The role of vitamin C in alteration of enzymes responsible of energy metabolism induced by administration of tamoxifen to mouse*

Zaina Zuhair, Hasan ALamri

Girls College of Education, King Abdulaziz University, Jeddah, Saudi Arabia. Email: Sala201018@gmail.com

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ABSTRACT

Tamoxifen is a synthetic non-steroidal ant estrogen. It was suggested to study the role of vitamin C in alteration of enzymes responsible of energy metabolism induced by administration of tamoxifen to mouse. The effect of tamoxifen and tamoxifen with vitamin C on some activity of enzymes in the mice representing glycolytic, gluconeogenic and glycogenolytic pathway and also, liver function enzymes represented by aspartate aminotransferase (AST), alanine amino-transferase (ALT), acid phosphatase (ACP) and alkaline phosphatase (ALP) were studied. The present results showed that a significant (p < 0.001) increase in glycolytic enzymes (HK, PK, GPI and PFK), gluconeogenic enzymes, G-6-Pase, acid phosphatase (ACP), alkaline phosphatase (ALP) and glucose, were observed in treated groups, while LDH, glycogen phosphorylase, AST and ALT enzymes activities showed significant (p < 0.01) reduction. The present results also, showed that significant reduction in glycogen, total protein, total cholesterol, uric acid, urea, and creatinine in treated mice as compared to the normal healthy control group. However, normal control mice treated with tamoxifen and vitamin C showed no side effects of most parameters compared to the normal healthy control group. It was concluded that vitamin C may prevent tamoxifen-induced testes toxicity in mice.

Keywords: Adult Male Albino Mice; Tamoxifen Vitamin C; Liver Tissue Homogenates

1. INTRODUCTION

Tamoxifen is one of the most effective synthetic non-steroidal ant estrogenic compounds, it is widely used in the treatment of advanced hormone-dependent breast cancer which is the most worldwide common form of cancer in women [1] by binding to estrogen receptors and suppressing epithelial proliferation [2,3] and as adjuvant therapy following surgery in early stages of the disease. Tamoxifen is also, proposed for the prevention of cancer amongst high risk women [4]. Such an approach requires objective and accurate evolution of the side effects which could result from the administration of this drug.

Some studies showed that tamoxifen has adverse side effects on the cardiovascular system, bone metabolism and liver. Also, tamoxifen caused cytotoxicity on primary cells from human multiple organs: Kidney, liver and lung [5].

Vitamin C is an antioxidant agent that limits the injury produced by drugs. Vitamin C is an essential nutrient that functions as a non-enzymatic antioxidant in the cytosol. The various experimental studies indicated that this vitamin is effective in preventing the oxidative renal damage and stress [6].

It is well known that the liver is one of the major target organs affected by drug, where the most metabolic processes are usually located. The most important metabolic pathways are glycolysis, gluconeogenesis and glycogenolysis contributions of these pathways elucidate the metabolic relationship between glucose, glycogen and energy release. The characteristic pattern of change in some enzymes activity representing glycolytic pathways as (hexokinase (HK), pyruvate kinase (PK), phosphofructokinase (PFK), lactate dehydrogenase (LDH), and glucose phosphate isomerase (GPI); glycogenolytic pathways as glycogen phosphorlase, glucose-6-phosphatase (G-6-Pase); gluconeogenic pathways as fructose-1-6 diphosphatase (F-D-P ase), phosphoenolpyruvate carboxykinase (PEPCK), as well as glycogen content in soft tissue and glucose in serum as bioenergetics parameters of critical importance in reflecting the physiological alteration of animals under the stress [7].

Carbohydrates and amino acids are one of the important parameters which could be used as another indicator...
of the stress by molluscicides [8].

The aim of the present work is to cast more light on the toxic effect of tamoxifen on some liver enzymes in mice representing metabolic pathways are glycolysis, gluconeogenesis and glycogenolysis and the role of vitamin C in minimizing the toxicity induced by tamoxifen.

