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# Protective effect of dietary flaxseed oil on arsenic-induced nephrotoxicity and oxidative damage in rat kidney

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ABSTRACT

Arsenic, a naturally occurring metalloid, is capable of causing acute renal failure as well as chronic renal insufficiency. Arsenic is known to exert its toxicity through oxidative stress by generating reactive oxygen species (ROS). Flaxseed, richest plant based dietary source of $\omega$-3 polyunsaturated fatty acids (PUFAs) and lignans have shown numerous health benefits. Present study investigates the protective effect of flaxseed oil (FXO) on sodium arsenate (NaAs) induced renal damage. Rats prefed with experimental diets (Normal/FXO diet) for 14 days, were administered NaAs (20 mg/kg body weight i.p.) once daily for 4 days while still on the experimental diets. NaAs nephrotoxicity was characterized by increased serum creatinine and blood urea nitrogen. Administration of NaAs led to a significant decline in the specific activities of brush border membrane (BBM) enzymes both in kidney tissue homogenates and in the isolated membrane vesicles. Lipid peroxidation and total sulfhydryl groups were altered upon NaAs treatment, indicating the generation of oxidative stress. NaAs also decreased the activities of metabolic enzymes and antioxidant defence system. Histopathological studies supported the biochemical findings showing extensive damage to the kidney by NaAs. In contrast, dietary supplementation of FXO prior to and alongwith NaAs treatment significantly attenuated the NaAs-induced changes.



## 1. Introduction

Human exposure to metals/metalloids such as uranium, lead, arsenic (As) and cadmium in both occupational and environmental settings is a common occurrence. Arsenic is a metalloid found in water, soil and air from natural and anthropogenic sources and exists in inorganic as well as organic forms (Flora et al., 2009). The major inorganic forms of arsenic include trivalent arsenite ($As^{III}$) and pentavalent arsenate ($As^{V}$). Arsenic typically arsenate enters the body mainly via consumption of contaminated drinking water (Shi et al., 2004). Prolonged ingestion of water contaminated with arsenic result in the manifestations of toxicity in practically all systems of the body (Mandal and Suzuki, 2002).

Kidney is a known target organ for arsenic and is critical for both arsenic biotransformation and elimination. Epidemiologic investigations and animal experiments have demonstrated that acute and chronic exposure to arsenic can cause injury to the kidney and increase the risk of renal cancer (Waalkes et al., 2004). The epithelial cells of proximal convoluted tubules are found to be more sensitive to arsenic induced toxicity due to their high reabsorptive activity and anatomical positions as the first renal tubular epithelial cells to be exposed to filtered toxicants (Peraza et al., 2006). Combined ultrastructural/biochemical studies (Brown et al., 1976) conducted in kidneys of rats exposed to arsenate have shown in situ swelling of mitochondria associated with decreased respiratory functions. Moreover, arsenic has been reported to increase the number of lysosomes (Brown et al., 1976).

Increasing evidence indicate that multifactorial mechanisms might be involved in metal induced toxicity and that one of the well known mechanism involves metal induced reactive oxygen species (ROS) generation (Abdel Moneim et al., 2011; Kokilavani et al., 2005). Arsenic is one of the most extensively studied metals that induce ROS generation and results in oxidative stress (Roy et al., 2009). Interference of toxic metals with glutathione (GSH) metabolism is found to be an essential part of the toxic response of many metals (Scott et al., 1993). Depletion of cellular sulfhydryl reserves seems to be an important indirect mechanism for arsenic-induced oxidative stress (Stohs and Bagchi, 1993). Consequently in studies aimed at abrogation of arsenic toxicity, the generation of oxidative stress has been targeted and the beneficial effects of various ROS scavengers (Flora, 1999; Ramanathan et al., 2005) have been explored. Certain complexing and metal chelating agents were also used to reduce arsenic induced toxicity (Gupta et al.,

