous disease pathologies such as cancer, ischemia, and degenerative diseases such as aging, atherosclerosis, arthritis and neurodegeneratio ⁷,⁸.

The liver, an important organ in the body, used for bio-transformation and detoxification of materials is particularly susceptible to oxidative stress. The degree of oxidative damage to the liver can be estimated by these marker enzymes; ALP, ALT and AST, which under diseased state, are released
from the liver into the bloodstream (9). The antioxidant system, which consists of enzymatic and non-enzymatic antioxidants, is an important mechanism provided by the body to modulate/reduce the effect of free radicals and oxidative stress (4). Endogenous antioxidants of biological importance include SOD, CAT, and GSH, which are predominantly located in the liver (10). Exogenous sources of antioxidants include diet and drug supplements which enhance the functions of the endogenous antioxidants (11).

Theobroma cacao, also known as cocoa tree, is a small but economically important tree belonging to the Sterculiaceae family. It is native to the deep tropical region of South America. The seeds of Theobroma cacao (popularly known as cocoa) are a rich source of polyphenols (12). Plant polyphenols are non-nutritive, hydrophilic components found in small amounts (micrograms) in all kinds of plant-derived food sources such as fruits and vegetables, drinks (wine, coffee, juices) and cereals (13). Numerous studies have attributed the health promoting effects of Theobroma cacao to its rich polyphenols content (14, 15). Some of the reported health promoting effects of the seeds include; its antioxidant capacity, such as the attenuation of copper-mediated and endothelial cell-mediated oxidation of LDL (16), protection against erythrocyte hemolysis (17) and enhancement of cardiovascular health (18).

This study was undertaken to ascertain the efficiency of the water extract of cocoa in preventing or minimizing the biochemical disorders associated with irradiation of rats.

MATERIALS AND METHODS

Material:
Commercial cocoa powder (Theobroma cacao L.), as well as standard commercial rodent diet were purchased from a local market in Cairo, Egypt

Preparation of CAE:
10 g of cocoa powder was dissolved in 20 ml of deionized water, boiled for 10 min in order to avoid lumps, cooled at room temperature and administrated orally to the rats by gavage at a dose of 1g/ kg body weight (19).

Radiation Facility:
Whole body gamma irradiation of rats at a dose level of 6.0 Gy was performed using a Canadian gamma cell-40, (137Cs) at the NCRRT, Cairo, Egypt. The irradiation dose rate was 0.44 Gy/min at the time of the experimentation.

Experimental Animals:
Male albino rats, reared in the NCRRT animal house, Egypt, were used in the present work. Matched weight animals (125-150 g) were selected and housed in plastic cages under controlled condition and fed on a standard commercial rodent diet and received water ad-libitum.

Experimental Design:
Animals (28 rats) were randomly divided into 4 groups each of seven rats as follows:

Group 1: Rats remain untreated, (control group).

Group 2: Rats were exposed to whole body gamma-radiation (6.0Gy) at the beginning of the experiment (irradiated group).

Group 3: Rats received cocoa, 1g/ kg b.w., orally for 6 weeks (cocoa-treated group).
Group 4: Rats were whole body gamma irradiated (6.0Gy) at the beginning of the experiment and administered cocoa, 1 g/ kg b.w., orally for 6 weeks (irradiated & cocoa-treated group).

At the end of the experiment, the animals were sacrificed and blood samples were collected through heart puncture after light anesthesia. Blood samples were allowed to coagulate and centrifuged to obtain serum for biochemical analysis. The liver was quickly removed then it was homogenated in saline solution. The homogenate was centrifuged at 3000 rpm for 15 min and the supernatant was used for the biochemical analysis.

Biochemical Analysis:

The determination of serum AST and ALT activity was done using the method of Reitman and Frankel (20). ALP activity was estimated according to Kind and King (21), γ- GT was determined using the method of Szasz (22). In addition, the concentration of TC, TG and HDL-C was determined according to procedure described by Allain et al. (23), Fossati and Prencipe, (24) and Demacker et al. (25), respectively while LDL-C was evaluated according to Friedwald et al. (26) formula by the following equation: LDL-C (mg/dl) = TC - (TG/5+HDL-C). The extent of lipid peroxidation was assayed by the measurement of TBARS according to Yoshioka et al. (27). SOD and CAT activity was determined according to Minami and Yoshikawa et al (28), and Aebi (29), respectively. The content of GSH was determined according to Beutler et al. (30).

