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Comparative study of antioxidant activity of α-eleostearic acid and punicic acid against oxidative stress generated by sodium arsenite

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ABSTRACT

The present study was undertaken to evaluate the antioxidant efficacy of α-eleostearic acid and punicic acid, two isomers of conjugated linolenic acid, in terms of normalization of altered biochemical parameters of oxidative stress following sodium arsenite treatment in rats. Animals were divided into four groups. The first group used as control. While, group 2, 3 and 4 were orally treated with α-eleostearic acid (0.5% of total lipid given) plus sodium arsenite (Sa; 10 mg/kg BW), punicic acid (0.5% of total lipid given) plus sodium arsenite (Sa; 10 mg/kg BW) and sodium arsenite (Sa; 10 mg/kg BW), respectively. Results showed that activities of antioxidant enzymes decreased significantly due to oxidative stress generated by sodium arsenite. Lipid peroxidation also increased due to sodium arsenite administration. α-Eleostearic acid and punicic acid acted as antioxidant and caused mostly all the altered parameters restored to normal level. Results also showed that antioxidant activity of α-eleostearic acid was more predominant than that of punicic acid.

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1. Introduction

Arsenic is a naturally occurring element that is present in food, soil and water; it is released in the environment from both natural and man-made sources. Oxidative stress has been identified as an important mechanism of arsenic toxicity and carcinogenicity. The most common forms of arsenic are water-soluble arsenite (the trivalent form, As III) and arsenate. Trivalent arsenic is more toxic than pentavalent arsenic and its inorganic forms are more toxic than organic forms (Bertolero et al., 1987). Because arsenic targets ubiquitous enzyme reactions, it affects nearly all organ systems in humans and other animals (Guha Majumdar, 2005). Epidemiological studies of populations from arsenic exposed areas confirm a wide variety of adverse health outcomes linked to arsenic exposure.

The dissolved arsenic compounds are readily absorbed through the gastrointestinal tract after ingestion and distributed in the blood to the liver, brain, kidney and many other organs, i.e., it affects nearly entire organ systems of the body. In particular, arsenic induces oxidative DNA damage and lipid peroxidation. A number of studies have shown arsenic-induced formation of reactive oxygen and nitrogen species as well as elevated DNA oxidation. Thus, arsenite increases the generation of superoxide anions (O_2^-) and hydrogen peroxide (H_2O_2) in diverse cellular systems (Shi et al., 2004). Liver is an important target organ for arsenic toxicity during its cycles between different oxidation states; arsenic generates reactive oxygen species (ROS) and causes organ-toxicity. ROS directly react with cellular biomolecules, damage lipids, proteins and DNA in cells and that can ultimately lead to cell death (Mo et al., 2006). Arsenic binds with thiol groups on functional proteins and causes a primary imbalance between pro-oxidant and antioxidant homeostasis in biological systems (Aposhian and Aposhian, 1989). It also induces oxidative tissue damage through interference with glutathione (GSH) utilization (Bhadauria and Flora, 2007). Inorganic arsenic has been shown to inhibit several of the antioxidant systems in the body, such as catalase, glutathione peroxidase and superoxide dismutase (Ramanathan et al., 2002). Thus, increasing the antioxidant levels in the body may protect against arsenic-induced toxicity. There are few reports in which certain antioxidant compounds like α-tocopherol, ascorbic acid and quercetin were used to repair or inhibit the oxidative damage induced by sodium arsenite (Kadirvel et al., 2007; Ghosh et al., 2009).

Abbreviations: As, arsenic; As (V), arsenate; As(III), arsenite; CAT, catalase; CLnA, conjugated linolenic acid; CLA, conjugated linoleic acid; EM, erythrocyte membrane; GSH, reduced glutathione; GPx, glutathione peroxidase; MDA, malondialdehyde; NOS, nitric oxide synthase; PPARγ, peroxisome proliferator activated receptor gamma; RBC, red blood cell; ROS, reactive oxygen species; Sa, sodium arsenite; SH-groups, sulfhydryl groups; SOD, superoxide dismutase; TBA, 2-thiobarbituric acid; TCA, trichloro acetic acid.

