Hepato Protective and Antioxidant Activity of *Aerva Lanata* Against D-Galactosamine Induced Oxidative Stress in Rats

Sk. Abdul Saleem[1], D. Rathna Kumari[2], O. Mahendra varma[2], S. Bhargavi[2], S.Ramesh[2], Y. Vyshnavi[2], J.N.Suresh Kumar[3]

[1:] *Associate Professor, Department of Pharmacology, Narasaraopeta Institute of Pharmaceutical Sciences, Kotappakonda road, Yallamanda(P)Narasaraopet(M), Palnadu(Dt.) 522601*

[2:] *Research Scholar, Narasaraopeta Institute of Pharmaceutical Sciences, Kotappakonda road, Yallamanda(P)Narasaraopet(M), Palnadu(Dt.) 522601*

[3:] *Principal, Professor, Narasaraopeta Institute of Pharmaceutical Sciences, Kotappakonda road, Yallamanda(P)Narasaraopet(M), Palnadu(Dt.) 522601*

Abstract: Plants have a large number of bioactive compounds with high antioxidant activity. Studies for the determination of the Hepatoprotective and antioxidant activity of different plant species could contribute to revealing the value of these species as a source of new antioxidant compounds. There is alarge variety of in vitro methods to quantify hepatoprotective and antioxidant activity, and it is important to select the proper method to determine which species have the highest antioxidant activity.Their extracts were analyzed using the following methods: 2,2-di-phenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay, ferric reducing (FRAP) assay, Trolox equivalent antioxidant capacity (ABTS) assay, and reducing power (RP) assay. The four methods selected could quantify the antioxidant capacity(1). The Another difference established between these methods was the sensitivity obtained with each of them.

Keywords: ABTS; DPPH; FRAP; antioxidant activity methods; Hepato protective; reducing power assay.

INTRODUCTION

In a sense, there has always magic in plants, an unknown Genie, mysterious and omnipotent, an all pervading powerful force. In last five decades, these plants have been extensively studied by advanced scientific techniques and reported for various medicinal properties viz, anticancer activity, antibacterial activity, antifungal activity, antidiabetic activity, antioxidant activity, hepatoprotective activity, haemolytic activity, larvicidal activity and anti-inflammatory activity etc.

TYPES OF FREE RADICALS

Most free radicals are coming from oxygen atoms and are called Reactive Oxygen Species (ROS), such as superoxide ion, hydroxyl radical, hydrogen peroxide and singlet oxygen species.

1) Endogenous antioxidant –those which are physiological in origin
2) Exogenous antioxidant- those which cannot be produced by the human body.

MECHANISM OF FREE RADICALS

Prevention of ROS formation

1) Interception of ROS attack by scavenging the reactive metabolites and converting them to less reactive molecule and by enhancing the resistivity of sensitive biological targets to ROS attack(2)
2) Facilitating the repair of damage caused by ROS
3) Providing(e.g as a cofactor by acting to maintain a suitable redox status)

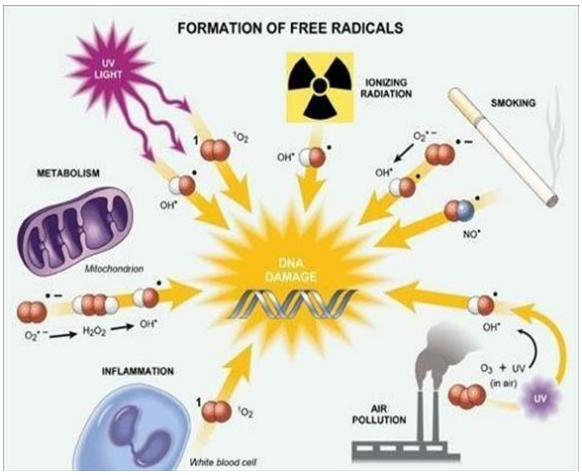

Fig 1: Formation of free radicals

MATERIALS AND METHODS

ANIMALS : Albino wistar rats (180-220gm)
CHEMICALS: D- galactosamine
: Vitamin C
: Ethanolic extract of *Aerva lanata.*

METHADOLOGY

Treatment protocol

The acclimatized animals were divided into 5 groups of each 6 animals, designated as

- Group 1: Served as normal control and receive normal diet and water.
- Group 2: Toxic control received 25mg/kg of D-galactosamine through I.P for21days.
- Group 3: Standard control received 25mg/kg of vitamin E orally for 21 days.
- Group 4: The treatment control received 200mg/kg of Ethanolic extract of *Aerva lanata* for 21 days.
- Group5: The treatment control received 400m/kg of Ethanolic extractof Aerva lanata

PLANT PROFILE

Description:

Mountain knotgrass is an annual with a branching, somewhat woody root system. The stems aremostly straggling and sprawling and spread widely, sometimes as much as 6 feet (1.8 m) in length. The often stalkless leaves are alternate, oval and 0.5 to 1.5 in (13 to 38 mm) long. They grow from whitish papery stipules with two lobes and red bases[3]. The tiny clusters of two or three flowers grow in the leaf axils. The flowers are about 0.1 in (2.5 mm) long, pink, green or dull white. The flowers are normally self-pollinated. Flowering time is from May to October.

