The effect of date palm fruit (*Phoenix dactylifera* L.) on the hormone 17-β-estradiol in 7,12-dimethylbenz(α)anthracene-induced mammary cancer in rats

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Abstract. The effect of date palm fruit feeding (to female Sprague-Dawley rats with 7,12-dimethylbenz(α)anthracene–induced mammary cancer) on the hormone 17-β-estradiol concentration was compared to that of feeding soybean seeds and injection with two drugs (one preventive against and the other curative for 7,12-dimethylbenz(α)anthracene-induced mammary cancer i.e. tamoxifen and the hormone 17-β-estradiol). The date palm fruit raised the hormone concentration significantly (*p*<0.05). The hormone concentration was positively correlated with the palpable tumor latency, and negatively correlated with tumor incidence rate and multiplicity. It is concluded that the preventive effect of the date palm fruit against 7,12-dimethylbenz(α)anthracene-induced mammary cancer is related to the effect on the hormone 17-β-estradiol.

Keywords: Date palm fruit, 17-β-estradiol, 7,12-dimethylbenz(α)anthracene-induced mammary cancer, sprague dawley rats

1. Introduction

In 2008, the World Health Organization (WHO) [1] reported 7.6 million deaths from cancer (about 13% of total worldwide deaths). Mammary cancer caused 460000 deaths in 2008 [1]. In 2009, breast cancer was ranked as the number one among the ten most common cancers in Jordanian females [2]. Mammary cancer is a neoplastic change in the epithelial cells of the mammary tissue [3]. Mammary cancer may be invasive or non-invasive [3]. According to the cell proliferation requirements and neoplastic cell response to hormonal therapy, mammary cancer can be classified into 2 classes: the hormone-dependent mammary cancer (HDMC) and the hormone–independent mammary cancer (HIMC) [4]. Most mammary cancers are HDMC where the development of this type of cancers depends mainly on the cell hormonal environment [5].

Estrogen–dependent mammary cancer is initiated when chemical carcinogen molecules combine with the nuclear estrogen receptors [6]. The carcinogen-receptor complex interacts with the cell DNA at the estrogen response elements. This binding induces irreversible alteration in the cell genetic makeup leading to the activation of proto-
oncogenes and suppression of tumor suppressor genes. The result is an initiated cell. However, DNA changes may occur due to the action of other physical or biological carcinogens [7].

Mammary tissue development is a continuous process that starts from the intrauterine life, continues during puberty, maximizes during pregnancy, and regresses during menopause [5]. It is controlled mainly by the ovaries [5]. Ovarian steroids, thyroid hormones, pituitary gonadotropins, and adrenal hormones are the major contributors in the growth and the development of mammary tissue [5].

The hormone 17-β-estradiol stimulates the growth, elongation, and differentiation of mammary ductal system and regulates the mammary alveolar system [8]. Furthermore, estradiol stimulates the synthesis and secretion of prolactin (PRL) which induces the transcription of estrogen receptors [9].

2. Materials and methods

The effect of date palm fruit on the hormone concentration was compared to that of feeding soybean seeds and injection with two drugs (one preventive against and the other curative for 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary cancer i.e. tamoxifen and the hormone 17-β-estradiol. Date palm fruit at two edible maturity stages were used in the experiment: Rutab (incompletely mature) and Tamr (mature fruit). Test meals, animal experimentation, and the administration of drugs and carcinogen details can be reviewed in our previous work [10].

Four weeks after ending estradiol injection, all animals were fasted for 16 hours and killed by chloroform anesthesia. Five milliliters blood was drawn from the right heart ventricle. Blood samples were centrifuged (Hermle, Z 200A, Germany) at 3000 rpm/15 minutes to separate serum. Serum was removed and kept in conical tubes in the freezer at −20°C until used for the determination of the hormone 17-β-estradiol (E2) concentration.

Analysis of serum concentration of 17-β-estradiol was carried out by microparticle enzyme immunoassay technique (ARCHITECH i 1000 sr®, New Zealand) in Al–Khalidi Medical Laboratories.

2.1. Statistical analysis

The statistical analysis was carried out using the Statistical Analysis System (SAS). The hormone data were analyzed by one way analysis of variance (ANOVA) test. Comparison of different treatment groups was performed to detect the statistical significance. Fisher’s (F-test) protected least significant difference (LSD) was used for mean separation and to detect significant differences between the treatment means. Differences were considered significant at $p < 0.05$. Pearson’s correlation coefficients were calculated between the data of serum concentration of E2, hepatic glutathione-S-transferase (GST) activity, and the tumor-related data (data can be reviewed in our previous work [10, 11]. Correlations were considered significant at $p < 0.05$.

