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Research Article

Antioxidant Activity of Phenolic Fractions of *Terminalia Catappa* in Ela Propagated Swiss Albino Mice

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ABSTRACT

This investigation aims to evaluate the antioxidant potential of Phenolic fractions of *Terminalia catappa* in ELA propagated Swiss albino mice. The levels of enzymic antioxidants such as catalase, superoxide dismutase and glutathione peroxidase and non enzymic antioxidants such as vitamin A, vitamin E and reduced glutathione were increased on administration with phenolic fractions of *Terminalia catappa* in ELA induced mice. This result suggests that phenolic fractions of *Terminalia catappa* possess antioxidant activity.

Keywords: *Terminalia catappa*, Ehrlich's Lymphoma Ascite, Phenolic fractions, Antioxidants

1. INTRODUCTION

Medicinal and aromatic plants, a gift of nature are being used against various infections and diseases in the world. Natural products serve as an excellent source of bioactive molecules used for treating a wide range of different human diseases [1]. Natural product drugs include aromatic polyketides, polyethers, coumarins, flavanoids, terpenoids, alkaloids and aminoglycosides [2]. Cancer is a major cause of mortality worldwide and cancer incidents rapidly increase from year to year. Recent studies have shown strong evidence that biological reactive oxygen species (ROS) such as hydroxyl radical and superoxide anion are involved in the development of cancer. As ROS are involved in cancer development, compounds with high ROS reduction activity are able to prevent cancer incidence. Antioxidants have been reported to prevent oxidative damage caused by ROS and may prevent the occurrence of cancer [3]. Flavanoids and phenolic compounds widely distributed in plants which have been reported to exert multiple biological effect including, antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic, etc. Phenol and flavanoid contents of *Melliiotus officinalis* extract and phenolics from *Scutellaria baicalensis* exhibited the free radical scavenging and antioxidant activities [4-5] have been reported. Azizah Othman *et al* investigate the phenolic content of coca beans extracts possess antioxidant activity [6]. *Terminalia catappa* is (family - combretaceae) widely grown in tropical regions of the world as an ornamental tree. The phytochemicals of this plant include tannins, flavanoids and triterpenoids. Aqueous and ethanolic extracts of leaves were reported for their heapatoprotective activity. More and more pharmacological studies have reported that the extract of *T. catappa* leaves and fruits have anticancer, antioxidant, anti-HIV reverse transcriptase, anti-inflammatory, antidiabetic effects and hepatoprotective activities [7]. Recently, it has been reported that *Terminalia catappa* leaf protein has antioxidant activity against ELA implanted Swiss albino mice [8]. Therefore the aim of the present study was to determine the antioxidant potential of phenolic fraction of *Terminalia catappa* against ELA implanted Swiss albino mice.

2. MATERIALS AND METHODS

2.1 Plant material

Fresh leaves of *Terminalia catappa* were collected in area free of pesticides and other contaminants from the area surrounding of Tiruchengode, Namakkal district, Tamilnadu. The collected leaves were washed thoroughly and blotted dry with filter paper and used for the phenolic fraction preparation.

2.2 Preparation of Methanol Extract

Terminalia catappa leaves were collected and dried and made into powder form. 10 gm of sample were packed in a Soxhlet apparatus and extracted using methanol for 4 hours. The Marc was discarded and the filtrate was dried in a desiccators. The Paste obtained was stored at 4^0 c in cold room.

2.3 Animals

Seven to eight weeks old Swiss albino male mice weighing about 25-30 g were brought from small animals breeding station, Thrissur, Kerala. The animals were acclimatized for 15 days under standard laboratory conditions and fed with standard diet with water ad libitum (889/ac/05/CPCSEA).

2.4 Propagation of ELA cell lines

Ehrlich's Lymphoma Ascites (ELA) tumour 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 $1x10^6$ cells in 100µl of PBS. After 15 days, the cells were drawn from the intraperitoneal cavity and used for the *in vitro* cytotoxic studies by trypan blue exclusion

method [9]. *In vitro* cytotoxic studies were carried out to find out the 50% effective dose (ED_{50}) of *Terminalia catappa* phenol extract which was 75µg/100µl determined by trypan blue exclusion method. The fraction which showed minimum ED_{50} was selected for the *in vivo* studies.

