

Anticancer effect of Ellagic acid and paclitaxel on Lipid Peroxidation and Antioxidant status in female Sprague dawley rats

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Abstract: There has been growing interest in studying the role played by lipid peroxidation and antioxidant status in breast cancer. Free radicals are found to be involved in both initiation and promotion of multistage carcinogenesis. In the present study, effects of both ellagic acid and paclitaxel on the levels of lipid peroxides (LPO) and status of antioxidants in several tissues were studied in mammary carcinoma rats. The levels of reactive oxygen species (ROS) were also measured in control and experimental animals. A significant increase in the levels of LPO, ROS and a decreased levels of antioxidants observed in mammary carcinoma bearing rats were found to be reverted back to near normal levels on treatment with both paclitaxel and ellagic acid. These results suggest that the free radical mediated damage during mammary carcinoma could have been controlled by both ellagic acid and paclitaxel by its free radical quenching and antioxidative potential. The above results also show that both paclitaxel and ellagic acid exert its anticancer effect on the development of breast cancer.

Keywords: Ellagic acid, Breast cancer, LPO, Reactive oxygen species.

I. Introduction

Breast cancer is the second most prevalent cancer worldwide and their incidence increases gradually. Women who have stage IV breast cancer receive chemotherapy and/or hormonal therapy to destroy cancer cells and control the disease. The goal of chemotherapy is to shrink primary tumors, slow the tumor growth, and to kill cancer cells that may have spread (metastasized) to other parts of the body from the original tumor. Chemotherapeutic drugs elicit some toxicity towards normal cells also, that limits its usage.

Breast cancer incidence is increasing and it is currently estimated that one in eight women develop the disease during lifetime. It is characterized pathologically by a proliferation of malignant epithelial cells within the mammary ductal-lobular system (Dickson and Clarks 1996). Generally, worldwide trends show that in developing countries going through rapid societal and economic changes, the shift towards lifestyles typical of industrialized countries leads to a rising burden of cancers associated with reproductive, dietary, and hormonal risk factors. Incidence has been increasing in most regions of the world, but there are huge inequalities between rich and poor countries. Incidence rates remain highest in more developed regions, but mortality is relatively much higher in less developed countries due to a lack of early detection and access to treatment facilities. For example, in western Europe, breast cancer incidence has reached more than 90 new cases per 100 000 women annually, compared with 30 per 100 000 in eastern Africa. In contrast, breast cancer mortality rates in these two regions are almost identical, at about 15 per 100 000, which clearly points to a later diagnosis and much poorer survival. Breast cancer is the second leading cause of cancer deaths –about 16% in women after that of cancer of lungs (Greenlee et al. 2001). About 30% of the tumors develop in the breast during her 39 years of life and one in every 231 women faces this disease (Landis SH et al. 2001). In the United States more than 20,000 women are diagnosed with breast cancer every year, however very few risk prediction tests are known. Approximately 8% of American women can expect to be stricken with this disease some time during their life time (Moore et al. 1983).

Selective destruction of tumor cells without damaging normal cells is an important goal for cancer chemotherapy. The problem of cancer in the elderly will become increasingly more apparent. Plants, sources of phytochemicals with anti-cancer potential are reported to interfere with targets implicated in carcinogenesis and tumor cell biology makes them interesting tools in cancer research (Morse et al 1993). The carcinogen used in the present study was Dimethyl Benz (a) anthracene. It has been reported to cause cancer in many cancer models like skin, mammary gland, oral, ovarian and salivary gland (Kim et al. 2003). Huggins et al. (1961), described the rat mammary tumor model which is also referred as a 'Huggins tumor model' and the histogenesis of the model has been studied by Russo (Russo et al. 1996, 2001). It is an indirect acting carcinogen that requires metabolic activation to yield an ultimate carcinogenic form (Moore et al. 2006).

Flavonoids may interfere in several of the steps that lead to the development of malignant tumors, including protecting DNA from oxidative damage, inhibiting carcinogen activation and activating carcinogen

detoxifying systems (Ren et al. 2003; Birt et al. 2001). Researchers have become interested in flavonoids for their medicinal properties, especially their potential role in the prevention of cancers.

