

Molecular Mechanism Underling the Chemopreventive Effect of Turmeric and Banana Peel Extracts On 7, 12-Dimethylbenzeneanthracene (DMBA) - Induced Mammary Carcinogenesis in Female Mice

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Abstract

Evaluate the anticarcinogenic effect of turmeric and banana peel extracts versus vitamins supplementation on 7, 12-dimethylbenzeneanthracene (DMBA)-induced breast cancer in female mice.

Phytochemical composition of turmeric and banana peel extracts was estimated colorimetrically. Plasma vitamins C, E and β -carotene were determined by high-performance liquid chromatography (HPLC). Agarose gel electrophoresis was used to analyze DNA from the tissues. Prostate specific antigen (PSA) determined by ELISA was used as a tumor marker. Crude extract of banana peel and turmeric extract were evaluated versus vitamin C, E and A supplementation in reversing DMBA induced carcinogenesis.

DMBA-induced changes were reversed by turmeric extract and to a lesser extent by banana peel extract or vitamins supplementation as evidenced by improved oxidants- antioxidants imbalance, apoptotic DNA fragmentation and decreased PSA.

Keywords: Turmeric; Banana; DMBA; Breast Cancer

Introduction

Breast cancer represents the most common malignancy in females associated with high mortality [1]. Turmeric extract is a natural non-toxic antioxidant, Banana peel contains bioactive compounds such as polyphenols, carotenoids and possess antioxidant capacity against hydrogen peroxide-induced damages [2,3]. Vitamin C, E

and A are potent free radical scavengers that can reduce DNA damage and cell membrane peroxidation [4].

Prostatic specific antigen (PSA) a protein produced almost exclusively by prostate, used for the diagnosis and follow up of prostatic cancer and recently in breast and ovarian cancer [5,6].

Aim of the Work

- To study Phytochemical composition of turmeric and banana peel extracts.
- To determine antioxidant levels (vitamins C, E and β -carotene) in plasma following administration of turmeric or banana peel extracts and vitamin supplementation.
- To evaluate the chemopreventive effect of turmeric and banana peel extracts.

Materials and Methods

For six weeks, in a randomized complete block design(RCBD), thirty adult female albino mice (12 weeks of age, weighing between 21 and 28 g ,housed in clean cages at 25°C from animal house in faculty of medicine, Assiut university, Egypt) were divided into 5 groups, each group had 6 mice (after approval of the Assiut university ethics committee):

Group I: Mice treated with distilled water only.

Group II: Mice treated with 20 mg/ml/week DMBA only to induce breast cancer (the dose as described by Minari and Okeke (2014) [7,8].

Group III: Mice treated with 20 mg/ml/week DMBA +100 mg/ml/day of turmeric extract (DMBA and extract were given intragastrically).

Group IV: Mice treated with 20 mg/ml/week DMBA + 100 mg/ml/day of dried and powdered banana peel extract.

Group V: Mice treated with 20 mg/ml/week of DMBA + Vitamin C (purity: 99%) + vitamin E (purity: 95%, 200 mg/kg/day in 1 ml of normal saline administered orally as previously described) + β -carotene (purity: 97%) (20 mg/kg/day in 1 ml of normal saline administered orally as reported) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Each mouse had 12 mammary glands checked by inspection; plasma samples and breast tissues were obtained for agarose gel electrophoresis [9-11].

Determination of Vitamins in Plasma by HPLC

Vitamin C: plasma proteins were precipitated (100 μ l plasma + 400 μ l of 60% methanol/EDTA, incubated for 10min at 4°C then centrifuged at 12,000 rpm for 8min), the clear phase was evaporated to dryness and dissolved in 100 μ l of methanol. -Vitamin E and β -carotene: 100 μ l of plasma was deproteinized with 100 μ l of ethanol, extracted with 600 μ l chloroform then shaken for 5min and centrifuged. The organic layer was extracted and

evaporated to dryness and dissolved in 100 μ l of methanol [12].

Tannin Determination

0.2 powdered sample in 10 ml actone (70%) shaken for 2hrs and centrifuged then 0.2ml supernatant+0.8ml DW are mixed, 1 ml standard tannin in 0.5 ml DW . 0.5 ml Folin Ciocalteu reagent and 2.5 ml Na₂CO₃(20%) were added to both, incubated for 40 min ,absorbance read at 725nm against blank [13].

Alkaloid Determination

5 gm sample +200ml of 10% acetic acid+50 ml ethanol filtered and concentrated in water bath till 1/4 volume , ammonium hydroxide was added drop wise till precipitation and filtered and weighed [14].

Total Flavonoid Determination

10 gm powdered sample in 100ml of 80% aqueous methanol filtered, evaporated in water bath to dryness and weighed [15].

Determination of Phenol

sample was boiled with 50ml of ether for 15 min.5 ml extract +10 ml DW+2ml ammonium hydroxide+5 ml amyl alcohol left to react for 30 minutes for color development; the absorbance was measured at 550nm [16].

