Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading doi:10.1016/S2221-1691(12)60309-8

Evaluation of antioxidant activity of *Ammania baccifera* (L.) Whole plant extract in rats

Subash vijayakumar[*], Ramaiyan Dhanapal[1], , I. Sarathchandran[2], A. Saravana Kumar and J.Vijaya Ratna[4]

[*]*Department of Clinical Pharmacy Service, MGM Hosptial, Vaagdevi College of Pharmacy, Warangal, Andhra Pradesh, India-506 001*
[1]*Department of Pharmaceutics, Kakatiya Institute of Pharmaceutical Sciences, Pembarthi (V), Hasanparthy (M), Warangal, Andhra Pradesh, India-506 371.*
[2]*Department of Pharmaceutics, Gokula Krishna College of Pharmacy, Sullurpet, Nellore, Andhra Pradesh, India-524 121.*
[3]*Department of Pharmacology and Toxicology; Department of Pharmaceutical Chemistry, SVCP Sri Sainathnagar, Tirupati, Andhra Pradesh, India-517 102.*
[4]*Pharmaceutical Technology Division, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India-530 003.*

ARTICLE INFO

Article history:
Received 15 June 2012
Received in revised from 5 July 2012
Accepted 7 August 2012
Available online 28 August 2012

Keywords:
Antioxidant enzymes
Carbon tetrachloride
Lipid peroxidation
Ammania baccifera

ABSTRACT

Objective: To study the effect of antioxidant activity of the ethanol extract of whole plant of *Ammania baccifera*, Linn (EEAB). **Methods:** To investigate in rats against carbon tetrachloride (CCl4) induced erythrocyte damage. Simultaneous intraperitoneal administration of the crude extracts (600 or 800 mg/kg body weight/day) with carbon tetrachloride (1ml/kg of body weight) to rats for alternate days of two weeks protected the loss of functional integrity and membrane lipid alteration in red blood cells induced by oxidative stress. **Results:** EEAB inhibited the accumulation of lipid peroxidation products in the plasma as well as maintained the activities of antioxidant enzymes such as superoxide dismutase (SOD) and catalase. The extracts further had the ability to decrease the membrane fluidity induced by carbon tetrachloride. **Conclusion:** It can therefore be suggested that the whole plant of *Ammania baccifera*, Linn possess an erythrocyte protective activity against drug induced oxidative stress. These findings also provide a rationale for further studies on isolation of active principles and its pharmacological evaluation.

1. Introduction

Nature is and will still serve as the man's primary source for the cure of his ailments. However, the potential of higher plants as sources for new drugs is still largely unexplored. It is widely accepted that antioxidants are radical scavengers, which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neuro-degenertion, Parkinson's diseases, mongolism, ageing process and perhaps dementias [1,2].

Free radicals such as hydroxy radicals, superoxide anion radicals and singlet oxygens are agents that attack the unsaturated fatty acids in the biomembranes resulting in membrane lipid peroxidation, a decrease in membrane fluidity, loss of enzymes and receptor activity and damage to membrane proteins leading to cell inactivation. [3] Lipid peroxidation is also strongly associated with aging and carcinogenesis. [4] However, living systems are protected from active oxygen species by enzymes such as superoxide dismutase, glutathione peroxidase and catalase. These living systems have also been reported to receive non-enzymatic protection by endogenous antioxidants such as α-tocopherol, ascorbic acid, β-carotene, and uric acid. Generally, food antioxidants act as reducing agents, reversing oxidation by donating electrons and hydrogen ions. There is a worldwide trend toward the use of natural antioxidants. The commercial development of plants as sources of antioxidants to enhance health and food preservation is of current interest.

Ammania baccifera Linn (Family: Lythraceae) is a glabrous, erect branching herb, found as weed in rice-fields and marshy localities throughout India. The leaves are acrid and used in the treatment of rheumatic pain, as laxative, rubifacient and external remedy for ringworm.[6,7] This plant was found to possess hypothermic, hypertensive, antiurolithiasis, antibacterial and CNS depressant activities. [8-10] In our previous study, we reported analgesic [11] and antisteroidogenic activities [5,12] of this plant. The herb is rich in vitamin C. [13] Steroid, triterpenes, coumarins, flavonol and tannins were previously isolated from various

*Corresponding author: Subash vijaya kumar, Mpharm, Ph.D., Associate Professor & Head, Department of Pharmacy Practice, Vaagdevi College of Pharmacy, Hanamkonda, Warangal, A.P, India. Email: vijayvijay66@yahoo.co.in

parts of the plant. [14]

In the present study, ethanol extract of A.baccifera whole plant (EEAB) were evaluated for antioxidant activity in on carbon tetrachloride induced rats models of erythrocyte damage using lipid peroxidation and the antioxidants superoxide dismutase (SOD) and catalase as biomarkers.