Statistical Analysis:

Analysis of variance (ANOVA) was conducted for all data using the general linear model (GLM) (SAS Institute31). Duncan's multiple-range test was used for comparison between treatments(32). Data were presented as means ± standard error. A value of P<0.05 was taken as criterion of significance.

RESULTS

The effect of gamma rays alone and the ameliorating effect of CAE after radiation exposure on some hepatic biochemical variables of various groups were assessed and are presented in (Table 1). It is clear from the results that treatment of rats with γ-rays caused a significant elevation in the activity of AST, ALT, ALP and γ-GT compared to that of control group animals. The level of the liver marker enzymes was significantly suppressed by the administration of rats with CAE (p< 0.05 vs. irradiated rats).

The level of TC, TG and LDL-C in serum was significantly higher in irradiated group than that of the control group. Treatment of rats with CAE post irradiation significantly ameliorated the elevation in the level of lipid profile (Table 2). On the other hand, and in comparison with normal conditions, radiation exposure of rats resulted in a significant decrease in serum HDL-C level. This effect was significantly improved by treatment with CAE (Table 2).

Table (1): Effect of CAE administration to rats on the activity of serum hepatic marker enzymes.

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (U/ml)</th>
<th>ALT (U/ml)</th>
<th>ALP (U/l)</th>
<th>γ-GT (U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>69.18 ± 1.83c</td>
<td>26.51 ± 2.23b</td>
<td>73.06 ± 3.47c</td>
<td>1.74 ± 0.002b</td>
</tr>
<tr>
<td>CAE</td>
<td>68.11 ± 2.17c</td>
<td>25.84 ± 1.45b</td>
<td>73.68 ± 1.19c</td>
<td>1.63 ± 0.006b</td>
</tr>
<tr>
<td>Irrad.</td>
<td>157.26 ± 3.28a</td>
<td>67.22 ± 2.77a</td>
<td>124.13 ± 2.9a</td>
<td>4.95 ± 0.018a</td>
</tr>
<tr>
<td>CAE + Irrad.</td>
<td>79.54 ± 1.56b</td>
<td>30.43 ± 1.34b</td>
<td>82.76 ± 1.47b</td>
<td>2.08 ± 0.009b</td>
</tr>
</tbody>
</table>

Data are expressed as means ± S.E. of seven rats per group. Values with different superscript in the same columns are significantly different at P ≤ 0.05.
Table (2): Effect of CAE administration to rats on serum lipid profile.

<table>
<thead>
<tr>
<th>Groups</th>
<th>T-C (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>57.56±1.42</td>
<td>74.65±0.67</td>
<td>51.49 ±0.74</td>
<td>9.06±0.33</td>
</tr>
<tr>
<td>CAE</td>
<td>54.62±1.07</td>
<td>74.03±1.18</td>
<td>51.47±1.12</td>
<td>10.15±1.07</td>
</tr>
<tr>
<td>Irrad.</td>
<td>69.09±1.25</td>
<td>107.86±0.92</td>
<td>33.68±0.63</td>
<td>13.31±0.52</td>
</tr>
<tr>
<td>CAE + Irrad.</td>
<td>54.53±1.13</td>
<td>92.10±1.02</td>
<td>49.16±0.82</td>
<td>11.22±1.21</td>
</tr>
</tbody>
</table>

Legend as table 1

The effect of gamma rays alone and the ameliorating effect of CAE on LPO and the antioxidant related parameters in liver of various animal groups were assessed and are presented in (Table 3). Whole body gamma irradiation of rats led to a significant (p< 0.05) increase in the level of liver TBARS. Whereas, a significant decrease (p< 0.05) in SOD and CAT activities, and GSH content of rats were observed in irradiated animals, in comparison with those of control group. However, treatment of rats with CAE produced a significant reduction (p<0.05) in the hepatic LPO level when compared with gamma irradiated group. A significant increase (p<0.05) in SOD, CAT activity and GSH content was observed in rats treated with CAE, compared to those irradiated only.

