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RESEARCH ARTICLE

Antioxidative Activity of *Melia azedarach* Linn Leaf Extract

MOHD. FAZIL AHMED, MOHD. ASHWAQ AHMED, HAMEED THAYYIL, KHAJA ZAMEERUD-DIN and MOHD. IBRAHIM

For author affiliations, see end of text.

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ABSTRACT

The antioxidant activity of the *melia azedarach* was investigated in rats with ethanol-induced erythrocyte damage. Chronic administration of ethanol (20% w/v, 2 g/kg.p.o., daily for four weeks) increased the level of lipid peroxidation (LPO), decreased the activity of superoxide dismutase (SOD) and catalase and reduced the content of glutathione (GSH). The concurrent treatment of ethanol-administered rats with *melia azedarach* (500mg/kg, p.o.) prevented the ethanol-induced changes and the effect was compared with combination of vitamin E and C. It can, therefore, be suggested that the leaves of *melia azedarach* posses an erythrocyte protective activity against drug-induced oxidative stress.

Keywords: *Melia azedarach, Antioxidant activity, Ethanol, Oxidative stress.*

Within the human body, millions of chemical reactions are occurring constantly .These processes require oxygen. Reactive oxygen spices (ROS), sometimes called active oxygen species, are various from of activated oxygen, which include free radicals such as super oxide ions(O_2^-) and hydroxyl radicals (OH^-), as well as non-free-radicals species such as hydrogen peroxide (H_2O_2) [1,2]. In living organisms, various ROS can form in different ways, including normal aerobic respiration and stimulated peroxisomes in polymorph nuclear leukocytes and macrophages,. These appear to be the main endogenous sources of most of the oxidants produced by cells. Exogenous sources of free radicals are tobacco smoke, ionizing radiation, certain pollutants, organic solvents, and pesticides [3-5].

Free radicals are the compounds generated from normal body processes and also from environmental pollutions .They tend to attack the healthy cells DNA as well as proteins and fats, causing them to deteriorate.

Anti-oxidants are compounds that protect cells against the damaging effects of reactive oxygen specious, such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Oxidative stress has linked to cancer, ageing, atherosclerosis, ischemia injury, inflammation and neurodegenerative diseases (Parkinson's and Alzheimer's) [6]. Lipid peroxidation is also strongly associated with aging and carcinogenesis [7].

Certain antioxidant enzymes are produced within the body .The most commonly recognized of naturally-occurring antioxidants are superoxide Dismutase, catalase, and Glutathione. Superoxide Dismutase changes the structure of oxidants and breaks them down into hydrogen peroxide. Catalase in turn breaks down hydrogen peroxide into water and tiny oxygen particles or gasses. Glutathione is a detoxifying agent, which binds with different toxins to change their form so that they are able to leave the body as waste. Other antioxidant agents are found in foods, such as green leafy vegetables. Items high in vitamin A, vitamin E, and beta-carotene are believed to be the most beneficial.

Melia azedarach linn (meliaceae; Neem) is an indigenous plant possessing several medicinal properties. *Melia azedarach linn* (synonym: media dubia Cav, Indian lilac, Persian lilac) belongs to the family meliaceae and is a tree found in India. It is popular as Indian lilac. Different phytochemicals have been isolated from fruit include melianoninol (I), melianol (II), melianone (III), meliandiol (IV), vanillin (V) and vanillic acid (VI) [8]. The plant is traditionally used for the treatment of leprosy, inflammations, and cardiac disorders. Its fruits extracts possess ovicidal [9] and larvicidal activity [10]. The leaf extracts also possess antiviral [11].and antifertility activity [12]. As the role of free radicals has been

Table 1. Effect of ethanolic extract of *Melia azedarach* Linn on oxidative stress in rats (mean±SEM, n=6).

