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

Hiba F. Al-Sayyed[a,*], Hamed R. Takruri[a], Maha S. Shomaf[b] and AbdulRahman Al-Saleh[c]

[a]*Department of Nutrition and Food Technology, Faculty of Agriculture, The University of Jordan, Amman, Jordan*
[b]*Department of Pathology and Microbiology and Forensic Medicine, Faculty of Medicine, The University of Jordan, Amman, Jordan*
[c]*Division of Food Microbiology, Department of Food Laboratories, Directory of Laboratories, Jordan Food and Drug Administration, Amman, Jordan*

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-

*Corresponding author: Hiba F. Al-Sayyed, Department of Nutrition and Food Technology, Faculty of Agriculture, The University of Jordan, Amman, Jordan. E-mail: hibaalsayyed@yahoo.com.

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(α)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 (E_2) 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 E_2, 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 ± E_2, 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 + E_2, 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:

Table 1

The concentration of serum hormone E_2 for the experimental groups[1]

Group (n)[2]	$[E_2]$ (pg/ml)[3]
Negative/DMBA (10)	32.17 ± 6.78^c
AIN-93G + Sesame oil (10)	38.50 ± 5.87^{bc}
AIN-93G + TAM (13)	35.40 ± 5.25^c
Positive/DMBA control (10)	43.63 ± 5.87^{bc}
AIN-93G + E_2 (12)	36.00 ± 5.25^c
AIN-93G + Soyabean (10)	52.50 ± 5.87^{ab}
AIN-93G+ "Rutab" (8)	66.50 ± 6.78^a
AIN-93G+ "Tamr" (10)	53.38 ± 5.87^{ab}

[1]Values are expressed as mean $\pm$ SEM; values with different letters within a column differ significantly at ($p < 0.05$) using Fischer"s protected LSD test. [2]Abbreviations are as follows: AIN-93G: diets recommended by the American Institute of Nutrition-1993 for growing rats; n: number of rats used within each experimental group; TAM: tamoxifen, E_2: 17-β-estradiol, DMBA: 7,12-dimethylbenza(α)anthracene; the concentration of the hormone 17-β-estradiol is expressed as pigogram per milliliter of serum), Rutab (incompletely mature date palm fruit) and Tamr (mature date palm fruit).

Table 2

Correlation coefficients between the concentration of serum 17-β-estradiol, hepatic GST activity, and tumor-related data[1,2]

Parameter	Hepatic GST[1] activity	IMDAC[1] incidence	PIMDAC[1] incidence	NPIMDAC[1] incidence	Concentration of serum 17-β-estradiol	PIMDAC[1] multiplicity	Tumor latency	PIMDAC[1] Diameter	PIMDAC[1] Weight
Hepatic GST[1] activity	–	$r = -0.216$ $p = 0.641$	$r = 0.116$ $p = 0.807$	$r = -0.334$ $p = 0.456$	$r = 0.205$ $p = 0.659$	$r = 0.197$ $p = 0.701$	$r = 0.080$ $p = 0.880$	$r = -0.221$ $p = 0.674$	$r = -0.385$ $p = 0.451$
IMDAC[1] incidence	$r = -0.216$ $p = 0.641$	–	$r = 0.207$ $p = 0.657$	$r = 0.494$ $p = 0.260$	$r = -0.780$ $p = 0.039$	$r = 0.863$ $p = 0.012$	$r = -0.636$ $p = 0.175$	$r = 0.204$ $p = 0.698$	$r = 0.084$ $p = 0.875$
PIMDAC[1] vincidence	$r = 0.116$ $p = 0.807$	$r = 0.207$ $p = 0.657$	–	$r = -0.270$ $p = 0.558$	$r = -0.396$ $p = 0.380$	$r = 0.178$ $p = 0.702$	$r = -0.434$ $p = 0.389$	$r = -0.271$ $p = 0.603$	$r = -0.111$ $p = 0.834$
NPIMDAC[1] incidence	$r = -0.334$ $p = 0.465$	$r = 0.494$ $p = 0.260$	$r = -0.270$ $p = 0.558$	–	$r = -0.351$ $p = 0.440$	$r = 0.234$ $p = 0.614$	$r = -0.455$ $p = 0.364$	$r = 0.709$ $p = 0.115$	$r = 0.709$ $p = 0.115$
Concentration of serum 17-β-estradiol	$r = 0.205$ $p = 0.659$	$r = -0.780$ $p = 0.039$	$r = -0.396$ $p = 0.380$	$r = -0.351$ $p = 0.440$	–	$r = -0.797$ $p = 0.032$	$r = 0.942$ $p = 0.008$	$r = -0.242$ $p = 0.644$	$r = -0.404$ $p = 0.427$
PIMDAC[1] multiplicity	$r = 0.179$ $p = 0.701$	$r = 0.863$ $p = 0.012$	$r = 0.178$ $p = 0.702$	$r = 0.234$ $p = 0.614$	$r = -0.797$ $p = 0.032$	–	$r = -0.690$ $p = 0.129$	$r = 0.115$ $p = 0.828$	$r = 0.358$ $p = 0.486$
Tumor latency	$r = 0.080$ $p = 0.880$	$r = -0.636$ $p = 0.175$	$r = -0.434$ $p = 0.389$	$r = -0.455$ $p = 0.364$	$r = 0.942$ $p = 0.008$	$r = -0.690$ $p = 0.129$	–	$r = 0.780$ $p = 0.068$	$r = -0.692$ $p = 0.128$
PIMDAC[1] Diameter	$r = -0.221$ $p = 0.674$	$r = 0.204$ $p = 0.698$	$r = -0.271$ $p = 0.603$	$r = 0.709$ $p = 0.115$	$r = -0.242$ $p = 0.644$	$r = 0.115$ $p = 0.828$	$r = 0.780$ $p = 0.068$	–	$r = 0.899$ $p = 0.015$
PIMDAC[1] Weight	$r = -0.385$ $p = 0.451$	$r = 0.084$ $p = 0.875$	$r = -0.111$ $p = 0.834$	$r = 0.709$ $p = 0.115$	$r = -0.404$ $p = 0.427$	$r = 0.358$ $p = 0.486$	$r = -0.692$ $p = 0.128$	$r = 0.899$ $p = 0.015$	–

