Biomedicine & Aging Pathology 4 (2014) 137–145

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Original article

# Liver damage associated with exposure to aspirin and diazinon in male rats and the ameliorative effect of selenium

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ARTICLE INFO

*Article history:*
Received 3 December 2013
Accepted 3 January 2014
Available online 4 February 2014

*Keywords:*
Acetyl salicylic acid
Diazinon
Liver enzymes
Histopathology
Immunohistochemistry antioxidant
Rat

ABSTRACT

*Purpose:* The widespread use of organophosphorus insecticides (OPIs) consequently leads to the exposure of manufacturing workers, field applicators, the ecosystem, and finally the public to the possible toxic effects of OPIs. In addition, drugs or pharmaceutical products, which are used to cure diseases, are also xenobiotics with both therapeutic/toxic potentials. It is evident from the literature, which is very limited, that drug/insecticide interactions can result in altered response/toxicity, which is of clinical relevance. The aim of the present study was designed to assess the adverse effects of exposure to aspirin and diazinon and their combination on liver of male rats and hepatoprotective potential of selenium (Se).
*Methods:* Rats were oral administered with vehicle, acetyl salicylic acid (ASA) at the maximum administration dose (1350 mg/personal/day = 22.5 mg/kg. b.wt.), diazinon (DIA) at 20 mg/kg. b.wt. and Se at a dose of 200 μg/kg b.wt./day and their combinations for 28 consecutive days. Serum liver biomarkers, e.g. ALT&AST, ALP, ChE, LDH, albumin, total protein were determined as well as histological and histochemical studies.
*Results:* Body weight was statistically ($P \leq 0.05$) decreased, while relative liver weight was statistically ($P \leq 0.05$) increased in DIA and ASA + DIA-treated groups. The activities of serum aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were statistically ($P \leq 0.05$) increased, while the activity of cholinesterase (ChE) was decreased in rats exposed to DIA, ASA and DIA + ASA. In addition, administration of DIA, ASA and their combination resulted in damage of liver structures and increase in the immunoreactivity of caspase-3 expression in the cytoplasm of the hepatocytes as compared to the control group. Combination therapy with Se significantly ($P \leq 0.05$) restored these alterations to within the normal limits and prevents disruptions of liver structures.
*Conclusions:* The present study indicates that liver enzymes, histopathology and immunoreactivity of caspase-3 would trigger ASA- and DIA-induced liver injury. The severities of such observations were more pronounced in their combined exposure. Combination therapy with Se restored these alterations to within the normal limits and prevents disruptions of liver structures. The data throw light on the problem of simultaneous exposure to OPIs and commonly used drugs especially that are metabolised by CYP450. Accordingly, ASA should be avoided since many of the adverse effects associated with these drugs are similar to the complications of chronic liver disease especially of agricultural workers in developing countries, where the handling of drugs without medical prescription. We suppose that antioxidant supplementation may be beneficial for the people using ASA for longer periods.


## 1. Introduction

Organophosphorus insecticides (OPIs) are one of the main classes of insecticides in use since the mid 1940s. OPIs, widely used in agriculture, show several interesting features for environmental safety, such as limited persistence and selective toxicity to insects with respect to mammals. However, in spite of their selectivity of action, they are often highly toxic to humans and are responsible for most accidental intoxications in agriculture and the pesticide industry [1].

Diazinon (DIA: O, O-diethyl-O-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothionate), as an OPI, has been widely and effectively used throughout the world with applications in agriculture, horticulture and public health as a veterinary ectoparasiticide [2]. Upon entry into the body, phosphorothioate OPs (e.g. DIA)

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http://dx.doi.org/10.1016/j.biomag.2014.01.004

undergo a $P_{450}$-mediated desulfuration reaction to form an active, highly toxic oxon intermediate metabolite that is responsible for the inhibition of acetylcholinesterase, butyrylcholinesterase, and carboxylesterase [3]. DIA-induced tissues injury initially depends on development of oxidative stress and cell death, which can be directly induced by the parent pesticide or by toxic oxygenated metabolites [4,5]. DIA causes changes in liver enzymes and biochemical indices and swelling of mitochondria in hepatocytes [6]. It also causes toxic effects on blood cells, spleen, thymus and lymph nodes of rats [7] and other organisms [8]. DIA affects mitochondrial membrane transport in rat liver, and it disturbs cytochrome $P_{450}$ system in human liver [9].

Acetyl salicylic acid (ASA) or aspirin represents the prototype of non-steroidal anti-inflammatory drugs (NSAIDs), has been widely used as analgesic, antipyretic, and anti-inflammatory agent in the world [10]. Aspirin is stable in gastric and duodenal fluids, and is therefore absorbed by the gastrointestinal tract as unchanged aspirin and the major site of presystemic metabolism of aspirin in man is in the liver [11]. Epidemiological studies have shown that hepatotoxicity has been associated with intake of aspirin and some other NSAIDs, so for this reason hepatoxicity has been considered as a warning class for such group of drugs [12–14].

Aspirin is rapidly converted to salicylic acid, which is then further metabolised to five main metabolites: salicyluric acid, salicyl phenol glucuronide, gentisic acid, acyl glucuronide and gentisuric acid [15]. The pathways of salicyluric acid and salicyl phenol glucuronide involve saturable hepatic enzymes, so when a large amount of salicylate is ingested these enzymes quickly become saturated. In fact, drug metabolism can be affected by multiple factors, such as diet, age, genetics, underlying liver disease, and the presence of other drugs [16]. These factors must be considered when prescribing a possibly hepatotoxic drug to a patient who is at increased risk for developing drug-induced hepatotoxicity [17]. Moreover, in developing countries, the purchase of large quantities of commonly used drugs (e.g. aspirin) does not require a medical prescription. So, the non-medical use of prescription drugs is a complex issue especially between agriculture workers.