Lipid Peroxidation

1 ml of trichloroacetic acid 10% + 1 ml of thiobarbituric acid 0.67% in a boiling water bath for 30 min, absorbance at 535 nm [17].

Estimation of Reduced Glutathione

Reduction of Ellman's reagent (5,5` dithiobis, 2nitrobenzoic acid) with GSH to produce a yellow compound ,absorbance were measured at 405 nm [18].

Reducing Power Assay

1ml extract + 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml potassium ferricyanide (1%) were incubated for 20 minutes and 2.5 ml of trichloroacetic acid was added. The absorbance was read at 700 nm [19].

PSA Analysis

Sandwich ELISA was used for PSA quantitation (GenWay Biotech, Inc. Catalog No. 40-101-325050).

Results

Phytochemical studies revealed the presence of flavonoids, tannin, phenol and alkaloid in turmeric and banana peel extracts (Phenol was the highest in both extracts; all were higher in turmeric extract as compared to banana peel extract). DNA smears suggested possible DMBA-induced damage that was reversed to variable extent in various treatment modalities. As shown by DNA fragmentation (ladder formation on ethidium bromide-stained gel and UV illumination). DNA Fragments were marked in turmeric extract supplementation followed by banana peel extracts and lastly vitamins supplementation as compared to untreated DMBA-induced cancer samples

(Figure 1) indicating induction of apoptosis and attempt healing that was confirmed by improved oxidants-antioxidants imbalance and decrease in PSA level and improved overall survival (Figure 2).

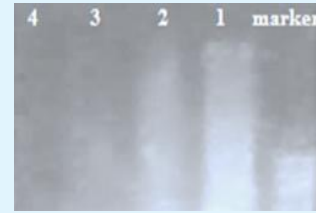


Figure 1: DNA fragmentation of DMBA induced breast cancer tissues exposed to various treatment modalities.

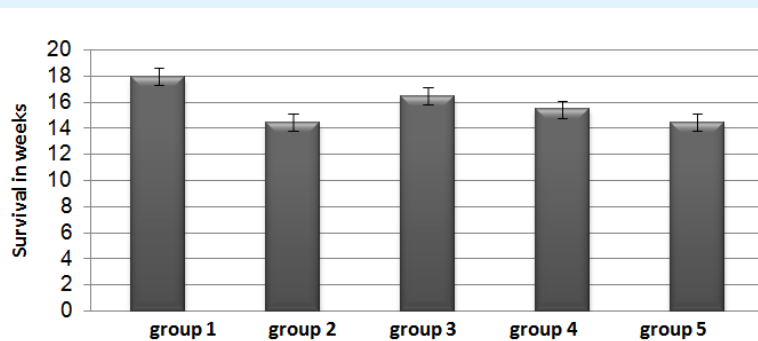


Figure 2: Mean survival in weeks in different groups (mean \pm SE).

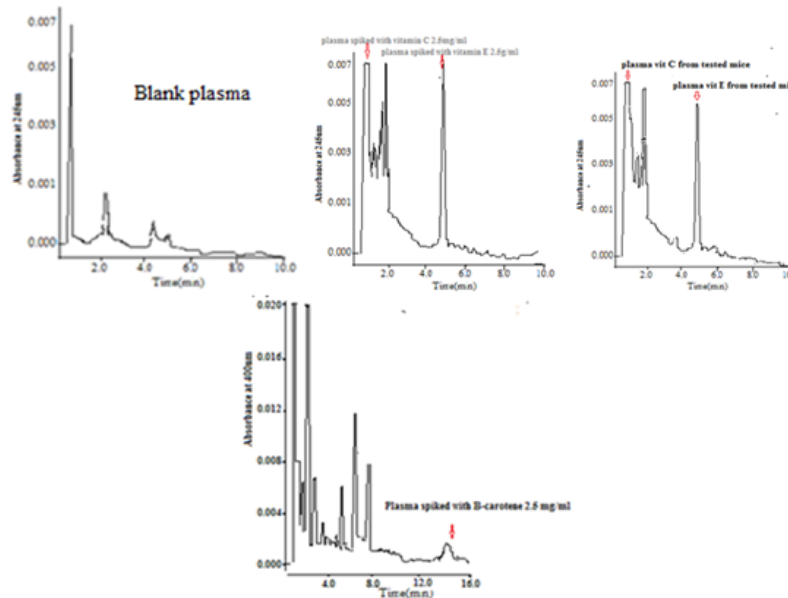


Figure 3: Determination of vitamins in plasma by HPLC in different studied groups.

Statistical Analysis

The data were analyzed by using SPSS 11.0 for Windows. Group results are expressed as means \pm SDs. Group means were compared by (ANOVA). Correlations of statistical significance between groups were done using Spearman Rank Correlation. A P-value < 0.05 was considered to be significant.