2. MATERIALS AND METHODS

2.1. Experimental Animals

Adult male albino mice in the present investigation were obtained from Schistosome Biological Supply Program (SBSP), Theodor Bilharz Research Institute, Cairo, Egypt. They were 3 months old and of an average weight of 25 - 30 g. They were fed ad labium with a standard diet. They were kept in cages and acclimatized in the laboratory for 7 days prior to experimentation.

2.2. Applied Drugs

Tamoxifen and vitamin C were purchased from Chemical Industries Development (CID) Company, Al-Harm, Giza, Egypt as tablets for oral administration, each tablet contains 40 mg and 500 mg of the active ingredients, respectively. The therapeutic dose of each drug for mice was calculated according to the table given by Paget and Barnes [9]. The therapeutic doses of tamoxifen and vitamin C were calculated as 0.1 mg/kg and 1.25 mg/kg for mice, respectively. The doses were given orally and estimated according to the body weight of the mouse.

2.3. Experimental Design

The mice were divided into three equal groups. The 1st group served as control and received distilled water. The 2nd group was daily administrated 0.1 mg tamoxifen. the 3th group were given daily 1.25 mg vitamin C simultaneously with the dose of 0.1 mg tamoxifen. All the doses were given daily for 28 days and sacrificed after month. Animals sacrifice and examination stared 4 weeks post-treatment; it was done on successive days. In each day only two animals from each group were sacrificed. Each liver was then taken and divided into 0.25 g portions. The liver portion were taken and covered with aluminum foil and stored at –4°C until used for homogenization and biochemical assays. All mouse were subjected to determine HK, PK, GPI, LDH, PFK, FD Pase, PEPCK, G-6-Pase, AST, ALT, ADP, ALP and glycogen phosphorylase enzyme activities as well as, glycogen and protein content in liver tissue. Glucose, total cholesterol, uric acid, urea, and creatinine in sera.

2.4. Preparation of Liver Tissue Homogenates

On the day of each the following enzyme parameter assay, one portion weighing 0.25 g from each liver aluminium package was taken and homogenized in 2.5 ml of the specific recorded solution to give 10% concentration and then used for assay. Similar periods elapsed between homogenization and enzyme was assayed in two livers from each group on the same days.

2.5. Bleeding and Preparation of Serum

Blood samples were collected, after 4 weeks post-treatment. Collected blood samples were centrifuged at 3000 rpm for 10 minutes and sera were stored immediately at –80°C until time of analysis.

2.6. Enzyme Assays

All physiological parameters determined in this study were determined spectrophotometrically, using reagent kits purchased from BioMerieux Company, France.

Hexokinase (HK) was assayed according to the method of Uyeda and Raker [10]. Pyruvatekinase (PK) according to the method of McManus and James [11]. Glucose phosphate isomerase (GPI) according to the method of King [12], Phosphofructokinase (PFK) according to the method of Zammit et al. [13] and Lactate dehydrogenase (LDH) activity according to the method of Cabaud and Wroblewski [14]. Phosphoenolpyruvate carboxykinase (PEPCK) according to the method of Suarez et al. [15] and Glucose-6-phosphatase according to the method of Swanson [16], Fructose-1, 6-diphosphatase (FD Pase) according to the method of Sand et al. [17] and Glycogen phosphorylase according to Hedrick and Fischer [18]. Aspartate and alanine aminotransferases according to the method of Reitman and Frankel [19]. Acid phosphatase and alkaline phosphatase activities according to Fishman and Ferner [20] and King and King [21] respectively. Total protein according to the method of Lowry et al. [22]. Glycogen according to the method of Nicholas et al. [23]. Glucose according to the method of Trinder [24].

Sera were used for measuring concentrations of glucose, total cholesterol, uric acid, urea and creatinine.

