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ORIGINAL ARTICLE

Antioxidant activity and hepatoprotective effect of pomegranate peel and whey powders in rats

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Abstract The antioxidant activity of pomegranate peel powder (PPP) and whey powder (WP) was evaluated, their hepatoprotective effect of each alone or in combination (PPWP) at equal levels was also evaluated in Wistar rats against carbon tetrachloride (CCL_4) induced liver injury. The hepatoprotective activity was assessed using various biochemical parameters and histopathological studies. The results indicated that both PPP and WP exhibited antioxidant activity. Also, rats fed on diets supplemented with 10% PPP, 10% WP or 10% of their mixture (PPWP) for 28 days showed a potential hepatoprotective effects compared to liver injury control group (IC). They succeeded to restore the biochemical parameters and improved the histological alteration of the liver. This improvement was pronounced in the group received PPWP. It could be concluded that whey powder should be incorporated with pomegranate peel powder when used as ingredients in functional foods for people suffering from liver diseases.

Introduction

The liver is an amazingly complex organ which virtually affects every physiological process of the body. Our body is protected from various injurious substances and toxic metabolic byproducts by the liver, which has been absorbed from intestinal tract. Xenobiotics are often reported to cause potential hepatic damage.

Liver diseases constitute a major problem of worldwide proportions. Carbon tetrachloride (CCl_4) is a well known hepatotoxin that is widely used to induce acute-toxic liver injury in a large range of laboratory animals (Sahu, 2007). The toxic effects of CCl_4 are the consequences of production of free radicals. These reactive free radicals initiate cell damage through two major mechanisms of covalent binding and lipid peroxidation. Lipid peroxidative process has been shown to augment collagen synthesis and fibrosis. Hence, antioxidants may play a role in inhibiting the liver injury induced during cell damage (Slater, 1984).

Foods rich in natural antioxidants have been proposed as a tool to prevent and cure liver damage (Morisco et al., 2008). The pomegranate is one of the important dietary sources of

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antioxidant phenolics Murthy et al. (2002) and Ozgen et al. (2008). Pomegranate peel is recognized for its many health-promoting qualities and apparent wound-healing properties (Chidambara et al., 2004), anticancer property (Jeune et al., 2005), antiatherosclerotic and antioxidative capacities (Tzulker et al., 2007).

Whey is a general term that typically denotes the translucent liquid part of milk that remains following the process (coagulation and curd removal) of cheese manufacturing. Whey also is one of two major protein groups of bovine milk, accounting for 20% of the milk while casein accounts for the remainder (Haffman and Falvo, 2004). Whey proteins are increasingly being used for nutritional purposes because they consistently received high score in traditional tests of protein quality. The content of total essential amino acids and branched-chain amino acids is superior in whey protein than in most dietary proteins (Helaine et al., 2001).

Because milk whey is an abundant by-product in cheese manufacture (9 l of whey are produced from 10 l of milk during cheese making (Manso and Lopez-Fandino, 2004) it is inexpensive source of high nutritional quality protein for uses as a physiologically functional food ingredient. So, the use of whey protein in formulating products is increasing due to the nutritional and health benefits attributed to these proteins (Onwulata et al., 2004). These proteins exhibit potent antioxidant activities by inducing cellular biosynthesis of glutathione (GSH), which can boost the immune system and detoxify potential carcinogens and its role in cancer treatment was reported by (Counous, 2000; Haug et al., 2007 and Bayram et al., 2008). In an earlier work, Eason et al. (2004) showed that whey powder reduced mammary tumor.

The objectives of the present study were to investigate the potential hepatoprotective of pomegranate peel powder (PPP) as a natural source for antioxidant and whey powder (WP) which is using in formulating products as well as their combination (PPWP) against CCL_4 induced liver injury in Wister rats.

Materials and methods

Materials

The full ripe pomegranate fruits (*Punica* granatum L.) wonderful variety was obtained from the local market. Whey powder containing 11% protein, was purchased from Green Land for Food Industries 10^{Th} of Ramadan City, Egypt. While, Commercial kits used for determining alanine aminotransferase (ALT), aspartate aminotransferase (AST), malondialdehyde (MDA) and reduced glutathione (GSH) were obtained from Biodiagnostic Co. Dokki, Egypt. Carbon tetrachloride, sodium carbonate and methanol were obtained from El-Gomhoreya Co., Cairo, Egypt. 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and Folin–Ciocalteus phenol reagent were purchased from Sigma–Aldrich Inc. (St. Louis, MO, USA).

Animals

Wistar rats with an average weight of 140 g were obtained from the Organization of Biological Products and Vaccines (Helwan Farm, Cairo, Egypt).

Preparation of pomegranate peels powder (PPP)

Pomegranate fruits were washed by distilled water then peeled and their edible portions were carefully separated. The peels were air dried in a ventilated oven at 40 °C for 48 h and ground to a fine powder.

Methods

Total phenolic content

The total phenolic content of the PPP was methanolic extracted and determined according to Singleton et al. (1999). The reaction mixture contained 0.5 ml of Folin–Ciocalteu reagent, 0.5 ml of 7.5% Na_2CO_3 and 0.5 ml of the PPP extract. The absorbance was measured at 765 nm after the mixture was stirred and allowed to stand for 30 min. The results were expressed as gallic acid equivalents (GAE) per gram PPP (mg GAE/g powder) by reference to the gallic acid standard curve.

DPPH radical scavenge activity %

The ability of the PPP methanolic extract to scavenge 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) free radicals were determined by the method described by Singh et al. (2002). While, the free radical scavenging activity of the whey powder aqueous extract was determined according to the method of Kennedy et al. (1995)

Biological experiment

The experiment was conducted on forty male Wistar rats, they were housed in screen-bottomed aluminum cages in rooms maintained at 25 ± 1 °C with alternating cycles of light and dark of 12 h duration. The animals were fed on basal diet according to AIN-93 guidelines (Reeves et al., 1993) and were provided with water ad libitum during the experimental period.

The animals were randomly divided into five groups with eight rats in each group. Group one was reserved as normal control (NC), groups two–five animals were administrated interperitoneal (IP) injection with single dose of 0.5 ml/kg body weight (2:5 v/v CCl_4/paraffin). Group two kept as injury control (IC), group three received basal diet with 10% pomegranate peel powder (PPP), group four received basal diet with 10% whey powder (WP) and group five received basal diet with a combination of 5% pomegranate peel and 5% whey powder (PPWP) for 28 days. The composition of the experimental diets is shown in Table 1, the changes in body weight

Table 1 Compositions of the experimental diets (%).

Component %	NC	IC	PPP	WP	PPWP
Corn starch	65.2	65.2	55.2	55.2	55.2
Casein	18	18	18	18	18
Corn oil	7	7	7	7	7
Cellulose	5	5	5	5	5
Salt mixture	3.5	3.5	3.5	3.5	3.5
Vitamin mixture	1	1	1	1	1
Choline bitartrate	0.3	0.3	0.3	0.3	0.3
Pomegranate peel powder	–	–	10	–	5
Whey powder	–	–	–	10	5