Antioxidant and anti-Inflammation Potentials of Moringa oleifera Aqueous Extract Against breast cancer chemotherapy drug Induced Kidney and Liver Toxicity in Experimental Rats

Essawy A.¹, Beeker H.M.¹, Ola N. Sayed ²Abdel-Wahhab K.G.³ and Saber S.R.²
1-Chemistry Department, Fayoum University, Fayoum, Egypt
2-Biochemistry Department, Fayoum University, Fayoum, Egypt
3- Medical Physiology Department, National Research Centre, Cairo, Egypt
*E. Mail : ons00@fayoum.edu.eg

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ABSTRACT
Breast cancer is the most common causing death in women. Tamoxifen, 1-[4-(2-dimethyl-aminoethoxy) phenyl]-1,2-diphenyl-1-butene), is a nonsteroidal antiestrogen breast cancer prevention drug causing liver toxicity. This study was carried out to elucidate the efficacy of Moringa oleifera aqueous extract against carcinogenic effect of Tamoxifen in male rats. 50 male albino rats were divided equally into five groups: control group (I); Moringa oleifera aqueous group (II) of dose (300mg/Kg/day for 6 weeks); group (III) received Tamoxifen (3mg/Kg/3 days for 6 weeks); rats received orally Tamoxifen and Moringa oleifera aqueous extract together for 6 weeks group (IV) and rats pretreated with Moringa oleifera aqueous extract then Tamoxifen with the same dose and period group (V). In group (III) there were elevate in serum activities of ALT, AST, LDH, and serum levels of TNF-α, IL-1β, IL-10, cholesterol, TG, LDL, HDL, Urea, Creatinine also liver MDA; NO; ATPase. There were decrease in serum Total protein, Albumin and liver antioxidants markers (TAC and GSH) compared to control. In conclusion, oral administration of Moringa oleifera aqueous extract improved the biochemical markers and provided antioxidant activity against the toxicity of Tamoxifen in liver tissues.

INTRODUCTION
Breast cancer is the second most common cancer worldwide and the leading cause of cancer death in women (Siegel et al., 2012 and Tomao et al., 2015). Approximately 85% of all breast cancers are hormonal. They can be treated with hormone therapies, including tamoxifen and aromatase inhibitors (AIs) as anastrozole (Arimidex), letrozole (Femara) and exemestane (Aromasin)(Anderson et al., 2002).

Tamoxifen has antiestrogen used in the prevention and treatment of breast cancer for both men and women through block the binding of estrogen, such as 17b-estradiol, to its receptor (McDonnell, 1999; Johnston, 2005 and Deroo et al., 2006).
Tamoxifen is metabolized in the liver by metabolizing enzymes into 4-hydroxytamoxifen (4-OHT) and endoxifen which have 100 times more affinity for the estrogen receptor than tamoxifen itself (Desta et al., 2004; Johnson et al., 2004 and Saladores et al., 2015). Tamoxifen has been shown to increase oxidative stress and induce apoptosis (Yang et al., 2013).

It was revealed that tamoxifen in high dose cause oxidative liver damage as it had been elucidated to be a hepatocarcinogen in rats (Ahotupa et al., 1994; Caballero et al., 2001; Galeone et al., 2006 and Ribeiro et al., 2014). It may be more toxic to liver because it has higher affinity to hepatic tissues than to any other tissues. A high frequency of p53 mutations was detected in hepatocarcinomas induced by tamoxifen exposure (Vancutsem et al., 1994).

*Moringa oleifera*, known as Moringa, is an Indian tree grows in Asia, South America and Africa (Palafox et al., 2012; Bhutada et al., 2016). It belongs to the *Moringaceae* family, enriched with effective flavonoids such as kaempferol, rhamnetin, chlorogenic acid, rutin, apigenin which exhibited anti-inflammatory, antimicrobial, anti-cancer and anti-diabetic effects (Karthivashan et al., 2016 and José et al., 2020). It provided a hepatoprotective effect (Upadhyay et al., 2015).

The objective of that study is to evaluate the improvement effect of *Moringa oleifera* extract against hepatotoxicity of tamoxifen in male rats.

**MATERIALS AND METHODS**

Adult fifty male Wistar albino rats (120-150g) were obtained from Animal
House, National Research Centre, Giza, Egypt. The animals were housed in suitable plastic cages for one week to the new room condition. Excess and fresh tap water and standard rodent food pellets were always available. All animals were received human care in compliance with the standard institutional criteria for the care and use of experimental animals.

