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Research Article

ANTINEOPLASTIC AND ANTIOXIDANT ACTIVITIES OF *ACORUS CALAMUS* L ON SWISS ALBINO MICE BEARING DALTON'S ASCITES LYMPHOMA

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ABSTRACT

Objective: Ethnobotanical alternates for synthetic allopathic drugs are soon gaining importance in the treatment of diseases due to their fewer side effects. *Acorus calamus* has been widely used globally due its richness in antioxidant phytoconstituents in the treatments yielding beneficial functions. The present study details the *in vivo* anticancerous effect of *A. calamus* against Dalton's ascites lymphoma (DAL).

Methods: The extract obtained by soxhlet extraction was screened for antioxidants by the enzymatic and non-enzymatic methods to indicate any inhibitory properties on DAL induced cells.

Results: Soxhlet extraction using organic solvents and water revealed methanol to yield a maximum percentage of the crude extract that was stored for antioxidant and antineoplastic analysis. DAL induced tumor cells in mice revealed decreased levels of superoxide dismutase, catalase, glutathione peroxidise, glutathione, vitamin C and also vitamin E on analysis of liver and kidney samples. The same organs, when analyzed on treatment with the methanolic extract of *A. calamus* revealed a reversal of these decreased values to normal range.

Conclusion: These results thus indicate the potential use of *A. calamus* for anticancer studies although the main phytoconstituent in the extract obtained is to be further analyzed.

Keywords: *Acorus calamus,* Dalton's ascites lymphoma, Anticancer, Phytoconstituents, Antioxidants.

INTRODUCTION

Indian systems of medicine, such as Ayurveda, Unani, and Siddha, have largely depended on a number of plant-derived extracts that are used and tested against diseases. This plant-based approach mainly is accountable by the immune modulatory and antioxidant potential leading to anticancer derivatives from medicinal plants. This immune modulatory activity is stimulated by both non-specific and specific immunity [1]. Dietary natural occurring substances that are absorbed by the human body due to consumption of large amounts of vegetables and fruits are also targeted and identified as potential chemopreventive agents [2].

Antioxidants are known free radical scavengers that prevent and cure cancer by protecting cells from the damage caused by highly reactive oxygen compounds. Humans are exposed to free radicals in the environment through radiation and pollution and in the body through various metabolic reactions. Antioxidants scavenge these free radicals and enable the cells to rejuvenate or stabilize for the process of life [3]. Phytochemical constituents such as the alkaloids, flavonoids, triterpenoids, phenolics, and steroids exert multiple biological effects due to their radical scavenging activity; thereby producing protective effects against diseases and pathologies [4].

Acorus calamus is used for several treatment conditions as an ethnobiological resource with a long history of usage not only in Asia but across the globe [5]. Historical ethnobotanical review of *A. calamus* dates back possibly to the time of Moses in old testament of the holy bible and in early Greek and Roman medicine. It is generally used from ancient and Vedic periods because of its ability to rejuvenate brain and nervous system and to normalize the appetite. Phytochemically, its richness in phenolics, alkaloids and flavanoids is indicative of the beneficial functions it harbors. Flavanoids, particularly, are secondary metabolites from aromatic plants and mainly provide protection from reactive oxygen species (ROS), photosynthetic stress and wound healing properties to the plants. Due to their high antioxidant potential, several flavanoids have been experimentally proved to inhibit cancer development [6,7].

Dalton's ascites lymphoma (DAL) tumorigenesis model in Balb/C/ Swiss albino mice provides a convenient model system to study antitumor activity within a short time [8]. DAL is referred as an undifferentiated hyperdiploid carcinoma with high no-regression, transplantable capability, shorter life span, rapid proliferation, 100% malignancy and functions without the tumor specific transplantation antigen [9]. Experiments with DAL cells following transplantation into the abdominal cavity of healthy mice indicates aggressive and immediate tumorigenesis. To our knowledge, no reports are available on the anticancer activities of the rhizome of *A. calamus* in DAL-bearing mice. Hence, this study was carried out to gain an insight into the antineoplastic and antioxidant potential of the methanolic extract of *A. calamus* (MEAC) against DAL cells.

METHODS

Collection and identification of the plant

A. calamus, the plant used for the present study was collected from Alappuzha district of Kerala, India. Identification of the plant was done in the Department of Botany, Sanatana Dharma College, Alappuzha. A voucher specimen is preserved as herbarium and submitted to the Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore.

Preparation of rhizome powder

The plant *A. calamus* with rhizome washed thoroughly to remove soil particles and adhered debris using sterile distilled water. Fresh rhizomes used for extraction were shade dried and powered using a mechanical grinder. Fine powder was obtained by sieving.

The powder was collected in two clean air tight containers. Powdered plant material in one container was used for determining the

physico-chemical parameters and the other was used for methanolic extraction.