2.7 Statistical Analysis

Data were analyzed by comparing values for different treatment groups with the values for individual controls. Results are expressed as mean ± S.D. The significant differences among values were analyzed using analysis by student’s t-test for comparing the means of experimental and control groups [25].

3. RESULTS

The present results in the Table 1 and Figure 1 showed that very highly significant (p < 0.01) reduction in Lactate (LDH) enzyme activity in mice treated with Ta-
moxifen (32.5 ± 2.3) as compared to the normal control (42.32 ± 2.6), while significant (p < 0.001) increase was noticed in other glycolytic enzymes hexokinase (HK), pyruvatekinase (PK), phosphofructokinase (PFK) and glucose phosphate isomerase (GPI) as compared to the normal healthy control. The enzymes activities in treated mice were 0.141 ± 0.13, 7.8 ± 2.1, 26.2 ± 2.2 and 1201 ± 2.1 and in control mice were (0.085 ± 0.05, 5.8 ± 2.1, 18.21 ± 1.4 and 88.6 ± 8.2) µ mol/min/mg protein, respectively. Moreover, treatment of mice with the tamoxifen and vitamin C recorded no significant difference in all glycolytic enzymes as compared to control group. A noticeable remark on the effect of tamoxifen with vitamin C pointed out to that there is no side effects on all glycolytic enzymes (LDH, HK, PK & GPI) as compared to the normal healthy control group.

The present results (Table 2 and Figure 2) showed that the effect of tamoxifen on some gluconeogenic enzymes. Significant increase (p < 0.001) in the levels of FDPase and PEPCK was noticed in treated group as compared to the normal group. The percentage of increases were 39.66% and 48.57%, respectively Figure 1, Table 2 showed a very highly significant increase (p < 0.001) in G-6-Pase, while a highly significant reduction (p < 0.001) in glycogen phosphorylase was noticed in treated group as compared to the normal healthy control group. The percentage of increases was 35.15% and 51.54% respectively.

Moreover, treatment of mice with the tamoxifen and vitamin C recorded no significant difference in all glycolytic, gluconeogenic and glycogenolytic enzymes as compared to control group. A noticeable remark on the effect of tamoxifen with vitamin C pointed out to that there is no side effects on all glycolytic, gluconeogenic and Glycogenolytic enzymes as compared to the normal healthy control group.

Table 3 and Figure 3 showed that highly significant reductions (p < 0.01) in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes in mice treated with tamoxifen while significant (p < 0.01) increases were observed in acid phosphatase (ACP) and alkaline phosphatase (ALP) levels as compared to the normal healthy control group.

Moreover, treatment of mice with the tamoxifen and vitamin C indicated that no significant difference in the level of liver function enzymes as compared to control group. Treatment of the normal healthy mice with the tamoxifen and vitamin C showed no side effects on the level of liver function enzymes.

Table 4 and Figure 4 showed that highly significant reductions (p < 0.01) in glycogen and total protein levels in treated mice with tamoxifen as compared to the normal healthy control group. The percentages of reductions were 31.01% and 37.56% respectively. The table shows a highly significant increase (p < 0.01) in glucose level (28.1%). The present results in the Table 4 showed that very highly significant (p < 0.01) reduction in total cholesterol, uric acid, urea and creatinine in mice treated with tamoxifen as compared to the normal healthy control. The percentages of reductions were 25.71%, 34.2%, 27.42% and 19.32%, respectively. Moreover, treatment of mice with the tamoxifen and vitamin C recorded no significant difference in the level of glucose, total cholesterol, uric acid, urea and creatinine as compared to control group. Treatment of the normal healthy mice with the tamoxifen and vitamin C showed no side effects on the level of glucose, total cholesterol, uric acid, urea and creatinine.