3. Results

Table 1 shows the serum concentration of the hormone 17-β-estradiol (pg/ml) of the experimental groups. The highest ($P < 0.05$) concentration was expressed in the AIN-93G+ “Rutab”, AIN-93G+ “Tamr”, and AIN-93G+ Soybean groups, followed by the positive/DMBA control, the AIN-93G+ Sesame oil groups, the AIN-93G + E2, AIN-93G + TAM, and the negative/DMBA control groups. Nonetheless, there were no significant differences ($P > 0.05$) among the AIN-93G+ “Tamr”, AIN-93G+ “Rutab”, and AIN-93G+ Soybean groups. Additionally, there were no significant ($p > 0.05$) differences among the AIN-93G + Soybean, positive/DMBA control, and AIN-93G + Sesame oil. Also, there were no significant ($p < 0.05$) differences among the positive/DMBA control, AIN-93G + Sesame oil, AIN-93G + E2, AIN-93G + TAM, and the negative/DMBA control groups.

3.1. Correlations

Table 2 shows the correlation coefficients between hepatic GST activity, serum 17-β-estradiol concentration, and the tumor-related data. Three significant ($p < 0.05$) correlations were found. These correlations are related to the concentration of the serum 17-β-estradiol as follows:
The concentration of serum hormone E2 for the experimental groups¹

<table>
<thead>
<tr>
<th>Group (cx)</th>
<th>[E2] (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative/DMBA (10)</td>
<td>32.17 ± 6.79a</td>
</tr>
<tr>
<td>AIN+3G + Sesame oil (10)</td>
<td>38.50 ± 5.87bc</td>
</tr>
<tr>
<td>AIN+3G + TAM (13)</td>
<td>35.40 ± 5.25b</td>
</tr>
<tr>
<td>Positive/DMBA control (10)</td>
<td>43.63 ± 5.87bc</td>
</tr>
<tr>
<td>AIN+3G + E2 (12)</td>
<td>36.00 ± 5.25b</td>
</tr>
<tr>
<td>AIN+3G + Soyabean (10)</td>
<td>52.50 ± 5.87ab</td>
</tr>
<tr>
<td>AIN+3G + &quot;Rutab&quot; (8)</td>
<td>66.50 ± 6.78b</td>
</tr>
<tr>
<td>AIN+3G + &quot;Tamr&quot; (10)</td>
<td>53.38 ± 5.87ab</td>
</tr>
</tbody>
</table>

¹Values are expressed as mean ± SEM; values with different letters within a column differ significantly at (p<0.05) using Fischer’s protected LSD test. ²Abbreviations are as follows: GST: glutathione-S-transferase, IMDA: invasive mammary ductal adenocarcinoma, PIMDAC: palpable mammary ductal adenocarcinoma, NMIMDAC: nonpalpable mammary ductal adenocarcinoma, Rutab (incompletely mature date palm fruit) and Tamr (mature date palm fruit).

