Biomedical & Pharmacology Journal Vol. **2**(2), 431-435 (2009)

In vitro antioxidant and anticancer studies on the leaf of *Acalypha indica*

S. KAVITHA[1], T. KALAI KOVAN[2] and R. VIJAYA BHARATHI[3]

[1]Department of Pharmacology, Krupanidhi College of Pharmacy, Banaglore (India).
[2]Department of Pharmacology, Krupanidhi College of Pharmacy, Bangaolre (India).
[3]Department of Pharmacognosy, C.LBaid Metha College of Pharmacy, Chennai (India).

(Received: June 18, 2009; Accepted: August 13, 2009)

ABSTRACT

Major diseases like cancer, athresclerosis,hypertension etc are caused because of inheritance, life style, food style and environmental factors , also stress play a major role in developing all diseases. Free radicals are generated due to stress and develop cancer and heart diseases etc. these free radicals are quenched by antioxidants such as vitamin-c and e also occur as polyphenols, phenolic compounds and flavanoids. Acalypha indica was found to have antioxidant compounds such as flavonoids and tannins.Hence antioxidant and in vitro anticancer (cytotoxicity) activities were carried out on the leaf extract of *A. indica.* Aim of this study to provide an introduction to the principles of drug treatment for solid tumours in cancer treatment. Antioxidant activity was studied by conducting lipid peroxidation assay, the total antioxidant capacity of Et OH and water extracts was found to be 442 and 338 nmol/g respectively thus establishing Et OH and water extracts of *A.indica* possess antioxidant activity. In vitro cytotoxicity(anticancer) activity was studied by growing Agrobacterium tumefacians on yeast extract media for 48 hrs at 28°. Russet potatoes were disinfected by scrubbing under running water with a brush, then immersed in 10% Clorox for 20 min. Suspension of A. tumifaciens in phosphate buffered saline (PBS) were standardized to 1 $\times 10^9$ colony forming units, as determined by an absorbance value of 0.96± 0.22 at 600 nm. Cisplatin and different Et OH and water extracts of *A.Indica* were dissolved in DMSO. The test solution consisted of 1 ml of drug + 0.25 ml of water. The control solution consisting of 1.25 ml of water + 1 ml of bacterial suspension. The stained potato dishes were viewed under a dissecting microscope and the mean number of tumors was counted. The results of the study showed that the extracts of *A. indica* produced maximum reduction in the mean number of tumors compared to control. These above studies showed that the antioxidant activity and related anticancer activity of Acalypha indica , because of the presence of flavanoids and tannins present in the Et OH and water extracts of *Acalypha indica.*

Key words: Antioxidant and anticancer studies, *Acalypha indica.*

INTRODUCTION

Cancer is a group of more than 100 different diseases, it develops gradually as a result of a complex mix of factors related environment, lifestyle, food style, stress and hereditary. Inherited risk factors are unavoidable where as other factors are unavoidable. Free radicals are generated due to stress, damaging any part of the cell inducing DNA, free radical damage may cause cancer. These free radicals are found in pollutants, chemicals (fertilizers and pesticides, prescription drugs, processed foods, cigarette smoke, environmental pollution, alcohol and electromagnetic radiation). Free radicals are quenched by antioxidants occur as phenols, polyphenols ,flavanoids and tannins.

A young healthy body can repair body most oxidative damage. But overtime the repair mechanisms falter or become overwhelmed by

radical-formers such as smoking and a high fat diet. When that happens oxidative damage can play havoc with DNA and lead to cancer. Thus maintaining high blood levels of antioxidants can reduce risk of cancer, heart disease and stroke by as much as 50%, 20% and 59% respectively. This present study on antioxidant and in vitro anticancer activity of leaf extract of Acalypha.indica^{O} would be useful in future for isolating the respective active constituent and for yielding a potential drug to mankind in treating cancer without adverse effects.

MATERIAL AND METHODS

Acalypha indica occurs as a weed in gardens, waste places and along the road-sides throughout the hotter parts of India from Bihar to Assam.

The plants were collected from the road sides of Perungudi and Thoraipakkam and was authenticated by Plant Anatomy Res. Centre, Medicinal Plant Res. Unit, W.Tambaram, Chennai.