After the animals being acclimatized with experimental room conditions, they were divided randomly into five groups (10 animals each) as:

**Group I)** comprised of normal animals fed diet and acting as control.

**Group II)** Animals fed diet and subjected to daily oral administration of *Moringa oleifera* aqueous extract (300mg/Kg/day) for consecutive six weeks (Jaiswl et al., 2009).

**Group III)** comprised of normal animals fed diet and subjected to oral administration of Tamoxifen (3mg/Kg/3days) for consecutive six weeks (pala et al., 2015).

**Group IV)** rats received orally aqueous extract of *Moringa oleifera* (300mg/kg/day) in combination with Tamoxifen (3mg/kg/3days) for consecutive six weeks.

**Group V)** rats treated daily via oral administration of *Moringa oleifera* aqueous extract (300mg/kg/day) for consecutive six weeks before they were intoxicated orally with Tamoxifen (3mg/kg/3days) for a similar period.

**Blood Sampling:**

At the end of the study period, animals fasted overnight, and following diethyl ether anesthesia, blood specimens (3-7 ml) were drawn from the retro-orbital plexus using non-heparinized blood was withdrawn into open vacutainer collecting tubes (Paget & Thosmon, 1979). Blood specimens were centrifuged at 3000 rpm for 10 minutes using centrifuge to separate sera and then stored at -70°C until biochemical measurements.

**Tissue Sampling:**

After blood collection, all animals were rapidly sacrificed then liver and kidney of each animal were dissected and washed with saline, dried, rolled in a piece of aluminum foil and stored at -70 °C until homogenization and biochemical determinations.

**Preparation of Liver and Kidney Homogenate:**

A specific weight from each organ (liver or kidney) was subjected to homogenization in ice-cold phosphate buffer (50 mM, pH 7.4) to give 10% homogenate (w/v) and dilution factor equal 10; then homogenate was centrifuged at 9000 rpm for 20 min to remove the nuclear and mitochondrial fractions. The supernatant was divided into aliquots and stored at -70°C till the determination of the biochemical measurement.

**Aqueous Extract of Moringa oleifera:**

*Moringa* (*Moringa oleifera*) aqueous extract was carried out according to the method of Berkovich et al. (2013). 50 g of the dry-powdered leaves were mixed with 500 ml boiling distilled water for 15minutes. The mixture was then filtered through sterile Whatman filter paper number 42 using Buchner funnels. The filtrate was subjected to lyophilization process through freeze drier under pressure, 0.1-0.5 m Bar and temperature -35-41°C conditions. The dry extract was stored in a dark bottle at -20°C.

**Histo-Pathological Examination:**

The livers and kidneys from each animal of the study groups were fixed in 10% formalin-saline buffer for 24 hours; then washing in tap water followed by dehydration in graded alcohol, clearing in xylene for 20 minutes and embedded in paraffin wax. Transverse serial sections were then cut at 5 micrometers thickness and mounted on albumenized slide. Sections were stained with hematoxylin and eosin then investigated (Drury & Wallington, 1980).

**Biochemical Analysis:**

**Liver Function Biomarkers Assays:**

ALT, AST and LDH activities
were evaluated according to Schumann and Klauke (2003) and Tietz (1983) respectively. Total protein and albumin were performed according to Berth and Delanghe (2004).

**Kidney Function Biomarkers Assays:**
Creatinine and Urea were evaluated according to the method of Allen (1982) and Eisenwiener (1976) respectively.

**Oxidative Stress Biomarkers Assays:**
Lipid peroxidation was performed by measuring MDA levels in the supernatant of liver and kidney homogenate according to (Draper & Hadley, 1990). ATPase activity was measured according to the chemical method described by Tsakiris et al. (2004). Nitric oxide level was determined according to the method of Montgomery and Dymock (1961).

Total antioxidant capacity and Glutathione level of tissue homogenate determined according to the method of Koracevic et al. (2001) and Beutler et al. (1963) respectively.

**Anti-Inflammatory Biomarkers Assays:**
Serum levels of IL-18; IL-10 and TNF-α were determined using Enzyme-Linked Immunosorbent Assay technique with ELISA kit as described by (Allan et al., 2005 and Brouckaert et al. (1993) respectively.

**Lipid Biomarkers Assays:**
Serum total cholesterol level; triglycerides; HDL and LDL were determined according to the photometric system described by Berth and Delanghe (2004) and Naito (2003) respectively.

**Statistical Analysis:**
The obtained data were subjected to one-way ANOVA followed by a post hoc test (Duncan). All data were expressed as mean ± standard error (SE). Differences were considered significant at $P \leq 0.05$.