Table (3) Effect of CAE administration to rats on hepatic level of TBARS, GSH, SOD and CAT.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (n mol/ g tissue)</th>
<th>GSH (mg/ g tissue)</th>
<th>SOD (U/ mg protein)</th>
<th>CAT (U/ mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>146.75 ± 1.91</td>
<td>47.17± 1.52</td>
<td>54.61 ± 2.17</td>
<td>15.33 ± 0.24</td>
</tr>
<tr>
<td>CAE</td>
<td>140.79 ± 4.20</td>
<td>48.23 ± 2.67</td>
<td>55.54 ± 1.33</td>
<td>16.10 ± 0.18</td>
</tr>
<tr>
<td>Irrad.</td>
<td>205.73 ± 5.36</td>
<td>22.31 ± 2.72</td>
<td>32.47 ± 1.86</td>
<td>9.70 ± 0.27</td>
</tr>
<tr>
<td>CAE + Irrad.</td>
<td>163.53 ± 5.12</td>
<td>38.13 ± 2.12</td>
<td>51.40 ± 2.64</td>
<td>12.66 ± 0.11</td>
</tr>
</tbody>
</table>

Legend as table 1

The results of the present study showed that the rats received CAE exhibited insignificant changes in all estimated parameters of control rats (Tables 1-3).

DISCUSSION

The present study was undertaken to investigate the possible effect of water extract of Theobroma cacao on some biochemical disorders induced in irradiated rats. Exposure of rats to γ-radiation causes oxidative stress through the generation of highly oxidizing, tissue-damaging radicals such as hydroxyl radicals (OH•) or closely related species which are the final mediators of most free radical induced tissue damage (33). Almost all reactive oxygen species (ROS) exert their pathological effects by giving rise to hydroxyl radicals which initiate the peroxidation reactions by interacting with available polyunsaturated fatty acids of the cells and tissues (34). The oxidative stress is characterized by disrupted structural integrity as well increased membrane permeability (35). Hepatic damage induced by gamma rays is evaluated by assaying serum enzymes activity such as ALP, AST and ALT, and the increase in their activities reflected occurrence of acute liver damage and inflammatory hepatocellular disorders (33). The liver which is popularly known for its bio-transforming and detoxifying activities, is protected from oxidative damage by the antioxidant system.
It was observed that γ-irradiation of rats led to elevated levels of serum AST, ALT, ALP and γ-GT when compared to the control group (Table 1). These findings stand in well agreement with those of El-Gawish et al. (36), Ighodaro and Omole (37), who reported a significant increase in the activity of serum ALP, ALT and AST due to the damage of structural integrity of the liver which resulted in the leakage of these enzymes from the cytosol into the blood stream. Administration of the CAE to irradiated rats decreased the serum liver enzymes, indicating hepato-protective properties of the extract. The significant (P≤0.05) decrease in the total cholesterol levels of the rats drank cocoa in the work being reported, resides in the well-established role of oxidative stress in the pathophysiological processes of liver injury (39). Despite recent skepticism, literature abounds with the in vivo anti-oxidative benefits of the polyphenols in cocoa (40, 41).

