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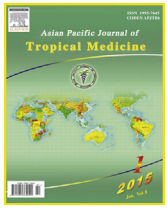

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Pistacia chinensis: Strong antioxidant and potent testicular toxicity amelioration agent

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ABSTRACT

Objectives: To evaluate *in vitro* and *in vivo* antioxidant potency of *Pistacia chinensis* (*P. chinensis*) bark and leaves extracts along with its protective role against CCl_4 induced toxicity in testis of the rat.

Methods: Various *in vitro* models such as DPPH, ABTS, hydrogen peroxide, superoxide, hydroxyl and nitric oxide scavenging activities, anti-lipid peroxidation activity, phospho-molybdenum activity, β carotene bleaching assay was used for analysis of antioxidant potential. Experimental groups for *in vivo* study were: Group Ⅰ (control) untreated, Group Ⅱ (Vehicle control), Group Ⅲ (1 mL/kg b.w 30% CCl_4), Group Ⅳ (1 mL/kg b.w CCl_4 + Silymarin), Group Ⅴ (200 mg/kg b.w PCBE + CCl_4), Group Ⅵ (400 mg/kg b.w PCBE + CCl_4) and Group Ⅶ (400 mg/kg b.w PCBE alone).

Results: *In vitro* antioxidant assays displayed significant results and the highest activity was not specified to a specific extract. However, ethyl acetate extract of bark (PCBE) showed highest results in most of the antioxidant assays *i.e.* beta-carotene bleaching, hydroxyl radical scavenging, ABTS, lipid peroxidation and superoxide radical scavenging activity. On this base, this fraction was selected for *in vivo* antioxidant experiment. Testis tissues were analyzed to observe the protective effects of PCBE on antioxidant enzymes; catalase, superoxide dismutase, peroxidase, glutathione-S-transferase, glutathione reductase, glutathione peroxidase and quinone reductase activities and glutathione (GSH) as well as nitrite content. Profile of plasma testosterone was also compared to various treatments. Observation suggests a protective role of *P. chinensis* against CCl_4 induced toxicity.

Conclusions: It is concluded that some bioactive antioxidants of *P. chinensis* bark might be a good source to isolate the potent antioxidant components.

1. Introduction

Oxidative stress occurs as an outcome of an imbalance between the formations of reactive oxygen species (ROS) and the available antioxidant defense against them. ROS are produced consistently by many physiologic and metabolic processes [1,2]. Spermatogenesis is an extremely coordinated process which takes place in the seminiferous tubules of the testis [3]. Spermatozoa are very sensitive to ROS as their plasma membrane is comprised of polyunsaturated fatty acids (PUFA), which can be easily oxidized and they also lack cytoplasm to produce a robust preventive and repair mechanism against ROS [4].

Although carbon tetrachloride (CCl_4) is known to be hepatotoxic as well as nephrotoxic to humans and experimental animals. It is still being used in the fumigation of grains, in dry cleaning, in filling fire extinguishers, and as an insecticide. The mechanism of CCl_4-induced liver injury is well studied in the rat model. Accidental ingestion of CCl_4 results in the accumulation of CCl_4 in the testes and causes damage to this organ. Testes have a great affinity for CCl_4 and contain cytochrome P450 which activates converse of CCl_4 to toxic metabolites. The initial step in the tissue injury induced by CCl_4 is its cytochrome

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P450-mediated formation of trichloromethyl radical (CCl_3) and trichloromethyl peroxyl (CCl_3OO) free radicals. The overproduction of CCl_3 free radicals initiates membrane lipid and protein oxidation, eventually leading to various pathological changes. Free radical reactions have been implicated in the pathology of many disease conditions like atherosclerosis, ischemic heart diseases, the aging process, inflammation, diabetes, immunosuppression, and neurodegenerative disease. Inadequate antioxidant defenses lead to disturbances in redox homeostasis causing damage to lipids, proteins, carbohydrates, and DNA. Exogenous antioxidants such as polyphenolic compounds in medicinal plants may constitute an antioxidative defense by scavenging free radicals and possibly increase the longevity of biological systems in such conditions [5].

In the recent scientific progress throughout the world, the medicinal qualities of the plants have been studied due to their fewer side effects, potent antioxidant activities and commercial viability [6]. Phenolics and flavonoids compounds of plants show manifold biological benefits by exerting antioxidative scavenging role [7].

The present study aim was to determine the antioxidant potential of *Pistacia chinensis* (*P. chinensis*) plant by using multiple *in vitro* antioxidant assays and *in vivo* models. *In vitro* studies were conducted to evaluate the antioxidant capacity of the leaves and bark. The potent antioxidant component was evaluated by *in vivo* experiment against CCl_4 induced toxicity in rat testis.