4. DISCUSSION

In the present study, significant increase in glycolytic enzymes HK, PK, GPI and PFK were observed in treated group with tamoxifen, while LDH enzyme activity showed significant reduction. The enhancement in the activities of glycolytic enzymes in treated mice could be attributed to increase metabolic activities of treated liver tissues to compensate the inhibition of host Krebs’ cycle of host caused by treatment with tamoxifen [26,27]. LDH inhibition revealed the aerobic–anaerobic switch induced by treatment with tamoxifen [27]. Kuser et al. [28] indicated that lactate is accumulated and glycogen depleted confirming inhibition of aerobic respiration and stimulation of anaerobic glycolysis through hexokinase, a rate limiting enzymes of glycolysis. Some authors reported that tissue damage followed the release of cellular enzymes such as LDH [29,30]. Besides in spite of the decrease in LDH activity, there was insignificant change in D-lactate and pyruvate level as compared to untreated snails, as reported by Reddy et al. [31].

Concerning gluconeogenic enzymes activities (Fructose-1,6-diphosphatase and Phosphoenolpyruvate Carboxykinase), the present results showed significant elevations in treated mice, where the significant increase in gluconeogenic enzymes fructose-1,6-diphosphatase is due to depletion of glucose in the treated mice, where the ratio of glycogen to glucose levels in liver is known to be regulated by the balance between glycogen synthesis and degradation capacities. The increase influx of glucose into glycolytic flux and enhanced glycogen stored lead to stimulation of the enzymes [32]. The nature of the end product formed is dependent on the competition for PEP by the two enzymes pyruvate kinase (PK) and phosphoenolpyruvate carboxykinase (PEPCK). Stimulation of PK in treated animals ascertained the stimulation of the glycolytic flux previously reported by Horemans et al. [33] and Ahmed and Gad [26]. With respect to G-6-
Table 1. Effect of tamoxifen and vitamin C on some glycolytic enzyme, in mice liver.

<table>
<thead>
<tr>
<th>Glycolytic enzymes</th>
<th>HK</th>
<th>PK</th>
<th>PFK</th>
<th>LDH</th>
<th>GPI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>% change</td>
<td>Mean ± SD</td>
<td>% change</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Contol</td>
<td>0.0 85 ± 0.05</td>
<td>5.8 ± 2.1</td>
<td>18.21 ± 1.4</td>
<td>42.32 ± 2.6</td>
<td>88.6 ± 8.2</td>
</tr>
<tr>
<td>Mice treated with tamoxifen</td>
<td>0.141 ± 0.13***</td>
<td>64.7%</td>
<td>7.8 ± 2.1***</td>
<td>34.48%</td>
<td>26.2 ± 2.2***</td>
</tr>
<tr>
<td>Mice treated with tamoxifen &amp; vitamin C</td>
<td>0.082±0.0.072</td>
<td>3.53%</td>
<td>5.6 ± 1.5</td>
<td>3.4%</td>
<td>18.8 ± 2.1</td>
</tr>
</tbody>
</table>

Data are means ±S.D. of five mice in each test; All values are expressed as µ mol/min/mg protein; ** p < 0.01 & ***p < 0.001.

Figure 1. Percentage change in the activity of glycolytic enzyme of mice treated with Tamoxifen and Tamoxifen with vitamin C.

Table 2. Effect of tamoxifen and vitamin C on some gluconeogenic and glycogenolytic enzyme, in mice liver.

<table>
<thead>
<tr>
<th>Gluconeogenic enzymes</th>
<th>FD Pase</th>
<th>PEPCK</th>
<th>Glycogenolytic enzymes</th>
<th>G-6-Pase</th>
<th>Glycogen phosphorylase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>% change</td>
<td>Mean±SD</td>
<td>% change</td>
<td></td>
</tr>
<tr>
<td>Contol</td>
<td>11.6.1 ± 0.21</td>
<td>3.5 ± 1.8</td>
<td>18.212.1</td>
<td>1.3 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>mice Treated with tamoxifen</td>
<td>16.2 ± 1.8 ***</td>
<td>39.66%</td>
<td>5.2 ± 0.22***</td>
<td>48.57%</td>
<td>24.6 ± 3.1 ***</td>
</tr>
<tr>
<td>mice Treated with tamoxifen &amp; vitamin C</td>
<td>12.1 ± 2.2</td>
<td>4.31%</td>
<td>3.6 ± 0.62</td>
<td>2.86%</td>
<td>18.621 ± 0.72</td>
</tr>
</tbody>
</table>