In the present work, the level of TC, TG and LDL-C in serum was significantly higher in irradiated group than that of the control group. Whereas, a remarkable decreases was observed in the concentration of HDL-C in the serum of γ-irradiated rats. This justifies a state of liver dysfunction, as the liver is the major organ of cholesterol synthesis and excretion. These data are in accordance with previous results of Ragab and Ashry (42) and Abou-Safi et al. (43) who observed that the elevation in serum lipid fractions might result from ionizing radiation ability to accelerate other pathways of cholesterol formation like increasing its rate of biosynthesis in the liver and other tissues, or destruction of cell membrane by radiation and also to disturbance of LDL cholesterol receptors, leading to hypercholesterolemia. It is also, suggested by Osman (45) and Abou-Safi et al. (46), who observed an increase in insulin level after radiation exposure, that the oxidative stress might be an important determinant of altered lipid metabolism (44). The recorded elevated level of triglycerides correlates previous findings of and synthesis of triglycerides was increased in both adipose tissues and liver which was accompanied by an acceleration of fatty acids mobilization from fat depots to blood. Similarly, the present study showed that cocoa extract might have the potential to reduce TC, TG and LDL-C levels in irradiated rats, which suggests a hypocholesteromic effect of the cocoa. This report collaborates that of Jalil and Ismail (47), who recorded a decrease in lipid profile of rats at the supplementation of 3% and 15% cocoa powder that contained 56 and 265 mg theobromine respectively in hyperlipidaemic rats. Corti et al. (48) also reported that consumption of flavanol-rich cocoa lowered the plasma levels of LDL and oxidized LDL, while increasing the HDL levels of hypercholesteremic patients. The significant decrease in the total cholesterol levels of the experimental rats (Table 1) may have resulted from the antioxidant properties of the polyphenols in cocoa (49). Previous studies have indicated that polyphenols could exert their lipid lowering properties through various mechanisms, namely by slowing down triacylglycerol absorption through inhibition of pancreatic lipase, increasing cholesterol excretion in faeces, attenuating hepatic lipid accumulation through activation of adenosine monophosphate (AMP)-activated protein kinase suppressing hepatic secretion of apo-lipoprotein B100 and increasing expression of LDL receptors in the liver (50-52). In addition to that, polyphenols in cocoa have the ability to increase the synthesis of nitric oxide (NO) which has the ability to cause vasodilation, resulting in the clearance and prevention of the deposition of excess cholesterol in the blood vessels (53). The beneficial effects of NO modulation include the regulation of blood pressure, lowering of NO-affected hypercholesterolemia and monocyte adhesion, all of which are involved in the progression of atherosclerosis (53). Stearic acid, which is a saturated fatty acid abundant in cocoa, is reported to cause reduction in plasma cholesterol by limiting its absorption and enhancing the excretion of endogenous cholesterol (54).

The present work shows that exposure of rats to γ-radiation resulted in a significant increase in lipid peroxidation, as measured by the formation of TBARS in the liver (Table 3).
increase in the hepatic TBARS level of the irradiated group could be due to the generation of free radicals resulting to the peroxidation of membrane lipids \(^{(2, 55)}\). Several investigators reported that irradiation of rat induced oxidative damage in several organs \(^{(56, 57)}\). The significant reduction in TBARS obtained at the treatment of rats with the cocoa extract is indicative of the anti-lipid peroxidative property of the cocoa extract. The report by Keen et al. \(^{(58)}\), indicated that consumption of flavanol-rich cocoa product among healthy subjects resulted in the inhibition of LDL oxidation. They also reported the in vitro attenuation of copper-mediated and endothelial cell-mediated oxidation of LDL with cocoa extract supplementation.

In the current investigation, the irradiated rats without cocoa extract treatment showed a reduced SOD and CAT activity and GSH level relative to that of normal control rats. The decreased activity of these antioxidant enzymes is indicative of the oxidative stress occurring in the liver (Table 3). The antioxidant enzymes constitute a mutually supportive system of defense against free radicals \(^{(59)}\). GSH serves as co-factor for the enzyme glutathione peroxidase, while SOD represents the first line of defense against free radicals. SOD catalyses the dismutation of superoxide radicals to hydrogen peroxide (\(H_2O_2\)), with the release of oxygen molecule. The \(H_2O_2\) generated is removed by the action of either CAT or GPx \(^{(60)}\). Increased generation of free radicals distorts antioxidant mechanism, thus reducing cellular antioxidant levels as well as their activity. The reduced activities of the antioxidants; SOD, CAT and GSH in the liver homogenates could be attributed to the scavenging action of these enzymes on radicals produced by radiation during oxidative damage and lipid peroxidation. Reduced activities of the antioxidant enzymes could also be due to radiation induced liver injury, as the ability of the hepatocytes to replenish the enzymes is reduced during liver damage. Treatment of the irradiated animals with the cocoa extract enhanced the activities of the antioxidant enzymes, and increased GSH level. This is consistent with the report of Noori et al. \(^{(19)}\), which showed a significantly increased antioxidant (SOD, GSH and CAT) levels in the liver of rats after a 21 day treatment with cocoa powder (1g/ kg).

**CONCLUSION**

The results of the present study show that the daily administration of the water extract of natural cocoa powder to rats six weeks post whole body \(\gamma\)-irradiation would decrease the radiation induced toxicity and associiative tissue damages, possible due to antioxidative and free radicals scavenging properties.

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