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In vivo study using Amygdalin against mammalian tumors in a mice model

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ABSTRACT

The present study aimed to evaluate the positive effects of amygdalin against mammalian tumors *in vivo*. Twenty female albino mice, 4-6 weeks old age, were equally divided into four groups: group I (control) received no drugs; group II (carcinogenic group) received oral 7,12-dimethylbenz[a]anthracene (DMBA) (50 mg/kg b.wt. once a weak) dissolved in sesame oil for 4 weeks; group III (treatment group) received oral amygdalin (0.6 mg/kg b.wt./day) dissolved in 100% corn oil for 4 weeks after DMBA-induced tumor as in group II; group IV (protection group) received amygdalin as in group III prior DMBA administration. The results showed that DMBA-induced mammalian tumors caused a significant increase in serum bilirubin (total and direct). Still, a substantial decrease in serum albumin as a hepatic function was recorded. Serum uric acid as a kidney marker revealed an insignificant increase. In addition, mammalian tissue L-malondialdehyde (L-MDA) concentration showed substantial and a considerable decrease in mammalian tissue total antioxidant capacity (TAC) concentration compared with the control group. However, the administration of amygdalin was able to mitigate DMBA-induced mammalian tumors through decreasing total and direct bilirubin, uric acid concentration, as well as L-MDA concentration, along with a significant increase of TAC concentration in mammalian tissue. Additionally, hematological parameters of amygdalin-administrated mice showed a substantial increase compared to mice with mammalian tumors induced by DMBA. Thus, it can be concluded that amygdalin may successfully protect mammary tumors.

1. INTRODUCTION

Breast cancer has many hereditary and clinical variations (Stingl and Caldas, 2009). In addition to being the cancer with the highest mortality rate, it is also the cancer with the highest incidence. Death rates from breast cancer have risen in emerging nations during the previous quarter century (Juanjuan et al., 2017). Breast cancer stands alone among oncogenes as a major health concern in Egypt (NCRPE, 2012). Cancer deaths among Egyptian women accounted for almost 29.1% of all cancer deaths in the country in 2010 (Jemal et al., 2011). Surgery, radiation therapy, and chemotherapy are generally unsuccessful against late cancer stages, and they can have detrimental effects on vital organs like the liver and kidneys. The development of therapeutic regimens with no or minimal effects on normal organs is urgently needed to prevent such occurrences (Niedzwiecki et al., 2016).

Amygdalin is an antiquated drug with many uses, including cancer prevention. Easily extracted from the pits of apricots, almonds, cherries, peaches, and plums, riboflavin is a plant glucoside of the Rosaceae family known as vitamin B17. Laetrile (D-mandelonitrile—D-glucoside-6—glucoside), another name for amygdalin, is a cyanogenic aromatic molecule with the formula $C_{20}H_{10}NO_{11}$ with a molecular weight of 457.42 Dalton (Santos et al., 2014). Hydrocyanic acid, an anticancer chemical, and benzaldehyde, which can cause an analgesic effect, were produced from amygdalin. That's why it's effective against cancer and pain (Chang et al., 2006).

Recent years have seen a surge in interest in amygdalin's potential anticancer effects. Laetrile's anticancer properties are governed by carcinogenic chemicals that break down in the body and kill tumor cells, cutting off cancerous cells' access to nutrition and halting their growth. Traditional cancer treatments in Italy, Japan, China, and the USA have been processed and used (Do et al., 2006). This research set out to determine if amygdalin has any anticancer effects in mice with cancer of mammalian tissue.

2. MATERIAL AND METHODS

2.1. Chemicals

2.1.1. Amygdaline: Chemical prodrug in the form of crystalline powder and dissolved in 100% corn oil, purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA); ID. NO. 24891046; it is used at an oral dose level (0.6 mg/kg.bw/day) (Bromley et al., 2005).

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2.1.2. 7,12-Dimethylbenz[a]anthracene:
The most commonly utilized chemical carcinogen**;** it is known as DMBA with chemical formula C20H16, molecular weight 256.341 g/mol, in the form of powder and dissolved in 100% sesame oil, purchased from Sigma-Aldrich Chemical Co., ID. NO. 24891046, and provided by Egyptian International Center for Import Cairo, Egypt). It is used at an oral dose level (50 mg/kg b. wt. once a week) for four weeks (Minari and Okeke, 2014).

