Ameliorative Role of Oleuropein Extracted from Olive Leaf on Tamoxifen-Induced Hepatic 8-Hydroxydeoxyguanosine in DNA of Balb/C Mice

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Abstract: One of the most attractive approaches to disease prevention involves the use of natural antioxidants to protect tissue against toxic injury. Tamoxifen (TAM), a medication used as anti-neoplastic drug for the treatment of breast cancer, often induces menopausal symptoms. The aim of this study was to investigate the toxicity of this drug by measuring the ability of TAM to induce oxidative DNA damage through the formation of 8-hydroxydeoxyguanosine (8-OH-dG) using HPLC with electrochemical detection as well as investigating the effect of oleuropein as antioxidant to ameliorate its toxicity. TAM initiates reactive oxygen species (ROS) formation followed by damage to DNA and other cellular compartments. Oleuropein, the phytoalexin extracted from olive leaf and proved to be a strong free radical scavenger, markedly inhibited the formation of 8-OH-dG. Results showed that oleuropein reduced the toxicity of TAM by 9 fold. These findings are consistent with conclusion that oleuropein has highly protective power against TAM toxicity and the protective action relates, at least in part to its direct free radical scavenging ability.

Key words: Oleuropein - ROS - 8-Hydroxydeoxyguanosin - DNA Damage

INTRODUCTION

Tamoxifen (TAM), a triphenylethylene derivative, is a selective estrogen receptor modulator (SERM) [1,2] that has become the treatment of choice for women diagnosed with all stages of hormone-responsive breast cancer [3,4].

There are unquestionable benefits in the use of this drug for the treatment of women with breast cancer. Its use as a chemopreventive agent in healthy women is not clear-cut [5].

There is no doubt that tamoxifen damages liver DNA, resulting in adduct formation [6]. Tamoxifen has also been shown to bind covalently to the liver DNA [7], proposed tamoxifen DNA adducts include the bridge epoxide [8] and an adduct formed from hydroxyethyl tamoxifen [9]. The increase in cell proliferation in the liver could be due to an estrogenic effect of tamoxifen in the liver; however, there is also the possibility that tamoxifen can cause toxicity in the liver resulting in cell death [10]. It was postulated by others [4-6] that it is working via epigenetic mechanism, the hepaticarcinogenic effect in mice being in same way related to estrogenic/antiestrogenic pitency.

Recent evidence suggested that generation of reactive oxygen species (ROS) and oxidative stress also played a role in the TAM toxicity [11]. Recently, a number of natural antioxidants have been evaluated for their chemoprotective effects in various pathological states [12-15]. Oleuropein extracted from olive leaf is a flavonoid complexes showed very potent radical scavenger. The aim of this study was to investigate the toxicity of TAM and the role of oleuropein in the protection against TAM-induced liver oxidative damage in Balb/c mice.

MATERIALS AND METHODS

Chemicals: All chemicals used in this study were of analytical grade and purchased from Sigma Co./USA.
**Extraction of Oleuropein:** 500 g of healthy olive leaves were collected from Jordan. Oleuropein was extracted and purified according to the methods described before [16,17].

**Experimental Design:** Forty male Balb/c mice (28-34 g body weight) were fed standard chow and maintained at 22-24°C, 12-12 h dark/light periods and water ad libitum. The animals were divided into four groups (10 mice each), the first group was control group (C) without any supplementation. The second group was oleuropein (O), mice of this group were received oral dose of oleuropein (30 mg/kg bw daily for 30 consecutive days).

The vehicle used for oleuropein was carbopol 0.5% as recommended in literatures [18]. The third group was tamoxifen group (TAM), mice were supplemented with 20 mg/kg/day TAM orally, daily for 4 weeks prior to scarification. 4th group was the experimental group (TAMO), mice were supplemented daily orally for 4 weeks with 30 mg/kg of oleuropein as well as 20 mg/kg of TAM then sacrificed.