Ellagic acid is a nutraceutical and has very strong anti-oxidant properties. It is a potent anti-carcinogen and anti-mutagenic. It is also considered to be a cancer inhibitor which has the ability to restore apoptosis or normal cell death in cancer cells (Daniel et al. 2001). Paclitaxel is a novel antimicrotubule agent that promotes the assembly of microtubules from tubulin dimers and stabilizes microtubules by preventing depolymerization. This stability results in the inhibition of the normal dynamic reorganization of the microtubule network that is essential for vital interphase and mitotic cellular functions. The purpose of the present study is to evaluate the combined effect of Paclitaxel and Ellagic acid against the DMBA induced mammary carcinogenesis.

II. Materials and methods

Animals:

Aged Female Sprague Dawley rats weighing (150-200 g) were purchased from National Institute of Nutrition, Hyderabad, India and were used throughout the study and they were maintained in the controlled environmental conditions of temperature and humidity on alternative 12 hr light/dark cycles. All the animals were fed standard pelleted diet (Gold Mohur rat feed, M/s. Hindustan Lever Ltd., Mumbai) and water ad libitum. Experimental animals were handled according to the university and institutional legislation, regulated by the committee for the purpose of control and supervision of experiments on Animals [CPCSEA, IAEC NO 02/068/06], Ministry of Social Justice and Empowerment, Government of India.

Experimental Protocol :

The animals were divided into five groups of six animals each.

Group I: Control animals treated with Saline.

Group II: Dimethyl Benz (a) anthracene DMBA treated animals (20 mg /kg body wt., one injection by mammary Pad Air pouch technique).

Group III: Breast cancer bearing animals treated with Paclitaxel (33 mg/kg body wt., i.p) for 16 weeks (once in a week).

Group IV: Breast cancer bearing animals treated with Ellagic acid (100 mg/kg body wt., orally) for 16 weeks (twice in a week).

Group V: Breast cancer bearing animals treated with both paclitaxel and Ellagic acid (as in Group III and Group IV) for 16 weeks .

Production of Air Pouch

Air pouch was produced in the Sprague-Dawley rats by the method of Arun et al. (1984). About 2 ml of air was drawn in to the 5 ml syringe. It was autoclaved at 15 psi for 20 minutes in an airtight condition. The sterile air in the syringe was injected carefully just beneath the mammary fat pad subcutaneously so as to produce sterile air pouch. The air inside the pouch was allowed to stabilize for a day before administration of the carcinogen.

Administration of Carcinogen

20 mg of DMBA was weighed in a sterile vial and 0.5 ml of sterile saline and 0.5 ml of sunflower oil were added. The vial was stoppered and vortexed vigorously to obtain a uniformly dispersed emulsion. The single dose of DMBA was injected in to the air pouch. The growth of the tumor was measured at frequent intervals up to the 90th day when it attained maximum size. At the end of the experimental period, the animals were killed by cervical decapitation. The breast and liver tissues were used for further analyses.

Biochemical analysis

Total protein was estimated by the method of Lowry et al . The antioxidant enzymes Superoxide dismutase was analyzed by Marklund and Marklund , Catalase and Glutathione Peroxidase by Sinha and Rotruck respectively. Nonenzymic antioxidants Reduced Glutathione was analyzed by Morom et al , Vitamin E and C by Desai and Omaye et al respectively. Lipid peroxidation was analysed by Ohkawa .

Statistical analysis

For statistical analysis, one way analysis of analysis of Variance (ANOVA) was used, followed by the Newman-Keuls Multiple Comparison test.

III. Results

Table I shows the levels of lipid peroxidation, cellular antioxidant enzymes like SOD, CAT and GPx and non-enzymatic antioxidants GSH Vit E and C in Breast tissues of various experimental groups. Highly significant reductions in the activity of enzymic and non-enzymatic antioxidants, with a concomitant increase in the lipid peroxidation in the tumor bearing rats (Group II) were observed. These adverse changes were reversed to near normal levels in Ellagic acid (100 mg/Kg body wt.) and Paclitaxel (33 mg/Kg body wt.) treated animals (group V) than the animals treated with Paclitaxel (group III) and Ellagic acid alone (group IV) alone.