2.2. Experimental animals

Benha University, Faculty of Veterinary Medicine's Ethical Committee approved all experiments under No. (BUFVTM 18-01-23). Twenty female albino mice aged 4-6 weeks and weights from 18-35 g were utilized in this investigation. Mice were acquired from the animal facility at Faculty of Veterinary Medicine, Benha University. They were kept in individual metal cages with a controlled atmosphere and diet throughout the experiment. Regular rations of food were provided, and animals had access to water at all times. Seven days were given to the animals for acclimation before the experiment began.

2.3. Experimental design

Mice were randomly divided into four main groups, placed in individual cages, and classified as follows:

Group I (control group): comprised 5 female albino mice received no drugs.

Group II (carcinogenic group): comprised of 5 mice, received oral DMBA at a dose of (50 mg/kg b. wt. orally once a week) in sesame oil at 5 weeks age for 4 weeks (Minari and Okeke 2014).

Group III (treatment group): comprised 5 mice, administrated with DMBA (50 mg/kg b. wt. orally once a week) at 5 weeks age for 4 weeks (Minari and Okeke, 2014) then treated orally with amygdalin (0.6 mg/kg. b. wt./day) in 100% corn oil for 4 weeks (Bromley et al., 2005).

Group IV (protection group): comprised 5 mice, received oral amygdalin at a dose level (of 0.6 mg/kg b. wt./day) in 100% corn oil for 4 weeks (Bromley et al., 2005) then administrated with DMBA (50 mg/kg orally once a week) at 5 weeks age for 4 weeks (Minari and Okeke 2014) along with amygdalin dose administration.

2.4. Blood sampling:

Blood samples were taken from mice at the end of the experiment and divided into two parts: one put in EDTA tube for hematological parameters analysis, and the other put in plain tube to obtain the serum after centrifugation at 3000 rpm for 15 min. The clear, serum was collected using an automated pipette, placed in a dry sterile samples tube, and stored in the freezer at -20 ℃ till do the biochemical analysis for bilirubin (total and direct), albumin, and uric acid levels.

2.5. Tissue Samples (mammary tissues):

Mice were anesthetized prior to sacrifice according to Marquardt et al., (2018) when their experimentation time was up. To analyze oxidative stress indicators L-MDA and TAC, the mammary tissues were rapidly removed and frozen at -80°C.

2.6. Assay methods

2.6.1. Hematological Analysis

An automated hematology analyzer was used for the hematological tests (Abbott Cell-Dyn 3500 Hematology Analyzer). Blood parameters were measured, such as hemoglobin (Hb), red blood cell (RBC) count, hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, white blood cell (WBC) count, and its differential counts (neutrophils, lymphocytes, monocytes, eosinophils, and basophils) (Charles et al., 2007).

2.6.2. Serum biochemical analysis:

Bilirubin (total and direct), albumin, and uric acid analyses were carried out using JENWAY 6051 Colorimeter U.K device with (Spectrum GmbH Company kits; CAT. NO. 222001; 211001; 323000) according to methods formerly (Balistreri and Shaw 1987; Doumas et al., 1971; Tiffany et al., 1972; respectively).

2.6.3. Tissue oxidative stress:

Mammary tissues were cut and washed with a PBS (phosphate buffered saline) solution, pH 7.4 containing 0.16 mg/ml heparin to remove any red blood cells and clots. Homogenization of one gram mammary tissue took place in 5 ml cold buffer (i.e., 50mM potassium phosphate, PH 7.5 1mM EDTA), using sonicator homogenizer. Aliquots of tissue homogenates was centrifuged by cooling centrifuge 4000rpm for 20min then the supernatant was removed and stored at -20˚C till do the oxidative stress. TAC and L-MDA concentrations were determined by Spectro nanodrop using commercial kits (Biodiagnostic, Cairo, Egypt; CAT. NO. TA2513; MD2529) according to (Korcevic et al., 2011) (Satoh, 1978) using the supernatants from the homogenates centrifuged at 8000 rpm for 20 minutes at 4 °C.

2.7. Statistical analysis

A one-way analysis of variance (ANOVA) and the Duncan multiple test on the collected data were used for statistical analysis. The social science statistics package was used for all studies (SPSS, 13.0 software, 2005). P-values below 0.05 were deemed statistically insignificant; all data are presented as mean ± SE.