The whole plant was collected and after removing the foreign particles it is dried in the shad. Then the dried plant is powdered and sieved by using the sieve no. 16 and this is used for further extraction.

Ferrous sulphate (Fischer inorganics & Aromatics limited, Madras), Thiobarbituric acid (Kiran light laboratories), Trichloroacetic acid(Paxmy laboratories), Dimethyl sulphoxide (s.d. fine chem. Limited), Trisodium phosphate (E.merck (India) limited, Mumbai), Ethanol (Changshu yangyuan chemical, China), Ascorbic acid, Ammonium molybdate, Butylated hydroxy toluene, Lugal's reagent, Phosphate buffer solution Acetic acid, Hydrochloric acid, 10% Chlorax, Acetocarmine dye, Agrobacterium tumifaciens Cisplatin and Ferric acetate.

In-vitro antioxidant studies

The total antioxidant activity was determined according to spectrophotometric method. 0.1 ml of the fraction (10 mg/ml) dissolved in 10 % DMSO was combined in an ependroff tube with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The tubes were capped and incubated in a thermal block nat 95°C for 90 mins. After cooling to room temperature, the absorbance was measured at 695 nm against a blank. Ascorbic acid was used as the standard and the total antioxidant capacity was expressed as equivalents of ascorbic acid. This experiment was performed in triplicate.

Antilipidperoxidation

This method[2,] involves the preparation of rat brain homogenate, further processed to get 10% homogenate in 0.15 M KCl using a Teflon homogenizer. , this homogenate was filtered to get a clear solution of polysaturated fatty acids for determining the extent of lipid peroxidation.. The extent of lipid peroxidation in rat brain homogenate was measured in vitro in terms of formation of thiobarbituric acid reactive substances (TBARS). Different concentrations of the extract (100-500ìg/Ml) were made up with ethanol. These samples were individually added to the bran homogenate (0.5 ml). This mixture was incubated with 0.15 M KCl (100µl). Lipid peroxidation was initiated by adding 100µl of 15 mM FeSO4 solution. The reaction mixture was incubated at 37°C for 30 min. 1 ml of TBA-TCA was added to the above solution followed by the addition of 1 ml BHT. The final mixture was heated on a water bath for 20 min at 80°C and cooled, centrifuged and absorbance read at 532 nm using spectrophotpmeter (Schimaszu 160 IPC). The percentage inhibition of lipid peroxidation was calculated by comparing the results of the test with those of controls not treated with the extract as per the formula, Inhibition (%) = (Control- test) x 100/ Control.

In-vitro cytotoxicity studies

Agrobacterium tumefaciens was grown on Yeast Extract Media (YEM) for 48 hrs at 28°C, Russet potatoes were disinfected by scrubbing under running water with a brush, then immersing in 10% Chlorax for 20 min. Potatoes were removed from the Chlorax, blotted on sterile paper towels, and each side removed allowing for a flat surface without the skin. The trimmed sections were placed in Chlorax (20%) for 15 min. Cylinders cut from the disinfected sections using a sterile cork borer (10 mm). Each cylinder segment was placed in sterile distilled water. After rinsing, each of the cylinder

was excised and discarded and the remaining cylinder was rinsed again in sterile distilled water. Disks (0.5-cm thick) were cut aseptically from the cylinders. These disks were placed in a 24 well culture plate containing 15% water agar. Suspensions of A. tumifaciens in phosphate buffered saline (PBS) were standardized to 1x109 Colony forming Units, as determined by an absorbance value of 0.96±0.22 at 600 nm. Cisplatin and different EtOH and water extracts of A.indica were dissolved in DMSO.

The test solution consisted of 1 ml of drug + 1 ml of Bacterial suspension + 0.25 ml of water. The control solution consisting of 1.25 ml of water + 1 ml of bacterial suspension.

Each disc in the 24 well culture plate was overlaid with 50µl of the appropriate extract/water/ bacteria mix and incubated at room temperature for 12 days. On day 12, the discs were stained with lugol's Reagent (I_2 KI; 5% I_2 plus 10% KI in distilled water). Lugol's solution stains with starch in the potato tissue a dark blue to dark brown colour, but the tumors produced by A.tumifaciens will not take up the stain, appears creamy to orange. The stained potato disks were viewed under a dissecting microscope and the mean number of tumors was counted. All the experiments were carried out in triplicate.

