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Original article

Protective efficacy of dietary D-Pinitol on hepatic and renal tissues during experimental breast cancer in rats challenged with 7,12-Dimethylbenz (*a*) anthracene: A biochemical approach

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ABSTRACT

Traditionally, dietary compounds have been practiced to eliminate toxins and to strengthen the defense system towards resistance to many disease. D-Pinitol is one of a natural dietary compound predominantly found in soy products. It has been reported to possess diverse biological properties and their therapeutic properties propel them to evaluate against various experimental diseases. We have reported here the protective role of D-Pinitol on renal and hepatic tissue against oxidative stress mediated experimental breast cancer model. DMBA, the polycyclic aromatic hydrocarbon, was used to induce breast cancer in female Sprague Dawley rats with single oral dose of 20 mg/kg/body weight diluted in corn oil. The cancer-bearing rats were treated with the natural dietary compound D-Pinitol at the concentration of 200 mg/kg/body weight orally for 45 days. We observed that D-Pinitol significantly restored the elevated levels of marker enzymes such as ALT, AST and LDH and efficiently down regulates lipid per-oxidation and lysosomal enzymes. The antioxidants levels were found to be significantly enhanced and the carbohydrate key metabolizing enzymes were excellently modulated towards normal range. These biochemical alterations were well reflected in the histopathological studies of liver and kidney tissues of control and experimental groups. Thus, the hepatic and renal tissue protective property might be due to the antioxidant activity which might reduce the per-oxidation reaction that were induced by DMBA and also render protection to the membrane integrity through its firm antioxidant property and modulatory role with prominent intervention strategies in gluconeogenesis process and lysosomal enzymes.

1. Introduction

Polycyclic aromatic hydrocarbons are reported as a ubiquitous environmental contaminant. 7,12-Dimethylbenz (a) anthracene (DMBA) is one of the polycyclic aromatic hydrocarbon (PAH) produced during the incomplete combustion of carbon-containing compounds, and predominantly found in tobacco smoke and motor vehicle exhaust emissions [1]. Reactive oxygen species such as superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$) are highly produced during metabolic activation of DMBA which may lead to oxidative damage to cells and thereby decreasing the efficiency of antioxidant defense mechanism [2]. Epidemiological and experimental studies demonstrated that high consumption of natural products that are rich in antioxidants reduces the risk of many oxidative stress related diseases [3]. Bioactive compounds from plant origins have the potential to subside the biochemical imbalances induced by various toxins associated with free radicals. They provide protection without causing any side effects, therefore the development of drugs from plant products is desired. D-Pinitol (3-O-methyl-chiro-inositol) is a naturally occurring compound found in soyabean seeds, pinewood, alfalfa and legumes. It has been reported to possess several interesting multi-functional properties such as inhibition of the T-helpercell-1 response [4], antiviral [5], larvicidal [6], anti-inflammatory [7], antihyperlipidemic [8], cardioprotective [9] and inhibition of ovalbumin-induced airway inflammation [4]. Furthermore, D-Pinitol is an active ingredient of *Talisapatra*, a traditional Ayurvedic medicine and has been shown to exhibit antidiabetic activities [10] and antioxidant activity [11]. The changes of rat liver enzymes are reported to be far more reproducible and reliable than that of mammary enzymes during 7,12-Dimethylbenz (*a*) anthracene (DMBA)-induced mammary carcinoma [12]. In this context, Bishayee and colleagues have suggested that liver enzymes provide much more sensitive indication of a distant neoplasm in rats [13]. Although D-Pinitol has been reported to have diverse biological properties, we have particularly interested to investigate

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whether D-Pinitol has any protective role in liver and kidney tissues against oxidative stress induced by the DMBA. Moreover, it is worthy to point out that the role of liver and kidney tissues in experimental breast cancer, since minor changes reflect in these tissues, which perhaps is helpful to assess any disease condition in a successful manner. Hence, the present investigation was undertaken to evaluate the role of D-Pinitol in the context of oxidative damage inflicted by DMBA-induced experimental mammary carcinoma model with special reference to renal and liver tissue biochemical parameters such as antioxidants, lipid per-oxidation, status of marker enzymes in addition to lysosomal and glucose metabolizing enzymes.

2. Materials and methods

2.1. Chemicals

D-Pinitol and DMBA were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). All the other chemicals used in this study were of analytical grade available commercially.

