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Biochemical alterations associated with experimentally induced breast cancer in rats

Zeweil[a], M.M.,Taha[b], N.M., Sadek [a,*], K.M., El- Sayed[c],Y.S.,Nasr[d], S.M.

[a]Department of Biochemistry, Faculty of Veterinary Medicine, Damanhour University, Egypt
[b]Department of Biochemistry, Faculty of Veterinary Medicine, Alexandria University, Egypt
[c]Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Damanhour University, Egypt
[d]Department of Animal Husbandry and Wealth Development, Faculty of Veterinary Medicine, Damanhour University, Egypt

A B S T R A C T

This study was conducted to demonstrate the preventive graviola effect on breast cancer induced by 7,12-dimethylbenz [a] anthracene (DMBA) in fifty female rats distributed into four groups. Group I: Control group injected orally by physiological saline, group II: DMBA induced-breast cancer, injected orally a single dose of DMBA (50mg/kg) diluted in sesame oil (1 ml), group III: Graviola 200 mg/kg two times per week given orally by gavage from the first day of the experiment till the end plus a single dose of DMBA (50mg/kg) diluted in sesame oil (1mL) given orally at age of 57 days and group IV: rats treated with single dose of DMBA (50mg/kg) diluted in sesame oil (1mL) given orally plus graviola 200 mg/kg two times weekly both at age of 57 days till the end. After 30w the animals were anaesthetized to collect blood samples to determine the hepatic and renal protection of graviola. Treatment with graviola significantly ($p< 0.05$) reduced ALT activity and creatinine level. We can conclude that graviola mitigated hepatic and kidney functions.

Keywords: Breast cancer; Graviola; Liver; Kidney, Histopathology, Body weight

1. Introduction

Incidence rates of breast cancer in females remain highest in more developed regions, but mortality is relatively much higher in less developed countries due to a lack of early detection and access to treatment facilities (Ferlay et al., 2015).

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants that are carcinogenic and immune-suppressive. Both toxicological and epidemiological data have linked exposure to PAHs with various forms of cancer (lung, skin, breast, esophageal, and bladder), cardiovascular diseases, asthma/immunological effects, neurological effects, reproductive and developmental effects. 7,12-dimethylbenz(a) anthracene (DMBA) is frequently used as model of PAHs because of its carcinogenic activities (Dean et al., 1986)

Breast tissues of several rat strains, mainly Sprague-Dawley and Wistar-Furth, are susceptible to transformation induced by the two most common chemical carcinogens, DMBA and N-methylnitrosurea (Dias et al., 2000)

Radiation, chemotherapy and surgery as treatment methods all have side effects. In locally advanced breast cancer, 50% of the cases are observed with resistance to radiotherapy due to the hypoxic tumor microenvironment (Bendinelli et al., 2009).

Many synthetic drugs cause severe side effects that are not acceptable except as treatments of last resort for terminal diseases such as cancer (Valko et al., 2007).

* Corresponding author:
E-mail address: ksaadek@yahoo.com, kadry.sadek@vetmed.dmu.edu.eg
Department of Biochemistry, Faculty of Veterinary Medicine, Damanhour University, Egypt

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Phytochemicals are among the most promising chemo-preventive treatment options for the management of breast cancer and the metabolites discovered in medicinal plants may avoid the side effect of synthetic drugs. Improved adjuvant treatment in early breast cancer has resulted in better prognosis (Moghadamtousi et al., 2015)

It is clear that under such circumstances there is an urgent need for new and effective drugs. On the other hand, such drugs should be used with caution as they may be associated with severe deterioration of the quality of life of the patients (Stenvang et al., 2013).

Annona muricata "Graviola" exhibit a broad range of biological properties, such as cytotoxic, immunosuppressive, pesticidal, antiparasitic and antimicrobial activities, and their potential to inhibit cells that are multiple drug-resistant has attracted increasing interest (Bermejo et al., 2005).

2. Material and methods

2.1. Animals

Fifty female Wistar rats (Rattus norvegicus) weighing approximately 80-90 g and aging 5 weeks were purchased from the College of Science, Cairo, Egypt. The animals were housed in standard stainless-steel cages and acclimatized for 10 days at 21 ± 2 ^{o} C with alternating 12 hours light/dark cycle and were fed a standard diet and allowed access to water ad libitum. The study protocol was approved by the local authorities and all animals were receiving care in compliance with the National Institutes of Health criteria for care of laboratory animals.

2.2. *Animal grouping*

Group I (control): physiological saline P.O. Group II (DMBA): At the age of 57 days, gastro-gavaged a single dose of DMBA (50 mg/kg) diluted in sesame oil (1 ml). Group III (DMBA+G1): At the age of 37 days-old till the end of the experiment, graviola 200 mg/kg two times per week p.o. plus a single dose of DMBA at the age of 57 days-old. Group IV (DMBA+G2): At the age of 57 days-old till the end of the experiment, graviola 200 mg/kg two times per week p.o. plus a single dose of DMBA at the age of 57 days-old.