**RESULTS**
This study was carried out to evaluate the ameliorative effect of *Moringa oleifera* aqueous extract against the toxicity of tamoxifen in male rats. The obtained data showed that daily oral administration of rats with moringa aqueous extract had no effect on the activities of serum ALT; AST and LDH (-2.34; -4.22 and -4.3%) respectively; while oral administration with Tamoxifen led to a significant increase (46.0; 77.17 and 42%, respectively) in their activities compared to control group. Animals treated orally with moringa in group IV showed a significant decrease in their activities than group V comparing with rats treated with tamoxifen (-32.95; -37.38 and -21.39%, respectively) (Table 1).

Our results showed that rats that received daily moringa extract resulted in non-significant changes (3.03 and 2.94% respectively) in serum total proteins and albumin while oral intoxication with Tamoxifen led to a significant decrease in their levels when compared with the control group (-10.60 and -14.70% respectively); while there improvement in their levels in group IV and group V (7.2; 3.6; 6.6 and 3.3 g/dl respectively) when compared with group III (Table 2).

There was a significant increase in creatinine and urea levels in rats of group III which are treated with tamoxifen when compared to control (40.84 and 112.28% respectively). Administration of moringa extract improved their levels in both group IV and group V but receiving moringa extract with tamoxifen effective than group V (Table 2).
Antioxidant and anti-Inflammation Potentials of Moringa oleifera Extract

Table 1: Mean values and percentage of change of serum ALT; AST and LDH activities in all studying groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>ALT (IU/L)</th>
<th>AST (IU/L)</th>
<th>LDH U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (I)</td>
<td>M±SE</td>
<td>72.6±2.98</td>
<td>125.3±4.44</td>
<td>1162±55</td>
</tr>
<tr>
<td>MAE (II)</td>
<td>M±SE</td>
<td>70.9±2.87</td>
<td>120±4.24</td>
<td>1112±53</td>
</tr>
<tr>
<td></td>
<td>% A</td>
<td>-2.34%</td>
<td>-4.22</td>
<td>-4.30</td>
</tr>
<tr>
<td>TMX (III)</td>
<td>M±SE</td>
<td>106.2±13.01</td>
<td>222±21.45</td>
<td>1650±78a</td>
</tr>
<tr>
<td></td>
<td>% A</td>
<td>46.0%</td>
<td>77.17</td>
<td>42.00</td>
</tr>
<tr>
<td>MAE+TMX (IV)</td>
<td>M±SE</td>
<td>71.200±13.3b</td>
<td>139±8.09b</td>
<td>1297±61b</td>
</tr>
<tr>
<td></td>
<td>% A</td>
<td>-32.95%</td>
<td>-37.38</td>
<td>-21.39</td>
</tr>
<tr>
<td>MAE→TMX (V)</td>
<td>M±SE</td>
<td>83.600±5.7b</td>
<td>180±11.81db</td>
<td>1468±70ab</td>
</tr>
<tr>
<td></td>
<td>% B</td>
<td>-21.28%</td>
<td>-18.91</td>
<td>-11.00</td>
</tr>
</tbody>
</table>

* All data are expressed as mean ± standard error (M±SE); each group of 10 animals (n=10).
* MAE (Moringa aqueous extract) and TMX (Tamoxifen drug).
* %A (percent of change calculated from control group) and % B is percent of change calculated from Tamoxifen drug group.
a Significant at P value ≤ 0.01 versus control group and b Significant at P value ≤ 0.01 versus Tamoxifen group.

Table 2: Mean values and percentage of change of serum Total.Proteins; Albumin; Creatinine and Urea levels in all studying groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Total Proteins g/dl</th>
<th>Albumin g/dl</th>
<th>Creatinine mg/dl</th>
<th>Urea mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (I)</td>
<td>M±SE</td>
<td>6.6±0.11</td>
<td>3.4±0.04</td>
<td>0.71±0.06</td>
<td>57±2.8</td>
</tr>
<tr>
<td>MAE (II)</td>
<td>M±SE</td>
<td>6.8±0.12b</td>
<td>3.5±0.04b</td>
<td>0.72±0.06b</td>
<td>53±2.8b</td>
</tr>
<tr>
<td></td>
<td>% A</td>
<td>3.03</td>
<td>2.94</td>
<td>1.40</td>
<td>-7.02</td>
</tr>
<tr>
<td>TMX (III)</td>
<td>M±SE</td>
<td>5.9±0.18a</td>
<td>2.9±0.09a</td>
<td>1.0±0.08a</td>
<td>121±14.0a</td>
</tr>
<tr>
<td></td>
<td>% A</td>
<td>-10.60</td>
<td>-14.70</td>
<td>40.84</td>
<td>112.28</td>
</tr>
<tr>
<td>MAE+TMX (IV)</td>
<td>M±SE</td>
<td>7.2±0.14a</td>
<td>3.6±0.05b</td>
<td>0.8±0.02b</td>
<td>92±11.0ab</td>
</tr>
<tr>
<td></td>
<td>% B</td>
<td>22.0</td>
<td>24.14</td>
<td>-20.0</td>
<td>-23.96</td>
</tr>
<tr>
<td>MAE→TMX (V)</td>
<td>M±SE</td>
<td>6.6±0.11ab</td>
<td>3.3±0.032ab</td>
<td>0.9±0.07ab</td>
<td>108±12.8ab</td>
</tr>
<tr>
<td></td>
<td>% B</td>
<td>11.86</td>
<td>13.79</td>
<td>-10.0</td>
<td>-10.74</td>
</tr>
</tbody>
</table>