Fig 1,2,3,4 shows the levels of Lipid peroxidation and enzymic and non- enzymatic antioxidants in liver of various experimental groups. Increase in Lipid peroxidation with significant decrease in antioxidant activities was observed in cancer bearing rats (group II). These changes were reversed in the treatment groups.

IV. Discussion

Polyunsaturated Fatty acid present in the cell membrane undergoes spontaneous free radical chain reaction in the presence of trace of transition metal ions and produces free radicals (Gutteridge 1990). Lipid peroxidation and free radicals are closely associated with various human diseases such as atherosclerosis, diabetes, neurodegenerative diseases and cancer. Flavonoids for a long time have been part of the herbal treatment that act at the initiation stage of LPO as scavengers which react with peroxy radicals of PUFA breaking the chain reaction (Ratty 1998).

Significant increase in lipid peroxidation associated with various forms of carcinogenesis has been documented widely and the scavengers are known to play an important role in cancer prevention or treatment. In the present study also significant increase in the levels of lipid peroxidation was observed in the breast and liver tissues of cancer bearing rats. This may be due to the enormous production of reactive oxygen species and free radicals, which are produced in response to exposure to the carcinogen.

One of the prominent and medically most useful properties of flavonoids is their ability to scavenge the free radicals (Cholbi 1991). Paclitaxel eliminates the tumor cells and reduces the tumor burden in group III cancer bearing rats. Also Ellagic acid when given in combination with any drug increases the potential of the accompanying drug (Agarwal 1981). Hence group V animals treated with both Paclitaxel and Ellagic acid show significant decrease in Lipid peroxidation by modulating the glutathione peroxidase- glutathione reductase system. Gallic acid is a structural product of Ellagic acid contributes to the antilipid peroxidative activity. The present study indicates that Ellagic acid with paclitaxel significantly suppress the LPO in breast and liver tissues of cancer bearing rats.

The survival of any organism may depend on its ability to overcome the toxic effect of oxygen species. Antioxidants are the free radical scavengers that provide a natural defense system against free radicals (Sun 1990). The activities of antioxidant enzymes like SOD, CAT and GPx were significantly reduced in the group II cancer bearing rats.

This is due to the increased levels of oxygen radicals which themselves reduce the activity and availability of antioxidants (Wiseman 1996)). Several authors have cited the decreased activities of these antioxidant enzymes in various types of tumors (Winkler 1992)). Group III cancer bearing animals treated with Paclitaxel alone showed increase in the activities of these enzymes when compared to group II animals. This might be due to the cytotoxicity exerted by Paclitaxel towards cancer cells, which inhibits the cell division hence the utilization of these enzymes by the tumor cells. In Paclitaxel and Ellagic acid treated Group V animals the activities were significantly inclined towards near normal due to the combined cytotoxic and antioxidant effect of the drugs used in our present study.

The presence of nonenzymic antioxidants like Reduced glutathione (GSH) Vitamin C and E are also essential for the removal of radicals (Moron 1979). GPx the major antioxidant enzymes decomposes hydroxyl radicals at the expense of GSH. Hence any decrease in GPx concentration can attribute to the decline in glutathione concentration. In the present study the decreased level of GSH in cancer bearing rats might be due to its irreversible loss following oxidative stress and or use of GSH for free radical scavenging excess utilization of these antioxidant for cell proliferation by tumor cells also. Vitamin E is a chain breaking antioxidant that donates its labile hydrogen atom from phenolic hydroxyl groups to propagating lipid peroxy and alkoxy radical intermediates of LPO, thus terminating the chain reaction (Desai 1984). Reduced glutathione keeps up the cellular levels of ascorbic acid and tocopherol in active form. Hence GSH Vitamin C and E are tightly linked to each other. Reduced glutathione replenish vitamin E from α tocopherol radical and vitamin C also supports the recycling of tocopherol. When there is reduction in the levels of GSH cellular levels of nonenzymic antioxidants are also lowered. There is extensive evidence that administration of ellagic acid for 16 weeks can increase the GSH level.

