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Original article

Honey has a protective effect against chlorpyrifos-induced toxicity on lipid peroxidation, diagnostic markers and hepatic histoarchitecture

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Abstract

Introduction: Currently, there is increasing interest in the identification of natural sources of antioxidants that have minimal side effects and can be used as preventive medicine. In this study, the protective role of honey against oxidative damage and hepatotoxicity induced by sub-chronic exposure to chlorpyrifos (CPF) was investigated.

Methods: Female Wistar rats ($n = 24$) were randomly divided into the following four groups: (1) control, (2) honey-treated (3.0 g/kg), (3) CPF-treated (5.4 mg/kg) and (4) honey (3.0 g/kg) + CPF (5.4 mg/kg) treatments. All of the doses were administered daily via oral gavage for 4 weeks.

Results: Oral exposure to CPF caused hepato- and nephrotoxicity as indicated by the marked elevation in the serum hepatic marker enzymes (the transaminases and alkaline phosphatase activities), the total bilirubin, total cholesterol, triglycerides, urea, creatinine and hepatic lipid peroxidation levels, whereas the serum total protein level was significantly lower compared with the control. A significant decrease in the body weight gain and an increase in the absolute and relative liver weights were observed in the CPF-treated animals compared with the control. The biochemical alterations observed were accompanied by histopathological changes marked by the appearance of degenerative necrosis, congestion, inflammation and edema in the liver section. However, co-treatment with honey ameliorated the changes in the investigated biochemical parameters and the changes in the body and liver weights as revealed by the inhibition of lipid peroxidation and the improvement observed histopathologically.

Conclusions: The present study suggests that honey has a protective potential for the alleviation of hepatic and renal toxicities induced by CPF.

Keywords: Honey; Chlorpyrifos; Hepatotoxicity; Lipid peroxidation; Histoarchitecture

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Introduction

Pesticides are chemical substances used to control pests, and exposure to these substances has become a major public health issue due to their indiscriminate and extensive utilization in agricultural and domestic settings [1,2]. Among the most common pesticides, organophosphate (OP) insecticides pose the greatest risk and are a major cause of morbidity and mortality in the third world countries [3,4]. Residual amounts of OP pesticides have been detected in several types of domestic vegetables, grains and water sources [1,5,6].

Chlorpyrifos (CPF) [O,O-diethyl O-(3,5,6-trichloro-2-pyridinol) phosphorothionate] belongs to the OP insecticides and is widely used for agricultural and public health applications [7]. The biological activation of CPF to its potential toxic form, CPF-oxon, is achieved through oxidative desulfuration by specific cytochrome P450 oxidases present primarily in the liver, which can lead to the inhibition of acetylcholinesterase, a major cause of CPF toxicity [8,9]. Recent reports have revealed that one of the molecular mechanisms of CPF poisoning is the generation of cytotoxic reactive oxygen species (ROS), as evidenced by the enhanced level of thiobarbituric acid reactive substances (TBARS) and the consequent oxidative stress in their target tissues [10,11]. ROS, including superoxide, hydrogen peroxide and hydroxyl radicals, produced during the metabolic reactions interact with cellular proteins, lipids and DNA, resulting in alterations in cellular functions [12,13]. Moreover, CPF, which is lipophilic, binds extensively to biological membranes, especially phospholipid bilayers, and may cause membrane damage by inducing lipid peroxidation (LPO) [8,14,15]. Because the generation of free radicals is expected to induce organ toxicities, including hepatotoxicity, supplementation of antioxidants is considered as an alternative to chelating therapy [11,16]. Accordingly, to date, safe and effective natural products that may confer free radical scavenging activities are in global demand as an additional armamentarium to protect against oxidative damage [11,17].

Honey, a natural product of honey bees, is a mixture of sugars, proteins, minerals and vitamins and many other bioactive compounds [18,19]. Recent studies have indicated that honey is an important health-promoting dietary supplement and have revealed information on antioxidant and non-peroxide dependent properties [20,21]. The antioxidant constituents of honey include phenolics, flavonoids, ascorbic acid, proteins [22], certain enzymes (glucose oxidase, catalase, and peroxidase), carotenoids and the products of the Maillard reactions [23,24]. However, honey is a unique compound because of its highly variable composition, and its phenolic composition is largely dependent on the floral and geographical sources in which a correlation between its content and antioxidant activity has been successfully established [18,25,26].

Recently, the antioxidant properties of honey collected from Sundarban, Bangladesh, which is the largest mangrove forest in the world, have been reported to contain among the highest levels of phenolics (688.50 mg Gallic acid/kg), total flavonoids (155 mg Catechin/kg) and ascorbic acid [19]. In addition, the presence of a number of phenolic acids, including gallic, benzoic, caffeic, and trans-cinnamic, along with chlorogenic acids and flavonoid compounds, such as catechin, kaempferol and naringenin, in honey [25] further confirms its scientific importance to human health. However, currently, insufficient information is available on the protective effect of honey against chronic or sub-chronic OP poisoning. Therefore, the present study was designed to investigate the protective roles of honey against CPF-induced hepatotoxicity in an experimental rat model.

Materials and methods

Chemicals

The chlorpyrifos insecticide (purity 99.0%) was obtained from Dr. Ehrenstorfer GmbH, Augsburg, Germany. Florisil (magnesium silicate, mesh size 60–100) was purchased from Sigma, St. Louis, MO, USA and 1,1,3,3-tetraethoxy propane was purchased from Nacalai Tesque, Japan. The solvents, such as n-hexane (Merck, Germany), ethyl acetate and diethyl ether (BDH, England), and all of the chemicals were of analytical grade, whereas acetonitrile (ACN) (Scharlau, EU) was of high performance liquid chromatography (HPLC) grade.

Honey samples

The multi-floral honey samples were collected from Sundarban, Bangladesh in May 2013 during the summer season.

Animals

Healthy female Wistar rats at the ages of 16–24 weeks (160–180 g) were used in this study. The animals were bred and reared in the animal house facility of the Department of Biochemistry and Molecular Biology of Jahangirnagar University at a constant room temperature ($23 \pm 2\,^{\circ}C$) and in an environment with the humidity ranging from 40 to 70%. The rats were housed in clean plastic cages and received a natural 12-h day-night cycle. The rats were provided with a standard laboratory pellet diet and water *ad libitum*. The experiments were conducted according to the ethical guidelines approved by the Bangladesh Association for Laboratory Animal Science. The experimental protocol was approved by the Biosafety, Biosecurity & Ethical Committee of Jahangirnagar University (Approval No. BBEC, JU M 2013.2b).

Animal treatment schedule

All of the rats were acclimatized 1 week prior to the experiment. The rats were divided into the control ($n = 6$) and treatment groups ($n = 18$). The control group (Group A) received only corn oil at a dose of 0.2 ml per rat, which was given only once a day via oral gavage. The rats in the treatment group were further divided into three groups. In Group B, the rats ($n = 6$) were treated only with honey at a dose of 3 g/kg per day via oral gavage. The dose of honey used in this study was based on previous studies [27,28]. In Group C, the rats ($n = 6$) were treated only with CPF in corn oil via gavage once daily at a dose of 5.4 mg/kg [~1/25