* All data are expressed as mean ± standard error (M±SE); each group of 10 animals (n=10).
* MAE (Moringa aqueous extract) and TMX (Tamoxifen drug).
* %A (percent of change calculated from control group) and % B is percent of change calculated from Tamoxifen drug group.
a Significant at P value ≤ 0.01 versus control group and b Significant at P value ≤ 0.01 versus Tamoxifen group.

The obtained data showed that daily oral administration of rats with moringa extract resulted in non-significant changes (1.17, -6.41&-2.56% respectively) in the concentration of serum total cholesterol, triglycerides and low-density lipoprotein cholesterol (LDL-c), respectively with a non-significant increase (6.66%) in serum high-density lipoproteins cholesterol (HDL-c) level. In contrast, oral administration with Tamoxifen causes a significant increase (44.70,55.13&71.79% respectively) in the serum of total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-c) levels matched with a significant decrease (-33.33%) in serum high-density lipoproteins cholesterol (HDL-c) level compared to control group. (Table 3).

Animals treated with moringa extract with tamoxifen (group IV) showed a significant decrease (-22.76,-15.70&-
19.40% respectively) in serum total cholesterol, triglycerides and low-density lipoprotein cholesterol (LDL-c) levels concomitant a significant raise (30.0%) in serum high-density lipoproteins cholesterol (HDL-c) level more than group V when compared with control group (Table 3).

Table 3: Mean values and percentage of change of serum Cholesterol; TG; LDL and HDL levels in all studying groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol</th>
<th>TG</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (I)</td>
<td>M±SE 85±3.9</td>
<td>78±2.7</td>
<td>39±2.7</td>
<td>30±3.9</td>
</tr>
<tr>
<td>MAE (II)</td>
<td>M±SE 86±3.9</td>
<td>73±2.5</td>
<td>38±2.6</td>
<td>32±4.0</td>
</tr>
<tr>
<td>TMX (III)</td>
<td>M±SE 123±6.6</td>
<td>121±4.2a</td>
<td>67±4.5a</td>
<td>20±1.4a</td>
</tr>
<tr>
<td>MAE+TMX (IV)</td>
<td>M±SE 95±4.3a</td>
<td>102±3.5b</td>
<td>54±3.7ab</td>
<td>26±2.6a</td>
</tr>
<tr>
<td>MAE→TMX (V)</td>
<td>M±SE 113±5.2ab</td>
<td>116±3.4ab</td>
<td>61±4.1ab</td>
<td>18±0.8ab</td>
</tr>
</tbody>
</table>

* All data are expressed as mean ± standard error (M±SE); each group of 10 animals (n=10).
* MAE (Moringa aqueous extract) and TMX (Tamoxifen drug).
* %A (percent of change calculated from control group) and % B is percent of change calculated from Tamoxifen drug group.
* a Significant at P value ≤ 0.01 versus control group and b Significant at P value ≤ 0.01 versus Tamoxifen group.

Oral administration with Tamoxifen (group III) showed a significant elevation (75.47&34.76&24.46% respectively) in serum TNF-α, IL1β and IL 10 levels, respectively when compared with control. On the other side, animals treated with moringa aqueous extract in combination with Tamoxifen (group IV) showed a significant decrease (-22.58&-21.98&-18.80% respectively) in their levels more than group V when compared with group III (Table 4).