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2005). However, most of these agents have been reported to exhibit toxic manifestations (Shi et al., 2004). This has led to an increased interest in utilizing the therapeutic potential of naturally occurring dietary nutrients having free radical scavenging and/or antioxidant properties to counteract free radical mediated arsenic toxicity (Manna et al., 2008). One such source of dietary nutrient is flaxseed. Flaxseed meal and flaxseed oil (FXO) have been used as/in food for centuries in Asia, Europe and Africa. Recently, flaxseed (*Linum usitatissimum*) has been identified as the richest plant based dietary source of omega-3 ($\omega$-3/*n*-3) polyunsaturated fatty acids (PUFAs), specifically α-linolenic acid (ALA). Nutritional recommendations have emphasized the need to consume $\omega$-3 PUFAs owing to their several beneficial effects on human health. Further, a number of animal studies involving dietary supplementation of flaxseed/FXO have reported inhibition of arrhythmogenesis during ischemia–reperfusion (Ander et al., 2004), inhibition of atherogenesis (Prasad, 2005) and protection against vascular dysfunction during hypercholesterolemic conditions (Dupasquier et al., 2006). The activity of constituent ALA itself or of its longer-chain PUFA derivatives viz. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) might be playing an important role in these healing effects (Sinclair et al., 2000; Burdge and Calder, 2005). Flaxseed is also a good source of dietary fiber and phytoestrogenic lignans that are believed to have antioxidant properties (Newairy and Abdou, 2009; Zanwar et al., 2010). The oxygen radical scavenging properties of flaxseed lignans were shown in vitro by either direct hydroxyl radical scavenging activity or inhibiting lipid peroxidation (Kitts et al., 1999). We have recently reported that FXO mitigates lead induced nephrotoxic effects (Rizwan et al., 2013). However, the efficacy of FXO to protect against arsenic-induced nephrotoxicity has not yet been evaluated.

In view of this, the present work was undertaken to study detailed biochemical events/cellular response/mechanisms of sodium arsenate (NaAs) nephropathy and its possible mitigation by FXO. We hypothesized that FXO would prevent NaAs-induced adverse effects on kidney due to its intrinsic biochemical and antioxidant properties that would result in improved metabolism and antioxidant defense mechanism in the kidney.

## 2. Materials and methods

### 2.1. Chemicals and drugs

Flaxseed oil: Omega Nutrition Canada Inc (Vancouver, BC, Canada), sodium arsenate (Merck, Mumbai, India). All other chemicals used were of analytical grade and were purchased either from Sigma Chemical Corp. or SRL (Mumbai, India).

### 2.2. Diet

A nutritionally adequate laboratory pellet diet was obtained from Aashirwaad Industries, Chandigarh (1544, Sector 38-B, Chandigarh, India). Normal diet (ND) was prepared by crushing the pellets finely and adding vitamin E as DL-α-tocopherol (270 mg/kg chow) to the crushed diet. Flaxseed oil (FXO) diet was prepared by adding 15% flaxseed oil by weight to the normal diet. The diet was stored in airtight containers. Vitamin E was added in order to meet the increased metabolic requirement for antioxidants on a diet high in polyunsaturated fatty acids.

### 2.3. Experimental design

The animal experiments were conducted according to the guidelines of Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India. Adult male Wistar rats (10–12 rats/group) weighing between 150 and 200 g were used in the study. Animals were acclimatized to the animal facility for a week on standard rat chow and allowed water ad libitum under controlled conditions of 25 ± 2 °C temperature, 50 ± 15% relative humidity and normal photoperiod (12 h dark and light). Four groups of rats entered the study after acclimatization (Fig. 1). They were fed on either normal diet (control and NaAs groups) or FXO diet (AsFXO and FXO groups). After 14 days, rats in the two groups (NaAs and AsFXO) were injected with sodium arsenate (NaAs) in distilled water intraperitoneally (20 mg/kg bwt/day) every day for 4 days (four sodium arsenate injections in total). The dose of sodium arsenate selected for this study was much less than the reported LD50 value of sodium arsenate for rats (Franke and Moxon, 1936), and was comparable to the dose used in several previous studies to induce the toxic effects (Hood et al., 1988; Hood et al., 1978, 1987). Since our objective was to study the acute effects of arsenic exposure, hence sodium arsenate was injected in 4 doses, over a short period of time. Animals in the control and FXO group received an equivalent amount of distilled water. The rats were sacrificed on the sixth day after the last NaAs injection under light ether anesthesia. Blood and urine samples were collected and kidneys were removed and processed for the preparation of homogenates and brush border membrane vesicles (BBMV) as described below. Each preparation of BBM/homogenate was made by pooling the tissue (cortex/medulla) from 2 to 3 animals in each group. Thus, the number of animals mentioned above contributed to 4–5 different preparations for each group. Analyses of various parameters were performed simultaneously under similar experimental conditions to avoid any day to day variations.

