

## RESEARCH ARTICLE

**ANTIOXIDATIVE ROLE OF *TERMINALIA CATAPPA* LEAF PROTEIN AGAINST ELA INDUCED MICE**

R.SANTHI\* AND S.ANNAPOORANI

\*LECTURER, DEPARTMENT OF BIOCHEMISTRY, VIVEKANANDHA COLLEGE OF ARTS AND SCIENCES FOR WOMEN, ELAYAMPALAYAM, TIRUCHENGODE – 637 205, TAMILNADU.

READER, DEPARTMENT OF BIOCHEMISTRY, AVINASHILINGAM UNIVERSITY, COIMBATORE – 641 043, TAMILNADU.

**ABSTRACT**

*The antioxidant activities of Terminalia catappa were quantified using non-enzymic, enzymic antioxidants and liver marker enzyme. The activity of enzymic and non-enzymic antioxidants increased significantly by the intraperitoneal injection of plant protein fraction compared to ELA induced mice whereas it was reversed for liver marker enzymes. Terminalia catappa protein fraction can be considered as a potential free radical scavenging antioxidant.*

**KEY WORDS:** *Terminalia catappa, Ehrlich's lymphoma ascites, Antioxidants, Liver marker enzymes, Phosphate buffered saline, Tcpf.*

**INTRODUCTION**

The mother earth is a rich reservoir of natural resources. All through evolution, nature has provided us with all our needs. Through out the evolutionary process man was afflicted by diseases. Now man has no time to go in search of leaves and roots of plants of medicinal importance even through they are harmless. He even neglects the medicinal plants around him. The majority of the world's population in developing countries still relies on herbal medicines to meet their health needs in cases when synthetic medicine could not relieve patients who suffer from hard to cure illnesses like cancer<sup>[5]</sup>.

There is increasing interest in the use of herbs for the treatment of human diseases including cancer. Plants

contain a wide variety of compounds that may have biological activities including anticancer effect<sup>[10]</sup>. Nature had always been an inspiration and guidance for human existence form time immemorial. Beginning with AD 1500 there was a continuous activity in this area and many of the well known medicinal plants were chemically analyzed and characterized for their active principles<sup>[8]</sup>.

Cancer is a major public health burden in both developed and developing countries. Plant derived agents are being used for the treatment of cancer. Several anticancer agents including taxol, vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan and etoposide derived from epipodophyllotoxin are in clinical use all over the world<sup>[4]</sup>. In India, rates for oral and oesophageal cancers

are some of the highest in the world. In contrast the rates for colorectal, prostate and lung cancer are one of the lowest<sup>[7]</sup>.

Free radicals involve tumor promotion by different ways.

1. Through growth of cancer cells by enhancing the expression of somatic mutation.
2. Other growth promoting process between initiation and the development of clinically recognizable tumor.
3. By interference in the extra cellular processes that normally inhibit cancer cell growth, for example by preferentially killing non-cancer cells<sup>[11]</sup>.

*Terminalia catappa* is a large deciduous stately tree, originally from India, growing up to 9.0 feet tall with horizontal whorls of branches offering clusters of foot long, obovate leaves that turn pink-red to red-yellow before falling. Some of the pigments responsible for this are violanxanthin, lutein and zeaxanthin. In addition to these pigments leaves also contain violeoxanthin, epoxide lutein and  $\beta$ -cryptoxanthin<sup>[3]</sup>. The tannins isolated from leaves include coliragin and ellagic acid<sup>[6]</sup>, terflavins A and B, tergalagin, tercatatin, punicalin, punicalagin, chebulic acid, geranin, granatin and desgalloyleugenin<sup>[9]</sup>.<sup>[2]</sup> isolated flavone glycosides viz, apigenin 6-C-(2'-O-galloyl)- $\beta$ -D glucopyranoside, pigenin-8-C-(2'-O-galloyl)- $\beta$ -D glucopyranoside, isovitexin, vitexin, isoorientin and rutin from the dried fallen leaves of *T.catappa*. The present study is an attempt to evaluate the antitumorogenic effect of *T.catappa* against Ehrlich's Lymphoma Ascites.