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Nutritional antioxidants in diseases related to oxidative stress have gained immense interest in recent years. Researchers are looking forward in search of protective antioxidant in order to combat against arsenic-induced organ-toxicity. Beneficial effect of conjugated fatty acids in relation to the prevention and recovery from arsenic induced oxidative stress and poisoning is yet to be defined. Conjugated fatty acid is the general term of positional and geometric isomers of polyunsaturated fatty acids with double bonds in conjugation. Antioxidant activity of conjugated di-enoic fatty acid such as conjugated linoleic acid (CLA) has already been intensively studied. So, it was worth investigating the antioxidant activity of conjugated tri-enoic fatty acid against arsenic induced organ-toxicity. α-Eleostearic acid and punicic acid are two typical conjugated tri-enoic fatty acids found in seed oils, both are conjugated linolenic acid (CLnA) with systematic structure of *cis*-9, *trans*-11, *trans*-13-octadecatrienoic and *cis*-9-*trans*-11-*cis*-13-octadecatrienoic acid, respectively. Theoretically α-eleostearic acid consisting of 33% cis and 66% trans molecular composition and punicic acid has 66% cis and 33% trans configuration. It has been observed that seed fats of cucurbitaceae family, obtained from common vegetables like karela (*Momordica charantia*), usually consumed by people of all over India and Asia contains about 30–50% α-eleostearic acid in conjunction with linoleic, oleic and saturated fatty acids.

Punicic acid is a conjugated tri-enoic fatty acid of the constitution *cis*-9-*trans*-11-*cis*-13-octadecatrienoic fatty acid. It is found in the seed fat of snake gourd plant, *Trichosanthes anguina*, belonging to the natural order *Cucurbitaceae*. It may be stated that the natives consume the fruits and seeds of the snake gourd plant mostly as vegetables. The seeds contain about 40–70% oil, with punicic acid content to be more or less up to 40% (Takagi and Itabashi, 1981).

In case of polyunsaturated fatty acids (PUFA), lipid peroxidation can commence by ROS and other mechanisms that result in abstraction of an electron from PUFA, subsequently free radicals are constantly being generated, and antioxidant defense mechanism neutralizes them, making them ineffective.

Dhar et al. (1999) examined the antioxidant activity of conjugated octadecatrienoic fatty acid α-eleostearic acid (CLnA) of karela seed (*M. charantia*) on rats for 4 weeks. They concluded that the reduction of CLnA resulted in the formation of conjugated di-enoic fatty acids (CLA) that acted as antioxidants. Mukherjee et al. (2002) examined the activity of 9*c*, 11*t*, 13*c*-18: 3 fatty acid (punicic acid) upon administration in the diet of rats at various concentrations. It is also appeared to be efficient as an antioxidant.

Following the above observations, the present study has been designed to observe whether two isomers of CLnA could also act as antioxidant against arsenic induced oxidative stress and influence biochemical alterations in plasma, liver and brain of rats.

2. Materials and methods

2.1. Chemicals

Sodium arsenite ($NaAsO_2$) which was chosen as the source of arsenic, was purchased from s d Fine-Chem limited, Mumbai, India. All other chemicals used in the experiment were of analytical grade. The dose of Sa (10 mg/kg BW) was chosen on the basis of the previous studies of Kadirvel et al. (2007) and Rodriguez et al. (2001). The dose of CLnA isomers (0.5% of total lipid given) were used accordingly the previous studies made by Dhar et al. (1999) and Mukherjee et al. (2002).

2.1.1. Extraction and quantification of CLnA isomers

Authentic karela seeds and snake gourd seeds, obtained from the local market of Calcutta, India, were crushed into fine particles and oil was extracted from these crushed seeds with solvent petroleum ether. The extracts of the sample were filtered and concentrated by evaporation in vacuum. Then free fatty acid content was measured. The free fatty acids present in the oil were removed by miscella refining process (Bhattacharyya et al., 1986) The extracted oil containing hexane, known as miscella (hexane/oil, 2:1) was mixed with 10% NaOH solution (20% excess of the theoretical amount required) at 40 °C for 30 min to neutralize the free fatty acids. The soap formed was removed by centrifugation and the organic phase was washed with water. Deacidified oil was recovered after removing the solvent under vacuum distillation and drying under vacuum. The refined oil was then bleached with tonsil earth optimum (1% w/w) obtained from P.T. Sud-Chemic (Jakarta, Indonesia) and activated carbon (0.2% w/w), supplied by E. Merck India Pvt. Ltd. (Bombay, India) at 60 °C under vacuum for 20 min. After the bleaching operation, the oil was recovered by vacuum filtration and stored at −20 °C under nitrogen. The fatty acid compositions of the dietary oils were determined by gas liquid chromatography (GLC) techniques converting the oils (triglycerides) into their corresponding methyl esters. The *cis–trans* position of isomers were identified by dissolving them in cyclohexane and observed over wavelength range of 200–300 nm using a UV–Vis spectrophotometer (Lakshminarayana et al., 1982).