**Toxicological Studies:** After scarification of all mice, blood was collected, serum isolated and frozen in -80°C for further studies. The livers were immediately removed and perfused with Hanks-buffered saline to remove excess blood. To assay 8-OH-dG the method described before [19] was used. The assessment of the liver toxicity was performed by using serum enzymes levels as a biomarkers; alanine transaminase (ALT) [20], glutamyl-oxaloacetic transaminase (SGOT) [21] and glutamyl-pyruvic transaminase (SGPT) [22]. Activities of antioxidant enzymes, catalase (CAT) and superoxide dismutase (SOD) were determined using the method reported before [23] and Glutathione peroxidase (GSHpx) using the method recommended in literature [24]. For the measurement of lipid peroxidation, the thiobarbituric acid (TBA), method described before [26,27] was used by measuring the amount of malondialdehyde (MDA) present in the liver homogenate.

**Statistical Analysis:** Results are expressed as mean± standard deviation. For comparison between groups, data were analyzed by one-way ANOVA; P < 0.05 was considered statistically significant.

**RESULTS**

The present results revealed non significant alterations in the body weights of mice of the various treated groups. TAM treated mice liver showed a little increase in the liver body mass index ratio due to massive intra-hepatic hemorrhage and pooling of blood in the liver, making the liver appearance darker in colour when compared with the other groups, which were all within the normal values.

Serum values of ALT, SGOT and SGPT were utilized to evaluate liver injury. TAM administration increased serum values of ALT (7-fold), SGOT (5.5-fold) and SGPT (about 10 fold) compared to those in C mice, while pretreatment with oleuropein significantly (P < 0.05) inhibit the rise of these enzymes induced by TAM as shown in Table 1.

Antioxidant enzymes (CAT, SOD and GSHpx) activities were decreased significantly (P< 0.05) in mice treated with TAM only, while these activities were near the normal after pretreatment with oleuropein.

MDA is a product of oxidative damage to lipids and in this study, the concentration of MDA in liver homogenate is considered as a biomarker of TAM toxicity. Results in Table 1 shows that liver homogenate of mice exposed to TAM contained higher levels of MDA (about 24.5 fold increase) when compared with C values. These levels decreased significantly (P = 0.05) in liver homogenates of mice supplemented with oleuropein.

There was a significant increase (P < 0.05) in the level of 8-OH-dG in TAM treated mice. Table 1 shows that oleuropein inhibited TAM-induced formation of 8-OH-dG.

### Table 1: Summary of the results of the effects of oleuropein on the toxicity of TAM

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>O</th>
<th>TAM</th>
<th>TAMO</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/L)</td>
<td>60.77±0.443</td>
<td>56.66±0.661</td>
<td>428.22±3.112</td>
<td>97.56±1.370</td>
</tr>
<tr>
<td>LPO (MDA) µM</td>
<td>0.072±0.0.003</td>
<td>0.05±0.003</td>
<td>4.224±0.007</td>
<td>1.008±0.004</td>
</tr>
<tr>
<td>8-OH-dG/10 2dG</td>
<td>4±0.014</td>
<td>3±0.13</td>
<td>49±0.334</td>
<td>11±0.172</td>
</tr>
<tr>
<td>SGOT (U/L)</td>
<td>12.06±1.011</td>
<td>10.08±0.98</td>
<td>89.66±3.41</td>
<td>27.86±0.88</td>
</tr>
<tr>
<td>SGPT (U/L)</td>
<td>11.15±1.032</td>
<td>10.64±0.76</td>
<td>93.22±3.22</td>
<td>23.91±0.67</td>
</tr>
<tr>
<td>SOD (U/mg Protein)</td>
<td>68±2.970</td>
<td>71±3.033</td>
<td>18±1.002</td>
<td>41±2.012</td>
</tr>
<tr>
<td>CAT (U/min/ mg protein)</td>
<td>70±2.170</td>
<td>72±3.122</td>
<td>26±1.776</td>
<td>44±2.654</td>
</tr>
<tr>
<td>GSHpx (µmol/ Min/mg protein)</td>
<td>0.98±0.007</td>
<td>1.21±0.066</td>
<td>0.22±0.002</td>
<td>0.41±0.008</td>
</tr>
</tbody>
</table>
DISCUSSION