2.3. Chemicals

DMBA was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Graviola capsules were purchased from Inkanatural (Lima – Peru). Serum ALT and creatinine were determined by Biolabo Company.

2.4. DMBA preparation

Final concentration 50mg/kg B. W of DMBA dissolved in 1 mL sesame oil administered to all the rats except control group by gastric gavage for breast cancer induction (Lai and Singh, 2006). DMBA breast carcinogenesis is highly age-dependent, being maximal at sexual maturity (ages of 45-60 days) (Grubbs et al., 1986).

2.5. Graviola preparation

Graviola capsules consisted of 100% pure, finely milled Graviola leaf/stem powder with no binders or fillers. The powder contents were dissolved in distilled water (Florence et al., 2014) and administered by gastric gavage. Five rats were dead from DMBA group while three rats were dead from DMBA+G2 and two from DMBA+G1.

2.6. Body weights

Body weights were recorded twice throughout the experimental period.

2.7. Serum sample preparation

Under anesthesia of ether at the end of the experiment, blood was collected by heart puncture and centrifuged at 3000 rpm for 15 min.

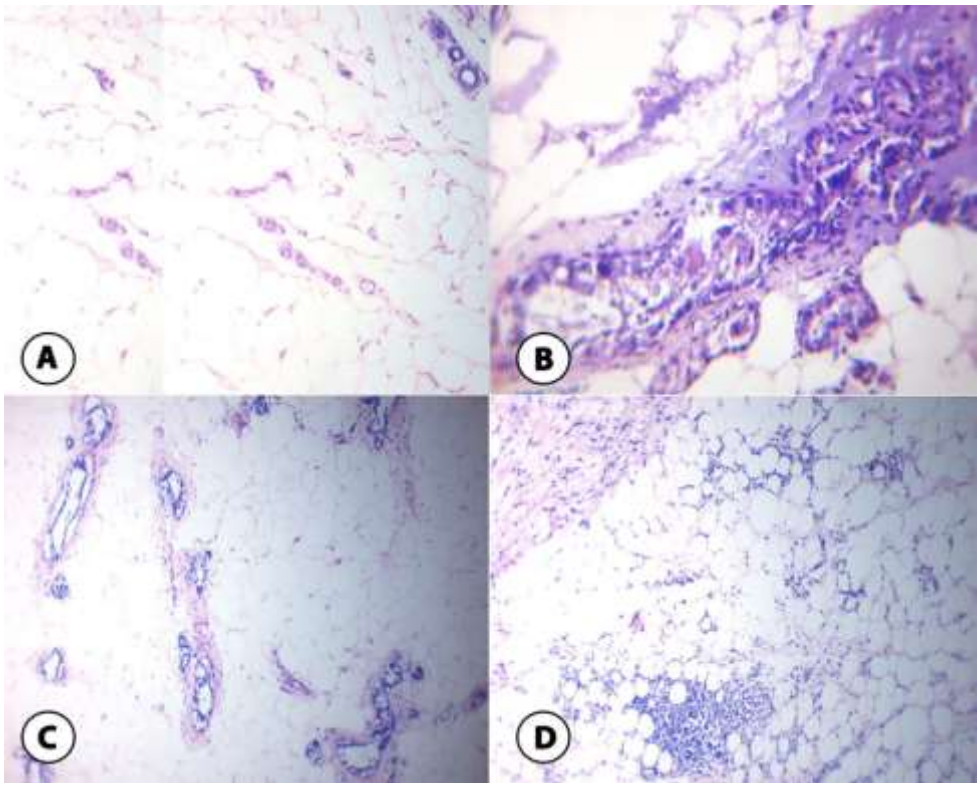

Figure 1: Tissue section of mammary gland of a rat stained with hematoxylin and eosin. (A) Control (X400), (B) DMBA-treated (X400), (C) group III (DMBA+ G1) (X100) and (D) group IV: DMBA+G2 (X160).

to obtain the serum which stored at -20°C for further determination of ALT activity and creatinine levels. Brifely kinetic determination of ALT activity was done according to the following reaction:

L-Alanine + 2 –oxoglutarate $\overset{ALT}{\Leftrightarrow}$ pyruvate + L-glutamate

Pyruvate + NADH+H+ $\overset{LDH}{\Leftrightarrow}$ L-Lactate + NAD+

The decrease in absorbance due to the conversion of $NADH+H^+$ into NAD^+ was proportional to ALT activity in the specimen at 340nm. Creatinine in alkaline solution reacted with picric acid to form a colored complex. The amount of the complex formed was directly proportional to the creatinine concentration.

2.8. Breast tissue preparation

Mammary gland tissues were kept in neutral buffered formalin solution (10%) for histolopathological examination.

2.9. Statistical analysis

The results are expressed as the mean ±SE. The statistical analysis of variance (ANOVA) was performed using *Duncan's* multiple range tests (SPSS Inc). Values of P <0.05 were statistically significant.