2.5 Grouping of animals

The animals were divided into 5 groups and each group consisted of 6 mice. The **Group I**: Received 0.1 ml of Dimethyl sulphoxide (DMSO) every day, intraperitoneally and served as a vehicle control for the experimental groups 2 to 4. **Group II:** Positive control group fed with (0.18 mg/kg body weight) standard antioxidant silymarin. **Group III:** Received $1x10^6$ ELA tumour cells, intraperitoneally and treated as ELA control. **Group IV:** Received ED_{50} of Phenol extract of *T. catappa* (*T.cattapa* phenol 75µg in 100µl of DMSO) intraperitoneally. **Group V:** *T. catappa* extract and ELA tumour cells were administered on the same day and phenol extract administration was continued for 15 days. After 15 days the mice were sacrificed after an overnight fasting. The liver was dissected , washed with PBS at pH 7.2 and homogenate was prepared using PBS and used for the determination of catalase [10], superoxide dismutase [11], glutathione peroxidise [12] and the non enzymic antioxidants such as Vitamin A [13], vitamin E [14], reduced glutathione [15] and lipid peroxidation [16] and the results were presented as the mean ± standard deviation of 6 animals.

3. RESULT AND DISCUSSION

Table I shows the levels enzymic antioxidants Catalase, Superoxide dismutase and Glutathione peroxidase in the liver homogenate of control and experimental groups. The levels of CAT, SOD, GPx were decreased in ELA induced mice when compared to control groups. Table II shows the levels of non enzymic antioxidants such as vitamin A, vitamin E, reduced glutathione and lipid peroxidation in the liver homogenate of control and experimental groups. The levels non enzymic antioxidants Vitamin A, Vitamin E and Reduced Glutathione were decreased in ELA induced mice when compared to control groups. The level of lipid peroxidation was increased in ELA induced mice when compared to control group.

TABLE 1: ACTIVITIES OF ENZYMIC ANTIOXIDANTS IN THE LIVER OF CONTROL AND EXPERIMENTAL SWISS ALBINO MICE

Control and Experimental Groups	CAT (U/mg Phenol) (a)	SOD (U/mg Phenol) (b)	GPx (U/mg Phenol) (c)
DMSO	6.65 ± 0.18	2.28 ± 0.26	0.34 ± 0.04
SILYMARIN	6.61 ± 0.14	2.30 ± 0.18	0.22 ± 0.01
ELA	3.47 ± 0.24	0.85 ± 0.06	0.05 ± 0.01
Terminalia phenol extract	7.58 ± 0.20	2.40 ± 0.14	0.41 ± 0.03
Terminalia phenol extract + ELA	5.25± 0.247	1.91 ± 0.08	0.45 ± 0.05

The values are the means ± standard deviation of six animals.

Where,a – n moles H_2O_2 decompse / seconds / mg phenol

b – amount of enzyme that gives 50% inhibition of extent of NBT reduction

c – micrograms of GSH utilized per minute per milligram phenol.

TABLE 2: ACTIVITIES OF NONENZYMIC ANTIOXIDANTS IN THE LIVER OF CONTROL AND EXPERIMENTAL SWISS ALBINO MICE

Control and Experimental Groups	Vitamin.A (a)	Vitamin.E (b)	GSH (Reduced Glutathione) (c)	LPO (d)
DMSO	0.80±0.11	2.77 ±0.09	11.44 ±0.17	0.17 ± 0.01
SILYMARIN	0.70 ± 0.00	2.24 ± 0.01	10.85 ± 0.20	0.12 ± 0.02
ELA	0.63 ± 0.07	2.56 ± 0.15	8.76 ± 0.02	0.23 ± 0.01
Terminalia phenol extract	0.92 ± 0.07	3.31 ± 0.09	10.50 ± 0.61	0.19 ± 0.02
Terminalia phenol extract+ ELA	0.90 ± 0.12	3.11 ± 0.10	9.35 ± 0.37	0.19 ± 0.01

The values are the means and standard deviation of six animals.

a - µg / g tissue, b - µg / g tissue, c- n moles /g tissue, d - n moles /g tissue

Flavonoids and phenolic compounds widely distributed in plants which have been reported to exert multiple biological effect including, antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic, etc. Polyphenolic compounds, are defined as substances possessing an aromatic ring with one or more hydroxyl substituent and are reported to quench oxygen derived free

radicals by donating a hydrogen atom or an electron to the free radical [17-18]. Likewise administration of phenolic fraction of *Terminalia catappa* increases the levels enzymic antioxidants Catalase, Superoxide dismutase and Glutathione peroxidise and non enzymic antioxidants Vitamin A, Vitamin E and Reduced Glutathione in the liver homogenate of ELA induced mice compared with to ELA control mice. The generation ROS scavenged by antioxidants, lipid peroxidation was decreased when administration with plant extract compared to ELA control mice. The enzymic antioxidants and enzymic antioxidants activity also increased when administered with Silymarin. The result of the study confirmed that administration of phenol extract of *Terminalia catappa* inhibited ELA tumour cell multiplicity can be recommended as an antitumorogenic agent.

4. REFERENCES

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