Materials and methods

Plant material

The whole plant of A. baccifera (L) was collected from Trichy, Tamilnadu, India and was identified and authenticated by Prof. Sri. Ganesh, Botanist Madurai College, Madurai, Tamil nadu. A voucher specimen MG-3 has been kept in out laboratory for future reference. The whole plant was dried under shade, powdered by a mechanical grinder and was passed through 40-mesh sieve and stored in airtight container for further use.

Preparation of Extract

About 1kg of the powdered plant material was successively extracted using petroleum ether (40° -60 ℃), chloroform, and then ethanol (90%) in a Soxhlet extraction apparatus. The various extracts were concentrated and the traces of the solvent were completely removed under reduced pressure and were stored in a vacuum desiccator for further use. The yield was found to be petroleum ether extract (0.9%), chloroform extract (1.7%) and ethanol extract (3.6%) w/w with respect to dried powder, preliminary qualitative chemical rests indicates the presence of steroids, triterpenoids, flavonoids and tannins. The further investigation was carried out using ethanol extract.

Animals

Mature Albino male rats (Wistar) weighing 180-195g were used for the present study. They were supplied with standard pellet diet (Hindustan Lever) and water ad libitum. The rats were divided into nine groups and housed in wire-meshed cages for 6 days to acclimatize them to the experimental environment before the start of the experiment. The Experiment was performed under the guidance of Ethical Committee (Registration No: 129/99/CPCSEA).

Experimental design

Body weight of animals was recorded and then they were divided into 6 groups of 6 rats each Propylene glycol (PG) was used as a carrier of EEAB extracts (600 and 800 mg/kg body weight/day) as well as for carbon tetrachloride (1ml/kg body weight), administered intraperitoneally alternate days for 14 days. The following experimental groups were used: Group 1, 2, 3, 4, 5 and 6 were received distilled water + PG (Normal control), EEAB 600mg/kg + PG (Herb control), EEAB 800mg/kg + PG (Herb control), Carbon tetrachloride in PG, EEAB600mg/kg + carbon tetrachloride in PG, EEAB800mg/kg + carbon tetrachloride in PG respectively. On the 15th day rats were kept fasting for 12 hours and sacrificed by cervical dislocation. Blood was collected from the jugular vein into tubes containing heparin, centrifuged at 3000 rpm for 15 min and the resulting buffy coat removed. The packed cells were washed three times with physiological saline (0.9% NaCl), lysed by suspending them in cold distilled water, and then centrifuged at 7000 rpm for 30 min. The resulting pellet contained the erythrocyte membrane and the supernatant represented the haemolysate.

Biochemical estimation

Plasma resulting from the initial centrifugation was used for measuring lipid peroxidation following the method of Gutteridge and Wilkins [15] while the haemolysate was used for the estimation of superoxide dismutase [16] and catalase [17] activities. Lipids from the erythrocyte membrane were extracted using the method of Folch *et al*. [18]

The concentration of cholesterol and phospholipids were determined using previously established methods. The cholesterol/phospholipid ratio was then calculated.

Statistical analysis

The data, presented as means standard deviation, were analysed using analysed using ANOVA. Duncan's Multiple Range Test (DMRT) was used to determine significant differences between means. The results were considered statistically significant if the P values were 0.05 or less.

Results

Table-1 shows the effect of EEAB on carbon tetrachloride induced oxidative stress. Treatments with the extracts significantly (P <0.05) prevented the accumulation of lipid peroxidation products in the plasma. Intoxication of the rats with carbon tetrachloride also led to significant increases in superoxide dismutase and catalase activities, while simultaneous administration of carbon tetrachloride with the extracts significantly (P <0.05) decreased these activities.

Intoxication with carbon tetrachloride causes an increase in membrane cholesterol, a decrease in membrane phospholipid and a subsequent increase in the cholesterol to phospholipid ratio (Table 2).

Discussion and conclusion

The results obtained in this study indicate the rigidity of the membranes. Administration of EEAB extracts prevented changes in membrane lipids as well as those in membrane fluidity. It is well recognized that free radicals are critically involved in various pathological conditions such as cancer, cardiovascular disorders, arthritis, inflammation and liver diseases. [19,20,27,28] Under normal physiological conditions low concentrations of lipid peroxidation products are found in tissues and cells. In the presence of oxidative stress more lipid peroxidation products are formed due to cell

Table 1

The effects of *Ammania baccifera* extract on lipid peroxidation products and primary antioxidant enzymes of the erythrocytes of carbon tetrachloride –intoxicated rats.

Design of treatments	Lipid peroxidation× 10^{-6} (units)	Enzyme activities (Units/mg protein)	
		Superoxide dismutase	Catalase
(Control, Propylene glycol)	0.28 ± 0.03	192.6 ± 9.3	1.9 ± 0.2
(EEAB600mg/kg + Propylene glycol)	0.27 ± 0.05	193.2 ± 6.7	1.8 ± 0.1
(EEAB800mg/kg + Propylene glycol)	0.28 ± 0.02	190.7 ± 10.2	1.8 ± 0.2
(Carbon tetrachloride + Propylene glycol)	0.47 ± 0.03c	272.4 ± 1.7c	4.7 ± 0.6c
(Carbon tetrachloride + EEAB600mg/kg)	0.39 ± 0.04ce	228.1 ± 2.3ce	3.8 ± 0.2ce
(Carbon tetrachloride + EEAB800mg/kg)	0.34 ± 0.06ce	214.0 ± 1.8ce	3.3 ± 0.6ce

Values are means ± standard deviation for six rats per group. Means in the same column having different superscript are significantly different (P <0.05). EEAB=Ethanol extract of *A.baccifera* (L) whole plant.

