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Alteration of DMBA-induced oxidative stress by additive action of a modified indigenous preparation—Kalpaamruthaa

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Abstract

The present study investigated the protective efficacy of the novel preparation named as Kalpaamruthaa (KA, includes *Semecarpus anacardium* Linn nut milk extract (SA), dried powder of *Phyllanthus emblica* fruit and honey) on the peroxidative damage and abnormal antioxidant levels in the hepatic mitochondrial fraction of 7,12-dimethylbenz(*a*)anthracene (DMBA)-induced mammary carcinoma rats. Female Sprague–Dawley rats of weight 180 ± 10 g were categorized into six groups. Three groups were administered DMBA (25 mg/rat dissolved in olive oil, orally) to induce mammary carcinoma. One of these groups received KA treatment (300 mg/kg b.wt., orally) and other group received SA (200 mg/kg b.wt., orally) for 14 days after 90 days of DMBA induction. Vehicle-treated control and drug control groups were also included. The hepatic mitochondrial fraction of untreated DMBA rats showed 2.96-fold increase in MDA content when compared to control rats and abnormal changes in the activities/levels of mitochondrial enzymic (superoxide dismutase, glutathione peroxidase and glutathione reductase) and non-enzymic (glutathione, vitamin C and vitamin E) antioxidants were observed. DMBA-treated rats also showed decline in the activities of mitochondrial enzymes such as succinate dehydrogenase, α-ketoglutarate dehydrogenase, malate dehydrogenase and isocitrate dehydrogenase. In contrast, rats treated with SA and KA showed normal lipid peroxidation antioxidant defenses and mitochondrial enzymes, thereby showing the protection rendered by SA and KA. Although, KA treatment exhibited more profound effect in inhibiting DMBA-induced oxidative stress than sole SA treatment. Results of the study indicate that the anticarcinogenic activity of KA during DMBA-initiated mammary carcinogenesis is mediated through alteration of hepatic antioxidant status as well as modulation of TCA cycle enzymes. On the basis of the observed results, KA can be considered as a readily accessible, promising and novel cancer chemopreventive agent.

Keywords: 7,12-Dimethylbenz(*a*)anthracene; Reactive oxygen species; *Semecarpus anacardium*; *Phyllanthus emblica*; Kalpaamruthaa; Mitochondria

1. Introduction

Cancer is one of the major human diseases and causes considerable suffering and economic loss worldwide. The changes of rat liver enzymes are reported to be far more reproducible and reliable than that of mam-

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mary enzymes during 7,12-dimethylbenz(*a*)anthracene (DMBA)-induced mammary carcinoma [1]. Hence, the present article designed to study the changes in enzyme activities/levels were measured in the liver because it is known that liver enzymes provide much more sensitive indication of a distant neoplasm in rats [2].

The mitochondria are an important site for the production of cellular reactive oxygen species (ROS). The mitochondrial electron transport chain consumes oxygen through oxidative phosphorylation to form cellular energy in the form of ATP. During this process, 2% of the consumed oxygen is released in the mitochondria as superoxide free radicals [3]. ROS formed during DMBA metabolism can diffuse from the site of generation to other targets within the cells or even propagate the injury outside to intact cells. These ROS produce deleterious effects by initiating lipid peroxidation directly or by acting as second messengers for the primary free radicals that initiate lipid peroxidation [4]. Thus, it is essential to provide a suitable range of antioxidants to quench the ROS before they could spread and cause serious damage to mitochondrial DNA, protein and lipids leading to degenerative diseases like cancer [5].

Numerous studies have demonstrated the protective properties of polyphenolic flavonoids. Flavonoids as effective antioxidants may provide protection against cancer [6]. Also *in vitro* they were found to be antiproliferative against broad spectrum of cancer cells [7]. Tannins are also plant polyphenols comprising a heterogeneous group of compounds and as active constituents are responsible for many pharmacological activities [8]. An increasing body of experimental evidence supports the hypothesis that tannins exert antioxidant and anticarcinogenic activity in chemically induced cancers in animal models [9]. The antitumour activities of a series of naturally occurring tannins have been demonstrated on several tumor cell lines [10]. Gallic acid and ellagic acid has also been reported to have antioxidant activity and cancer chemopreventive properties [11]. Besides, interactions between flavonoids and ascorbic acid have been documented [12], ascorbate is reported to have flavonoid protective [13] and flavonoid-enhancing activities [14]. Also, stabilization of vitamin C at sub-optimal concentrations by flavonoids is well established [15]. Moreover, a protective relationship between vitamin C and breast cancer was reported [16].

The combined interactions of these phytochemicals improve effectiveness in several ways by modifying the pharmacological effects of the drug either potentiating the effect of bioactive phytochemicals or interfering with their activity. Hence, in the present study, in order to improve the bioactivity of *Semecarpus anacardium* Linn nut milk extract (SA) which has been reported for the presence of carbohydrate, phenols and flavonoids [17] has combined with *Phyllanthus emblica* fruit (PE) constituting vitamin C, hydrolysable tannins (Emblicannin-A, Emblicannin-B, Punigluconin, Pedunculagin), Gallo-ellagitannoids, flavonoid (rutin) [18] trigalloyl glucose [19], and phyllemblic acid [20]. Kalpaamruthaa (KA) a modified indigenous Siddha preparation constituting SA and PE were formulated and tried for the first time with a view to promote the curative potency. The synergistic effect of these phytochemicals suggests that the combination of SA and PE might act as a strong chemomodulator and potentiate more intense anticancer property. In order to promote intellect and prevent senility and for longevity, honey was also added in Kalpaamruthaa [21]. Moreover, SA and PE have been itself highly preferred for their wide range of pharmacological activities such as antioxidants, anti-inflammatory, membrane stabilizing and immunomodulatory properties [22,23]. In addition to that, they also exhibit anticancer effect against various types of cancer [24–26]. Therefore, this study was undertaken to assess the result of KA on DMBA induced mitochondrial lipid peroxidative changes and antioxidant status of hepatic mitochondrial fractions.

2. Materials and methods

2.1. Drugs and chemicals

S. anacardium Linn nut milk extract has been prepared according to Formulary of Siddha medicine [27]. To this, honey and fresh dried powder of *P. emblica* fruits was added. 7,12-Dimethylbenz(*a*)anthracene (DMBA) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and solvents used were of analytical grade.

2.2. Animals

Adult female albino rats of Sprague–Dawley strain weighing 180 ± 10 g were purchased from National Institute of Nutrition, Hyderabad, India. The animals were maintained under standard conditions of humidity, temperature ($25 \pm 2\,^{\circ}C$) and light (12 h light/dark). They were fed with standard rat pelleted diet (M/s Pranav Agro Industries Ltd., India) under the trade name Amrut rat/mice feed and had free access to water. Experimental animals were handled according to the University and Institutional Legislation, regulated by the Committee for the Purpose of Control and Supervision of Experiments