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ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACTS OF *CALLICARPA LINATA* LEAF

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Abstract

Antioxidants are our first line of defense against free radical damage, and are critical for maintaining optimum health and wellbeing. Catalase (CAT), superoxide dismutase (SOD), and thiobarbituric acid reactive Substances (TBARS) estimated using liver homogenate. Dose dependent antioxidant activity was obtained against DPPH radical. Levels of serum markers AST, ALT, ALP, TB and total protein were significantly increased in CCl_4 treated rats. Increase in antioxidant enzymes CAT and SOD and a decrease in the level of TBARS was observed.

Key Words: Callicarpa linata, antioxidant, Flavonoids, DPPH, free radical scavenging activity.

Introduction

The term antioxidant refers to a broad range of substances, which have the ability to neutralize free radicals by donating one of their own electrons. Antioxidants act as scavengers preventing cellular and membrane damage. This process is called anti-oxidation. Antioxidants act in different ways by preventing free radical formation (metal chelation), by scavenging free radicals, by preventing the propagation of the oxidative chain reaction, by being part of the redox antioxidant network, or by regulating gene expression[1-4].

In response to the increased popularity and greater demand for medicinal plants, a number of conservation groups are recommending that wild medicinal plants be brought into cultivation. Ethno-pharmacological surveys conducted among herbal practitioners of traditional Arab medicine in Palestine and the Middle East have revealed that a large number of indigenous plant species are being used as a source of herbal therapies [5]. Overall, free radicals have been implicated in the pathogenesis of at least 50 diseases [6,7]. Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer [8].

Callicarpa Linata. Family: Verbenaceae, an plant known worldwide as *Tomex tomentosa* L. *Callicarpa tomentosa* L. is widely used in tropical countries as a source of ethno medicines. It is recommended in a wide range of ailments including fever, Hepatic obstruction, hepatic eruption, skin diseases and used as a wash for aphthae in the mouth [9]. *Callicarpa Linata* leaves contain β- sitosterol, maslinic, oleanolic and ursolic acids and their methyl ester acetates, lupeol acetate and β-amyrin acetate; heartwood contains β-sitosterol and oleanolic acid. Bark contains methyl betulinate, baurenol and β-sitosterol acetate [10]. *Callicarpa Linata* EtOH (50%) extract of root and stem is antivirous and antifungal and plant extract is anti-inflammatory in rats [11].

The present investigation was therefore carried out to study the antioxidant actitivity of ethanol extract and chloroform extract of *Callicarpa linata* in rats.

Materials and Methods

Chemicals and Drugs

DPPH, gallic acid, 2,4,6-tripyridyl-s-triazine (TPTZ), 6-hydroxy-2,5,7,8 tetramethyl chroman-2-carboxylic acid (Trolox) and ferric chloride were obtained from Sigma Chemical Inc., India. All other reagents and chemicals used were of analytical grade procured from local sources.

Collection and Authentication of Plant

The whole plant of *Callicarpa linata* was collected in January 2012 from Thiruvallur district, Tamil Nadu, identified by Dr. Kumareshan, Botanist, authenticated by Dr. Marimuthu and the voucher specimen of *Callicarpa linata* was deposited at the herbarium of PRIST University, Thanjavur (Ref. No: 216/COL/2012) for future reference.

Preparation of Extract

The leaves was dried under shade at room temperature and then powdered with a mechanical grinder. The powdered material was passed through sieve No 40 and stored in an airtight container for future use. About 500 g of powdered leaves of *Callicarpa linata* was defatted with petroleum ether (60-80°C) and extracted with ethanol in a Soxhlet apparatus for 72 h. The extracted material was filtered and the filtrate was concentrated under reduced pressure using a rotary flash evaporator to obtain a semisolid mass [12].

Preliminary Phytochemical Screening

Preliminary phytochemical screening of the extracts was carried out using standard methods [13].

In vitro antioxidant activity using DPPH method

The hydrogen donating ability of ethanol extract of *Callicarpa linata* was examined in the presence of DPPH stable radical [14]. An aliquot of 1 mL 0.3 mM DPPH ethanol solution was added to 2.5 mL sample solution of different concentrations and allowed to react at room temperature. After 30 min the absorbance values were measured at 517 nm. Ethanol (1 mL) plus plant extract solution (2.5 mL) was used as a blank. DPPH solution (1 mL, 0.3 mM) plus ethanol (2.5 mL) served as negative control. Ascorbic acid was used as positive control. The concentration (mg/mL) of the extract required to scavenge 50% of the radicals was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated using the formula.

$$I\% = (Ac-As)/Ac \times 100$$

Where Ac is the absorbance of the control and As is

the absorbance of the sample [15].

The rats were sacrificed and liver was removed and washed immediately with ice cold saline to remove as much as blood possible. It was homogenized (5% w/v) in cold potassium phosphate buffer (50 mM, pH 7.4) using a Remi homogenizer. The unbroken cells and cell debris was removed by centrifugation at 3000 rpm for 10 min, using Remi C-24 refrigerated centrifuge. The supernatant obtained was used for the estimation of catalase (CAT), superoxide dismutase (SOD), and thiobarbituric acid reactive substances (TBARS).