Soxhlet extraction

10 g of the powder was weighed using an electrical balance (Denver 210) and made in to 8 packets using xerohaze filter paper (10 A grade SD's). The powder was subjected to sequential soxhlet extraction using different solvents such as petroleum ether, chloroform, ethanol, methanol, and water to get respective extracts. All the extracts were filtered and evaporated to dryness and percentage yields of the extracts were determined. From this, the maximum percentage yield was noted and that extract alone was stored in a refrigerator and used for the further analysis.

Experimental animals

Healthy Swiss albino mice, *Mus musculus* (20±5 g) were used for the study. The animals were obtained from Amala Cancer Institute, Thrissur, Kerala and brought to the laboratory. Animals were kept in polypropylene cages with Sawdust bedding and maintained in laboratory conditions. Standard pellets were given as diet and water was provided *ad libitum*. The animals were acclimatized to laboratory condition for about 1 week before commencement of the experiment. The experiments were performed after the approval from the Institution of Animal Ethical Committee and in accordance with the recommendation for the proper care and use of the laboratory animals.

Treatment procedure

Animals were divided into five groups each comprising six animals. One group served as the control while the remaining four groups were injected with Dalton's ascites lymphoma (1×10^6 cells/mouse) to induce tumor. The treatments were given intraperitonially at 24 hrs after the tumor inoculation and continued for 14 consecutive days.

The designation of the animal groups and treatment details were as follows:

Group I → Normal control
Group II → DAL control
Group III → DAL+positive control (5-FU: 10 mg/kg)
Group IV → DAL+MEAC 100 mg/kg
Group V → DAL+MEAC 200 mg/kg

Antioxidant studies

The antioxidant assay was performed with liver and kidney tissues. All the tissue preparations were frozen on dry ice and then transferred to a –80°C freezer. The isolated organs were divided into 2 parts for the preparation of homogenates.

One part was used for the preparation of 10% w/v homogenate in potassium chloride (0.15 M). It was centrifuged at 8000 rpm for 10 minutes and the supernatant thus obtained was used for estimation of catalase (CAT) and malondialdehyde (MDA).

The second part was used for the preparation of 10% w/v homogenate in 0.25% w/v sucrose in phosphate buffer (5 M, pH 7.4) and was centrifuged at 8000 rpm for 10 minutes. The supernatant thus obtained was used for estimation of superoxide dismutase (SOD) and glutathione peroxidase (GSH). All the other estimations were done according to the manufacturer manual of standard enzymatic kits procured from piramal healthcare limited, lab diagnostic division, Mumbai, India by using semi auto analyzer (photometer 5010 v_{5+}).

Statistical Analysis

The values measured in triplicates were expressed as mean ± standard deviation. The statistical analysis was performed using one-way Analysis of Variance followed by Dunnett's test using Graph pad prism software. The results showing $p<0.01$ was considered significant.

RESULTS

The soxhlet processed results are expressed as percentage yield of crude extract (Table 1). The experimental data shows that the yield obtained by using methanol as organic solvent produced a significant more percentage of crude extract when compared to other organic solvents. On comparison with water, however, methanol is indicated to yield more than double the volume of crude extract. Among the organic solvents, chloroform was found to produce marginally less crude extract when compared to petroleum ether and ethanol.

The levels of various tissue antioxidants were summarized in Figs. 1 and 2. The level of MDA in the tumor control animal was found to be

Table 1: Yield (%) of extraction of *A. calamus* using water and four organic solvents

S. No	Solvent	Empty weight of petri plate	Weight of petri plate+ residue	Actual weight of residue	
				Extraction volume	% yield
1	Petroleum ether	52.3	52.67	0.37	3.7
2	Chloroform	45.6	45.92	0.32	3.2
3	Ethanol	53.1	53.43	0.33	3.3
4	Methanol	56.0	56.5	0.5	5
5	Water	46.7	46.9	0.2	2

