ANTI-LIPID PEROXIDATIVE ACTIVITIES OF *Cynodon dactylon* AND *Moringa oleifera* AGAINST ELA INDUCED MICE

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Summary

Anti-lipid peroxidative activities of *Cynodon dactylon* and *Moringa oleifera* were quantified by measuring the production of Malondialdehyde during peroxidation of lipids. The lipid peroxide level decreased significantly by the intraperitoneal injection of plant leaf and seed protein extracts as compared to ELA induced mice. When compared to these two plant proteins, *Cynodon* protein can be considered as a potential anti-lipid peroxidative agent.

Key words: *Cynodon dactylon, Moringa oleifera*, Ehrlich's lymphoma ascites, liver marker enzymes, phosphate buffered saline, protein fractions

Introduction

Cancer is a genetic disease, arising from an accumulation of mutations that promote clonal selection of cells with increasingly aggressive behaviour [1]. A number of medicinal plants have been screened for anticancerous property [2]. Plant derived compounds are important sources used as anti-cancer agents [3]. Antioxidants play a major role in deactivation of oxygen whereas high activities of protective enzymes are mainly responsible for detoxification of long living oxygen products such as oxygen and hydrogen peroxide [4]. An active constituent of *Asparagus arcemosus* has anticarcinogenic activity in an animal model [5]. *Moringa oleifera* is a highly valued plant and important for its medicinal value. It is a draught tolerant and provides a rich and rare combination of quercetin and saponin [6&7]. *Cynodon dactylon* contains several constituents such as β-sitosterol, β.carotene, vitamin C, palmitic acid, triterpenoids, alkaloids, furfural, glucose, fructose selenium. It possesses antimicrobial [8] and antioxidant [9] activities. Antioxidant potential of *Chlorophytum tuberpsum* has been investigated for their ability to scavenge 1, 1-diphenyl picryl hydrazyl (DPPH), no radical with their capacity to reduce lipid peroxidation in rat liver homogenate [10]. The present study is an attempt to evaluate the anti-lipid peroxidative activities of *Moringa oleifera* and *Cynodon dactylon* against Ehrlich's Lymphoma Ascites.

Materials and Methods

Plant material

Fresh leaves of *Cynodon dactylon* (*C. dactylon*) and seeds of *Moringa oleifera* (*M. oleifera*) were collected in an area free of pesticides and other contaminants from the surrounding of Tiruchengode. The collected leaves and seeds were washed thoroughly and blotted dry with filter paper and used for the protein preparation.

Preparation of proteins

Using Phosphate Buffered Saline (PBS), 20% extract of *C. dactylon* fresh leaves and *M. oleifera* seeds were prepared and centrifuged at 5000 rpm for 10 minutes. The supernatant was subjected to the ammonium sulphate fractionation using 10-100% saturation of ammonium sulphate and the precipitates were dissolved in a known amount of PBS. Dialysis was done to desalt the protein fractions.

Animals

Swiss albino mice weighing 25-30g of either sex were used in this study. They were procured from Perundurai Medical College, Perundurai. The animals were acclimatized for 15 days under laboratory conditions. They were housed in polypropylene cages and maintained at 27ºC ± 2ºC. They were fed with standard mice feed and water *ad libitum* was provided. The litter in the cages was renewed thrice a week to ensure hygeinicity and maximum comfort for animals. Ethical clearance for handling the animals was obtained from the Institutional Animals Ethical Committee prior to the beginning of the project work (889/ac/05/CPCSEA).

Tumor cell lines maintenance

Ehrlich's Lymphoma Ascites (ELA) tumor cell lines were procured from Amala Cancer Research Centre, Thrissur, Kerala. The mice were acclimatized for two weeks and cells were propagated by intraperitoneal transplantation of 1 x 10^6 cells in 100 µl of PBS. After 10-15 days, the cells were drawn from the intraperitoneal cavity and used for the *in vitro* cytotoxic studies by trypan blue exclusion method. *In vivo* studies were carried out using 70% ammonium sulphate C. protein and 10% of ammonium sulphate M. protein.