3. Results

3.1. Graviola effects on body weights

Table (1) reported that DMBA group lost body weight in respect to control group. Treatment with graviola significantly ($p < 0.05$) increased body weight when compared to non-treated DMBA rats.

3.2. Graviola effects on hepatic and kidney functions

Serum ALT activity and creatinine levels in DMBA group were significantly higher ($p<0.05$) than control group. After graviola treatment, the serum ALT activity and creatinine levels significantly decreased ($p< 0.05$) than non- treated group. Table (2)

3.3. Histopathological examinations of rat mammary glands

Histopatological examination of breast tissue of control showed normal epithelial cells surrounding the lumen of the alveoli without any cell debris. DMBA group showed development of carcinoma with focal alveolar hyperplasia. In Graviola-DMBA group showed no appearance of cancer with many normal mammary alveoli in particular group III consisted of two cell layers with clear lumen (Fig. 1).

4. Discussion

Breast carcinoma is a common female cancer (Ahmad et al., 2013). It is likely that dietary derived agents would have effects throughout the carcinogenic process (Yu and Kong, 2007). Therefore, it is necessary to enhance the naturally occurring phytochemicals effects in prevention of cancer in experimental studies to elevate their possible protective role in humans especially they have less side effects in respect to conventional ALT activity, these results agree with (Florence et al., 2014; Syahida et al., 2012). Also, DMBA significantly increased serum creatinine levels than control (Sharmila Banu et al., 2009; Singh et al., 2011) while chemotherapeutic drugs. (Chakraborty et al., 2011). DMBA administration activate phase I enzymes leading to active oxygen species generation such as peroxides and superoxide anion radicals causing lipid peroxidation and

Table (1): Effects of graviola on b.wt (g) in control, DMBA induced breast cancer and treated rats during 15th and 30th weeks

	Period	
Group	**15th week**	**30th week**
Control	184.2 ± 1.16^{a}	249.6 ± 1.50^{a}
DMBA induced breast cancer	165.0 ± 1.39^{c}	219.4 ± 1.36^{d}
DMBA+G1	174.6 ± 1.78^{b}	237.0 ± 0.7^{b}
DMBA+G2	171.3 ± 0.86^{b}	228.6 ± 1.03^{c}

Means within the same column carry different superscript are significantly different (P <0.05).

Table 2: Means and their standard errors of serum ALT activities (IU/L) & creatinine (mg/dl) in control, DMBA induced breast cancer and treated rats

	Hepatic marker	Renal function test
Group	**ALT**	**Creatinine**
Control	29.86 ± 3.32^{b}	0.9 ± 0.07^{b}
DMBA induced breast cancer	59.80 ± 2.46^{a}	1.51 ± 0.12^{a}
DMBA+G1	35.83 ± 3.14^{b}	1.11 ± 0.18^{b}
DMBA+G2	37.02 ± 2.95^{b}	1.09 ± 0.095^{b}

oxidative stress (Bishayee et al., 2000; Priyadarsini and Nagini, 2012). The results of the present study showed that exposure to DMBA caused ROS generation that caused significant decrease in the total body weights in respect to control group. Our findings desperately agree with the findings of (Moselhy and Al mslmani, 2008) who attributed this to oxidative damage caused by DMBA affecting protein synthesis while the body weights increased significantly in graviola treated groups compared to DMBA group. This is consistent with (Florence et al., 2014) who attributed this to graviola antioxidant content which prevent tissue damage and maintained cell physiology. Graviola flavonoids inhibit LPO by donating hydrogen atoms to the radicals thus terminating the propagating chain by forming a flavonoid radical which in turn reacts with free radicals (El-khawaga et al., 2003; Robak and Gryglewski, 1988). Since Graviola is a complex mixture, its biological action in several instances not due to one compound but to a synergistic action of several components (Vickers, 2002). The present study reported significantly increased ALT activity in group receiving DMBA than control, this result supported by (Dakrory et al., 2015) while administration of the plant extract significantly reduced administration of graviola significantly decreased its level, this agrees with (Florence et al., 2014; Syahida et al., 2012). Increased serum ALT activities and creatinine levels indicating the hepatic and kidney damage

resulting from ROS due to breast cancer. DMBA damaged the membrane through lipid peroxidation of unsaturated fatty acids and alter its function (Memisogullari and Bakan, 2004). The plant extract significantly suppressed the increase of ALT activities and creatinine levels suggesting a protective role of the plant extract against oxidative stress, hepatic and kidney injuries. Protection may be achieved because of enhanced detoxification of reactive electrophiles and free radical scavenging, as well as induction of repair pathways (Adewole and Ojewole, 2008; Mukhtar and Ahmad, 2000; Valko et al., 2007)

5. Conclusion

We can conclude that graviola mitigated hepatic plus renal functions in experimentally induced breast cancer.

Conflict of interests

The authors have not declared any conflict of interests.

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