TAM or triphenylene compounds undergo metabolic activation reactions, such as 4-hydroxylation, 3-hydroxylation, \(\alpha\)-hydroxylation, \(N\)-demethylation, etc., in animal tissues [4-5], which raised the production of reactive oxygen species (ROS) [16]. Great attention now focused on the clinical usage of this drug because of its several diverse effects, mainly idiosyncratic hepatotoxicity [28]. The level of serum ALT, SGOT and SGPT activities reflect damage to hepatocytes and indicates the increased cellular permeability [26, 27] and are considered to be highly sensitive and fairly specific preclinical and clinical biomarkers of hepatotoxicity [29]. Our results show that oleuropein extracted from olive leaf provides a hepatoprotective effect by reversing the changes produced by TAM.

Our results show that TAM increase lipid peroxidation with high level of malondialdehyde (MDA) as a main product of lipid break down, but oleuropein has been proved by others [30-34] to be a powerful antioxidants, reduce the formation of MDA likely via its ability to scavenge free radicals. Peroxidation reaction of tamoxifen could yield metabolites reactive with DNA [35]. The generation of 8-OH-dG resulted from oxidative modification of DNA [36]. A strong correlation between higher amounts of 8-OH-dG and greater degree of oxidative stress, DNA strand break, or DNA damage has been reported [37]. As expected, the antioxidant oleuropein reduced the level of 8-OH-dG.

\(\text{H}_2\text{O}_2\) is a normal metabolite in the cell; its steady state concentrations range from \(10^{-14}\) to \(10^{-9}\) [38]. Although \(\text{H}_2\text{O}_2\) may not cause DNA damage under physiological conditions, it participates in the metal ion-catalyzed Haber-Weiss reaction and generates the highly reactive hydroxyl radical, which can target DNA resulting in oxidative DNA damage [39].

In this study, the reduction or prevention of oxidative hepatic injury caused by TAM metabolites has been proposed to be achieved through increasing host antioxidant defense system by supplementation with oleuropein [40]. The body has defense mechanisms of antioxidants to limit damage caused by different species of ROS. These protective mechanisms consist of enzymatic species, SOD, CAT and GSHpx and our results showed that the activities were affected by TAM, but supplementation of oleuropein attenuated their activities to the about normal. SOD is considered as the first line of defense to scavenge superoxide anions generated in cytosolic and mitochondrial compartments of the cell [41]. CAT and GSHpx causes direct breakdown of hydrogen peroxide to oxygen and water [42]. Thus, our results indicate that TAM treatment disrupted the antioxidant defense mechanism of the liver cells causing oxidative damage.

Oleuropein is a heterosidic ester which yields in hydrolysis elenolic acid glucoside and hydroxytyrosol [43], both are known to be efficient antioxidants in vivo, as well as in vitro [44]. This study showed that oleuropein presents significant anti-tamoxifen toxicity which is essentially due to its antioxidant potential. In fact, oleuropein and its derivative hydroxytyrosol has been shown to be scavengers of superoxide anions and inhibitors of the respiratory burst of neutrophils and hydrochlorous acid-derived radicals [45], both compounds also scavenged hydroxyl radicals [46].

This study demonstrated a potential and beneficial effect of oleuropein in attenuating oxidative stress and enhancing the antioxidant defenses in experimental mice.

CONCLUSION

In conclusion, we demonstrate that polyphenols recovered from olive leaf extract, oleuropein, exhibited a pronounced a potent antioxidant effect, reduced the lipid peroxidation process and enhanced the antioxidant defense system in an experimental model. These effects highlighted once again the olive tree byproduct as a source of antioxidants able to reduce the frequency of oxidative stress-related toxicity of some medications.

REFERENCES