A. calamus: Acorus calamus

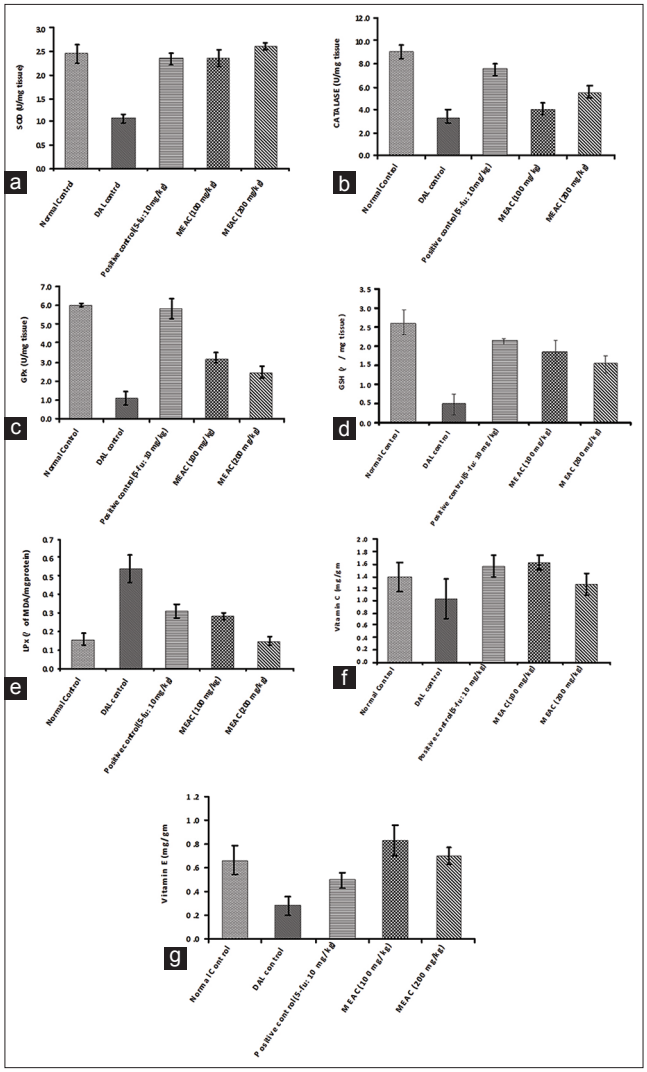

Fig. 1: Effect of methanolic extract of *Acorus calamus* on liver antioxidant enzyme level, (a) Superoxide dismutase, (b) Catalase, (c) Glutathione peroxidase, (d) Glutathione, (e) Lipoperoxidation, (f) Vitamin C, and (g) Vitamin E. Values are significantly different from cancer control at p<0.01

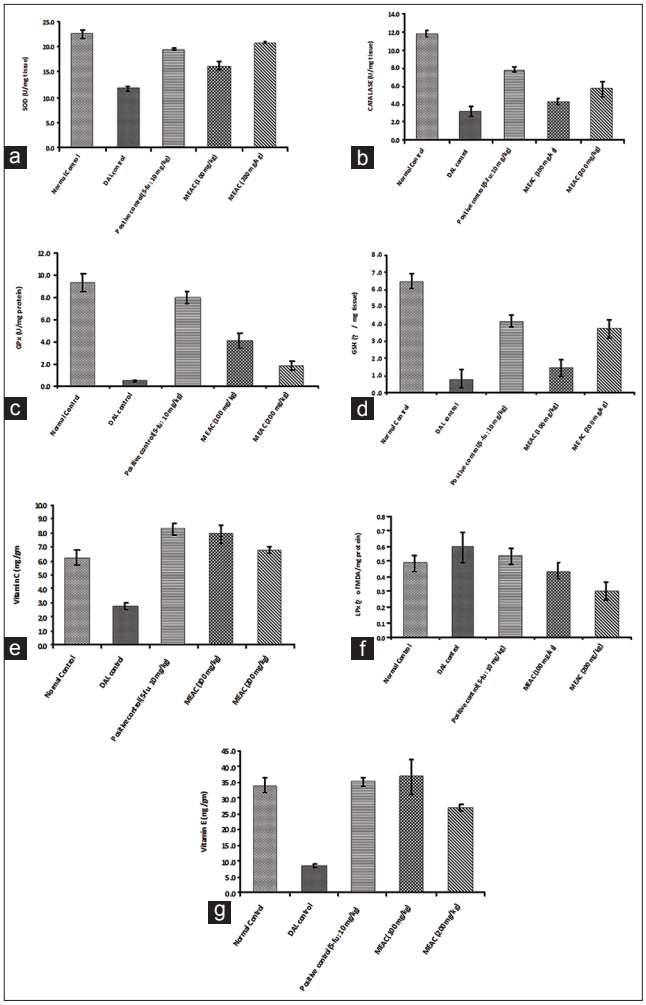

Fig. 2: Effect of methanolic extract of *Acorus calamus* on kidney antioxidant enzyme level (a) Superoxide dismutase, (b) Catalase, (c) Glutathione peroxidase, (d) Glutathione, (e) Lipoperoxidation, (f) Vitamin C, and (g) Vitamin E. Values are significantly different from cancer control at p<0.01

increased when compared to normal and MEAC treatment animals. The inoculation of DAL cells to tumor control animals caused significant (p<0.001) decrease in the levels of SOD, CAT, GPx, GSH, vitamin C and also vitamin E levels in the liver and kidney samples. The treatment with MEAC at doses 100 and 200 mg/kg bodyweight reversed these changes to near normal values. Most of the results were found to be significant. Almost similar results were observed with 5-FU treatment.