Fig 2:leaves of Aerva lanata plant

Fig 3: flowering part of *Aerva lanata*

Fig 4:Seeds of *Aerva lanata*

Fig 5: *Aerva lanata* plant Fig 6: Roots of *Aerva lanata*

MORPHOLOGY

Herb, erect or prostrate with a long tap-root, branched from near the base; branches many, pubescent or wolly- tomentose, striate.

Leaves alternate, 2-2 × 1-1.6 cm on the main stem, 6-10 x 5-6 mm on the branches, elliptic or obovate,or subotbicular, obtuse or acute, entire, pubescent above, more or less white with cottony hairs beneath; petioles 3-6 mm long, often obscure.

Flowers greenish white, very small, sessile, often bisexual, in small dense subsessile axillary heads or spikes 6-13 mm long, often closely crowded and forming globose clusters; bracteoles 1.25 mm, long, membranous, broadly ovate, concave, apiculate. Perianth 1.5-1.25 mm long; sepals oblong, obtuse, sometimes apiculate, silky-hairy on the back. Utricle broadly ovoid, acute; stigmas two, seed 0.85 mm in diameter, smooth and polished, black.[11]

PHYTOCONSTITUENTS

Alkaloids:

Plant contains biological active canthin-6-one alkaloids such as 10-methoxy-canthin-6-one, 10-hydroxy-canthin-6-one, 10-O-β-D-glucopyranosyloxycanthin-6-one, 10-hydroxycanthin-6-one (ervine), 10-methoxycanthine-6-one (methylervine), 10-β-D-glucopyranosyloxycanthin-6-one (ervoside), aervine (10-hydroxycanthin-6-one), methylaervine (10-methoxycanthin-6-one) and aervoside (10-β-D-glucopyranosyloxycanthin-6-one)[4]. Plant also contains alkaloids like β-carboline-1-propionic acid, 6-methoxy-β carboline-1-propionic acid, 6-methoxy-β-carbolin-l-ylpropionic acid (ervolanine), and aervolanine (3-(6-methyoxy-β-carbolin-1-yl) propionic acid).[12]

Flavanoids:

Aerva lanata is a rich source of flavanoids such as kaempferol, quercetin, isorhamnetin, isorhamnetin 3-O-β-[4-p-coumaroyl-α-rhamnosyl(1→6) galactoside and flavanone glucoside persinol, persinosides A and B, 5, 4'- pentamethoxyl flavone, 3,3',5,7-trihydroxy-4'-methoxyflavone, apigenin 7-O-β-D- glucoside and 7-O-β-D-hydroxy-3, 6, 7-trimethoxyflavone, 5-hydroxy-3, 6, 7, 4-tetramethoxyflavone, 5-hydroxy 2', 3,5', 6, 7- glucopyranoside.

Miscellaneous phytoconstituents

Aerva lanata also contains methyl grevillate, lupeol, lupeol acetate benzoic acid, β-sitosteryl acetateand tannic acid.[13]

PHYTOCHEMICAL SCREENING

Detection of alkaloids:

A small portion of the solvent free extract was stirred saperately with a few drops of dilutehydrochloric acid and filtered. The filtrate was tested with various agents.

Dragendrof's test:

To the 1 ml of extract add 1ml of reagent (potassium bismuth iodide). An orange red precipitateindicates the presence of Alkaloids.

Mayer's test:

To the 1ml of extract add 1ml of reagent (potassium mercuric iodide) whitish yellow or cream coloured precipitate indicates presence of Alkaloids.

Hager's test:

To the add 1ml of extract add 1ml of saturated

aqueous solution of picric acid yellow colour precipitate indicates the presence of Alkaloids.[14]

Wagner's test :

To the 1ml of extract add 1ml of reagent (iodine in potassium iodide). Reddish brown precipitate indicates the presence of carbohydrates.

Detection of flavanoids:

Shinoda test:

The alcoholic extract was treated with magnesium foil and concentrated sulphuric acid(3ml) gives intense cherry red colour, indicates the presence of flavones.[5]

The Ethanolic extract is treated with 10% sodium hydroxide solution and ammonium was added. Dark yellow colour indicates the presence of flavanoids.[15]

Detection of proteins:

Biurets test:

Add 1ml of 40 % sodium hydroxide solution and 2 drops of 1% copper sulphate solution till a blue colour is produced and then add to the 1ml of extract. Formation of pink or purple violet colour indicates the presence of proteins.[16]

Detection of carbohydrates:

Benedict's test:

To 0.5ml of filtrate add 0.5ml sodium citrate, sodium carbonate , pentahydrate of copper sulfate was added. The mixture was heated on a boiling water bath for 2min[17]. A characteristic coloured precipitate indicates the presence of sugars.