3. RESULTS

The obtained hematological results indicated a significant decrease in RBCs count, Hb level, Hct, and platelets count in DMBA-bearing mice with a significant increase in WBCs and their absolute differential counts (lymphocytes, monocytes, eosinophils, and basophils) compared with the control group; but MCV, MCH and MCHC not significantly altered. On the other hand, protecting DMBA-bearing mice with amygdalin significantly improved Hb, RBCs, Hct and platelets parameters compared with DMBA-administrated mice. Treating DMBA-bearing mice with amygdalin insignificantly improved Hb level and not significantly altered MCV, MCH and MCHC. WBCs didn't improve by amygdalin administrations, but their absolute differential counts (neutrophils, monocytes, and basophils) reached control values in amygdalin treated mice. All these data are illustrated in Tables (1 & 2)

Table 1 Effect of Amygdalin on hematological parameters (CBC) in DMBA-induced tumor mice.

	Hb (g/dL)	RBCs ($\times10^6$/µL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (%)	Platelets ($\times10^3$ /µl)
Control	9.87±0.20[a]	3.37±0.09[a]	28.47±0.58[a]	84.67±0.52	29.27±0.17	34.60±0.00[a]	513.33±14.53[a]
DMBA	7.30±0.23[c]	2.50±0.12[b]	21.43±1.00[b]	85.80±0.75	29.70±0.25	34.60±0.00[a]	141.67±10.14[b]
Treatment	8.93±0.09[b]	3.10±0.05[a]	26.17±0.62[a]	84.33±0.68	29.13±0.23	34.60±0.00[a]	502.33±30.31[a]
Protection	9.70±0.12[a]	3.33±0.09[a]	28.28±0.59[a]	84.93±0.43	29.33±0.15	32.93±1.67[a]	507.33±10.11[a]

Differences between column means denoted by different subscript letters are statistically significant at 0.05. (*P*<0.05*)*

Table 2 Effect of Amygdalin on hematological parameters (WBCs and their differential counts) in DMBA-induced tumor mice.

	WBCs ($\times10^3$ /µL)	Neutrophils (10^3/µL)	Lymphocytes (10^3/µL)	Monocytes (10^3/µL)	Eosinophils (10^3/µL)	Basophils (10^3/µL)
Control	5.00±0.35[c]	1.43±0.10[a]	3.03±0.25[c]	0.28±0.03[b]	0.13±0.01[b]	0.00±0.00[b]
DMBA	6.70±0.06[a]	0.67±0.04[b]	5.29±0.04[a]	0.42±0.02[a]	0.04±0.02[c]	0.26±0.04[a]
Treatment	6.00±0.17[b]	1.66±0.10[a]	3.85±0.03[b]	0.26±0.03[b]	0.20±0.02[a]	0.02±0.02[b]
Protection	2.93±0.09[d]	0.51±0.04[b]	2.19±0.05[d]	0.12±0.01[c]	0.07±0.01[c]	0.01±0.01[b]

Differences between column means denoted by different subscript letters are statistically significant at 0.05. (*P*<0.05*)*

According to biochemical markers, the results revealed that DMBA-induced mammary tumors showed a significantly increased bilirubin (total and direct) compared to the control, and significantly decreased in serum albumin level. However, insignificant changes of uric acid were recorded in DMBA-mice. Interestingly, it was found that the amygdalin administration significantly improved their hepatic functions, but insignificantly with a kidney marker level (Table 3)

Regarding oxidative stress**,** mammary tissue L-malondialdehyde (L-MDA) significantly increased by DMBA compared to the control group and grossly decreased by amygdalin administrations with the DMBA-induced tumor. Total antioxidant capacity (TAC) was significantly declined by given DMBA and remained at control value in amygdalin protected mice, while amygdalin treated mice revealed insignificant change mammalian tissue TAC (Table 4)

Table 3 Effect of Amygdalin on bilirubin (total and direct), albumin, and uric acid concentrations in DMBA-induced tumor mice.

	Albumin (g/dl)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Uric acid (mg/dl)
Control	3.47±0.03[ab]	0.89±0.02[b]	0.28±0.01[c]	2.73±0.18
DMBA	3.27±0.06[c]	1.20±0.06[a]	0.40±0.01[a]	3.00±0.00
Treatment	3.60±0.06[a]	0.85±0.01[b]	0.27±0.01[c]	2.93±0.07
Protection	3.38±0.01[bc]	0.92±0.01[b]	0.31±0.01[b]	2.73±0.18

Differences between column means denoted by different subscript letters are statistically significant at 0.05. (*P*<0.05*)*

Table 4 Effect of Amygdalin on L-MDA and TAC concentrations in DMBA-induced tumor mice.