Treatment	Lipid preoxidation nmDA/g Hb	Glutathione μm DTNB conjugated/g Hb	Superoxidation dismutase units/ mg protein	Catalase units/mg protein
Normal control	70.1±1.29	4.42±0.17	29.34±1.36	287.17±3.57
Ethanol	169.93±1.49*	2.51±0.19*	16.41±0.54*	191±2.50*
Ethanol + *Melia azedarach*	70.51±2.72**	4.32±0.53**	19.53±2.44**	233.68±2.82**
Ethanol +Vitamins E and C	98.19±2.63***	4.48±0.26***	20.02±1.39***	251.95±2.69***
Melia azedarach	63.20±2.83	4.68±0.27	29.63±1.07	279.42±1.11
Vitamins E and C	68.97±3.29	4.76±0.22	30.89±0.81	279.28±2.35
One way Anavo F	270.73	7,78	37.78	23.67
FD	5,30	5,30	5,30	5,30
P value	<0.0001	<0.0001	<0.0001	<0.0001

*$p< 0.05$ when compared with respective control group. **$p< 0.05$ when compared with vehicle treated experimental group. ***$p<0.05$ when compared with RC treated experimental group.

documented in many of these conditions, the present study was directed to investigate the antioxidant activity of *Melia azedarach* leaf extract in rats.

MATERIALS AND METHOD

Plant materials

The leaves of melia azedarach linn were collected in the month of January 2007 from botanical garden, Annamalai University, Annamalainagar, Chidambaram. The plant was identified and authenticated by Department of Botany, Research Officer (botanist) Annamalai University, Annamalainagar, Chidambaram-608 002.

The leaves were dried under shade and powdered in a mechanical grinder. The powdered material was extracted with methanol using soxhlet apparatus. The methanol extract was concentrated in vacuo and kept in a vacuum dessicator for complete removal of solvent. The yield was 9.1% w/w with respect to dried powder. Preliminary qualitative analysis of the methanol extract showed the presence of alkaloids, tannins, glycosides and saponins.

Chemicals

Pyrogallol and hydrogen peroxide were obtained from S.D. fine chemicals Ltd., India. Thiobarbituric acid(TBA), Trichloroacetic acid(TCA),5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), phosphate buffer and Tris buffer were obtained from Sigma, USA. All other reagents used were of analytical grade.

Animals

The approval of Institute's Animal Ethics Committee was obtained. Male albino Wistar rats (100-150 g, 4-6 weeks old) were maintained under controlled conditions of light/dark (12/12) and temperature (23±1°C). Food pellets and top water were provided ad libitum. For experimental purposes animals were fasted overnight but were allowed free access to water.

Methods

Six groups of rats, six in each, received the following treatment schedule:
Group I: Normal control (saline)
Group II: Ethanol (20% w/v, 2 g/kg.p.o.)
Group III: Ethanol + *Melia azedarach* leaf extract (500 mg/kg, p.o.)
Group IV: Ethanol + Vitamin C and E (100 mg/kg, p.o.)
Group V: *Melia azedarach* leaf extract (500 mg/kg, p.o.)
Group V I: Vitamin C and E (100 mg/kg, p.o.)

The experimental groups III and IV was divided in such a manner that all the rats concurrently received ethanol (20% w/v, 2 g/kg.p.o) [13] and either the leaf extract of *Melia azedarach* (500mg/kg, p.o) or the Vitamin C (100mg/kg, p.o) and Vitamin E (100mg/kg, p.o) [14]. The above treatments were given daily for four weeks. At the end of 4th week, i.e. on 29th day, blood sample was collected from retro-orbital plexus under ether anesthesia.

Measurement of lipid peroxidation (LPO)

To 2 ml, 5% suspension of separated RBC in 0.1 M phosphate buffered saline, 2 ml of 28% trichloroacetic acid was added and centrifuged. One ml of 1% thiobarbituric acid was added to 4 ml of supernatant, heated in boiling water for 60 min and cooled immediately. The absorbance was measured spectrophotometrically at 532 nm. The lipid peroxidation was calculated on the basis of the molar extinction coefficient of malondialdehyde (MDA)(1.56x 105), and expressed in terms of nanomoles of MDA/g Hb [15].