DISCUSSION

Although the causes of cancer are largely divided into three groups - physical (e.g. radiation), chemical (carcinogens), and viral (e.g. oncogenes), the mechanism for the action of the three groups have been believed to be mediated largely by free radicals. Substantial experimental evidence so far has provided the strong implication that free radicals are involved in both the initiation and promotion stages of carcinogenesis [10]. Although studies clearly indicate antioxidant enzymes to scavenge radicals, the mechanisms and implications are still unclear; although substantiative results indicate SOD, H_2O_2, and CAT break down water to stop the formation of ROS. The present study carried out to evaluate the effect of MEAC on the antioxidant status in different organs in DAL bearing mice indicates certain degrees of antioxidant potential by scavenging free radicals.

SOD plays an important role in the antioxidant enzyme defense system. SODs convert superoxide radicals in to hydrogen peroxide. In eukaryotic cells, two intracellular SOD exist: The Cu, ZnSOD and the MnSOD [11]. Cu, ZnSOD is the major intracellular SOD. It exists as a 32 kDa homodimer and is present in the cytoplasm and nucleus of every cell type examined, where it acts as a bulk scavenger of superoxide [12]. The MnSOD is a 96 kDa homotetramer and is located primarily in the mitochondrial matrix. The loss or disfunction of either Cu, ZnSOD or MnSOD has been associated with ROS mediated pathologies. For example, mutated Cu, ZnSOD proteins have been linked to instances of amyotrophic lateral sclerosis while loss of MnSOD has been associated with neonatal death [13]. MEAC enhances the activity of SOD in mice by acceleration of SOD mediated catalyzing reaction. Therefore, our study revealed that increased in level of SOD and protective effect on liver and kidney from cancer induced ROS.

Oxidative stress triggers detoxification pathway that involves multiple enzymes. SOD is studied to catalyze the first step followed by CAT and various peroxidases that effectively remove hydrogen peroxide from cell to prevent the formation of free radicals. These enzymes protect DNA from oxidative stress and prevent the individual's risk of cancer susceptibility. These observations are in accordance with this study as a reduction of CAT enzyme level in DAL control group was observed that was normalized by MEAC treatment.

Glutathione is a natural tripeptide found within almost all cells with a vital role in the maintenance of the redox state, detoxification of xenobiotics, and modulation of the immune response. With respect to cancer, glutathione metabolism is able to play both protective and pathogenic roles [14] although elevated levels of glutathione in cancer treatment are able to protect the other cell from violent ROS. Our research also showed that MEAC treatment group showed an elevation of GSH in liver and kidney.

Much attention has been given to the problems of measuring lipid oxidation in biological systems [15]. Many reviews on the biological effects of lipid oxidation products and their relevance to cancer implicate lipid oxidation products in the disruption of biological membranes, the inactivation of enzymes and damage to proteins, the formation of age pigments in damaged membranes, oxidative damage to lungs by atmospheric pollutants and cancer. The formation of fluorescent conjugated Schiff bases by the interaction of amino acids, esters, and amines with MDA has been suggested for a long time as a measure of *in vivo* lipid oxidation. The significantly elevated levels of MDA in liver and kidney of tumor inoculated animals indicated that unsaturated chain of membrane fatty acids can readily react with free radicals and undergo peroxidation.

The most notable defense mechanisms that the body has against *in vivo* lipid oxidation include vitamin E and other natural antioxidants, and protective enzymes such as glutathione peroxides and SOD. Vitamin E is the most effective *in vivo* inhibitor of lipid oxidation [16]. In addition to its dual effects as a free radical and, oxygen scavenger vitamin E may have other cellular effects in protecting the integrity of the membranes [17]. The significantly elevated levels of vitamin E in MEAC treatment groups probably suggests that a comparatively stronger effect of vitamin E and dosages could play an important role in the inhibitory properties of DAL cells.

Vitamin C is one of the most popular and least toxic antioxidants in food, and is widely reputed to have chemopreventive effects at dosages higher than the current RDA of 60 mg/day. Vitamin C has been used as a dietary supplement to prevent chronic oxidative stress-mediated diseases such as cancer. It is found in high concentrations in immune cells, and is consumed quickly during infections. Similar results were obtained with the present study.

CONCLUSION

The present study concluded that the methanol extract of *A. calamus* exhibited anticancer potential against DAL in Swiss albino mice. Both the doses of MEAC protect the liver and kidney enzymes in cancer

bearing mice. The possible mechanism of anticancer effect may be due to its antioxidant effect. It may be possible that the natural antioxidants strengthen the endogenous antioxidant defense from ROS ravage and restore the optimal balance by neutralizing the reactive species.

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