Table 4: Mean values and percentage of change of serum TNF-α; IL 1β; IL 10 and levels in all studying groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>TNF-α</th>
<th>IL 1β</th>
<th>IL 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (I)</td>
<td>M±SE 53±3.9</td>
<td>837±2.4</td>
<td>188±0.8</td>
<td></td>
</tr>
<tr>
<td>MAE (II)</td>
<td>M±SE 52±3.9</td>
<td>811±2.4</td>
<td>183±0.8</td>
<td></td>
</tr>
<tr>
<td>% A</td>
<td>-1.89</td>
<td>-3.11</td>
<td>-2.65</td>
<td></td>
</tr>
<tr>
<td>TMX (III)</td>
<td>M±SE 93±6.9a</td>
<td>1128±3.2a</td>
<td>234±0.9a</td>
<td></td>
</tr>
<tr>
<td>%A</td>
<td>75.47</td>
<td>34.76</td>
<td>24.46</td>
<td></td>
</tr>
<tr>
<td>MAE+TMX (IV)</td>
<td>M±SE 72±5.3a</td>
<td>880±2.6ab</td>
<td>190±0.9ab</td>
<td></td>
</tr>
<tr>
<td>% B</td>
<td>-22.58</td>
<td>-21.98</td>
<td>-18.80</td>
<td></td>
</tr>
<tr>
<td>MAE→TMX (V)</td>
<td>M±SE 89±6.6a</td>
<td>1054±3.1b</td>
<td>222±0.9a</td>
<td></td>
</tr>
<tr>
<td>% B</td>
<td>-2.30</td>
<td>-6.56</td>
<td>-5.13</td>
<td></td>
</tr>
</tbody>
</table>

* All data are expressed as mean ± standard error (M±SE); each group of 10 animals (n=10).
* MAE (Moringa aqueous extract) and TMX (Tamoxifen drug).
* %A (percent of change calculated from control group) and % B is percent of change calculated from Tamoxifen drug group.
* a Significant at P value ≤ 0.01 versus control group and b Significant at P value ≤ 0.01 versus Tamoxifen group.
Antioxidant and anti-Inflammation Potentials of *Moringa oleifera* Extract

From our study rats treated with Tamoxifen showed a significant increase (43.0; 35.19; 37.73 and 30.18% respectively) in the concentration of hepatic and renal MDA and NO levels with a significant decrease in ATPase activity (-28.73 and -26.95% respectively) when compared with control group. Comparing with Tamoxifen-intoxicated group, animals treated orally with moringa aqueous extract in combination with Tamoxifen (group IV) showed a significant decrease (-22.88, -21.92, -20.55 and -27.54% respectively) in the levels of hepatic and renal MDA and NO coupled with a significant increase (23.32 and 26.48% respectively) in ATPase activity better than group V (Tables 5 & 6).

In group III where rats treated with tamoxifen showed a significant decrease in hepatic& renal TAC and GSH levels (-35.7,-23.8; -36 and -15.52%, respectively) when compared with the control group with improvement in their levels in groups IV &V (Tables 5&6).

Table 5: Mean values and percentage of change of ATPase; MDA; NO; TAC and GSH levels in liver homogenate of all studying groups:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>ATPase</th>
<th>MDA</th>
<th>NO</th>
<th>TAC</th>
<th>GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (I)</td>
<td>M±SE</td>
<td>355±1.3</td>
<td>107±4.1</td>
<td>54±3.7</td>
<td>28±0.5</td>
<td>63±2.2</td>
</tr>
<tr>
<td>MAE (II)</td>
<td>M±SE</td>
<td>318±1.3</td>
<td>105±4.1</td>
<td>52±3.5</td>
<td>29±0.5</td>
<td>65±2.2</td>
</tr>
<tr>
<td>% A</td>
<td>-10.42</td>
<td>-1.86</td>
<td>-3.70</td>
<td>3.6</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>TMX (III)</td>
<td>M±SE</td>
<td>253±1.0</td>
<td>153±5.9</td>
<td>73±5.0</td>
<td>18±0.3</td>
<td>48±1.7</td>
</tr>
<tr>
<td>% A</td>
<td>-28.73</td>
<td>43.00</td>
<td>35.19</td>
<td>-35.7</td>
<td>-23.8</td>
<td></td>
</tr>
<tr>
<td>MAE+TMX (IV)</td>
<td>M±SE</td>
<td>312±1.2</td>
<td>118±4.5</td>
<td>57±3.9</td>
<td>22±0.4</td>
<td>82±3.0</td>
</tr>
<tr>
<td>% A</td>
<td>-26.95</td>
<td>37.73</td>
<td>30.18</td>
<td>-36.0</td>
<td>-15.5</td>
<td></td>
</tr>
<tr>
<td>MAE→TMX (V)</td>
<td>M±SE</td>
<td>290±1.2</td>
<td>141±5.5</td>
<td>69±4.6</td>
<td>19±0.4</td>
<td>54±2.1</td>
</tr>
<tr>
<td>% B</td>
<td>14.62</td>
<td>-7.84</td>
<td>-5.48</td>
<td>5.55</td>
<td>12.5</td>
<td></td>
</tr>
</tbody>
</table>

