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Amelioration of paracetamol hepatotoxicity and oxidative stress on mice liver with silymarin and *Nigella sativa* extract supplements

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ABSTRACT

Objective: To evaluate the ameliorator property of silymarin or/and *Nigella sativa* (*N. sativa*) extract against *N*-acetyl-*p*-aminophenol (APAP)-induced injury in male mice at the biochemical, histological and ultrastructural levels.

Methods: The mice were divided into seven groups (10/group). The first group was served as control. While, the second group was treated with dose of APAP. The third and fourth groups were treated with silymarin alone and *N. sativa* extract alone respectively. The fifth and sixth groups were treated with combination of APAP with silymarin and APAP with *N. sativa* extract respectively. The seventh group was treated with combination of both ameliorative compounds (silymarin and *N. sativa* extract) with APAP and all animals were treated for a period of 30 days.

Results: Exposure to APAP at the treated dose to mice led to an alteration of liver functions, increased the alanine transaminase, aspartate aminotransferase and lactate dehydrogenase levels, decreased total protein level as well as increasing the superoxide dismutase and malondialdehyde while decreased catalase, glutathione peroxidase and glutathione reduced activities. The levels of APAP on the biochemical parameters of mice were dose-dependent. Administration of silymarin or/and *N. sativa* extract to APAP-treated mice attenuates the toxicity of this compound, objectified by biochemical, histological and ultrastructural improvement of liver. But the alleviation was more pronounced with the both antioxidants.

Conclusions: The synergistic effect of silymarin and *N. sativa* extract is the most powerful in reducing the toxicity induced by APAP and improving the liver functions and antioxidant capacities of mice.

1. Introduction

The liver is the most significant organ of our body for detoxification; disorders to this organ remain some of the most serious health problems[1]. Liver diseases remain to be serious health problems and the management of liver disease is still a challenge to the modern medicine. Liver plays an essential role in regulation of physiological processes, involved in several vital functions such as storage, secretion and metabolism. It also detoxifies a variety of drugs and xenobiotics and plays a central role in transforming, clearing the chemicals and is susceptible to the toxicity from these agents[2].

Hepatotoxicity is a common cause of severe metabolic disorders and even death[3]. Hepatic damage occurs due to its multi-dimensional functions, various xenobiotics and oxidative stress leading to distortion of all of its functions[4].

Acetaminophen [paracetamol or *N*-acetyl-*p*-aminophenol (APAP)] is a derivative of para-aminophenol used as an analgesic and antipyretic drug belonging to the para-aminophenol class of the nonsteroidal anti-inflammatory drugs[5].

APAP (paracetamol) produces acute liver damage at very larger dose. The hepatotoxicity of APAP has been attributed to the formation of toxic highly reactive metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI) which causes oxidative stress and glutathione depletion[6]. It is a well-known antipyretic and analgesic agent, which produces hepatic necrosis at higher doses[7].

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Nanji *et al.* showed that APAP is mainly metabolized in the liver to excretable glucuronide and sulphate conjugates[8]. The hepatotoxicity of APAP has been attributed to the formation of toxic metabolites when part of it is activated by hepatic cytochrome P-450 to form the highly reactive metabolite (NAPQI). Accidental or intentional intake of high doses often causes acute hepatocellular necrosis with high morbidity and mortality[9].

Nigella sativa L. (Ranunculaceae) (*N. sativa*), commonly known as "black cumin", is an erect herbaceous annual plant. It grows in Mediterranean countries and is also cultivated in the north of Morocco. *N. sativa* seeds have traditionally been used in Middle Eastern folk medicine as a natural remedy for various diseases as well as a spice for over 2000 years. The seeds of *N. sativa* have been subjected to a range of pharmacological, phytochemical and nutritional investigations in recent years. It has been shown to contain more than 30% (w/w) of a fixed oil with 85% of total unsaturated fatty acid[10]. The protective role of the black seed oil against hepatotoxicity has been investigated in animal experiments[11]. *N. sativa* oil (0.27 g/100 g body weight/day) was administered into adult rats and the treated animals showed decrease in serum (gamma-glutamyl transferase) nuclear DNA content which was elevated in the control group of the animals.