Grouping of animals

The animals were divided into 8 groups and each group consisted of 6 mice.

Group 1 received (i.p) 0.1 ml of PBS every day and served as a vehicle control for the experimental groups 4 to 8.

Group 2 received (i.p) 0.1 ml of paraffin oil, which constituted the vehicle control for the standard antioxidant silymarin group.

Group 3 received (i.p) 25mg standard antioxidant silymarin in 100 µl of paraffin oil / kg body weight.

Group 4 received (i.p) ED_{50} of C. protein (52µg in 100µl of PBS).

Group 5 received (i.p) ED_{50} of M. protein (40µg in 100µl of PBS).

Group 6 received C.protein and $1x10^6$ ELA tumor cells (i.p) on the same day and Cdpf administration was continued for 30 days.

Group 7 received M. protein and $1x10^6$ ELA tumor cells (i.p) on the same day and M. protein administration was continued for 30 days.

Group 8 received $1x10^6$ ELA tumor cells (i.p) that served as ELA control.

After 30 days, the mice were sacrificed and the liver, lung, kidney, heart, spleen and brain were dissected, blotted of blood and washed with PBS and homogenate was prepared using PBS and used for the determination of lipid peroxide levels. The anti-lipid peroxidation effect was assessed by estimating the Malondialdehyde produced during lipid peroxidation [11].

Statistical analysis

Results were expressed as mean±SD. Statistical analysis was performed with one way analysis of variance (ANOVA).

Results

Protein contents in the PBS extracts of *C.dactylon* and *M. oleifera* were found to be 9.3mg/g leaf and 6mg/g seed respectively. The highest recorded protein content was in *C.dactylon* at 70% saturation of ammonium sulphate and *M. oleifera* at 10% saturation and they showed ED_{50} at minimum concentrations. The anti-lipid peroxidative role of protein fractions of the two plants were evaluated in the liver, lung, kidney, heart, spleen and brain. The anti-lipid peroxide effect of the two plants in the above organs of mice treated with protein fractions alone, combination with ELA tumor cells, control for PBS, paraffin oil and silymarin were shown in Table 1.

The lipid peroxide level is decreased significantly by the intraperitoneal administration of protein fractions of both the plants compared to the control and standard Silymarin. The decrease/increase lipid peroxide were found to be non significant between 15 days and 30 days treatment period.

A similar decrease in the level of lipid peroxide was observed in the rat liver by the administration of Mushroom extracts [12]. The hepatoprotective effects of protein fraction of various plants were found to possess good protective effect on carbon tetra chloride induced free radical toxicity in Swiss albino male mice [13]. The protein extracts of *C.dactylon* and *Momordica charantia* brought down the liver marker enzyme levels which were increased in ELA cell line alone [14].

It was noted that, the lipid peroxide levels in ELA induced mice were found to be significantly increased in all the selected organs where as all the protein fractions individually and Protein + ELA induced mice showed significant decreased level of lipid peroxide. It indicated their protective effect in the membranes of all the selected organs. So, the protein extracts of *C.dactylon* showed higher anti-lipid peroxide potential than *M.oleifera*. The results suggest that *C.dactylon* leaves are potential sources of anti-lipid peroxide.