Data are means ±S.D. of five mice in each test; All values are expressed as µ mol/min/mg protein; ** p < 0.01 & ***p < 0.001.
Table 3. Effect of tamoxifen and vitamin C on liver function enzymes in mice.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Enzyme activity μ mol/min/mg protein</th>
<th>Mean ± SD</th>
<th>% change</th>
<th>Mean ± SD</th>
<th>% Change</th>
<th>Mean ± SD</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aspartate amino transferase (AST)</td>
<td>31.6 ± 3.1</td>
<td></td>
<td>21.8 ± 2.11</td>
<td></td>
<td>5.8 ± 1.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alanine amino transferase (ALT)</td>
<td>20.5 ± 1.03**</td>
<td>–35.13 %</td>
<td>12.8 ± 0.75***</td>
<td>–41.28%</td>
<td>8.6 ± 0.31***</td>
<td>48.28%</td>
</tr>
<tr>
<td></td>
<td>Acid phosphatase (ADP)</td>
<td>30.7 ± 0.04</td>
<td>–2.85%</td>
<td>22.1 ± 0.86*</td>
<td>–1.38%</td>
<td>6.2 ± 0.38</td>
<td>6.9%</td>
</tr>
<tr>
<td></td>
<td>Alkaline phosphatase (ALKP)</td>
<td>31.6 ± 3.1</td>
<td></td>
<td>21.8 ± 2.11</td>
<td></td>
<td>5.8 ± 1.1</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

* p < 0.05, ** p < 0.01 & *** p < 0.001
Table 4. Effect of tamoxifen and vitamin C on biochemical parameters in mice.

<table>
<thead>
<tr>
<th>Treatment parameters</th>
<th>Control (Mean ± SD)</th>
<th>Mice treated with tamoxifen (Mean ± SD)</th>
<th>% Change</th>
<th>Mice treated with tamoxifen &amp; vitamin C (Mean ± SD)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% Change</td>
<td></td>
<td>% CHANGE</td>
<td></td>
</tr>
<tr>
<td>Total protein</td>
<td>31.6 ± 3.1</td>
<td>21.8 ± 2.11** −31.01%</td>
<td>30.5 ± 3.1</td>
<td>-3.48 %</td>
<td></td>
</tr>
<tr>
<td>Glycogen</td>
<td>20.5 ± 1.03</td>
<td>12.8 ± 0.75*** −37.56%</td>
<td>19.5 ± 3.31</td>
<td>-5.85 %</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>30.7 ± 0.04</td>
<td>22.1 ± 0.86** −28.1%</td>
<td>-29.8 ± 2.38</td>
<td>-2.93 %</td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>2.1 ± 1.2</td>
<td>1.6 ± 0.13** −25.71%</td>
<td>1.98 ± 0.06</td>
<td>-5.71 %</td>
<td></td>
</tr>
<tr>
<td>Uric Acid</td>
<td>52.13 ± 0.4</td>
<td>34.3 ± 4.1** −34.2%</td>
<td>50.8 ± 4.3</td>
<td>-2.55 %</td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>6.2 ± 0.4</td>
<td>4.5 ± 1.1** −27.42%</td>
<td>6.1 ± 0.73</td>
<td>-1.62 %</td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td>35.1 ± 2.1</td>
<td>28.4 ± 3.6* −19.32%</td>
<td>34.6 ± 1.8</td>
<td>-1.42 %</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4. Percentage change in biochemical parameters of mice treated with Tamoxifen and Tamoxifen with vitamin C.