#### MATERIALS AND METHODS

Fresh leaves of *T.catappa* were collected from pesticides free area of Tiruchengode. Using Phosphate Buffered Saline (PBS), 20% extract of *T.catappa* fresh leaves was 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. For a batch of seven to eight weeks old Swiss albino mice, weighing about 25-30g, ELA tumor cell lines were procured. The mice were acclimatized for two weeks and cells were propagated by intraperitoneal transplantation. 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 *T.catappa*.

The animals were divided into 6 groups and each group consisted of 6 mice. The groupings are ELA, PBS, paraffin oil, Silymarin, Tcpf, and Tcpf+ELA. After 15 days, the mice were sacrificed and the blood was collected by heart puncture. The separated serum was used for the estimation of liver marker enzymes such as AST, ALT and ALP. The free radical scavenging effect was assessed by the activity of enzymic antioxidants such as catalase, super oxide dismutase and glutathione peroxidase and the non-enzymic antioxidants such as vitamin A, E and reduced glutathione in the liver.

#### RESULTS AND DISCUSSION

Protein contents in the PBS extracts of *T.catappa* were found to be 7.5mg/g leaf, the highest recorded was at 70% saturation of ammonium sulphate and they showed ED<sub>50</sub> at minimum concentrations. Antioxidative role of eluted protein fractions of plant was evaluated in the liver. The enzymic, non-enzymic antioxidants and liver marker enzymes activities of the plant protein fraction in the liver of mice treated with Tcpf alone, combination with ELA tumour cells, control for PBS, paraffin oil and silymarin were shown in Table 1.

The activities of enzymic antioxidants such as catalase, super oxide dismutase and glutathione peroxidase elevated by the intraperitoneal administration of Tcpf compared to the control and standard antioxidant Silymarin. The levels of non-enzymic antioxidants were high in vitamin A which was followed by vitamin E and reduced glutathione.

## R.SANTHI AND S.ANNAPOORANI : ANTIOXIDATIVE ROLE OF TERMINALIA CATAPPA

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 <sup>[1]</sup>. The results suggest that the level of enzymic antioxidants were significantly reduced in mice treated with carbon tetra chloride. It was noted that the protein fraction brought down the liver marker enzyme

levels which were increased in ELA cell line alone. So, the protein fraction can be considered as a potential free radical scavenging antioxidants. The protein fraction of *T.catappa* showed higher antioxidant potential. The results suggest that *T.catappa* leaves are potential sources of antioxidant.

**Table 1: Activities of enzymic, non- enzymic antioxidants and liver marker enzymes in the liver of control and experimental Swiss albino mice**

Groups	ELA	PBS control	Paraffin oil control	Silymarin	Tcpf	Tcpf+ ELA
Catalase ( $\mu$ /mg protein) (a)	55.03 $\pm$ 0.05	73.43 $\pm$ 0.55	70.35 $\pm$ 0.20	75.10 $\pm$ 0.08	75.20 $\pm$ 0.11	68.23 $\pm$ 0.05
Super oxide dismutase ( $\mu$ /mg protein) (b)	2.47 $\pm$ 0.02	4.49 $\pm$ 0.04	4.51 $\pm$ 0.05	4.98 $\pm$ 0.03	5.31 $\pm$ 0.04	3.82 $\pm$ 0.09
Glutathione peroxidase ( $\mu$ /mg protein) (c)	36.98 $\pm$ 0.25	48.48 $\pm$ 0.24	48.94 $\pm$ 0.26	50.15 $\pm$ 0.17	53.51 $\pm$ 1.15	50.25 $\pm$ 0.00
Vitamin.A ( $\mu$ g / g tissue)	3.25 $\pm$ 0.12	7.18 $\pm$ 0.19	7.45 $\pm$ 0.13	8.05 $\pm$ 0.32	8.75 $\pm$ 0.08	5.51 $\pm$ 0.11
Vitamin.E ( $\mu$ g / g tissue)	3.18 $\pm$ 0.25	8.55 $\pm$ 0.24	8.78 $\pm$ 0.17	9.53 $\pm$ 0.21	10.63 $\pm$ 0.13	4.93 $\pm$ 0.08
Reduced glutathione (nmoles / g tissue)	1.65 $\pm$ 0.20	2.38 $\pm$ 0.21	2.20 $\pm$ 0.15	2.88 $\pm$ 0.27	5.03 $\pm$ 0.10	4.63 $\pm$ 0.08