2.2. Animals

Female Sprague Dawley rats at the age group of 45–48 days were procured from the Central Animal House Facility, Dr. ALM PG IBMS, University of Madras, Taramani. The animals were housed in well-ventilated large spacious polypropylene cages and had 12-h light and dark cycle throughout the experimental period. The animals received a balanced diet of commercially available pellet rat feed and water ad libitum. The Guidelines for Breeding and Experiments on Animals, 1998 defined by the Ministry of Social Justice and Empowerment of India were followed and the protocol was approved by the Institutional Animal Ethics Committee (IAEC No. 01/05/2011).

2.3. Tumor induction

7,12-Dimethylbenz (*a*) anthracene (DMBA) was used as a carcinogen for the present investigation. Mammary tumor was induced by a single dose of 20 mg of DMBA dissolved in corn oil (1 ml) given through an oral gavage [14].

2.4. Experimental design

The rats were divided into four groups and each group consisting six animals:

- Group I: animals received single dose of 1 ml of emulsion of corn oil given orally served as vehicle treated control;
- Group II: mammary carcinoma induced with single dose of 20 mg/kg body weight of DMBA dissolved in corn oil (1 ml) administered intragastrically;
- Group III: mammary carcinoma induced with single dose of 20 mg/kg body weight of DMBA dissolved in corn oil (1 ml) administered intragastrically and from the seventh week followed by D-Pinitol (200 mg/kg body weight) given for 45 days intragastrically;
- Group IV: animals received D-Pinitol alone at the concentration of 200 mg/kg body weight for 45 days intragastrically.

2.5. Collection of samples

At the end of the experimental period, all the rats were sacrificed by cervical dislocation. Blood was collected and the serum was separated by centrifugation. Kidney and liver tissues were dissected out and tissue homogenates were prepared in 0.1M Tris-HCl buffer pH 7.4 which was stored at −80° C, for further analysis.

2.6. Biochemical estimation

2.6.1. Estimation of antioxidants

The activity of Superoxide dismutase (SOD) was determined by the method of Marklund and Marklund [15]. The catalase (CAT) activity was measured by the method of Sinha [16]. Glutathione peroxidase (GPx) was assayed by the method of Rotruck et al. [17]. The level of reduced glutathione was estimated by the method of Moron et al. [18]. Vitamin E and Vitamin C (α-tocopherol) level were estimated by the method of Desai [19] and Omaye et al. [20] respectively.

2.6.2. Estimation of carbohydrate metabolizing enzymes

Phosphoglucoisomerase was assayed by the method of Horrocks et al. [21]. Glucose-6-phosphatase and Fructose-1,6-diphosphatease were assayed according to the method of Gancedo and Gancedo [22].

2.6.3. Lysosomal enzymes

The activity of β-D-Galactosidase was estimated by the method of Kawai and Anno [23]. The activity of β-D-Glucuronidase was estimated by the method of Delvin and Gianetoo [24]. The activity of N-Acetyl-β-D-Galactosaminidase was estimated by the method of Marhun [25].

2.6.4. Marker enzymes

The level of Aspartate transaminase (AST) and Alanine transaminase (ALT) were estimated by the method of King [26]. The Lactate dehydrogenase (LDH) was estimated by the method of King [27].

2.6.5. Lipid per-oxidation

Lipid per-oxidation as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) was assayed in plasma by the method of Yagi [28] and in tissues by the method of Ohkawa et al. [29]. The results were expressed as n moles of MDA formed per milligram of protein.

2.7. Histopathological analysis

The liver and kidney tissues of control and experimental animals were sliced and immersed at once in 10% buffered formalin solution for fixation and dehydrated with graded ethanol solutions from 50–100%, and then embedded in paraffin. Sections of 5 μm in thickness were cut and stained with haematoxylin and eosin and the slides were observed under the microscope.

2.8. Statistical analysis

The values are expressed as Mean ± SD for six rats in each group. The statistically significance between the groups were calculated using One-way Analysis of Variance (ANOVA) followed by the Student's Turkey's for multiple comparisons using Statistical Package for Social Sciences (SPSS) computer package. Values of $P < 0.05$ were considered to be significant.

3. Results

3.1. Effect of D-Pinitol on antioxidants status

The activities of enzymic and non-enzymic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), vitamin C and vitamin E in serum, liver and kidney of control and experimental animals