Pase as glycogenolytic enzyme, it showed an enhanced activity in treated mice which was attributed to either synthesis and/or degradation of glycogen [34], inhibition of translocase (T1) the glucose-6-phosphate transport protein [35] and to the elevation of cytosolic calcium that can trigger the conversion of the enzyme phosphorylase b (inactive form) to phosphorylase a (active form) which degrades glycogen into glucose [36].

Concerning AST and ALT enzymes activities, significant reduction was observed in both treated mice. The present result indicated that decrease in AST and ALT attributed to the hepatocellular damage where the transaminases level showed an intimate relationship to cell necrosis and/or increased cell membrane permeability led to discharge of the enzyme to blood stream [37,38]. The decrease in transaminases level providing additional support for the side effect of the toxic substance on mitochondria of the hepatic cells as it is the subcellular localization of transaminases.

In the present study, acid phosphatase (ACP) and alkaline phosphatase (ALP) showed that significant elevation in treated mice. Higher levels of acid phosphatase and alkaline phosphatase (ALP) in tissue was observed by El-Aasar et al. [37] and Abdel-Rahman et al. [39] which was attributed to the irritation of liver cells by toxins or due to increase loss of intracellular enzyme by diffusion through cell membrane which appear to act as a stimulus to the synthesis of more enzyme.

Tamoxifen was reported to alter the glutathione metabolizing enzymes [40]. Tamoxifen induces free radicals production in renal tissue, and at the same time decreases its ability to detoxify reactive oxygen species. TM intoxication leads to disruption of the activity of glutathione metabolizing and antioxidant enzymes [5].

There is no side effects on all glycolytic, gluconeogenic and glycogenolytic and liver function enzymes of

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mice treated with tamoxifen with vitamin C as compared to the normal healthy control group. The results of the present study indicated that the exogenously administered vitamin C may prevent tamoxifen-induced testes toxicity in mice. The protective effect of vitamin C is probably due to a counter action of free radicals by its antioxidant nature. Vitamin C may be recommended as an adjuvant therapy with certain anticancer. Protective effects of vitamin C against chemically-induced damage in various rodent organs have been demonstrated by many investigators [41,42]. Vitamin C was found to be effectively protecting chemically-induced oxidative renal damage in animals [43-45] reported that mega-dose of vitamins rendered significant protection of renal damage induced by anticancer and the effect of vitamin C was higher than that of vitamin E. The protective effects may be partially mediated by preventing the renal antioxidant status.

Increasing the glucose concentration stimulated glycogen synthesis and decreased the activity of glycogen phosphorylase. An inverse relationship was shown between the actual glycogen content and the rate of glycogenesis. So there is a substrate cycling that occurred between glucose-6-phosphate and glycogen content, i.e. glucose was incorporated into glycogen during period of net glycogen synthesis and vice versa; glycogen degradation occurred during periods of net glycogen synthesis which depends on glucose concentration [27]. Our data recorded enhancement levels of glucose-6-phosphatase and glucose.

The present results showed that significant reduction in total protein content in treated mice which could be attributed to cellular damage caused by toxins [30]. The main fraction of total protein content is albumin in turn may result from decrease anabolism or increase catabolism [45]. The significant decrease in total protein is mainly due to increase in messenger RNA degradation which is the possible cause for the hypoalbuminemia [46]. Also, the present results showed that very highly significant reduction in total cholesterol, uric acid, urea and creatinine in mice treated with tamoxifen as compared to the normal healthy control.

5. CONCLUSIONS

Normal control mice treated with tamoxifen showed side effects of most parameters compared to the normal healthy control group. Moreover, normal control mice treated with tamoxifen and vitamin C showed no side effects of most parameters compared to the normal healthy control group. Hence, vitamin C may prevent tamoxifen-induced testes toxicity in mice.

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