2. Materials and methods

2.1. Plant collection and extraction

A collection of *P. chinensis* plant leaves and bark samples were carried out from the surroundings of Quaid-i-Azam University, Islamabad in September 2012. The identification of the sample was carried out at Herbarium of Pakistan, Quaid-i-Azam University Islamabad. The plant leaves and stem bark were chopped into pieces, removed the dust particles and then shade dried at 25 °C for about two weeks. To prepare the dried powder of sample Willy Mill of 60-mesh size was used and a then powdered sample of leaves and bark were used separately for solvent extraction. One kg powder sample of leaves and bark was extracted two times with 2 L of crude methanol at 25 °C for 48 h. Filtration was carried out using Whatman No. 1 filter paper and rotary evaporator (Panchun Scientific Co., Kaohsiung, Taiwan) was used to concentrate the filtrate under reduced pressure at 40 °C. The same procedure was repeated to obtain the ethanol extract of each sample by using 80% ethanol instead of methanol. Both the extract were stored at 4 °C for *in vitro* and *in vivo* experiments.

2.2. In vitro antioxidant assays

2.2.1. 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity

The methodology of Sirajuddin [8] was followed to measure DPPH radical scavenging of methanol extract of *P. chinensis*. DPPH solution was prepared by mixing 12.5 mg of DPPH in 50 mL of methanol. The optical density of stock solution was measured at 517 nm. The absorbance of 0.9 was maintained by diluting the DPPH solution if needed with methanol. An aliquot of 100 μL of a test sample of different concentrations with (30–500) μg/mL prepared in methanol was added to 1 mL of DPPH solution. After vigorous shaking Eppendorf tubes were incubated at 37 °C for 20 min. The decrease in absorbance was determined at 517 nm. With the help of following formula of Sirajuddin [8], free radical activity was determined;

$$\text{DPPH scavenging activity}(\%) = \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs Control}} \times 100$$

2.2.2. Superoxide radical scavenging activity

Superoxide radical scavenging activity was carried out following the method of Saeed, Khan [9]. An aliquot of 150 μL of test sample of different concentrations with (30–500) μg/mL methanol was added to 250 μL of 50 mM potassium phosphate buffer (pH 7.6), 150 μL of 50 mM riboflavin, 126 μL of 20 mM PMS (potassium persulphate), 50 μL of 0.5 mM NBT (nitro blue tetrazolium). The reaction mixture was illuminated with a fluorescent lamp for 30 min to start a reaction. Using spectrophotometer optical density was determined at 560 nm. Ascorbic acid was used as a standard. Using the following formula [9], superoxide anion scavenging activity was determined;

$$\text{Super oxide scavenging activity}(\%) = \left(1 - \frac{\text{Abs Sample}}{\text{Abs Control}}\right) \times 100$$

2.2.3. Hydroxyl radical scavenging activity

The methodology of Halliwell, Gutteridge [11] was adopted to measure the hydroxyl radical scavenging activity of extracts of *P. chinensis*. An aliquot of 100 μL of test sample of different concentrations with (30–500) μg/mL prepared in methanol added to 100 μL of 100 mM $FeCl_3$ and 100 mM EDTA (1:1 ratio), 50 μL of 200 mM H_2O_2 and 250 μL of 2.8 mM 2-deoxyribose (prepared in 50 mM phosphate buffer). Fenton reaction was initiated by adding 100 μL of ascorbate (300 mM). After incubation for 1 h at room temperature, 500 μL of 1% thiobarbituric acid (prepared in 50 mM NAOH) and 500 μL of 2.9% TCA was added and reaction tubes were boiled on a water bath for 10 min. Optical density was determined at 532 nm after cooling of the reaction solution. Following formula [10] measured the hydroxyl radical scavenging activity;

$$\text{Hydroxyl radical scavenging activity}(\%) = \left(1 - \frac{\text{Abs Sample}}{\text{Abs Control}}\right) \times 100$$

2.2.4. H_2O_2 scavenging activity

The methodology of Shah, Khan [11] was followed to measure hydrogen peroxide scavenging activity of extracts of *P. chinensis*. An aliquot of 100 μL of a test sample of different concentrations with (30–500) μg/mL prepared in methanol was added to 300 μL of 50 mM phosphate buffer and 600 μL of 2 mM H_2O_2 (prepared in 50 mM phosphate buffer). Mixing was done by shaking and placed at room temperature for 15 min. Optical density observed at 230 nm using phosphate buffer as a blank. By following formula [12], scavenging of H_2O_2 activity was determined;

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