ALP (Units/Litre)	198.81	126.54	127.95	124.65	128.16	127.17
	±	±	±	±	±	±
	0.80	0.08	0.67	0.82	0.46	0.53
AST (Units/Litre)	83.87	50.77	50.44	49.16	49.08	50.06
	±	±	±	±	±	±
	0.75	3.01	2.46	0.73	0.94	2.22
ALT (Units/Litre)	99.48	95.15	96.08	93.15	89.21	96.65
	±	±	±	±	±	±
	0.50	0.38	0.57	0.38	0.54	1.20

a - nmoles H<sub>2</sub>O<sub>2</sub> decompose / sec / mg protein, b - amount of enzyme that gives 50% inhibition of extent of NBT reduction and c - µg of GSH utilized / minute / mg protein. (The values are the means of six animals)

#### REFERENCES

1. Annapoorani S, Hajara Sheereen N and Hema R. Hepatoprotective effects of protein fractions of selected medicinal plants against CCl<sub>4</sub> induced mice. *J. Ecotoxicol. Environ. Moni.* 2006; 16(1): 83 – 90.
2. Lin YI, Kuo YH, Shiao MS. and Chen CC. Flavonoid glycosides from *Terminalia catappa* L. *J. Chin. Chem., Soc.* 2000; 47(1): 253-256.
3. Lopez-Hernandez E, Ponce-Alquicira F and Gyerrerri-Legarreta I. Characterization and stability of pigments extracted from *Terminalia catappa* leaves. *J. Food Sci.* 2001; 66(6): 832-836.
4. Mohammad Shoeb. Anticancer agents from medicinal plants. *Bangladesh J Pharmacol.* 2006; 1: 35-41.
5. Raju Senthilkumar, Rangasamy Manivannan, Ayyasamy Balasubramaniam, Thangavel Sivakumar and Balasubramanian Raj Kapoor. Effects of ethanol extract of *Pisonia aculeate* Linn. On ehrlich Ascites carcinoma tumor bearing mice. *Journal of Green pharmacy* 2008; 2(1): 50-53.
6. Rayudu GVN and Rajadurai S. Polyphenols and carboxylic compounds of *Terminalia catappa*. *Leather Sci.* 1996; 13(10): 289-9.
7. Sinha R, Anderson DE, Macdonald SS and Greenwald P. Cancer risk and diet in India. *J Postgrad Med.* 2003; 49: 222-228.
8. Sukh Dev. Natural products in medicine - present status and future prospects. *Current Science* 1983; 52(20): 947-956.
9. Tanaka T, Nonaka G, and Nishioka I. Tannins and related compounds. XLII. Isolation and

characterization of four new hydrolysable tannins, terflavins A and B, tergallagin and tercatatin from the leaves of *Terminalia catappa* L. *Chem. Pharm. Bull.* 1986; 34(3): 1039-49.

10. Tatsuya Kaneshiro, Masumi Suzui, Reika Takamatsu, Akira Murakami, Hajime Ohigashi, Tetsuya Fujino and Naoki Yoshimi. Growth inhibitory activities of crude extracts obtained from herbal plants in the Ryukyu Islands on several human colon carcinoma cell lines. *Asian pacific J Cancer Prevention* 2005; 6: 353-358.
11. Yuspa SH, Hennings H, Sako T, Pettit GR, Hartly J, and Blumberg, P.M. In: *Anticarcinogenesis and radiation protection*, Cerruti PA, Nygaard OF and Simec MG, (eds) Plenum press, New york, 1987; 169.

\*\*\*\*\*

**Source of Support: NIL**  
**Conflict of Interest: NONE**