Table 1

The effect of date palm fruit (Phoenix dactylifera L.) on serum hormone E2 concentration, hepatic GST activity and tumor-related data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hepatic GST¹ activity</th>
<th>IMDA² incidence</th>
<th>PIMDAC³ incidence</th>
<th>NPIMDAC⁴ incidence</th>
<th>Concentration of serum hormone E2 (17-β-estradiol)</th>
<th>PIMDAC³ multiplicity</th>
<th>PIMDAC³ latency</th>
<th>PIMDAC³ Diameter</th>
<th>PIMDAC³ Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-β-estradiol</td>
<td>- r = -0.216</td>
<td>r = 0.116</td>
<td>r = -0.334</td>
<td>r = 0.205</td>
<td>r = 0.197</td>
<td>r = 0.090</td>
<td>r = -0.221</td>
<td>r = -0.385</td>
<td></td>
</tr>
<tr>
<td>activity</td>
<td>p = 0.641</td>
<td>p = 0.807</td>
<td>p = 0.456</td>
<td>p = 0.659</td>
<td>p = 0.701</td>
<td>p = 0.880</td>
<td>p = 0.674</td>
<td>p = 0.451</td>
<td></td>
</tr>
<tr>
<td>IMDA²</td>
<td>r = -0.216</td>
<td>- r = 0.207</td>
<td>r = 0.494</td>
<td>r = -0.740</td>
<td>r = 0.863</td>
<td>r = -0.636</td>
<td>r = 0.204</td>
<td>r = 0.084</td>
<td></td>
</tr>
<tr>
<td>incidence</td>
<td>p = 0.641</td>
<td>p = 0.657</td>
<td>p = 0.260</td>
<td>p = 0.039</td>
<td>p = 0.012</td>
<td>p = 0.175</td>
<td>p = 0.698</td>
<td>p = 0.875</td>
<td></td>
</tr>
<tr>
<td>PIMDAC³</td>
<td>r = 0.116</td>
<td>r = 0.207</td>
<td>- r = 0.270</td>
<td>r = -0.396</td>
<td>r = 0.178</td>
<td>r = -0.434</td>
<td>r = -0.271</td>
<td>r = 0.111</td>
<td></td>
</tr>
<tr>
<td>incidence</td>
<td>p = 0.807</td>
<td>p = 0.657</td>
<td>p = 0.558</td>
<td>p = 0.380</td>
<td>p = 0.702</td>
<td>p = 0.389</td>
<td>p = 0.603</td>
<td>p = 0.834</td>
<td></td>
</tr>
<tr>
<td>NPIMDAC⁴</td>
<td>r = -0.334</td>
<td>r = 0.494</td>
<td>r = -0.270</td>
<td>- r = 0.351</td>
<td>r = 0.234</td>
<td>r = -0.455</td>
<td>r = 0.709</td>
<td>r = 0.709</td>
<td></td>
</tr>
<tr>
<td>incidence</td>
<td>p = 0.465</td>
<td>p = 0.260</td>
<td>p = 0.558</td>
<td>p = 0.440</td>
<td>p = 0.614</td>
<td>p = 0.364</td>
<td>p = 0.115</td>
<td>p = 0.115</td>
<td></td>
</tr>
<tr>
<td>Concentration of serum hormone E2 (17-β-estradiol)</td>
<td>r = 0.205</td>
<td>r = -0.780</td>
<td>r = -0.396</td>
<td>r = -0.351</td>
<td>- r = -0.797</td>
<td>r = 0.942</td>
<td>r = -0.342</td>
<td>r = -0.404</td>
<td></td>
</tr>
<tr>
<td>of serum</td>
<td>p = 0.659</td>
<td>p = 0.039</td>
<td>p = 0.380</td>
<td>p = 0.440</td>
<td>p = 0.032</td>
<td>p = 0.088</td>
<td>p = 0.644</td>
<td>p = 0.427</td>
<td></td>
</tr>
<tr>
<td>PIMDAC³</td>
<td>r = 0.179</td>
<td>r = 0.863</td>
<td>r = 0.178</td>
<td>r = 0.234</td>
<td>r = -0.797</td>
<td>- r = -0.690</td>
<td>r = 0.115</td>
<td>r = 0.358</td>
<td></td>
</tr>
<tr>
<td>17-β-estradiol</td>
<td>p = 0.701</td>
<td>p = 0.012</td>
<td>p = 0.702</td>
<td>p = 0.634</td>
<td>p = 0.032</td>
<td>p = 0.129</td>
<td>p = 0.828</td>
<td>p = 0.486</td>
<td></td>
</tr>
<tr>
<td>Tumor latency</td>
<td>r = 0.080</td>
<td>r = -0.636</td>
<td>r = -0.434</td>
<td>r = -0.455</td>
<td>r = 0.942</td>
<td>r = -0.690</td>
<td>r = 0.088</td>
<td>r = 0.628</td>
<td></td>
</tr>
<tr>
<td>Diameter</td>
<td>p = 0.380</td>
<td>p = 0.175</td>
<td>p = 0.389</td>
<td>p = 0.364</td>
<td>p = 0.008</td>
<td>p = 0.129</td>
<td>p = 0.068</td>
<td>p = 0.128</td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>r = -0.231</td>
<td>r = 0.204</td>
<td>r = -0.271</td>
<td>r = 0.709</td>
<td>r = -0.242</td>
<td>r = 0.115</td>
<td>r = 0.780</td>
<td>- r = 0.709</td>
<td></td>
</tr>
<tr>
<td>PIMDAC³</td>
<td>r = -0.385</td>
<td>r = 0.084</td>
<td>r = -0.111</td>
<td>r = 0.709</td>
<td>r = 0.404</td>
<td>r = 0.358</td>
<td>r = 0.692</td>
<td>r = 0.999</td>
<td>-</td>
</tr>
</tbody>
</table>

¹Abbreviations as follows: GST: glutathione-S-transferase, IMDA: invasive mammary ductal adenocarcinoma, PIMDAC: palpable mammary ductal adenocarcinoma, NPIMDAC: nonpalpable mammary ductal adenocarcinoma, Rutab (incompletely mature date palm fruit) and Tamr (mature date palm fruit). ²Correlations were considered significant at p<0.05.
1. A negative correlation between the serum 17-β-estradiol concentration and IMDAC incidence rate ($r = -0.780$, $p = 0.039$).
2. A negative correlation between the serum 17-β-estradiol concentration and PIMDAC multiplicity ($r = -0.797$, $p = 0.032$).
3. A positive correlation between the serum 17-β-estradiol concentration and PIMDAC latency ($r = 0.942$, $p = 0.008$).