* All data are expressed as mean ± standard error (M±SE); each group of 10 animals (n=10).
* MAE (Moringa aqueous extract) and TMX (Tamoxifen drug).
* %A (percent of change calculated from control group) and % B is percent of change calculated from Tamoxifen drug group.

Table 6: Mean values and percentage of change of ATPase; MDA; NO; TAC and GSH levels in kidney homogenate of all studying groups:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>ATPase</th>
<th>MDA</th>
<th>NO</th>
<th>TAC</th>
<th>GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (I)</td>
<td>M±SE</td>
<td>460±7.2</td>
<td>53±2.4</td>
<td>53±1.8</td>
<td>25±0.8</td>
<td>58±2.2</td>
</tr>
<tr>
<td>MAE (II)</td>
<td>M±SE</td>
<td>427±6.7</td>
<td>51±2.4</td>
<td>51±1.8</td>
<td>25.1±0.8</td>
<td>61±2.2</td>
</tr>
<tr>
<td>% A</td>
<td>-7.17</td>
<td>-3.77</td>
<td>-3.77</td>
<td>0.4</td>
<td>5.17</td>
<td></td>
</tr>
<tr>
<td>TMX (III)</td>
<td>M±SE</td>
<td>336±5.3</td>
<td>73±3.4</td>
<td>69±2.4</td>
<td>16±0.5</td>
<td>49±1.8</td>
</tr>
<tr>
<td>% A</td>
<td>-26.95</td>
<td>37.73</td>
<td>30.18</td>
<td>-36.0</td>
<td>-15.5</td>
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</tr>
<tr>
<td>MAE+TMX (IV)</td>
<td>M±SE</td>
<td>425±6.7</td>
<td>58±2.7</td>
<td>49±1.7</td>
<td>19±0.6</td>
<td>74±2.6</td>
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<tr>
<td>% B</td>
<td>26.48</td>
<td>-20.55</td>
<td>-27.54</td>
<td>18.75</td>
<td>51.0</td>
<td></td>
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<tr>
<td>MAE→TMX (V)</td>
<td>M±SE</td>
<td>386±6.1</td>
<td>64±3.0</td>
<td>62±2.1</td>
<td>17.9±0.5</td>
<td>55±2.1</td>
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<tr>
<td>% B</td>
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<td>-12.33</td>
<td>-10.14</td>
<td>11.87</td>
<td>12.24</td>
<td></td>
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</table>

* All data are expressed as mean ± standard error (M±SE); each group of 10 animals (n=10).
* MAE (Moringa aqueous extract) and TMX (Tamoxifen drug).
* %A (percent of change calculated from control group) and % B is percent of change calculated from Tamoxifen drug group.
* a Significant at P value ≤ 0.01 versus control group and  b Significant at P value ≤ 0.01 versus Tamoxifen group.
The liver sections of normal showed histological structure of normal hepatic lobules and central vein and the cell corda were separated by narrow blood sinusoids lined by endothelial cells and kupffer cells. Liver sections of the rats treated with moringa aqueous extract only showed liver tissue more or less appeared normal (Figs. 3A&B).

The microscopic examination of liver sections of rats treated with Tamoxifen only demonstrated dilated and congested portal vessels. Dilated bile duct and massive cellular infiltration around it. Sings of degeneration in the form of pyknosis, karyolysis and fibrosis were seen. Hypertrophy of kupfer cells and vacuolar degeneration (Figs. 3C&D).

The microscopic examinations of liver sections of rats treated with tamoxifen along with moringa extract at the same time (group IV) induced an improvement in pathological changes in the form of no fibrosis, no inflammatory infiltrate, no signs of degeneration. Also, congestion of central vein, some hepatocyte appeared karyolysis and others necrosis (Fig. 3E). But rats treated with tamoxifen after administration of moringa extract (groupV) didn't show any improvement in pathological changes of liver sections showed fibrosis, dilated bile duct and blood sinusoid; degeneration in the form pyknosis, karyolysis, and vacuolar degeneration (Fig. 3F).