2.1.2. Dietary fat blends

Sunflower oil was obtained from I.T.C. Limited (Hyderabad, India). Sunflower oil was mixed with karela seed oil and snake gourd oil to give final oil mixtures containing 0.50% by weight of α-eleostearic acid and punicic acid, respectively. Table 1 shows the FA composition of dietary oil mixtures.

2.1.3. Animal experimental

Male albino rats of Charles Foster strain (selected for the authenticity of the strain) were housed in individual cages. The work was done under the supervision of the Animal Ethical Committee of the Department of Chemical Technology (University of Calcutta). The animals were divided into four groups (average body weight 70–80 g), each consisting of five animals. The first group served as control, received only vehicles (sunflower oil and deionized water) once per day. Rats in the group 2 were treated with α-eleostearic acid (0.5% of total lipid given) along with sodium arsenite (Sa; 10 mg/kg BW) by oral gavage once per day. Rats in the group 3 were treated with punicic acid (0.5% of total lipid given) along with sodium arsenite (Sa; 10 mg/kg BW) and rats in the group 4 were treated with sodium arsenite (Sa; 10 mg/kg BW) along with sunflower oil by oral gavage once per day.

The rats were fed experimental diets having the following composition: fat-free casein, 18%; fat, 20%; starch, 55%; salt mixture 4% [composition of salt mixture No. 12 (in g): NaCl, 292.5; KH_2PO_4, 816.6; $MgSO_4$, 120.3; $CaCO_3$, 800.8; $FeSO_4 \times 7H_2O$, 56.6; KCl, 1.66; $MnSO_4 \times 2H_2O$, 9.35; $ZnCl_2$, 0.5452; $CuSO_4 \times 5H_2O$, 0.9988; $CoCl_2 \times 6H_2O$, 0.0476] (Joanes and Foster, 1942); cellulose, 3%; and one multivitamin capsule (vitamin A I.P. 10,000 units, thiamine mononitrate I.P. 5 mg, vitamin B I.P. 5 mg, calcium pantothenate USP 5 mg, niacinamide I.P. 50 mg, ascorbic acid I.P. 400 units, cholecalciferol USP 15 units, menadione I.P. 9.1 mg, folic acid I.P. 1 mg, and vitamin E USP 0.1 mg) per kg of diet. The diets were adequate in all nutrients.

The animals were sacrificed after 15 days. Rats were fasted overnight for 12 h and then sacrificed under anesthesia; blood was collected, and liver and brain were immediately excised, blotted, and stored frozen (−40 °C) for analysis.

2.1.4. Lipid analysis

The total lipids were extracted from liver with chloroform/methanol mixture and estimated gravimetrically (Kates, 1972). The lipid component such as total cholesterol (Allain et al., 1974) of plasma, liver and brain was analysed using enzymatic kit supplied by Ranbaxy Diagnostics Ltd. (New Delhi, India). Phospholipid (Chen et al., 1956) of brain and liver tissues was estimated by standard method. Plasma peroxidation was measured by the assay of thiobarbituric acid-reactive substances (TBARS) according to the standard method (Wills, 1987). The amount of malondialdehyde (MDA) formed was calculated by taking the extinction coefficient of MDA to be $1.56 \times 105\ M^{-1}\ cm^{-1}$.

Table 1
Fatty acid composition of Dietary oils and oil mixtures.

Dietary fats	FA composition (area%)					
	16:0	18:0	18:1	18:2	18:3 (CLnA, 9*c*,11*t*,13*t*-18:3)	18:3 (CLnA, 9*c*,11*t*,13c-18:3)
Sunflower oil	6.3	3.5	32.4	57.8	–	–
Karela seed oil	1.8	31.6	7.5	6.8	52.3	–
Snake gourd oil	6.2	6.1	26.6	21.0	–	40.1
Sunflower + karela seed oil (99:1, w/w)	6.3	3.8	32.2	57.2	0.5	–
Sunflower + snake gourd oil (98.75:1.25, w/w)	6.3	3.5	32.3	57.4	–	0.5

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