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Original article

Hesperidin and tiger nut reduced carcinogenicity of DMBA in female rats

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ABSTRACT

Nutritional studies recommend the regular consumption of fruits and vegetables to favor a healthy quality of life. This study was carried out to evaluate the efficacy of hesperidin and tiger nut against the carcinogenic activity of DMBA in female rats. 72 adult Sprague Dawley female rats were divided equally into six groups: control group (I); Hesperidin treated group (II); Tiger Nut treated group (III); DMBA treated group (IV); HES-DMBA treated group (V); and TN-DMBA treated group (VI). There was a significant increase in serum levels of carcinoembryonic antigen, total sialic acid, progesterone, estradiol, ALT, AST, LDH, urea and creatinine, and significant decrease in reduced glutathione level, superoxide dismutase, catalase and glutathione peroxidase activities of DMBA treated group compared to control. In conclusion, our results suggested that supplementation of diets with hesperidin provided antioxidant and chemoprotective activities more significant than tiger nut against the toxicity of DMBA in breast, liver and kidney tissues.

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1. Introduction

DMBA (7,12-Dimethylbenz(a)anthracene) a polycyclic aromatic hydrocarbon produce during incomplete combustion of carbon compounds it found in tobacco smoke and motor vehicle exhaust emissions [1,2]. It is a main class of potent nephrotoxic, immunosuppressor and cytotoxic chemical carcinogen that increase breast cancer risk [3–5].

Hesperidin is one of flavanone glycoside found naturally in citrus fruits that exhibited antioxidant property by scavenging of free radicals [6,7]. It has anti-inflammatory, neuroprotective effects and modulation of drug-metabolizing enzymes without toxicity [8–10].

In experimental studies, hesperidin reduced cholesterol level and blood pressure in rats [11,12]. It exhibited a protective effect against sepsis, antiproliferation and anti-metastatic activities by suppressing the activity of matrix metalloproteinase-9 (MMP-9) [13,14].

Tiger nut tubers (*Cyperus esculentus*) are edible with a sweet and nutty flavor contains high qualities of fats and gluten with free cholesterol [15,16]. They are rich with vitamins E, C and other minerals such as potassium, calcium and magnesium which are necessary for bones and muscles [17]. Tiger nut contains alkaloids, saponins and tannins which exhibits antimicrobial and anti-inflammatory properties [18,19].

The objective of the present study is to evaluate the efficacy of hesperidin and tiger nut against toxicity of DMBA in female rats.

2. Materials and methods

Seventy-two healthy adult female virgin Sprague Dawley rats (70 ± 10 g), average 6 weeks old, purchased from the Egyptian Holding Company for Biological Products and Vaccines (Cairo, Egypt) were used. The animals were maintained under standard conditions of light, ventilation, temperature, and humidity and allowed to free access to standard pellet diet and tap water. All animals procedures were carried out in accordance with the Ethics Committee of the National Research Centre conformed to the "Guide for the care and use of Laboratory Animals" published by the US National Institutes of Health.

Abbreviations: HES, hesperidin; TN, tiger nut; DMBA, 7,12-dimethylbenz(a) anthracene; CEA, carcinoembryonic antigen; TSA, total sialic acid; CO, protein carbonyl; MDA, malondialdhyde; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; GSH, reduced glutathione; SOD, superoxide dismutase; CAT, catalase; GSH-Px, glutathione peroxidase.

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2.1. Animal groups

After an acclimation period of 2 weeks, rats were divided into 6 groups (12 rats/group). Group I: control; Group II: rats received HES (50 mg/Kg/day) via oral gavages [20]; GroupIII: rats fed on a standard diet mixed with 25% whole powder of tiger nut, TN [19]; Group IV: DMBA treated group were received 10 mg DMBA/rat [4] via oral gavages once a week for four weeks; Group V: rats treated with HES + DMBA where rats were received HES (50 mg/Kg/day) during 14 days before treating with DMBA and for 105 days after treatment; and Group VI: rats treated with TN + DMBA where rats were received TN (25% of diet) during 14 days before treating with DMBA and for 105 days after treatment.

At the end of the experiment, animals were sacrificed, under anesthesia of ether and blood was collected by heart puncture and centrifuged at 3000 rpm for 15 min to separate the serum and stored at −20 °C. Serum was used for the determination of TSA, CEA, progesterone, estradiol, CO, MDA, GSH, SOD, GSH-Px, CAT, ALT, AST, LDH, urea and creatinine. Mammary, liver and kidney tissues of each animal were taken for histopathological examination.