Fig. 3 A; Light micrograph showing liver sections of normal rat showed normal histological structure of hepatic lobules and central vein (star) (H&E100). B; Liver sections of the rats treated with moringa aqueous extract only showed liver tissue more or less appeared normal (H&E200). C; The microscopic examination of liver sections of rats treated with Tamoxifen only signs of degeneration in the form pyknosis (black arrow), karyolysis (white arrow) and cellular infiltration around bile duct (red curved arrow) (H&E400). D; A photomicrograph of the liver of Tamoxifen treated rats showing dilated and congested portal vessel (star) (H&E100). E; Photomicrograph of a cross sections in the liver of rat treated with tamoxifen along with moringa extract at the same time showing congestion of central vein (star) (H&E200). F; Section of the liver of tamoxifen after administration of moringa extract fibrosis (black arrow), dilated bile vessel (red arrow), aggregation of cellular infiltration (star) (H&E100).
DISCUSSION

Tamoxifen (TMX), a selective estrogen receptor modulator and non-steroidal antiestrogenic drug, is used in the chemotherapy of breast cancer (Kuo et al., 2012; Tsai et al., 2014; Pandey et al., 2016) but many studies reported its adverse effects, such as hot flashes, fatty liver, hepatotoxicity and hepatocarcinomas (Ribeiro et al., 2014; Wickramage et al., 2017). The present study was attempting to investigate the role of moringa oleifera extract, in reducing Tamoxifen side effects.

The present study pointed that intoxication with Tamoxifen® only induced a significant elevation in the activities of ALT; AST and LDH. This finding is agonist many previous studies (El-Beshbishy, 2005; Yang et al., 2016 and Wickramage et al., 2017). On the other hand, animals' groups treated with tamoxifen along with moringa extract at the same time (group IV) recorded a significant improvement in serum activities of ALT; AST and LDH in comparison to the animals' group treated with tamoxifen alone more than group V this finding is confirmed with that of Okwari et al. (2013); Sheikh et al. (2014) and Bahr&Farouk (2016).

The obtained data illustrated that tamoxifen alone induced a significant increase in hepatic and renal levels of oxidative markers (MDA and NO) matched with a significant reduction anti-oxidative marker (TAC and GSH) and ATPase activity as compared to the control group. These findings were concomitant with the reports of Nazarewicz et al. (2007); Tabassum et al. (2007) and Kumarappan et al. (2011).

Aministration of moringa extract at the same time with tamoxifen (group IV) recorded a significant improvement in hepatic and renal levels MDA, NO, ATPase, TAC and GSH in compare to the animals' group intoxicated with tamoxifen alone (group III) more than group V. This finding is confirmed with that of Saalu et al. (2012); Waterman et al. (2014) and Bibi et al. (2016).

Animals intoxicated with Tamoxifen only revealed a significant reduction in serum levels of total proteins and albumin compared to normal animals; this result reflecting that Tamoxifen manifested a severe inhibition in the hepatic protein synthetic capacity and overall liver damage, and confirm the depth and intensity of liver necrosis inconsistently with the report of Yuvaraj et al. (2007) and Kumarappan et al. (2011); while treatment with Moringa aqueous extract (group IV&V) attenuated the level of proteins as compared to those intoxicated with Tamoxifen. This finding agonist that of Ghasi et al. (2000).

The present study showed that rats treated with Tamoxifen alone showed a disturbance in lipid profile pointed with a significant elevation in the serum level of total cholesterol, triglycerides and LDL-c associated with a decrease in the serum level of HDL-c. These results agree with Yuvaraj et al. (2007) while administration of Tamoxifen and aqueous extract of Moringa oleifera (group IV) showed a marked improvement in serum lipograme in comparison to those intoxicated with Tamoxifen only. This finding is in accordance with many reports (Ghasi et al., 2000; Okwarie et al., 2013 and Sheikh et al., 2014).

On the other hand, animals intoxicated with Tamoxifen revealed a significant increase in serum levels of creatinine and urea as compared with control group with improvement in their levels with administration of aqueous extract of Moringa, confirming the nephrotoxicity effect of Moringa this agonist with Sato et al. (2004) and Tabassum et al. (2007).

Rats treated only with Tamoxifen (group III) revealed a significant increase level of TNF-α, IL1β and IL10 this result is conflicting that of Zuhair and ALamri
The animals treated with Tamoxifen and aqueous extract of *Moringa oleifera* showed a significant improvement in serum TNF-α, IL1β and IL10 levels in group IV than group V. These results are in accordance with Panda et al. (2013) and Waterman, et al. (2014).