Silymarin contains a number of active constituents called flavolignans which are also used to help protect the liver from poisoning[12]. *Silybum marianum* (milk thistle) has been used to treat liver diseases since the 16th century. Its major constituents are flavonoids, silibinin, silidianin, silichristin and isosilibinin of which silibinin is the biologically most active compound and used for standardisation of pharmaceutical products[13].

The pharmacological profile of silymarin has been well defined and hepatoprotective properties of silymarin were investigated both *in vitro* and *in vivo*. Experimental studies demonstrated antioxidant and free radical scavenging properties, improvement of the antioxidative defence by prevention of glutathione depletion and anti-fibrotic activity[13].

Therefore, the present study was undertaken to evaluate the ameliorator property of silymarin or/and *N. sativa* extract on toxic status during APAP-induced injury in male mice at the biochemical, histological and ultrastructure levels.

2. Materials and methods

2.1. Chemicals

APAP was purchased from the Egyptian International Pharmaceutical Industries Company; silymarin was obtained from Sedeco Pharmaceutical Co-6-october City, Egypt. The *N. sativa* seeds were purchased from a local herb store with a fair degree of quality assurance. Seeds were washed to remove sand and other debris and air-dried and finely powdered with an electric microniser according to traditional mode of preparation[14]. Crude extract was obtained by the maceration of 800 g of these seeds by boiling in distilled water (1200 mL) for 24 h and filtered through muslin[15] and each 1 mL of the extract will contain 0.6 g of *N. sativa*. After 24 h, the aqueous extract was filtered and concentrated at room temperature, then the dried extract was stored at 4 °C until use[16]. Other chemicals and reagents were of the highest analytical grade and were bought from standard commercial suppliers in Roche (Germany).

2.2. Animals

ICR male mice, weighing approximately 35–40 g were provided by Faculty of Veterinary Medicine, Zagazig University. The animals were maintained in solid bottom shoe box-type polycarbonate cages with stainless steel wire-bar lids, using a wooden dust-free litter as a bedding material. Animals were located in air-conditioned room and were allowed free access to pellet diet and tap water for a week before starting the experiment. The European Community Directive (86/609/EEC) and national rules on animal care have been followed. After 2 weeks of acclimation, animals were randomly divided into seven groups with 10 animals in each one as following: Group 1 was served as untreated control (1 mL/kg of physiological saline); Group 2 was treated with paracetamol (2 g/kg)[17]; Group 3 was treated with silymarin (50 mg/kg)[18]; Group 4 was treated with *N. sativa* extract (0.25 g/Kg) (0.5 mL of the prepared solution)[14]; Group 5 was treated with paracetamol and silymarin; Group 6 was treated with paracetamol and *N. sativa* and the final Group 7 was treated with combination of both ameliorative compounds (silymarin and *N. sativa* extract) with paracetamol. All the groups were treated orally for 30 successive days.

2.3. Collection of blood samples

At the end of experimental period, blood samples of the fasted mice were collected from the medial retro-orbital venous plexus immediately with capillary tubes (Micro Hematocrit Capillaries, Mucaps) under ether anesthesia[19]. Then, the blood was centrifuged at 3000 r/min for 15 min and serum was collected for different biochemical analyses.

2.4. Hepatic biomarkers determination

Serum aspartate transaminase (AST) and alanine transaminase (ALT) activities were determined with kits from Human Diagnostic Worldwide, Germany.

2.5. Preparation of tissues for measurement of oxidative/antioxidant parameters

The tissues of liver and kidney were used for the analysis of oxidative stress and antioxidant parameters. Prior to dissection, tissue was perfused with 50 mmol/L sodium phosphate buffer saline (100 mmol/L Na_2HPO_4/NaH_2PO_4) (pH 7.4) and 0.1 mmol/L ethylenediaminetetraacetic acid to remove any red blood cells and clots. Then tissues were homogenized in 5 mL cold buffer/g tissue by a Potter–Elvehjem type homogenizer. The homogenate was centrifuged at 10 000 r/min for 20 min at 4 °C, and the resultant supernatant was transferred into Eppendorf tubes and preserved in a deep freezer until used. The supernatant was used for the determination of some biochemical parameters of liver and kidney tissues.

2.6. Lipid peroxidation assay

The extent of lipid peroxidation was estimated as the concentration of thiobarbituric acid reactive product malondialdehyde (MDA) by using the method of Ohkawa *et al.*[20]. Malondialdehyde concentrations were determined using