	MDA (nmol/g)	TAC (µm/g)
Control	1173.17±12. 87[c]	1.86±0.03[a]
DMBA	2099.31±196.33[a]	1.54±0.07[b]
Treatment	1557.14±69.34[b]	1.61±0.01[b]
Protection	1323.55±72.86[bc]	1.93±0.07[a]

Differences between column means denoted by different subscript letters are statistically significant at 0.05. (*P*<0.05*)*

4. DISCUSSION

Researchers looked at amygdalin since it is a promising therapeutic plant extract inhibiting mammalian tumor cells. Amygdalin's role in the induction of DMBA carcinogens was investigated in vivo, focusing on its functional processes. Through oral treatment of DMBA, a tumor model in mice was successfully developed. The potential therapeutic and preventive effects of amygdalin treatment were studied by applying it.

Results highlight that hematological parameters nearly returned to normal after amygdalin treatment in DMBA-treated mice. This effectively raises Hb, RBCs, and HCT and in peripheral blood lymphocytes (PLTs) as reported in (Singh and Singh 2008). Anemia is indicated by a decrease in Hb, RBC, and HCT (Pilny, 2008), that is matching with DMBA mice group. Therefore, intoxication can cause anemia due to alterations in erythropoiesis, the inhibition of hematopoietic organs' activity, or the accelerated breakdown of red blood cells (RBCs) due to changes in RBC membrane permeability (Yuan et al., 2014). Furthermore, the obtained results showed a significant decrease in platelets count with DMBA that may be resulted from the suppression of bone marrow activity, decreased synthesis, increased consumption, or excessive platelet aggregation (Sirag, 2009). When blood vessels are damaged, platelets play a key role in forming a plan to stop bleeding (Campbell and Ellis, 2007). Multiple mechanisms have been proposed to account for this phenomenon, such as an increase in the osmotic fragility of red blood cells or a decline in the health of bone marrow cells. It is worth noting that changes in hematological parameters may result from treatment-induced exposure to natural substances (Rhiouani et al., 2008).

According to Adebayo et al. (2010), a rise in white blood cells (WBCs) is a typical immune response to pathogens. White blood cell counts across the board (lymphocytes, monocytes, eosinophils, and basophils) revealed a significant increase in DMBA mice group as a result of cancer obtained (Riley and Rupert, 2015). Amygdalin treatment showed absolute differential counts (neutrophils, monocytes, and basophils) within range of control values, that was in accordance with (Strati and Shanafelt, 2015).

In another aspect, amygdalin has hepatic ameliorative potential against DMBA carcinogen, with increases in albumin and decreases in total and direct bilirubin. Inhibiting bilirubin levels and increasing albumin expression are two mechanisms through which amygdalin has beneficial therapeutic effects on hepatocellular carcinoma, as revealed by (El-Desouky et al., 2020). Additionally, via modulating the NLRP3, NF-kappaB, and Nrf2/NQO1 signaling pathways, Amygdalin reduces acute liver injury generated by D-galactosamine and lipopolysaccharide (Tang et al., 2019). Bilirubin may indicate healthy liver cells and the biliary tree as a byproduct of heme breakdown. A surge in mass inhibition of the conjugation reaction and release of unconjugated bilirubin from damaged hepatocytes have been linked to

significant increases in serum total and direct bilirubin values in DMBA-intoxicated mice (Karabulut et al., 2014). Serum total and direct bilirubin levels were dramatically decreased by amygdalin, whereas serum albumin levels were significantly enhanced compared to the carcinogenic group. Hepatoprotective activity is shown, which treats liver injury by preventing enzyme leakage across membranes (Ali et al., 2019).

In addition, the present research showed that amygdalin effectively regulated the antioxidant defense system by increasing TAC levels. In contrast, decreased MDA levels indicate amygdalin's antioxidant capabilities and free radical scavenging capacity. Similarly, (Karabulut et al., 2014) demonstrated that amygdalin supplementation offered robust protection against the oxidative stress generated by DMBA by lowering oxidative stress and MDA levels and raising TAC levels. In a mouse model of 7,12-dimethylbenz[a]anthracene (DMBA)-induced carcino-genesis, the amygdalin-containing fraction showed anticancer efficacy in vivo by decreasing lipid peroxidation and boosting the antioxidant response as evaluated by total antioxidant capacity (TAC) and malondialdehyde (MDA). There was a correlation between the presence of amygdalin and the activity of the amygdalin-containing fraction. The latter could form HCN in malignant tissue, leading to cell death via an oxidative cascade (Hosny et al., 2021).

5. CONCLUSIONS

Amygdalin was found to be highly effective at preventing cancer in mammalian tissues. It restored the hematological parameters, liver and kidney indicators, and antioxidant levels that DMBA mice had enhanced.

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