Estimation of catalase (CAT)

The catalysis of H_2O_2 to H_2O in an incubation mixture adjusted to pH 7.0 was recorded at 254 nm. The reaction mixture contained 2.6 ml of 25 mM potassium phosphate buffer pH 7.0 and 0.1ml of tissue homogenate and was incubated at 37°C for 15 min and the reaction was started with the addition of 0.1 ml of 10 mM H_2O_2. The time required for the decrease in absorbance from 0.45 to 0.4 representing the linear portion of the curve was used for the calculation of enzyme activity. One unit of catalase activity was defined as the amount of enzyme causing the decomposition of µmol H_2O_2/mg protein/min at pH 7.0 at 25°C [16].

Estimation of superoxide dismutase (SOD)

SOD activity was determined by the inhibition of auto catalyzed adrenochrome formation in the presence of homogenate at 480 nm. The reaction mixture contained 150 µl of homogenate, 1.8 ml of carbonate buffer (30 mM, pH 10.2), and 0.7 ml of distilled water and 400 µl of epinephrine (45mM). Auto oxidation of epinephrine to adrenochrome was performed in a control tube without the homogenate. Activity was expressed as µmoles/min/mg protein [17].

Determination of lipid peroxidation (TBARS)

For TBARS, 0.1 ml of tissue homogenate (Tris-HCl buffer, pH 7.4) was mixed with 2.0 ml of TBA-TCA-HCl reagent (Thiobarbituric acid 0.37%, 0.25 N HCl and 15% TCA mixed in 1:1:1 ratio). The resultant solution was placed in water bath for 15 min, cooled and centrifuged at 1000 rpm at room temperature for 10 min. The absorbance of the clear supernatant was measured against reference blank at 535 nm. The results were expressed as nM/min/mg tissue protein [18].

Statistical analysis

The experimental results were expressed as the Mean ± SEM for six animals in each group. The results were analyzed statistically using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. P value of < 0.05 was considered as statistically significant**.**

Results and Discussion

DPPH radical scavenging activity of Ethanol extract of *Callicarpa linata* demonstrated in a concentration-dependent manner and the IC_{50} was found to be 139 mg/ml. A positive DPPH test suggests that the extract is a potential free radical scavenger. However, the activity was less when compared with the standard, ascorbic acid (Table No. 1). DPPH is a proton free radical that shows a maximum absorption at 517 nm. When DPPH encounters proton radical scavengers its purple color fades rapidly. Antioxidants, by providing a hydrogen atom or by donation of electrons, can quench DPPH• free radicals and convert them to a colour less bleached product resulting in a reduction in absorbance. The antioxidant activity was confirmed by a decrease in absorbance band upon increasing concentrations of the ethanolic extract of *Callicarpa linata* [19].

A significant decrease was observed in the activities of catalase and superoxide dismutase in liver homogenate of CCl_4 alone treated rats. Administration of ethanol extract of *Callicarpa linata* at doses of 1000, 2000 and 3000 mg/kg *b.w*, significantly elevated the levels of catalase and superoxide dismutase when compared to CCl_4 control group (Table No 2). The result was found to be statistically significant ($P<0.05$). Administration of *Callicarpa linata* increased the enzymatic antioxidant levels. There was significant ($P<0.05$) elevation in tissue TBARS in CCl_4 control rats. Administration of *Callicarpa linata* to CCl_4 treated rats for 7 days decreased the levels of tissue TBARS in the liver tissue. Catalase and superoxide dismutase are enzymatic antioxidants. Catalase and superoxide dismutase are considered primary enzymes since they are involved in the direct elimination of ROS. Superoxide dismutase, is an important defence enzyme which catalyses the dismutation of superoxide radicals and catalase is a haemoprotein which catalyses the reduction of H_2O and protects the tissue from hydroxyl radicals. The reduced activity of superoxide and catalase in the liver during hepatotoxicity is a result of deleterious effects which results in the accumulation of superoxide anion

radicals and H_2O_2 [16]. The activity of enzymatic antioxidants are increased significantly in extract treated animals ($P<0.05$). Marked increase in the concentration of TBARS was observed in the liver of CCl_4 control rats. Administration of ethanol extract of *Callicarpa linata* tends to bring the increased concentration of lipid peroxidation products to near normal level.

In conclusion, the present study demonstrated that ethanol extract of *Callicarpa linata* has antioxidant effect in CCl_4-induced liver damage. It is need of time to determine parameters like as oxidative stress markers and molecular biology assays to confirm our findings. However, Isolation and Purification works are in progress to isolate and purify the active principle involved in the antioxidant activity and to find mechanism of action of same compounds.

