ANTIOXIDANT ACTIVITY OF ASTERACANTHA LONGIFOLIA IN DMBA INDUCED MAMMARY CANCER IN SPRAGUE DAWLEY RATS

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Abstract: Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to human against infections and degenerative diseases. Current research is directed towards natural antioxidants originated from plants due to safe therapeutics. Asteracantha longifolia (Family-Acanthaceae), commonly known as ‘Hygrophila’ being used as folklore medicine. Plant has been reported to posses in vitro antioxidant activity and anticancer in nature. Hence the present study was evaluated for antioxidant activity of petroleum ether extract of A. longifolia by chemically inducing mammary tumor using 7, 12-Dimethylbenz (a) anthracene (DMBA) in Sprague Dawley rats. Test groups were treated with A. longifolia extract @ 600, 900 and 1200 mg/kg and the antioxidant activity is compared with the group of rats administered with Vitamin C (ascorbic acid) @ 200mg/kg body weight for 45 days. Mammary tumor, liver and kidney tissue were processed for antioxidant enzyme assay. The antioxidant activity were correlating with increased dose of plant extract, the data obtained in present study suggests that the extract of A. longifolia have potent antioxidant activity and prevent oxidative damage to major biomolecules and afford significant protection against oxidative damage seen in cancer.

Key words: Asteracantha longifolia, Mammary tumor, antioxidant.

INTRODUCTION

Antioxidants are defined as compounds that can delay, inhibit, or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. Oxidative stress is an imbalanced state where excessive quantities of reactive oxygen and/or nitrogen species (ROS/RNS, e.g., superoxide anion, hydrogen peroxide, hydroxyl radical, peroxynitrite) overcome endogenous antioxidant capacity, leading to oxidation of a varieties of biomacromolecules, such as enzymes, proteins, DNA and lipids. ROS, particularly $\text{H}_2\text{O}_2$, are potent regulators of cell replication and play an important role in signal transduction [1]. Hence, oxidative damage is considered a main factor contributing to carcinogenesis and evolution of cancer [2].

Cancer is a multi-step disease incorporating environmental, chemical, physical, metabolic, and genetic factors which play a direct and/or indirect role in the induction and deterioration of cancers. Plants with rich antioxidant activity significantly reduce the risk of many cancers, suggesting that certain phytoconstituents could be effective agents for the prevention of cancer incidence and mortality. These components present in the plant are a very promising group of compounds because of their safety, low toxicity, and general acceptance [3]. Flavonoids constitute one of the most numerous and ubiquitous...
group of plant metabolites. It was found that in addition to their primary antioxidant activity, this group of compounds displays a wide variety of biological functions which are mainly related to modulation of carcinogenesis. *A. longifolia* is one such plant has potent *in vitro* antioxidant activity, which might be due to rich flavonoid content of the plant [4]. The present study was aimed to evaluate the *in vivo* antioxidant activity by using chemically induced mammary tumors in Sprague Dawley rats as model animal.

**MATERIALS AND METHODS**

**Plant extract:** Aerial parts of *A. longifolia* were collected from Hassan District of Karnataka state, dried under shade (10 days), powdered and stored in air tight container until the preparation of extract. The powder (100g) was mixed in 1000 ml of petroleum ether, kept for 5 days and were periodically shaken using an electric shaker. After 5 days, contents were filtered by whatmann filter paper no.1 and it was further concentrated by rotary flask evaporator (Superfit India Ltd., Mumbai) at 39-40°C. The residual ether was evaporated after keeping the extract in a petri dish in a vacuum oven at 60°C for 3 min and it was further concentrated by rotary flask evaporator (Superfit India Ltd., Mumbai) at 39-40°C.

**Experimental design:** A total of 60, 45 days old Sprague Dawley (SD) female rats were procured from Central Animal Facility, Indian Institute of Sciences, Bangalore. The experiment was conducted with prior permission from the institutional ethical committee. All the sixty young virgin SD female rats were allowed to acclimatize to the laboratory environment and fed with *ad libitum* commercial pellet ration and potable water. The rats were divided in to normal control group with 6 animals and treatment group with 54 animals. The treatment group animals were administered with three doses of 60µg of estrodiol valerate (Cadila®) intramamillary at 4 days of interval prior to 7,12-dimethylbenz-(a)anthracene (DMBA) administration.

**Source of carcinogen:** For the induction of mammary gland tumors in rats, 7, 12-dimethylbenz (a) anthracene (DMBA) was used. DMBA was procured from Sigma Aldrich, USA (molecular weight: 256.34). For the administration of DMBA, soya oil was used as vehicle, which was procured from a commercial source (Fortune®: Soya refined oil).

**Administration of chemical carcinogen DMBA:** At the age of 45 days, all the animals, which weighed 185 g average, were administered by oral gavage with 1 ml of soya oil consisting of 10 mg of DMBA thrice at weekly interval followed by 5 mg of DMBA in soya oil as last dose intramammarily. Animals were palpated weekly and the locations of tumors were noticed. Tumors sizes were determined by palpation, comparing the volume of each tumor to that of preformed plasticine models. Tumor sizes were calculated using the formula: δ/6 × length (cm) × width² (cm) [5,6]. Then, the total weight of the tumors were calculated (assuming a density of 1gm/ml) after 12 weeks of induction (Fig. 1). The animals having suitable tumors (<6 g wt.) were randomly divided into different groups consisting of six animals per group. Group I served as negative control without any mammary tumor and gavaged with distilled water. Rats with mammary tumor were divided into group II as DMBA control gavaged with distilled water, group III administered with vitamin C (ascorbic acid) @ 200mg/kg body weight and group IV, V, VI were gavaged with *A. longifolia* extract at the dose level of 600, 900, 1200 mg/kg daily respectively for 45 days after induction of mammary tumors. Rats were sacrificed at the end of the study, mammary tumor (Fig. 2), liver and kidney were collected and subjected to antioxidant enzyme assay.