4. Discussion

4.1. The concentration of serum 17-β-estradiol

There is no reference data for the normal concentration of serum E$_2$. Additionally, the concentration depends on the hormonal status of the animal (stages of estrous cycle) [12, 13], and other external factors to which the animal is exposed such as diet [14, 15] and drug treatment(s) [12]. Similar studies in which E$_2$ concentration was measured showed that the concentration of the hormone in the serum of 15-wk old female SD rats fed AIN-93 G meals was 30.4 pg/ml [16]. This figure is close to that obtained in the negative/DMBA control group in this experiment (32.17 pg/ml).

All the rats which were administered DMBA tended to express higher serum concentration of the hormone. This result is consistent with that of [17] who found a significant elevated concentration of serum 17-β-estradiol (51.1 compared to 31 pg/ml in the negative/DMBA control group). The researchers attributed this increase to the fact that mammary carcinogenesis is related to hormonal disturbances [17]. Accordingly, DMBA seems to induce hormonal disturbances.

The administration of 17-β-estradiol increases the serum concentration of the hormone [13]. In the current study, the AIN-93G+E$_2$ group exhibited relatively low serum concentration of the hormone compared to the other rat groups. This might be explained with reference to the 4-week holding period. In this period, it is proposed that the hormone exerted its therapeutic effect and might have been reestablished to its basal level.

The insignificant difference between serum concentrations of 17-β-estradiol between the negative/DMBA and AIN-93G+TAM groups is in agreement with the result of [18]. In the aforementioned research, the administration of 50 μg TAM/day for 30 days to DMBA-administered female Wistar rats did not affect serum E$_2$.

Soybean contains phytoestrogens in amounts that are much greater than those in the date palm fruit. The presence of phytoestrogens in soybean seeds and the date palm fruit might be responsible for the high concentration of the hormone in these groups. Phytoestrogens compete with the hormone E$_2$ for estrogen receptors (ERs) and sex hormone binding globulin reducing the hormone availability in neoplastic cells, releasing it out to the circulating system [19]. Additionally, the consumption of isoflavones led to the reduction in the catabolism of estradiol by 24% [19] and compensated for E$_2$ in ovariectomized rats in a dose dependent manner [15].

4.2. Correlations

In estrogen receptor positive breast cancer, the estrogen level is the most important factor that determines the preventive effect of food constituents [20]. The elevation of serum E$_2$ is preventive against mammary cancer in human females [5, 19] and DMBA-induced cancer model [19, 21]. The results of the current research and those of our previous work [10] are in agreement with this fact. Interestingly, a significant negative correlation ($r = -0.780$, $p < 0.05$) was found between the serum concentration of the hormone and the incidence rate of invasive mammary ductal adenocarcinoma (IMDAC) in the rats. Additionally, a significant positive correlation ($r = 0.924$, $p < 0.01$) was found between the serum concentration of the hormone and the palpable mammary ductal adenocarcinoma (PIMDAC) latency. Furthermore, a significant negative correlation ($r = -0.797$, $p < 0.05$) has been found between the serum concentration of the hormone and the PIMDAC multiplicity in the rats.

5. Conclusions

According to the results obtained in the current research, it can be concluded that the administration of DMBA caused disturbance of serum 17-β-estradiol levels. Additionally, feeding female weanling SD rats with meals
containing the date palm fruit raised the serum concentration of the hormone. The serum concentration of the hormone was involved in the positive effects of the date palm fruit against DMBA-induced mammary cancer in the rats ($p < 0.05$).

6. Recommendations

Based on the results of the current research, it is recommended to increase the intake of this traditional food. Additionally, further studies taking into consideration different biomarkers are recommended to investigate the effect of the incorporation of different ratios of the date palm fruit and fruit extracts at different maturity stages on other hormones.

7. Conflict of interest

The authors declare that there is no conflict of interest among them in this manuscript.

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