[1]Abbreviations are as follows: GST: glutathione-S-transferase, IMDA: invasive mammary ductal adenocarcinoma, PIMDAC: palpable mammary ductal adenocarcinoma, NPMDAC: nonpalpable mammary ductal adenocarcinoma, Rutab (incompletely mature date palm fruit) and Tamr (mature date palm fruit). [2]Correlations 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 (stage 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 ovarectomized 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.

Acknowledgments

References

[1] WHO (World Health Organization) Cancer (Fact sheet No. 297). Date of access: December, 2011; Media Center, WHO, Rome.

[2] MoH (Ministry of Health), Jordan Cancer Registry. Non Communicable Diseases Directorate, MoH, Jordan, 2009.

[3] Gallager H. Classification of breast neoplasms. In Gallager H, Leis Herny, Syderman, Reuven, Urban J (eds), The breast. The C.V. Mosby Company, Saint Louis, 1978, pp. 49-72.

[4] Hieger I. Carcinogenesis. Academic Press, London and New York, 1961.

[5] El-Aaser A, E'l–Merzabani, M, Abudedair F. Etiology of human breast cancer. In Abdel Meguid L, Omar S (eds) Breast cancer: A comprehensive approach. Cairo Medical Syndicate, Egypt, 1982; pp. 25-46.

[6] Heldring N, Pike A, Andersson S, Matthews J, Cheng G, Hartman J, Tujague M, Storm A, Treuter, E, Warner M, Gustafsson J-A. Estrogen receptors: How do they signal and what are their targets. Physiol Rev. 2007;87:905-31.

[7] Poirier L. Stages in carcinogenesis: Alteration by diet. Am J Clin Nutr. 1987;45:185-91.

[8] Russo J, Hu Y-F, Yang X, Russo I. Developmental, cellular, and molecular basis of human breast cancer. J Nal Cancer Inst Monogr. 2000;27:17-37.

[9] Brisken C, O'Malley B. Hormone action in the mammary gland. Cold Spri Harbor Perspec in Biol. 2010;2:1-15. doi:10.1101/cshperspect.a003178

[10] Al-Sayyed H, Takruri H, Shomaf M. The effect of date palm fruit (*Phoenix dactylifera* L.) on the enzyme glutathione-S-transferase activity in Sprague-Dawley rats. PJN. 2013;12:410-5.

[11] Al-Sayyed H, Takruri H, Shomaf M. The effect of date palm fruit (*Phoenix dactylifera* L.) on 7,12-dimethylbenz(α)anthracene (DMBA)–induced mammary cancer in rats. ROAVS. 2014;4:11-8.

[12] Mitak M, Gojmerac T, Mandic B, Cvetnić Z. Changes in serum concentration of 17-β-estradiol in female rats during estrous cycle after treatment with atrazine and zearalenone. Veter Medi of Czech. 2001;5:145-8.

[13] Rajkumar L, Balasubramanian K, Arunakaran J, Govindarajulu P, Srinivasan N. Influence of estradiol on mammary tumor collagen solubility in DMBA-induced rat mammary tumors. Cell Biol Intern. 2006;30:164-8.

[14] Wayne S, Neuhouser M, Koprowski C, Ulrich C, Wiggins C, Gilliland F, Baumgartner K, Baumgartner R, McTiernan A, Bernstein L, Ballard-Barbash R. Breast cancer survivors who use estrogenic botanical supplements have lower serum estrogen levels than non-users. Br Cancer Res and Treat. 2009;117:111-9. doi: 10.1007/s10549-008-0224-x

[15] Taherianfard M, Tadjali M, Astaneh M. Can soy change serum estradiol concentration in neonate ovarectomized rat? Am Med J. 2012;3:47-9. doi: 10.3844/amjsp.2012.47.49

[16] Grill C, Cohick W, Sherman A. Postpubertal development of the rat mammary gland is preserved during iron deficiency. J Nutr. 2001;131:1444-8.

[17] Abd El-Aziz M, Hassan H, Mohamed M, Meki A. The biochemical and morphological alterations following administration of melatonin, retinoic acid and *Nigella sativa* in mammary carcinoma: An animal model. Inter J Exp Pathol. 2005;86:383-96.

[18] Hall D. Nutritional influences on estrogen metabolism. Appl Nutr Sci Reports. 2001;1:1-8.

[19] Yager J, Davidson N. Mechanisms of disease: Estrogen carcinogenesis in breast cancer. N Engl J of Med. 2006;354:270-82.

[20] Olsen A, Tjønneland A, Thomsen B, Loft S, Stripp C, Overvad K, Møller S, Olsen J. Fruits and vegetables intake differentially affects estrogen receptor negative and positive breast cancer incidence rates. J Nutri. 2003;133:2342-7.

[21] Hawkins R, Freedman B, Killen E. Oestradiol-17β and prolactin levels in rat peripheral plasma. Brit J Cancer. 1975;32:179-85.