Table 2

Effect of *Ammania baccifera* extract on erythrocyte membrane lipids and cholesterol/phospholipid ratio of carbon tetrachloride –intoxicated rats.

Design of treatments	Cholesterol (mg/100 μ l)	Phospholipids (mg/100 μ l)	Cholesterol /Phospholipid
Control, Propylene glycol	0.64 ± 0.03	1.08 ± 0.04	0.60 ± 0.04
EEAB600mg/kg + Propylene glycol	0.63 ± 0.02	1.09 ± 0.03	0.61 ± 0.02
EEAB800mg/kg + Propylene glycol	0.64 ± 0.04	1.07 ± 0.04	0.59 ± 0.05
Carbon tetrachloride + Propylène glycol	0.83 ± 0.05c	0.85 ± 0.02c	0.98 ± 0.03c
Carbon tetrachloride + EEAB600mg/kg	0.72 ± 0.02ce	0.94 ± 0.03ce	0. 77 ± 0.02ce
Carbon tetrachloride + EEAB800mg/kg	0.67 ± 0.03 ce	0.99 ±0.02 ce	0.70 ± 0.04 ce

Values are means ± standard deviation for six rats per group. Means in the same column having different superscript are significantly different (P <0.05). EEAB=Ethanol extract of *A.baccifera* (L) whole plant.

damage. Cellular antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase normally challenge oxidative stress. In this study, carbon tetrachloride damage to erythrocytes was confirmed by the increases in lipid peroxidation products, superoxide dismutase and catalase activities, and decreases in membrane fluidity. The increased superoxide dismutase activity resulted in the accumulation of hydrogen peroxide, which stimulated increases in catalase activity. Pre–treatment of experimental animals with the EEAB extracts exhibited an improved free radical scavenging resulting in decreased activities of superoxide dismutase and catalase, and the concentration of lipid peroxidation products towards normal. The cumulative effect of carbon tetrachloride resulted in increases in erythrocyte membrane peroxidation, which may also lead to hemolytic changes. It has been shown that micro–viscosity of a membrane increases markedly with increases in cholesterol to phospholipid ratio thus leading to cellular rigidity. [21] Intoxication of experimental animals with carbon tetrachloride altered membrane structure and function as shown by the increases in cholesterol and subsequent decreases in phospholipid concentrations, hence increased cholesterol to phospholipid ratio. Cooper *et al* [28] reported that alteration of bio–membrane lipid profile disturbs its fluidity, permeability, activity of associated enzymes and transport system. Thus *Ammania baccifera* plays a role in peroxidation by inhibiting the free radical attack on bio–membranes.

Phytochemical investigations on this plant by various authors demonstrated the presence of terpenoids, Vitamin C and flavonoids. [14–17] The terpenoids have been reported to protect lipids, blood and body fluids against the attack of reactive oxygen species like superoxide, peroxide and hydroxyl radicals. In experimental studies, terpenoids have prevented the occurrence of cancer in many tissues including, breast, colon, stomach, prostate, pancreas, liver and skin. [22–24] Vitamin C (ascorbate) acts as a potent water–soluble antioxidant in biological fluids [25] by scavenging physiologically relevant reactive oxygen species and reactive nitrogen species. [26] These include free radicals such as hydroxyl radicals, aqueous peroxyl radicals, and superoxide anion. Moreover, endogenous and exogenous vitamin C inhibits rather than promotes lipid peroxidation.] Flavonoids also reported to have antioxidant activity. [24] The presence of terpenoids, Vitamin C and flavonoids in A.baccifera extract might be responsible for their observed antioxidant activity.

Since reactive oxygen species are involved in stress and pathogenesis of cancer, diabetes mellitus, atherosclerosis, and dementia, the use of this plant may be beneficial in preventing initiation or progress of such disorders. Efforts are in progress in our laboratory to isolate and purify the active principle involved in the antioxidative efficacy of this medicinal plant. Whereas, other researcher reported that wound healing effect of this plant. [29–33]

Conflict of interest statement

The authors reports no conflicts of interest in this work. RD,ASK and VRJ drafted and revised the manuscript. All authors read and corrected draft versions of the manuscript and approved the final manuscript.

Acknowledgement

We extend our sincere thanks to vishwabhara educational society affiliated to kakatiya university for funding supported by grants VCOP -2004-C-007-042-PP3 throughout this work, The authors also thank . Principal, Vaagdevi College of Pharmacy, Warangal, Who permitted us to utilize the pharmacognosy department for this research work.

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