2.2. Biochemical analysis

2.2.1. Tumorigenicity biomarkers assays

Assay of CEA, Enzyme-linked immunosorbent assay (ELISA) was used using kit purchased from Elabscience Biotechnology Co.Ltd, according to Uotila et al. [21], TSA was determined according to the method described by Plucinsky et al. [22].

2.2.2. Hormonal assays

ELISA procedure was used for quantitative determination of Estradiol and progesterone concentrations using a kits purchased from Sigma Chemical Company (St Louis, MO, USA) according to Tietz [23,24].

2.2.3. Oxidative stress biomarkers assays

Lipid peroxidation was evaluated by measuring MDA levels according to the method of Yoshioka et al. [25]. Protein oxidation was evaluated by measuring protein carbonyl (CO) content as described by Levine et al. [26]. The activities of SOD, CAT, GSH-Px and Reduced glutathione content were determined according to the method of Minami and Yoshikawa [27], Aebi [28], Paglia and Valentine [29] and Beutler et al. [30] respectively.

2.2.4. Liver function biomarkers assays

ALT, AST and LDH were evaluated according to Sherwin [31] and Kachmar and Moss [32] respectively.

2.2.5. Kidney function biomarkers assays

Creatinine and Urea were evaluated according to the method of Tietz, [33,34].

2.2.6. Histopathological investigation

The mammary, liver and kidney tissues were fixed in 10% buffered formalin, embedded in pure paraffin wax (melting point of 58 °C) and then mounted into blocks and left at 4 °C until the time of use. The paraffin blocks were sectioned on the microtome at thickness of 5 mm and mounted on slides then left in the oven at 40 °C for dryness. The slides were deparafinized in xylol then immersed in alcohol (90–95%). The ordinary hematoxylin and eosin stain were used [35].

2.3. Statistical analysis

The Statistical Package for the Social Sciences (SPSS/PC) computer program was used for statistical analysis of the results. Data were analyzed using one way analysis of variance (ANOVA) followed by Post Hoc to determine significant differences between means. The data were expressed as mean ± standard deviation (SD). Differences were considered significant at $P \leq 0.05$.

3. Results

3.1. Biochemical results

No rats died in groups I–III, 6 of 12 rats died in Group IV. In Group V, 3 of 12 rats died, while in Group VI, 5 of 12 rats were died. There was an increase in the body weight of rats in all groups at the time of killing compared to the beginning of experiment, with percentage changes 69.3%, 122%, 87.4%, 18.7%, 57.6% and 38.5% for groups I–VI, respectively, (Table 1). At the end of the experiment, groups IV–VI were significantly decreased in body weight compared to control. There was a significant increased in body weight of rats group V compared with group IV. There was no significant change in body weight of rats in Group VI and group IV (Table 1).

Serum levels of CEA, TSA, progesterone and estradiol were significantly increased in groups IV–VI when compared with control. There were no significant changes in the levels of TSA, progesterone and estradiol between group IV and group VI, while their levels were significantly decreased in Group V when compared to group IV. Serum CEA level showed significantly decreased in group V and group VI when compared to group IV (Table 2).

Serum levels of both CO and MDA were significantly increased in groups IV–VI when compared with control. Their levels were significantly decrease in group V ($p < 0.001$) when compared to group IV, while there were no significant differences between groups IV and VI. Serum SOD, CAT, GSH-Px activities and the level of GSH were significantly decreased in groups IV–VI when compared to control, while they were significantly increased in group V when compared to group IV (Table 2), there were no significant differences between groups IV and VI.

Table 1
Mean ± S.D. of body weight at the start and end of the experiment in different groups.

			Control (Group I)	HES (Group II)	TN (Group III)	DMBA (Group IV)	HES-DMBA (Group V)	TN-DMBA (Group VI)
Body weight (g)	At start	N	12	12	12	12	12	12
		Mean ± S.D	129.3 ± 16.16	108.6 ± 13.29	117.8 ± 13.37	122.8 ± 14.18	114.6 ± 14.67	115.3 ± 13.76
	At sacrifice	N	12	12	12	6	9	7
		Mean + S.D	218.9 ± 28.5	241.4 ± 29.74	220.8 ± 24.19	145.8 ± 21.5	181 ± 25.29	159.7 ± 26.11
		P value		0.005[a]	0.819[a]	<0.001[a]	<0.001[a]	<0.001[a] 0.204[b]
Percentage change			↑69.3%	↑122%	↑87.4%	↑18.7%	↑57.6%	↑38.5%

Significant at *P* value ≤ 0.05. (TN)––Tiger nut; (HES)––Hesperidin; (DMBA)––7,12-Dimethylbenz (a) anthracene.
[a] *P* value versus control group (group I).
[b] *P* value versus DMBA group (group I).