**Antioxidant enzyme assay:** Part of collected organ samples was washed immediately in ice-cold saline and tissues were homogenized in 10 % 0.1M Tris-HCl buffer (pH 7.2) and centrifuged at 12,000 g for 30 min at 4°C. The supernatant obtained was used for the analysis of enzymatic as well as non-enzymatic antioxidants. Protein in each sample was estimated with coomassie brilliant blue G-250 using bovine serum albumin as a standard [7].

Superoxide dismutase (SOD) (EC 1.15.1.1) was determined by assay mixture consisted of 2 ml of 0.1M Tris Hcl, 0.5 ml of homogenate, 1.5 ml of distilled water and 0.5 ml of pyrogallol. OD value was taken for 3 min at 420 nm wave length and expressed as units/mg protein [8]. Catalase (CAT) (EC 1.11.1.6) was estimated by addition of 0.2 ml of homogenate and 1 ml of 30 mM H₂O₂ was added and the OD value was taken at 240 nm at an interval of 1 min for 3 min and enzyme activity was expressed as imol of H₂O₂ decomposed per min per mg of protein [9].

The level of thiobarbituric acid reactive substances
(TBARS) which are the indicators of lipid peroxidation was measured as per the modified method of Ramanarayan et al. [10]. The absorbance of chloroform phase having brilliant pink color was measured at 532 nm and the values were expressed as \( \text{imol L}^{-1} \text{ cm}^{-1} \) of malondialdehyde (MDA)/g tissue. Glutathione peroxidase (GPx) (EC 1.11.1.9) was determined by taking OD value at 412 nm and values expressed as \( \text{mM of glutathione utilized / min / mg protein} \) [11].

**Statistical analysis:** The data obtained from the present study were subjected to statistical analysis. The data were analyzed by using two-way ANOVA. \( P<0.05 \) was considered as significant. The analysis was carried out by Bonferroni’s multiple comparison test. Mean values and standard error of mean was calculated and all the values are expressed as mean ± SEM [12].

**RESULTS**

In antioxidant enzyme assay, the activities of SOD and CAT in plant extract treated animals (group IV, 

![Fig. 1](image1): Showing mammary tumor in the right caudal mammary gland in Sprague Dawley rat, 12 weeks post administration of DMBA.

![Fig. 2](image2): Showing gross mammary tumor in left caudal mammary gland during post-mortem examination, 45 days post administration of *A. longifolia* plant extract.
The petroleum ether extract of *A. longifolia* was administered to rats for duration of three weeks, there was significant increase in the level of SOD, CAT and GPx. Lipid peroxidation, measured by the formation of thiobarbituric acid reactive substances and in the present study there was significant increase in DMBA-treated groups, this was in agreement with earlier studies [15]. *A. longifolia* extract at lower doses did not considerably reduce the increased lipid peroxidation level, but higher doses of the extract showed considerable reduction in lipid peroxidation level which correlates with the earlier works [16].

DISCUSSION

The present investigation was carried out to evaluate the antioxidant activity of *A. longifolia* in DMBA induced mammary tumor in Sprague Dawley rats. The petroleum ether extract of *A. longifolia* exhibited significant antioxidant effect in a dose-dependent manner by increasing the levels of SOD, CAT, GPx and by decreasing the levels of lipid peroxidation suggesting the antioxidant property at higher dose rate and the antioxidant effects were similar to the effects of standard Vitamin C (ascorbic acid) at the dose of 200mg/kg body weight [13]. These results were in accordance with the findings of Vijaykumar et al. [14] which states that, ethanol extract of aerial parts of *A. longifolia* was administered to rats for duration of three weeks, there was significant increase in the level of SOD, CAT and GPx. Lipid peroxidation, measured by the formation of thiobarbituric acid reactive substances and in the present study there was significant increase in DMBA-treated groups, this was in agreement with earlier studies [15]. *A. longifolia* extract at lower doses did not considerably reduce the increased lipid peroxidation level, but higher doses of the extract showed considerable reduction in lipid peroxidation level which correlates with the earlier works [16].

The Phytochemical analysis of *Asteracantha longifolia* leaf extract was found positive for flavanoids, coumarins, bitter principles [4,13]. These phyto-constituents might be responsible for the biological activity of the *A. longifolia*. Among the chemical constituents, flavanoids are the most important biologically active compounds [17-19]. Sawadogo et al. [20] also found that methanolic extract of leaves of this plant contains phenolic and...
flavonoids showing promising antioxidant activity. Likewise, Dasgupta and De [21] reported that the aqueous extract of *A. longifolia* showed presence of flavonoids which has potent antioxidant property in various *in vitro* models. Due to their ability to scavenge and reduce the production of free radicals and act as transition metal chelators, natural flavonoids can exert a major chemopreventive activity [22]. Indeed, it has been shown that natural flavonoids can inhibit carcinogen/toxin-induced cellular oxidative damage, similar results have also been reported in oral carcinoma cell lines with epigallocatechin gallate [23].

Thus *A. longifolia* plant extract showed decrease in lipid peroxidation which is associated with increased activity of superoxide dismutase and catalase. Due to the free radical scavenging property, *A. longifolia* helps to improve the antioxidants defense system and prevent the damage induced by free radicals which is required to prevent the progression of cancer development [24].

**CONCLUSION**

On the basis of the results obtained in the present study, it is concluded that petroleum ether extract of *A. longifolia* exhibits high antioxidant activity. This *in vivo* assay indicates that this plant extract is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. However, the components responsible for the antioxidative activity are currently unclear. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract. Further study is in progress.

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**REFERENCES**