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Protective effect of Hesperidin and Tiger nut against Acrylamide toxicity in female rats

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ABSTRACT

Phytochemicals that have antioxidant effect play important role in protection against several diseases in humans. This study was carried out to evaluate the efficacy of hesperidin and tiger nut against the early changes that may be related to the toxicity of acrylamide in female rats. 72 Sprague Dawley female rats were divided into six groups (12 rat/group): control group (I); hesperidin (HES) treated group (II); tiger nut (TN) treated group (III); Acrylamide (ACR) treated group (IV); HES-ACR treated group (V); and TN-ACR treated group (VI). There was a significant increase in the levels of serum carcino embryonic antigen (CEA), malondialdehyde (MDA), protein carbonyls (CO), ALT, AST, LDH, urea and creatinine while no significant changes of serum total sialic acid, progesterone (prog) and estradiol (E2) levels, and significant decreases of body weights, catalase (Cat) activity, superoxide dismutase (SOD) activity, reduced glutathione (GSH) level, and glutathione peroxidase (GSH-Px) activity of ACR treated group compared with the control. Our results suggested that supplementation of a diet with hesperidin provided antioxidant defense more significant than tiger nut against the toxicity of ACR in breast, liver and kidney tissues.

1. Introduction

Acrylamide (ACR) is one of the most important contaminant in the environment. It is a water-soluble α,β-unsaturated amide and used extensively to manufacture polyacrylamides (EPA, 2009). It may be formed in foods, particularly plant-based foods rich in carbohydrate during cooking, frying, baking or roasting, at temperatures of 120 °C or higher (Rommens et al., 2008). Orally consumed ACR is absorbed into the circulation, then distributed to various organs, and reacts with DNA, neurons, hemoglobin, and essential enzymes causing several toxic effects (Baum et al., 2008; Rayburn and Friedman, 2010).

ACR is not genotoxic by itself but becomes activated to its primary epoxoide genotoxic metabolite glycidamide (GA) via epoxidation (Baum et al., 2008), by CYP2E1 which leads to the formation of GA-DNA and hemoglobin adducts (Doerge et al., 2005; Ghanayem et al., 2005). ACR has been reported to be carcinogenic in experimental animals (Hogervorst et al., 2010). Epidemiologic data are limited on the relation between dietary acrylamide intake and the risk of breast cancer in humans (Hogervorst et al., 2010).

Flavonoids are polyphenolic compounds and constitute an important group of antioxidants, which can directly quench free radicals and inhibit enzymes of oxygen reduction pathways (Kalpana et al., 2011). Hesperidin, a flavanone-type flavonoid, is found in sweet orange, lemon and by-product of citrus fruits (Wilmsen et al., 2005). It has anti-inflammatory, anti-allergic, hypolipidemic, vaso-protective and anti-carcinogenic actions (Kalpana et al., 2011). One of the main characteristics of HES is its radical scavenging property, which results in normalization of the redox profile of treated cells. In this regard, HES-treated cells showed less reactive oxygen species (ROS) and improved the antioxidant system (Roohbakhsh et al., 2015).

Tiger nut (*Cyperus esculentus L.*) is a lesser-known vegetable that produces sweet nut-like tubers known as “earth almonds” (Coşkuner et al., 2002). They are rich in sucrose (17.4–20.0%), fat (25.5%), protein (8.0%) and fatty oil (25–27%) (Kordyias, 1990), also rich in mineral contents such as sodium, calcium, potassium, magnesium, zinc and traces of copper (Oladele and Aina, 2007), vitamins E and C (Belewu and Belewu, 2007).

Tiger nut exhibit anti-inflammatory properties upon inflammation and immune-stimulatory effects (Salem et al., 2005). Moreover, Agbai and Nwanegwo (2013) reported that oral administration of tiger nut improve reproductive functions in adult male albino rats by altering the plasma levels of gonadotropins, testosterone and sperm functions in a

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

			Control (Group I)	HES (Group II)	TN (Group III)	ACR (Group IV)	HES-ACR (Group V)	TN-ACR (Group VI)
Body weight (g)	At start	N	12	12	12	12	12	12
		Range	105–154	89–130	95–138	102–136	102–148	104–136
		Mean ± S.D	129.3 ± 16.16	108.6 ± 13.29	117.8 ± 13.37	120.3 ± 9.27	123.5 ± 13.24	119.9 ± 10.24
	At sacrifice	N	12	12	12	11	12	11
		Range	170–260	194–284	186–260	135–200	160–240	170–241
		Mean + S.D	218.9 ± 28.5	241.4 ± 29.74	220.8 ± 24.19	159.3 ± 20.8	198.9 ± 24.8	201 ± 23.20
		P [(a)] value		0.005	0.819	< 0.001	0.013	0.031
		P [(b)] value		< 0.001	< 0.001		< 0.001	< 0.001
		P [(c)] value		< 0.001	0.007			0.782
Percentage change				↑69.3%	↑122%	↑87.4%	↑32.4%	↑61.1%	↑67.6%