In conclusion the antioxidant properties of Ellagic acid could very well be attributed to their free radical scavenging activity against alkoxy radicals. When given combined with Paclitaxel it reduces the toxic side effects of the later by its immunomodulatory activity and improves the treatment strategy. Our data

suggests that administration of Ellagic acid 100 mg/Kg with Paclitaxel significantly decrease the toxic implications of chemotherapy and increases the levels of free radical scavenging enzymes.

Table 1: Effect of Ellagic acid and Paclitaxel on non-enzymic antioxidants induced by DMBA in haemolysate, breast and liver of control and experimental animals

		Group I	Group II	Group III	Group IV	Group V
Reduced Glutathione (GSH)	Haemolysate	7.93±0.91	3.47±0.24a [#]	5.27±0.31b [#]	5.96±0.42b [#]	6.55±0.49b [#]
	Breast	6.50±0.53	3.43±0.27a [#]	4.50±0.44b [#]	5.63±0.45b [#]	6.06±0.53b [#]
	Liver	4.8±0.44	2.4±0.26a [#]	3.0±0.31b [@]	1.82±0.16b [@]	4.2±0.35b [#]
Vitamin C	Haemolysate	4.39±0.91	1.74±0.77a [#]	2.66±0.18b [#]	2.96±0.19b [#]	3.39±0.25b [#]
	Breast	3.70±0.26	1.45±0.13 [#]	2.06±0.10b [#]	2.72±0.22b [#]	3.10±0.17b [#]
	Liver	2.6±0.26	1.5±0.13a [#]	1.8±0.16b [#]	2.12±0.14b [#]	2.40±0.26b [#]
Vitamin E	Haemolysate	3.72±0.27	1.66±0.11a [#]	2.51±0.18b [#]	3.01±0.26b [#]	3.21±0.25b [#]
	Breast	10.1±0.90	6.50±0.44a [#]	7.40±0.62b [#]	8.26±0.67b [#]	9.20±0.80b [#]
	Liver	7.3±0.62	4.26±0.40a [#]	5.0±0.45b [#]	5.5±0.44b [#]	6.35±0.56b [#]

Each value is expressed as mean ±S.D for six rats in each group.

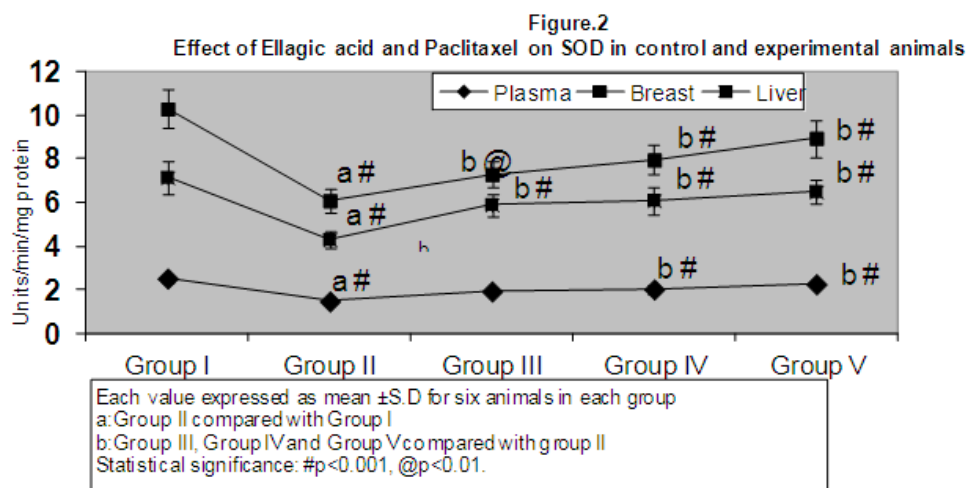
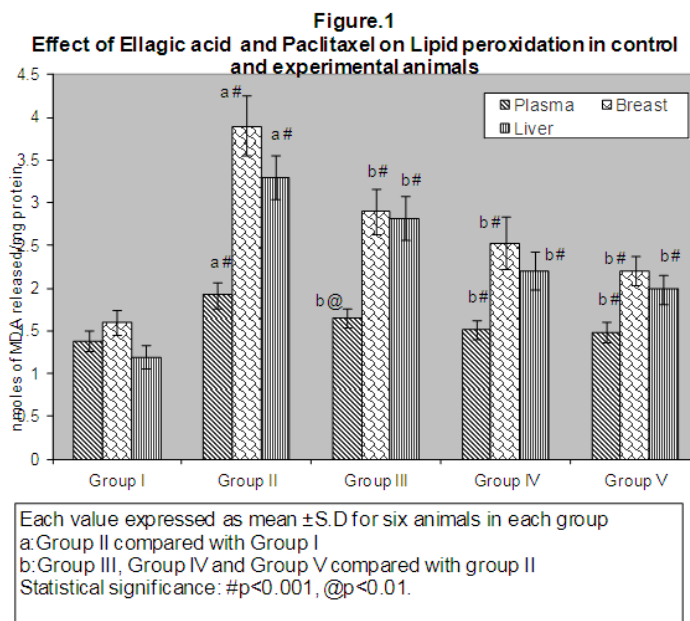
Units: Haemolysate: GSH, Vitamin C and E: µg/dl.