Discussion

The Phytochemical composition of turmeric extract and banana peel extract are shown in table 1. Both are rich in plant phenolics and flavonoids (higher in turmeric extract) that are highly effective free radical scavengers and antioxidants which explain their ability to react with reactive oxygen species (ROS) and reactive nitrogen species (RNS) [20,21].

| Phytochemical Analysis | Turmeric Extracts | Banana Peel Extract |
|--------------------------------|-------------------|---------------------|
| tnetnoc cilonehP latoT (g/gm) | 93.30 \pm 0.03* | 82.52 \pm 0.013 |
| Total flavonoid content (mg/g) | 43.6 \pm 0.3* | 33.43 \pm 0.06 |
| Reducing Power (μ g/ml) | 25 \pm 1.7* | 21 \pm 5.5 |
| Alkaloid (mg/100 g) | 0.76 \pm 0.01* | 13.60 \pm 0.51 |
| Tannin (mg/100 g) | 91.08 \pm 0.72 | 77.40 \pm 0.36 |

Table 1: Phytochemical analysis of turmeric extracts and banana leaves extract.

| Parameter | Group I (Control) | Group II (DMBA only) | Group III (turmeric extract) | Group IV (banana peel extract) | Group V (Vitamins intake) |
|--------------------------|-------------------|----------------------|------------------------------|--------------------------------|---------------------------|
| Vitamin C (mg/ml) | 0.35 \pm 0.02 | 0.16 \pm 0.08 | 0.76 \pm 0.04 | 1.60 \pm 0.03 | 1.9 \pm 0.5 |
| Vitamin E (μ g/ml) | 1.03 \pm 0.08 | 0.13 \pm 0.01 | 2.03 \pm 0.08* | 3.40 \pm 0.05* | 6.3 \pm 3.6* |
| B-carotene (μ g/ml) | 0.25 \pm 0.02 | 0.15 \pm 0.01 | 0.45 \pm 0.03 | 0.90 \pm 0.04 | 1.3 \pm 0.6* |
| GSH mmol | 0.73 \pm 0.25 | 0.23 \pm 0.02 | 1.73 \pm 0.25* | 1.56 \pm 0.33* | 0.48 \pm 0.02 |
| MDA (nmol) | 18.5 \pm 2.6 | 29.8 \pm 4.5** | 17.33 \pm 3.5** | 22.37 \pm 1.53* | 27.5 \pm 4.63* |
| PSA (ng/ml) | 12.1 \pm 0.2 | 22.3 \pm 1.3*** | 10.5 \pm 0.6** | 15.3 \pm 0.35* | 17.6 \pm 2.3* |

*P values < 0.05 , ** P values < 0.01

Table 2: Blood concentration of the studied parameters after intake of different treatments.

MDA as a marker for oxidative stress was significantly reduced associated with significant increase in reduced glutathione in plasma of all treated groups (marked in turmeric extract, less in banana peel extract and least in vitamins supplementation group) as compared to the untreated cancer group. It can be suggested that the observed marked anticancer effect of turmeric and banana peel extract in the present study is due to the presence of phenolics compounds and flavonoids in addition to the vitamins content rather than the vitamins content only as observed in the group that received vitamin supplementation alone.

Cancer therapy destroy cells through apoptosis evident as DNA fragmentation [22]. In the present study, DNA gel electrophoresis showed that DNA fragmentations was marked in turmeric extract supplementation followed by banana leaves extracts and lastly vitamins

supplementation compared to untreated DMBA-induced cancer tissues Also, PSA is a useful marker for monitoring breast cancer [23]. In the current study, it showed significant decline in all treated groups associated with improved survival that was marked in turmeric extract, less in banana peel extract and least in vitamins supplementation group as compared to the untreated cancer group. The antioxidant activity of turmeric extract is due to their chemical structure that exist in a stable enol form acting as a chain-breaking antioxidant [24]. It suppresses the activation of NF κ B, an inducible transcription factor that regulates expression of anti-apoptotic genes and induces metastasis thus increases apoptosis as observed in the current study [25].

Banana flower extract exhibited cytotoxic effects on cervical cancer cell line due to their phenol content that induced apoptosis as evident by increased caspase 9

activity [26]. The current results showed that banana peel extract produced a similar effect. Vitamin E, β -carotene, and vitamin C are antioxidants that protect cells from oxidative damage involved in carcinogenesis. Kirsh, et al. did not provide a strong support for population-wide supplementation of these antioxidants for the prevention of cancer. However, vitamin E and β -carotene supplementation were associated with reduced risk of cancer similar to the current study [27].

In conclusion, turmeric extract and less banana peel extract are beneficial anticarcinogenic agents possibly more effective than vitamins supplementation through induction of apoptosis.

Conflict of Interest

The author has no conflict of interest or financial, personal or other relationships with other people or organizations that could inappropriately influence this work.

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