References

1. Braca A, De Tommasi N, Bari LD, Pizza C, Politi M, Morelli I. Antioxidant principles from *Bauhinia terapotensis*. Journal of Natural Products 2001; 64:892–895.
2. Sies H. Oxidative stress, introductory remarks. In: Oxidative Stress.(Sies H, Ed.) Academic Press, London 1985; 1-8.
3. Toborek M, Wasik T, Drozdz M *et al*. Effect of hemodialysis on lipid peroxidation and antioxidant system in patients with chronic renal failure. Metabolism 1992; 41:1229-1232.
4. Klahr S. Oxygen radicals and renal diseases. Miner Electrolyte Metab1232; 23:140-143.
5. Nooman A. KHALAF, Ashok K. SHAKYA, Atif AL-OTHMAN, Zaha EL-AGBAR, Husni FARAH., Turkish Journal of Biology, 32 (2008) 51-55
6. Langseth, L. From the Editor: Antioxidants and Diseases of the Brain. Antioxidant Vitamins Newsletter 1993; 4:3.
7. Halliwell, B., Free Radicals, Antioxidants, and Human Disease: Curiosity, Cause, or Consequence? *Lancet* 1994; 344:721-724.
8. Devasagayam TPA, Tilak JC, Boloor KK et al. Review: Free radical and antioxidants in human health. Curr Stat Fut Pros JAPI 53: 794-804, 2004.
9. Nadkarni KM. Indian Materia Medica, 1975; 2: 253.
10. Ghani A. Medicinal Plants of Bangladesh with Chemical Constituents and Uses. 2nd Edn. Asiatic Society of Bangladesh, Dhaka, Bangladesh 267.
11. Asolkar LV, Kakkar and Chakre. Second Supplement to Glossary of Indian Medicinal Plants with active principles. Part-1 (A-K), CSIR, New Delhi 1992.
12. Zulfkar Latief Qadrie, R. Anandan, Beena Jacob, Humaira Ashraf: Liver Protective Activity of *Indoneesiella echioides* Against Carbon Tetrachloride (CCl_4)-Induced Hepatotoxicity in Rats. *Pharmacologyonline* 2011; 2: 416-429
13. Kokate CK. Preliminary phytochemical screening, Practical pharmacognosy. New Delhi: Vallabh Prakashan: 107-113, (1994).
14. Asokkumar K, Umamaheswari M, Sivashanmugam AT, Subhadradevi V, Subhashini N, Ravi TK: Free radical scavenging and antioxidant activities of *Glinus oppositifolius* (carpet weed) using different *in vitro* assay systems. *Pharm. Biol*, 2009; 47(6), 474–82.
15. Mensor LL, Menezes FS, Leitao GG, Reis AS, dos Santos TC, Coube CS and Leitao SG: Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res., 2001; 15: 127-130.
16. Aebi H: Catalase *in vitro*. *Meth. Enzymol*, 1984;105, 121–26.
17. Kakkar P, Das B, Viswanathan PN: A modified spectrophotometric assay of SOD. *Indian J. Biochem. Biophys*, 1984; 2, 130-32.
18. Asokkumar K, Umamaheswari M, Bahrudeen A, Sivashanmugam AT, Subhadradevi V, Ravi TK: Antioxidant and hepatoprotective activities of the fractions of *Ficus microcarpa* sing *in vitro* and *ex vivo* assay systems. *Functional Plant Science and Biotechnology*, 2010; 4(1), 17–27.
19. Halliwell B, Gutteridge JMC: Free radicals in biology and medicine, 1999; 3rd Ed, Oxford University Press, Oxford.

Table 1. *In vitro* antioxidant activity using DPPH method

Extract/ Standard	**DPPH**		
	Concentration (μg/ml)	**% Inhibition**	**IC_{50} μg/ml**
***Callicarpa linata* ethanolic leaf extract**	**1000**	72.66±2.78	139±0.85
	500	69.0±1.78	
	250	62.50±2.39	
	125	47.25±1.26	
	62	31.15±0.16	
Ascorbic Acid	**100**		269 ± 0.05

*Average of three determinations, values were mean ± S.E.M (n = 3). The results were found to be statistically significant P<0.05.

Table 2. Effect of ethanolic extract *Callicarpa linata* (EECL) Leaf on antioxidant enzymes and lipid peroxidation

Treatment	**Dose**	**Catalase (U/mg protein)**	**Superoxide dismutase (U/mg Protein)**	**Lipid peroxidation µmole of MDA/mg protein**
Control	**5ml/Kg**	354.51± 22.07	91.85±7.39	0.98±0.04
CCl_4	**1.25ml/Kg**	266.82±24.07[a]	57.35±4.22[a]	1.71±0.04[a]
EECL+ CCl_4	**1000mg/Kg**	282.17± 24.76[b]	60.21±5.21[b]	1.66±0.06[b]
EECL+ CCl_4	**2000mg/Kg**	305.25± 20.09[b]	67.42±6.24[b]	1.42±0.09[b]

Values are mean ± S.E.M. (n=6) Data were analyzed by using One-way ANOVA followed by Dunnett's test. Control group compared with intoxicated control ([a]P < 0.01)
Treatment groups compared with intoxicated control ([a]P < 0.01; [b]P < 0.05)