RESULTS

The total antioxidant capacity of the EtOH and water extracts of *A.indica* was calculated based on the formation of the phosphomolybdenum complex, which was measured spectrophotometrically at 695 nm. The total antioxidant capacity of the EtOH and water extracts was found to be 442 and 338 nmol/g respectively, thus establishing the EtOH and water extracts of Acalypha indica as an antioxidant. The various concentrations ranging from 100 – 500 µg/ml of the ethanolic and water extracts of Acalypha indica were tested for their antioxidant activity in vitro antilipid peroxidation model. Table-6 shows the effect of the extracts to scavenge free radicals. The maximum percentage inhibition of EtOH and water extracts was found to be 78.6 % and 70.2 % respectively at the concentration of 500 µg/ml level.

The anti-tumor activity of different concentrations of EtOH and water extracts of A.indica were studied on the Agrobacterium induced tumors in Potato discs. Three internal control treatments were included in this study.

Table 5: Effect of EtOH and water extracts of *A.indica* leaves on Total Antioxidant model

Drug	Percentage of Inhibition		
	100 µg/ml	200µg/ml	400µg/ml
EtOH extract	4.8	17	24.3
Water extract	19.12	24.3	36.5
Std drug-Ascorbic acid	4.8	7.3	12.1

Table 6: Effect of EtOH and water extract of A.indica leaves on lipid peroxidation model

Drug	Percentage inhibition				
	100µg/ml	200µg/ml	300µg/ml	400µg/ml	500µg/ml
EtOH extraxt of *A. indica*	57.3	57.7	69.6	72.3	78.6
Water extract of *A.indica*	58.4	59.6	60.2	63.9	70.2
Std drugs-ascorbic acid	61.3	64.5	71.8	73.3	97.0

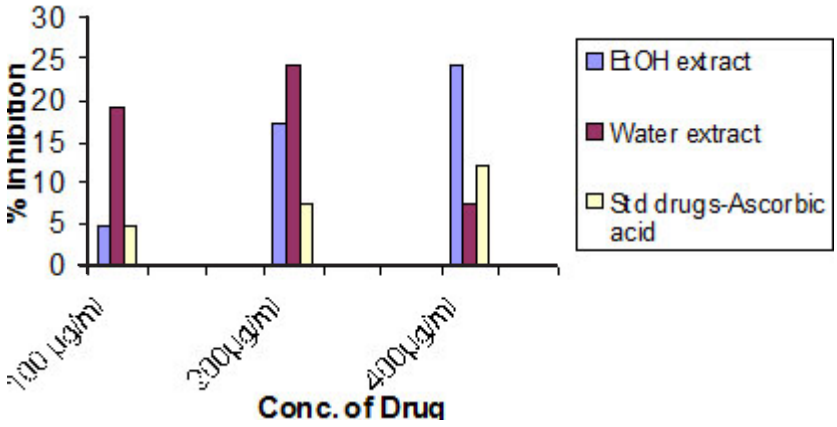

Fig. 2: Effect of EtOH and water extracts of *A. indica* leaves on antioxidant model

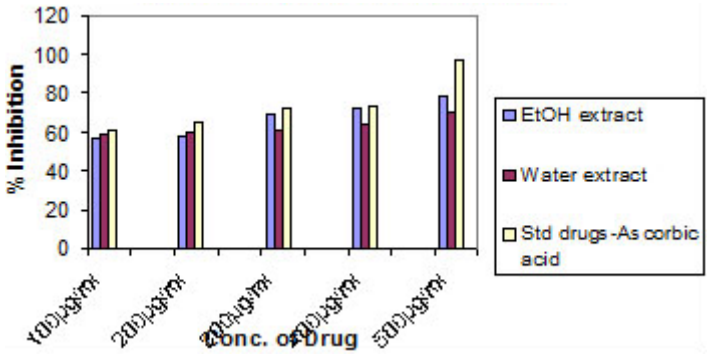

Fig. 2: Effect of EtOH and water extracts of *A. indica* leaves on lipid peroxidation model

Agrobacterium tumifaciens with DMSO induced an average of 24 tumors per potato disc. DMSO alone did not induce any tumor production at all concentrations.