Measurement of superoxide dismutase (SOD) activity

An erythrocyte lysate was prepared from the 5% RBC suspension [16]. To 50 µl of the lysate, 75 mM of Tris-HCl buffer (pH 8.2), 30 mM EDTA and 2 mM of pyrogallol were added. An increase in absorbance was recorded at 420 nm for 3 min by spectrophotometer (Schimadzu1601, Japan). One unit of enzyme activity is 50% inhibition of the rate of auto-oxidation of pyrogallol as determined by change in absorbance/min at 420 nm. The activity of SOD was expressed as units/mg protein.

Measurement of catalase (CAT) activity

Catalase activity was determined in erythrocyte lysate using Aebi's method with some modifications [17]. About 50 µl of the lysate was added to a cuvette containing 2 ml of phosphate buffer (pH 7.0) and 1ml of 30 mM H2O2. Catalase activity was measured at 240 nm for 1 min using spectrophotometer. The molar extinction coefficient of H2O2, 43.6 M cm-1 was used to determine the catalase activity. One unit of activity is equal to one millimoles of H_2O_2 degraded per minute and is expressed as units per milligram of protein.

Content of reduced glutathione (GSH)

Blood glutathione was measured by addition of 0.2 ml of whole blood to 1.8 ml distilled water followed by 3.0 ml of precipitating mixture (1.67 g metaphosphoric acid, 0.2 g EDTA and 30 g NaCl to make 100 ml of solution) [18]. It was centrifuged at 5000×g for 5 minutes and 1 ml of the filtrate was added to 1.5 ml of the phosphate solution, followed by the addition of 0.5 ml of DTNB reagent. The optical density was measured at 412 nm using spectrophotometer.

Statistical analysis

All data were analyzed with one-way ANOVA followed by Dunnett's multiple comparison test. $p<0.05$ was considered significant.

RESULTS

Table 1 shows the antioxidant activity of *Melia azedarach***.** The oxidation stress marker studies revealed that the chronic administration of ethanol increased the level of LPO, decreased the activity of SOD and CAT and reduced the content of GSH.

The concurrent treatment of ethanol–administered rats with *Melia azedarach* prevented the ethanol–induced changes in the markers of oxidative stress. Influences of *Melia azedarach* on ethanol–induced changes were similar and comparable with the effects of vitamins E and C treatment.

DISCUSSION

The present study has shown that the administration of ethanol, over a period of four weeks leads to oxidative stress, in rats, since the mixture of vitamins C and E significantly attenuated ethanol-induced changes. The literature has documented free radicals generation during the metabolism of ethanol [19]. The level of the markers of oxidative stress observed in ethanol-treated rats substantiates the possibility of extensive generation of free radicals. It was observed that administration of alcoholic extract of *Melia azedarach* prevented the ethanol-induced changes in oxidative stress parameters, and effect was comparable to that of vitamins E and C.

The mechanism of free radicals–induced impairment of immune system is not yet properly delineated. Reduced glutathione, a free radical scavenger, plays a key role in the activation of T cells and macrophages [20].

The present study has revealed that chronic treatment with ethanol depletes the glutathione level. It was further observed that treatment with leaf extract or the mixture of vitamins C and E prevented the above influence of ethanol on glutathione levels. Thus, the present study suggests that leaves of *Melia azedarach* linn might be a potential source of natural antioxidant.

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CURRENT AUTHOR ADDRESSES

Mohd. Fazil Ahmed, Nizam Institute of Pharmacy, Deshmukhi, Pochampally, Near Ramoji Film City, Nalgonda, (AP), India. E-mail: mohdfazil_pharma@yahoo.co.in (Corresponding author)

Mohd. Ibrahim, Nizam Institute of Pharmacy, Deshmukhi, Pochampally, Near Ramoji Film City, Nalgonda, (AP), India.

Mohd. Ashwaq Ahmed, Peoples Health Center, Bollaram (vill), Hyderabad, Andhra Pradesh, India.

Hameed Thayyil, Nizam Institute of Pharmacy, Deshmukhi, Pochampally, Near Ramoji Film City, Nalgonda, (AP), India.

Khaja Zameeruddin, Nizam Institute of Pharmacy, Deshmukhi, Pochampally, Near Ramoji Film City, Nalgonda, (AP), India.