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Evaluation of antioxidant activity of leaf and bark extracts of *Diospyros virginiana* in rats

S. Priya[1]* and S. Nethaji[2]

[1]P.G. Department of Biotechnology, S.T.E.T. Women's College, Mannargudi, Tamil Nadu, India.
[2]P.G & Research Department of Biochemistry, Marudupandiyar College, Vallam, Thanjavur, Tamil Nadu, India.

Abstract: The ethanolic extracts of *Diospyros virginiana* leaf and bark studied for their antioxidant effects on carbon tetra chloride (1.5 ml/kg) induced acute liver damage on swiss albino rats. The degree of protection was measured by using biochemical parameters such as Malondialdehyde (MDA/LPO), Reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and Ascorbic acid (Vitamin C) were estimated. *Diospyros virginiana* leaf and bark extracts at a dose level of 500mg/kg produce significant hepatoprotection by decreasing and increasing the activity of serum enzymes and vitamin. The effects of *Diospyros virginiana* leaf and bark were comparable to that of standard drug, Silymarin. From this study, it can be concluded that the ethanolic extract *D. virginiana* leaf and bark of is not only an effective hepatoprotective agent, but also possesses significant antioxidant activity.
Keywords : Antioxidant, *Diospyros virginiana,* carbon tetra chloride, liver damage.

Introduction

Medicinal plants are the local heritage with global importance. World is endowed with a rich wealth of medicinal plants. Herbs have always been the principal form of medicine in India and presently they are becoming popular throughout the developed world, as people strive to stay healthy in the face of chronic stress and pollution, and to treat illness with medicines that work in concert with the body's own defenses[1]. Medicinal plants also play an important role in the lives of rural people, particularly in remote parts of developing countries with few health facilities[2].

Many plant extracts and phytochemicals show antioxidant/free radical scavenging properties[3]. Secondary metabolites of plants serve as defense mechanisms against predation by many microorganisms, insects and herbivores[4].

Antioxidants are chemical substance that donate an electron to the free radical and convert it to a harmless molecule. In this way antioxidants intercept free radicals and protect cells from oxidative damage that leads to ageing and disease[5]. All plants having chemical compounds as a part of their normal metabolic activities. These are divided into primary metabolites and secondary metabolites. These compounds are responsible for all kinds of biological activities.

Diospyros virginiana is a persimmon species commonly called the American Persimmon, Common Persimmon, Eastern Persimmon, "'Simmon", "Possumwood", or "Sugar-plum". This is a well-known indigenous tree, growing in woods and fields. Persimmons have been used to lubricate the lungs and strengthen the spleen and pancreas[6]. They improve energy and contain enzymes that help damaged cells and foreign

microbes be broken down. Persimmons have a special affinity for the large intestines and heart. Persimmons have been used to treat bronchitis, catarrh, cough, diarrhea, dysentery, goiter, hangover, hemorrhoids and hiccoughs[7]. The bark has been used in intermittent and both it and the unripe fruit have been beneficial in various forms of disease of the bowels, chronic dysentery, and uterine hemorrhage; used in infusion, syrup, or vinous tincture[8].

Seeds and fruits are generally low in crude protein, crude fat, and calcium but high in nitrogen-free extract and tannin [9]. The inner bark and unripe fruit are sometimes used in treatment of fevers, diarrhea, and hemorrhage. Indelible ink is made from fruit. Persimmon is valued as an ornamental because of its hardiness, adaptability to a wide range of soils and climates, its lustrous leaves, its abundant crop of fruits, and its immunity from disease and insects.

Hence, the present study was undertaken to evaluate the possible antioxidant activity of the leaves and bark extract *D.virginiana* of which is used commonly in Indian traditional medicine, by using various validated models and to find out if the folk medicinal use has a scientifically justified basis.

Material and Methods

Plant collection and preparation of the extract

D.virginiana belongs to the family *Ebenaceae* was collected from Coonoor, Nilgiris District, Tamil Nadu, India and identified by the special key given Cambell flora [10]. The leaf and bark of *D.virginiana* were washed with sterile distilled water. After, the leaves and bark were shade dried and powdered by using pestle and mortar. 25g of powder was filled in the thimble and extracted successively with ethanol using a Soxhlet extractor for 48 h. The extracts were concentrated using rotary flash evaporator and preserved at 5°C in airtight bottle until further use. The ethanolic extracts of the plant was diluted with distilled water and was administered orally to mice.

Antioxidant activity

Swiss albino rats weighed about 100-130 g of either sex were divided into five groups of six animals each, kept on normal diet water ad libitum. The rats were used after an acclimatization period of 7 days to the laboratory environment. All the experiments were performed during morning according to CPCSEA guidelines for care of laboratory animals and the ethical guideline for investigations of experimental pain in conscious animals.

Experimental Design

Stomach homogenate obtained from antiulcer screening using *Diospyros virginiana* ethanol extracts were subjected to *in vivo* antioxidant activity.