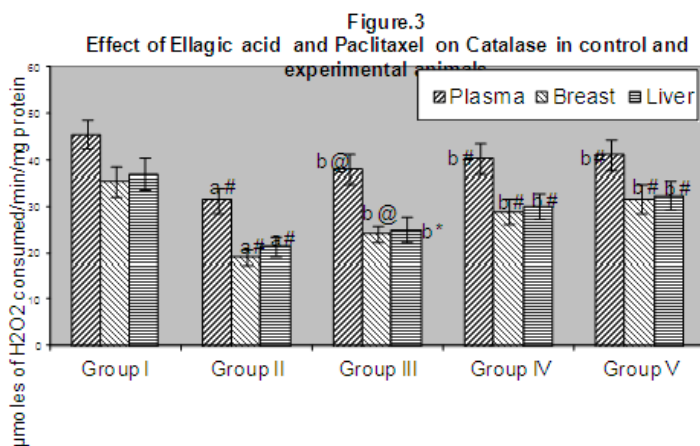
Breast and liver tissues: GSH, Vitamin C and E: µg/mg protein.

a: Group II compared with Group I;

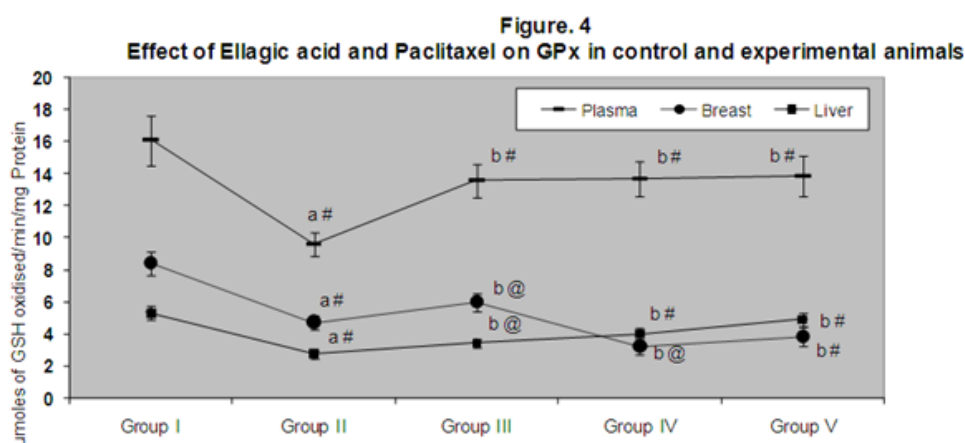
b: Group III, Group IV and Group V compared with Group II.

Statistical significance [#] P<0.001, [@]P< 0.01, *P<0.05.





Each value expressed as mean \pm S.D for six animals in each group
a: Group II compared with Group I
b: Group III, Group IV and Group V compared with group II



Each value expressed as mean \pm S.D for six animals in each group
a: Group II compared with Group I
b: Group III, Group IV and Group V compared with group II

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