Significant at P value ≤ 0.05.
$P^{(a)}$ value versus control group (group I).
$P^{(b)}$ value versus ACR treated group (group IV).
$P^{(c)}$ value versus HES-ACR treated group (group VI).
(TN)—Tiger nut − (HES)—Hesperidin − (ACR)–Acrylamide.

dose-dependent manner. But, there is awareness for increased utilization of tiger nut (Ade-Omowaye et al., 2008; Ukwuru et al., 2008).

The objective of the present study is to evaluate the efficacy of both Hesperidin and Tiger nut against toxicity of Acrylamide in female rat.

2. Materials and methods

2.1. Materials

Acrylamide with purity ≥ 99% and Hesperidin (HES) with purity ≥ 90%were purchased from Sigma Chemical Com. (St Louis, MO, USA) and Tiger nut (chufa) tubers (*Cyperusesculenuts*L) were obtained from the local market at Elfayoum city, Egypt.

2.2. Animals

Seventy two healthy adult female 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. All animals were housed in standard conditions of light, humidity, and temperature with food in pellets and tap water available. Procedures were carried out with the Ethics Committee of the National Research Centre and according to the "Guide for the care and use of Laboratory Animals" published by the US National Institutes of Health.

2.3. Study design

After an acclimation period of 2 weeks, the rats were randomly divided into 6 groups (12 rats/group). Group I: control; Group II: rats received HES (50 mg/Kg/day) via oral gavages (Hosseinimehr and Nemati, 2006); GroupIII: rats fed on a standard diet mixed with 25% whole powder of tiger nut (Salem et al., 2005); Group IV: ACR treated group received 15 mg acrylamide/kg/day (Klaunig and Kamendulis, 2005) via oral gavages during 4 months; Group V: rats treated with HES + ACR where rats were received HES (50 mg/Kg/day) during 14 successive days and then treated with ACR for 4 months; and Group VI: rats treated with TN + ACR where rats were received TN (25% of diet) during 14 successive days and then treated with ACR for 4 months.

At the end of the study, the animals were fasted overnight, then sacrificed using ether anesthesia and blood was collected by cardiac 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.

2.4. Biochemical analysis

2.4.1. Tumorigenicity biomarkers assays

Assay of CEA, Enzyme-linked immunosorbent assay (ELISA) was used and a kit purchased from Elabscience Biotechnology Co. Ltd, according to Uotila et al. (1981). TSA was determined according to the method described by Plucinsky et al. (1986).

2.4.2. Hormonal assays

ELISA procedure was used for quantitative determination of Estradiol and progesterone concentrations using a kit purchased from Sigma Chemical Company (St Louis, MO, USA) according to Tietz (1995a,b).

2.4.3. Oxidative stress biomarkers assays

MDA levels is used for determination of a non-specific lipid peroxidation, it was measured according to the method of Yoshioka et al. (1979). Protein oxidation was evaluated by measuring protein carbonyl (CO) content as described by Levine et al. (1990). The activities of SOD, CAT, GSH-Px and Reduced glutathione were determined according to the methods of Minami and Yoshikawa (1979), Aebi (1983), Paglia and Valentine (1967) and Beutler et al. (1963) respectively.

2.4.4. Liver function biomarkers assays

ALT and AST and LDH enzymes were evaluated according to Sherwin (1984) and Kachmar and Moss (1976) respectively.

2.4.5. Renal function biomarkers assays

Creatinine and Urea were evaluated according to assay of Tietz (1986, 1990).

2.5. Histopathological investigation

The mammary, liver and kidney tissues were taken and 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 clean glass slides then left in the oven at 40 °C for dryness. The slides were deparafinized in xylol then immersed in descending series of alcohol concentration (90–50%). The ordinary hematoxylin and eosin stain were used (Drury and Wallington, 1980).

2.6. Statistical analysis

The Statistical Package for the Social Sciences (SPSS/PC) computer program was used for statistical analysis of the results. Data were

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