Selenium, considered as an essential micronutrient for animals and humans [18], plays an important role in antioxidant defense systems, protects the structure and function of proteins, DNA and chromosomes against oxidation injury [19]. It is important in many biochemical and physiological processes including the biosynthesis of coenzyme Q (a component of mitochondrial electron transport systems), regulation of ion fluxes across membranes, maintenance of the integrity of keratins, stimulation of antibody synthesis, and activation of glutathione peroxidase [20]. This selenoenzyme-GSH-Px not only protects cells against damages by free radicals but also permits regeneration of a membrane lipid molecule through reacylation [21]. Se is a rational candidate element for the prevention of liver injury.

It is interesting to note that a large number of insecticides with liver toxicity are found to have clinically significant interactions with co-administered drugs [5]. The resulting liver toxicity therefore could be an interaction of the drug with endogenous or environmental agents. Also, it is postulated here that this group of drugs (NSAIDs) may exert their therapeutic effects by chelating various physiologically important metallic cations in the body. NSAIDs have a variety of adverse effects, including mainly gastrointestinal irritation, untoward and prolonged bleeding, renal function disturbance, skin eruptions, and otic effects [22].

To the best of our knowledge, there are no investigations carried out on the adverse effects of combined exposure to non-steroidal anti-inflammatory drugs "aspirin" and an organophosphorus insecticide "diazinon" as well as the protective effect of selenium in rats. Therefore, the aim of the present study was to:

- assess the adverse effects of exposure to aspirin and diazinon and there combination on liver in male rats;
- evaluate the antioxidant and hepatoprotective potential of Se against aspirin and diazinon-induced liver damage in rats.

## 2. Materials and methods

### 2.1. Animals

Healthy male Wister rats weighing $97 \pm 5$ g, were obtained from the Animal Breeding House of the National Research Centre (NRC), Dokki, Cairo, Egypt, and maintained in clean plastic cages in the laboratory animal room ($23 \pm 2\,^{\circ}C$). On standard pellet diet, tap water *ad libitum*, and daily dark/light cycle (12/12 h); the rats were acclimatized for 1 week prior to the start of experiments. The experimental work on rats was performed with the approval of the Animal Care & Experimental Committee, National Research Centre, Cairo, Egypt, and international guidelines for care and use of laboratory animals.

### 2.2. Chemicals

Acetyl salicylic acid (Aspocid® tablets, The Arab Drug Co., Egypt), each tablet contain 75 mg acetyl salicylic acid, was purchased from local pharmacies. Diazinon (Nasr-Cidol® 60% EC) was obtained from El-Nasr Mediate Chemical Co., Egypt. Sodium selenite ($Na_2SeO_3$) was purchased from Mallinckrodt. Inc. (Paris, France). Thiobarbituric acid (2, 6-dihydroxypyrimidine-2-thiol; TBA) was obtained from Merck (Germany). The assay kits used for biochemical measurements of aspartate aminotransferases (EC 2.6.1.1.), alanine aminotransferases (EC 2.6.1.2), alkaline phosphatase (EC 3.1.3.1), lactate dehydrogenase (EC 1.1.1.27), *cholinesterase* (*EC* 3.1.1.8), total protein and albumin were purchased from Biodiagnostic Company, Dokki, Giza, Egypt. All other chemicals were of reagent grades and were obtained from the local scientific distributors in Egypt.

### 2.3. Experimental design

#### 2.3.1. Groups and dosing

The animals were randomly divided into eight groups, each consisting of six rats. Diazinon, acetyl salicylic acid and selenium (sodium selenite, $Na_2SeO_3$) were prepared in distilled water and given via oral route for 28 consecutive days. Animals in Group 1 were served as control and given only distilled water (0.5 mL/rat). Animals in Group 2 were given Se ($Na_2SeO_3$) at a dose of 200 μg/kg b.wt./day [23]. Animals in Group 3 were given diazinon (DIA) at a dose of 20 mg/kg b.wt [24]. Animals in Group 4 were given acetyl salicylic acid (ASA) at a dose of 22.5 mg/kg b.wt. The selected dose of ASA was corresponded to the maximum administration dose (1350 mg/personal/day) based on the manufacture pamphlet. Animals in Group 5 were given simultaneously DIA (20 mg/kg b.wt.) and ASA (22.5 mg/kg b.wt.). Animals were co-administered Se with DIA, ASA and ASA + DIA for 5, 6 and 8 Groups, respectively.

#### 2.3.2. Body weight and blood collection

Body weight changes were recorded weekly during the experimental period (28 days). At the end of this period, blood samples were withdrawn from the animals under light ether anaesthesia by puncturing the retero-orbital venous plexus of the animals with a fine sterilized glass capillary. Blood samples were left to clot in clean dry tubes, and then centrifuged at 3000 rpm (600 g) for 10 minutes using Hereaeus Labofuge 400R, Kendro Laboratory Products GmbH, Germany, to obtain serum. The serum was then stored frozen at $-20\,^{\circ}C$ for the biochemical analysis (ALT, AST, ALP, LDH, ChE, total protein and albumin) within 1 week. After blood collection, rats were then killed by decapitation, livers were dissected out, cleaned