Table 1 Levels of lipid peroxide in the liver, lung, kidney, spleen, heart and brain control and experimental Swiss albino male mice

Groups/Days		ELA	PBS control	Paraffin oil control	Silymarin	CPF	MPF	CPF+ ELA	MPF+ ELA	#CD (0.05)
Liver	15	2.16 ± 0.01	1.23 ± 0.00	0.18 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.19 ± 0.01	0.62 ± 0.01	0.78 ± 0.02	0.016
	30	-	1.21 ± 0.01	0.17 ± 0.00	0.13 ± 0.01	0.18 ± 0.00	0.17 ± 0.01	0.61 ± 0.00	0.72 ± 0.01	
Lung	15	2.17 ± 0.01	1.83 ± 0.01	0.80 ± 0.01	0.77 ± 0.01	0.48 ± 0.01	0.57 ± 0.01	1.03 ± 0.01	0.97 ± 0.01	0.017
	30	-	1.84 ± 0.01	0.81 ± 0.01	0.81 ± 0.01	0.47 ± 0.01	0.58 ± 0.01	1.02 ± 0.01	0.95 ± 0.01	
Kidney	15	2.61 ± 0.01	1.62 ± 0.01	0.60 ± 0.01	0.56 ± 0.01	0.26 ± 0.01	0.36 ± 0.02	0.57 ± 0.01	0.52 ± 0.02	0.020
	30	-	1.58 ± 0.01	0.56 ± 0.01	0.55 ± 0.01	0.37 ± 0.01	0.46 ± 0.01	0.61 ± 0.01	0.66 ± 0.01	
Spleen	15	2.47 ± 0.01	1.65 ± 0.00	0.64 ± 0.01	0.61 ± 0.00	0.53 ± 0.01	0.55 ± 0.00	1.26 ± 0.01	1.28 ± 0.01	0.013
	30	-	1.63 ± 0.01	0.63 ± 0.01	0.60 ± 0.01	0.56 ± 0.01	0.57 ± 0.00	1.14 ± 0.01	1.18 ± 0.01	
Heart	15	2.47 ± 0.01	0.85 ± 0.00	0.84 ± 0.01	0.81 ± 0.00	0.73 ± 0.01	0.71 ± 0.00	0.96 ± 0.01	0.99 ± 0.01	0.013
	30	-	0.83 ± 0.01	0.83 ± 0.01	0.80 ± 0.01	0.74 ± 0.01	0.67 ± 0.00	0.94 ± 0.01	0.98 ± 0.01	
Brain	15	2.77 ± 0.01	1.71 ± 0.01	0.68 ± 0.01	0.66 ± 0.01	0.67 ± 0.01	0.86 ± 0.01	1.56 ± 0.02	1.45 ± 0.01	0.017
	30	-	1.71 ± 0.00	0.68 ± 0.00	0.65 ± 0.01	0.65 ± 0.01	0.81 ± 0.02	1.52 ± 0.01	1.40 ± 0.01	

The values are the mean±SD of six animals. #One way ANOVA

Discussion

Ayurveda is getting more popular as it has no side effects in which plants and plant products are the only ingredients intensive research is going on in the isolation or active principle from plants. Huge amounts are being spent by foreign countries for the research on medicinal plants for taking patent on a particular plant or a plant product. But no significant work is going on in India even though India has a good heritage of medicinal plants. This lacuna has to be filled up by involving in active research on medicinal plants.

During Cytotoxicity studies in ELA, we have found that *Cynodon dactylon* gave a significant Cytotoxicity against ELA with out affecting normal cells. Detailed study was carried out by preparing the protein from the plant. Mice were used as the experimental animals for the study. Lipid peroxidation was found be increased in cancer. But treatment with proteins could control this increase.

Treatment of the lymphomas with the protein of *Cynodon dactylon* and *Moringa oleifera* could control the changes in lipid peroxidation and the antioxidant deficiency, which might have resulted in the re-establishment of cellular metabolism and this, might have controlled the cellular damage. All these observations clearly support the antilipid peroxidative effect of *Cynodon dactylon* and *Moringa oleifera.*

Conclusion

In conclusion, the present study demonstrated that the leaf of *Cynodon dactylon* and seed of *Moringa oleifera* possess anti lipid peroxidative property of this plant. Further study is warranted to characterize and screen the protein that possess anti lipid peroxidative properties.

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