The results of the study showed (table: 2) that the extracts of A.indica and standard drug cisplatin at all concentrations showed maximum reduction in mean number of tumors produced by the Agrobacterium as compared to control. The EtOH and water extracts showed appreciable inhibition of tumor production. However, EtOH extract showed more inhibitory tumor production than water extract of A.indica. . The study reported herein affirms the use of this assay first screen in the search for new antitumor agents, whether their mode of action b inhibition of topoisomerase, interference with tubulin function or pre le reorganization.

DISCUSSION

Oxidative stress has been implicated in the pathology of many diseases and conditions including diabetes, cardiovascular diseases, inflammatory conditions, cancer and ageing[3]. Antioxidants may offer resistance against the oxidative stress by scavenging the free radical, inhibiting the lipid peroxidation and by many other mechanisms and thus prevent disease[4]. The peroxidation of membrane lipids initiated by oxygen radicals may lead to cell injury. Initiation of lipid peroxidation by

ferrous sulphate takes place either through ferryl-perferryl complex[5]. Or through OH radical by Fenton reaction[6] thereby initiating a cascade of oxidative reactions.

The results obtained in our studies may be attributed to several reasons viz., the inhibition of ferryl-perferryl complex formation; scavenging of OH of superoxide radical or by changing the ratio of Fe^{3+}/Fe^{2+}; reducing the rate of conversions of ferrous to ferric or by chelation of the iron itself[7]. The moderate activity of the extract may probably be due to the rapid and extensive degradation of the antioxidant principles in an exvivo state thereby corroborating the finding that was observed in a study carried out in Australia with a group of human volunteers[8].It is also known that the OH radical which initiates lipid peroxidation has a very short life time (10-9 s at 37°C) and hence very difficult to investigate by conventional methods[9].

CONCLUSION

The results obtained thus indicate that A.indica extracts have potent antioxidant activity achieved by scavenging abilities observed against lipid peroxidation and exhibiting potent total antioxidant capacity. The plant has been reported to contain phenolic compounds such as flavanoids and tannins which are known as antioxidants. Hence the alcoholic and water extracts of the plant also showed good antioxidant potential. The anti-tumor activity of different concentrations of EtOH and water extracts of A.indica were studied on the Agrobacterium induced tumors in Potato discs. The EtOH and water extracts showed appreciable inhibition of tumor production. Our study supports that the use of A.indica leaves in the Indian indigenous system of medicine as an anti cancer may be in part due to the antioxidant potentials of extracts. Further isolation of the compounds responsible for the anticancer activity has to be taken up which may result in new drug from this plant.

REFERENCES

1. Asima chatterjee & Satyesh Chandra Pakrashi., The treatise of Indian Medicinal Plants, vol-3. New Delhi: Publications & Information, 9 (1997)
2. Braugghler J M, Duncan C A & Chase L R., The involvement of iron in lipid peroxidation. Importance of ferrous to ferric ratio in initiation, *J Biol Chem,* **261**: 1028 (1986)
3. Gutteridge J M C., Age pigments and free radicals: fluorescent lipid complexes formed by iron and copper containing proteins, *Biochem and Biophys Acta,* **834**: 144 (1985)
4. Halliwell B, Superoxide-Dependent formation of hydroxy free radicals in the presence of iron chelates, FEBS Lett, **92**: 321 (1978)
5. Marx J L., Oxygen free radicals linked to many diseases, *Science* **235**: 259 (1987)
6. Ohkawa H, Ohishi N & Yagi K., Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal Biochem, 95: 351 (1979)
7. Pryor W A., Oxy-radicals and related species, their formation, lifetimes and reactions, *Annu Rev Physiol,* **48**: 657 (1986)
8. Record I R, Dreosti I E & Mc Inerney J K., Changes in plasma antioxidant status following consumption of diets high or low in fruits and vegetables or following dietary supplementation with an antioxidant mixture, *Brit J Nutr,* **85**: 459 (2000)
9. Youdium Ka & Joseph J A, A possible emerging role of phytochemicals in improving age-related neurological dysfunctions: a multiplicity of effects, *Free Rad Biol Med,* **30**: 583 (2001)