### 2.4. Histopathology

Animals were sacrificed under light ether anesthesia. Abdomen was opened and kidneys were removed, cut into pieces and kept in Karnovsky's fixative for one week (Immersion fixation). Small pieces of tissue samples were processed for paraffin embedding. Sections of 5 μm thickness were cut and stained with haematoxylin and eosin. Light microscopic observations were made under trinocular microscope (Olympus BX-40, Japan). Interesting findings were recorded at the initial magnification of 400×.

### 2.5. Preparation of homogenates

After the completion of the experiment, the kidneys were removed, decapsulated and kept in ice-cold buffered saline (154 mM NaCl, 5 mM Tris-HEPES, pH 7.5). The cortex was carefully separated from medulla as described earlier (Khundmiri et al., 2005). A 15% (w/v) homogenate was prepared in 0.1 M Tris–HCl buffer, pH 7.5, using Potter–Elvehjem homogenizer (Remi Motors, Mumbai, India); by passing five pulses. The homogenate was centrifuged at 3000×*g* at 4 °C for 15 min to remove cell debris and the supernatant was saved in aliquots and stored at −20 °C for assaying free-radical scavenging enzymes and for estimation of total-SH and lipid peroxidation (LPO).

### 2.6. Preparation of brush border membrane

BBMV were prepared from whole cortex using the $MgCl_2$ precipitation method as described previously (Khundmiri et al., 2004). Briefly, freshly minced cortical slices were homogenized in 50 mM mannitol and 5 mM Tris–HEPES buffer, pH 7.0 (20 ml/g), in a glass Teflon homogenizer with 4 complete strokes. The homogenate was then subjected to high speed Ultra-Turrex homogenizer (Type T-25, Janke & Kunkel GMBH & Co. KG. Staufen, Germany) for 3 strokes of 15 s each with an interval of 15 s between each stroke. $MgCl_2$ was added to the homogenate to a final concentration of 10 mM and the mixture stirred for 20 min on ice. The homogenate was centrifuged at 2000×*g* for 10 min in a Beckman centrifuge (J2 MI, Beckman instruments Inc Palo Alto, CA, USA) using JA-17 rotor and the supernatant was then recentrifuged at 35,000×*g* for 30 min. The pellet was resuspended in 300 mM mannitol and 5 mM Tris–HEPES, pH 7.4, with four passes by a loose fitting Dounce homogenizer (Wheaton, IL, USA) and centrifuged at 35,000×*g* for 20 min in a 15 ml corex tube. The outer white fluffy pellet of BBM was resuspended in small volume of buffered 300 mM mannitol. Aliquots of BBM and homogenates were saved and stored at −20 °C for BBM enzyme analysis.

### 2.7. Serum/urine chemistry

Serum samples were deproteinated with 3% trichloroacetic acid in a ratio of 1:3, left for 10 min and then centrifuged at 2000×*g* for 10 min. The protein free supernatant was used to determine inorganic phosphate (Pi) and creatinine. The precipitate was used to quantitate total phospholipids. Blood Urea Nitrogen (BUN) was determined directly in serum samples. All these parameters were determined by standard procedures as mentioned in a previous study (Khundmiri et al., 2005). Glucose was estimated by o-toluidene method using kit from Span diagnostics, Mumbai, India.

### 2.8. Assay of carbohydrate metabolism enzymes

The activities of the enzymes involving oxidation of NADH or reduction of NADP were determined spectrophotometrically on Cintra 5 fixed for 340 nm using 3 ml of assay buffer in a 1-cm cuvette at room temperature (28–30 °C). The enzyme activities of Lactate dehydrogenase (LDH, E.C.1.1.1.27), malate dehydrogenase (MDH, E.C.1.1.1.37), malic enzyme (ME, E.C.1.1.1.40), glucose-6-phosphate dehydrogenase (G6PDH, E.C.1.1.1.49), glucose-6-phosphatase (G6Pase, E.C.3.1.3.3) and fructose-1,6-bisphosphatase (FBPase, E.C.3.1.3.11) were assayed as described by Khundmiri et al., 2004. Hexokinase was estimated by the method of Crane and Sols, 1953 and the remaining glucose was measured by method of Nelson, 1944.