Liver sections of rats treated with Tamoxifen only showed signs of degeneration in the form of pyknosis, karyolysis, and cellular infiltration around the bile duct. Also, dilated blood sinusoid, congested portal vessel, dilated bile duct were observed. Additionally, a marked degree of fibrosis, hypertrophy of kupfer cells and vacuolar degeneration were noticed. These results are in agreement with Kumarap et al. (2011). Moreover, the section of the livers of rats treated with Moringa extract with Tamoxifen (group IV) exhibited remarkable preservation in the liver histological profiles comparable to the animals’ group intoxicated with Tamoxifen alone. This result is in accordance with Saaluet al. (2012); Yassar and Tohamy (2014); Bahr and Farouk (2016).

In conclusion from the present investigation offers strong evidence for the hepatoprotective, antifibrotic, anti-inflammatory and anti-oxidative efficiencies of the aqueous extract of *M. oleifera* leaves against tamoxifen hepatotoxicity in male rats.

**Abbreviation**

Alanine aminotransferase (ALT); Aspartate aminotransferase (AST); Lactate dehydrogenase (LDH); Tumor necrosis factor alpha (TNF-α); Interleukin one beta (IL-1β); Interleukin ten (IL-10); Malondialdehyde (MDA); Nitric Oxide (NO); Total antioxidant capacity (TAC); Triglyceride (TG); High density lipoprotein (HDL); Low density lipoprotein (LDL); Reduced glutathione (GSH).

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Antioxidant and anti-Inflammation Potentials of *Moringa oleifera* Extract

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Antioxidant and anti-Inflammation Potentials of Moringa oleifera Extract

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Antioxidant and anti-Inflammation Potentials of *Moringa oleifera* Extract

ARABIC SUMMARY

الدور الفعال لمستخلص المورينجا كمضاد للأكسدة والالتهاب ضد سمية الأدوية الكيميائية لعلاج سرطان الثدي في جذران التجربة

عبد النبي إبراهيم عيسوي 1، هدير محمد بكير 1، علاء نبيل سيد 2، خالد جمال الدين عبادو هاب 3، وشيماء ربيع صابر 1

1-قسم الكيمياء - كلية العلوم - جامعة الفيوم
2-قسم الكيمياء الحيوية - كلية العلوم - جامعة الفيوم
3-قسم الفسيولوجيا الطبية - المركز القومي للبحوث

سرطان الثدي هو أكثر أنواع السرطان شيوعاً بسبب الوفاة عند النساء. تاموكسيفين هو عقار مضاد للاستروجين لمنع سرطان الثدي السبب في الوفاة. أجريت هذه الدراسة لتوضيح فاعلية مستخلص المورينجا أوليفيرا المائي ضد تأثير عقار تاموكسيفين المسرطنة للكبد في ذكور الجرذان. تم تقسيم 50 من ذكور الجرذان البيضاء بالتساوي إلى خمس مجموعات: المجموعة الأولي وهي المجموعة الضابطة؛ مجموعه مورينجا أوليفيرا المائية المعالجة (المجموعة الثانية) بجرعة (300 مجم / كجم / يوم لمدة 6 أسابيع) ؛ المجموعة (الثالثة) تلقت عقار تاموكسيفين (3 مجم / كجم / 3 أيام لمدة 6 أسابيع) ؛ تلقت الجرذان مستخلص مائي تاموكسيفين ومورينجا أوليفيرا معاً لمدة 6 أسابيع للمجموعة (المجموعة الرابعة) وجرذان تمت معالجتها مسبقًا بمستخلص المورينجا أوليفيرا المائي ثم تاموكسيفين بنفس الجرعة والفترة (المجموعة الخامسة). في المجموعة الثالثة كان هناك ارتفاع كبير في أنشطة إنزيمات الامينات والالانين والاسبارتاك ومستويات الالتهاب والانترلوكينات وكذلك الكوليسترول والمدهون بالاضافة الى ارتفاع مستوي الأكسيد النmk (المالوندايالدهيد). كان هناك انخفاض معنوي في البروتينات الكلية والألبومين ومضادات الأكسدة مقارنة بجميع المجموعات. أدى تناول مستخلص المورينجا المائي عن طريق الفم إلى تحسين العلامات الكيميائية الحيوية وتوفير نشاط مضاد للأكسدة والالتهاب ضد سمية تاموكسيفين في نسيج الكبد وارتفاع ملحوظ في البروتينات الكلية والألبومين ومضادات الأكسدة وإنخفاض في أنشطة الإنزيمات الناقلة لملحوظة الأكسدة والاسبارتاك) وتحسين واضح في مستويات علامات الالتهاب.