Group I : Normal control
Group II : Toxic control, treated with CCl_4 (single dose of 1.5 ml/kg, i.p.)
Group III : CCl_4 + standard Silymarin (56 mg/kg, p.o)
Group IV : CCl_4 + *Diospyros virginiana* bark (500mg/kg)
Group V : CCl_4 + *Diospyros virginiana* leaves (500mg/kg)

On day 8 the rats were sacrificed by carotid bleeding and liver was rapidly excised, rinsed in ice-cold saline, and a 10% w/v homogenate was prepared using 0.15M KCI, centrifuged at 800 g for 10 min at 4°C. Further, the homogenate was centrifuged at 1000 g for 20 min at 4°C and the supernatant was used for biochemical estimation of enzymes, vitamins like Malondialdehyde (MDA/LPO), Reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and Ascorbic acid (Vitamin C) were estimated.

Results and Discussion

Free radicals are constantly generated resulting in extensive damage to tissues and biomolecules leading to various disease conditions. So the medicinal plants are employed as an alternative source of medicine to mitigate the diseases associated with oxidative stress [11].

Antioxidants ensure limited survival of reactive oxygen species such as superoxide and H_2O_2 in the extracellular fluid by binding the metals and thus diminishing their ability to accelerate lipid peroxidation [12].Secondary metabolites from medicinal plants function as small molecular weight antioxidants through direct antiradical,
chain-breaking of the free radical propagation and interaction with transition metals.Other mechanisms include the inhibition of ROS-generating enzymes such as Xanthine oxidase, inducing nitric oxide synthase, and improving the endogenous cellular antioxidant mechanisms such as the up-regulation of the activity of SOD [13].

It was carried to evaluate the *in vivo* antioxidant activity of *Diospyros virginiana* on CCl_4 induced oxidative stress in rats. The observations made on different groups of experiment and control animals. *In vivo* antioxidant activity of the leaf and bark extracts of *Diospyros virginiana* were recorded in table- 1. SOD, GSH and LPO levels were significantly regained in leaf and bark extracts treated animal groups. Reduced level of SOD (36.72±0.59), Catalase (10.1 ± 0.01), GSH (18.44±0.38) and Vitamin C (5.3 ±0.13) were noted in group II animals (diseased). Very high level of LPO was noted in tissues of group II animals (398.2±1.30). Leaf and bark extracts treated groups regained their level of antioxidant enzymes in a dose dependent manner (observed from group IV and V).

Table 1. Antioxidant activity of *D.virginiana* leaf and bark extracts in experimental animals

Groups	SOD	GSH	LPO	CAT	VIT- C
Group I	95.06±0.84	44.40±0.30	163.32±0.83	11.6±0.03	7.01±0.1
Group II	36.72±0.59	18.44±0.38	398.22±1.30	10.1±0.01	5.3±0.13
Group III	51.46±0.45	26.52±0.53	311.40±0.96	8.5±0.00	7.0±0.61
Group IV	93.56±0.51**	43.54±0.41	151.45±0.21	11.0±0.2	6.8±0.52
Group V	95.23±0.76	44.59±0.95	163.48±0.89	11.8±0.4	7.3±0.61

Values are given as mean ± standard deviation of six animals each.
* Values are statistically significant at $p<0.01$ level
**Values are statistically significant at $p<0.05$ level.

Crucial components of the antioxidant defense system in the body are cellular antioxidant enzymes (SOD and glutathione), which are involved in the reduction of reactive oxygen species (ROS) and peroxides produced in the living organism as well as in the detoxification of certain compounds of exogenous origin, thus playing a primary role in the maintenance of a balanced redox status [14].

Leaf extract treated groups effectively regained their antioxidant levels as in group I (normal) animals. 151.4±0.21 and 163.4±0.894 (both the values are significant at $p<0.05$ level) nM of MDA/mg protein of LPO was noted in leaf extract treated animal groups (Group - V) and bark (Group-IV) extracts.

Leaf (500mg/kg) treated animal group showed good regaining power of LPO, SOD, Catalase, GSH and Vitamin C than bark (500mg/kg). Leaf extract treated animal groups regained best SOD and LPO levels than Silymarin treated groups (Group - III) 51.46±0.456 (significant at $p<0.01$ level) and 311.4±0.9617 (significant at $p<0.05$ level) respectively). All the results were significantly different at $p<0.01$ and 0.05 levels. Level of LPO was high among CCl_4 induced animal tissues as well as in the samples of CCl_4 induced disease control groups and decreased level of SOD was noted in CCl_4 induced mice. Both the extracts of *Diospyros virginiana* effectively showed their antioxidant powers.

SOD plays an important role in the elimination of ROS and protects cells against the deleterious effects of super oxide anion derived from the peroxidative process in liver and kidney tissues. CAT considered as most important H_2O_2 removing enzyme and also a key component of anti oxidative defense system[15].Peroxidase is an enzyme that catalyzes the reduction of hydroperoxides, including hydrogen peroxides, and functions to protect the cell from peroxidative damage.

The antioxidant activities of medicinal plants may be due to the presence of phenolic compounds, containing the hydroxyl groups that confer the hydrogen donating ability and the flavonoids which are involved in free radical scavenging activity.

Conclusion

The findings of this study supports the view, that the ethanol extracts of the leaves and bark of *Diospyros virginiana* are promising sources of potential antioxidants and may be efficient as preventive agents in some diseases and can be considered as a natural herbal source in pharmaceutical industry. The medicinal plants which possess good antioxidant potential are the best supplements for the diseases associated with oxidative stress.

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