Fehling's test:

Mix 1ml of Fehling's and Fehling's B solutions. Boil for 1min , add equal volume of test solution. Heat in boiling water bath for 5-10 min. Orange red precipitate indicates the presence of carbohydrates.[18]

Detection of Terpenoids:

Salkowski test:

5ml of extract was mixed in 2 ml of chloroform and conc.H_2SO_4 (3ml) was carefully added to form a layer a reddish brown coloration of the interface was formed to show positive results for the presence of Terpenoids.[19]

Detection of Glycosides:

Keller-Killiani test:

4 ml of glacial acetic acid with 1 drop of 2% ferric chloride mixture mixed with 10ml of plant extract and 1ml of H_2SO_4. Brown ring is formed indicates the presence of glycosides.[20]

Fig no 7: Plant extract of *Aerva lanata*

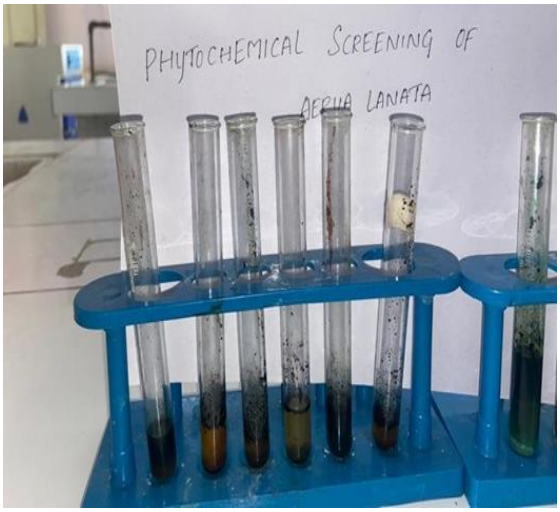

Fig no 8: Phytochemical screening

EXPERIMENTAL MODELS

For the study of hepatoprotective and antioxidant activity an animal modelwas addedthat would satisfy the following conditions.

- The animal should develop liver toxicity rapidly and reproducibly
- Pathological changes in the site of induction should result from liver damage.
- The symptoms should be ameliorated or prevented by a drug treatment effective in human beings[6] .
- The drug tested should be administered orally
- Drug dosage approximate the optimum therapeutic range for human, scaled the test animal weight.[21]

LABORATORY ANIMAL MODELS

EXPERIMENTAL PHARMACOLOGICAL STUDIES IN ANIMAL LIVER

To investigate and evaluate hepatoprotective substance, it is customary to subject animals to a range of toxic agents[7]. These hepatotoxicants include carbon tetrachloride, D- galactosamine, thioacetamide, ethanol, aflatoxin B1, alpha amanitine, phalloidin, cadmium, paracetamol, hydrazine, halothane, isoniazid etc that causes damage of rat liver, resulting in biochemical and histopathological changes. Different toxicants used for experimental liver damage with dose range, route, vehicle and detailed schedule of treatment.

Induced by ethanol

The basic mechanism in the induction of hepatotoxic by ethanol is principally metabolized to acetaldehyde in the liver and seldom in other tissue by alcohol dehydrogenase as well as CAT (catalase). Acetaldehyde is further oxidized into acetate by acetaldehyde dehydrogenase oxidase.,leading to the generation of ROS/free radical. Ethanol is also oxidised by a microsomal Ethanol oxidising system ($CYP2E_1$) to acetaldehyde and 1- hydroxyethyl radical especially following chronic ethanol consumption by which $CYP2E_1$ is induced[22]. Excessive alcohol intake results indisequilibriumin iron homeostasis and iron overload which further enhance oxidative stress by catalyzing the formationof more noxious hydroxyl free radical. Hence induction of $CYP2E_1$and iron overload by ethanol are critical path way by which ethanol generates a state of oxidative stress in hepatocytes.

Induced by paracetamol

The mechanism by which over dosage with paracetamol leads to hapatocellular injury and death involves its conversion to the toxic NAPQ1(N-acetyl – Para benzoquinone imines) metabolite. The glucoronide sulfa conjugation pathways become saturated and increasing amount undergo CYP-mediated N -hydroxylation to form NAPQI. This is eliminated rapidly by conjugation with GSH and then further metabolized to a mercapturic acid and excreted into urine. In the setting of paracetamol overdose, hepatocellular level of GSH become depleted[23]. The highly reactive NAPQ1 metabolite binds covalently to cell macromolecules leading to dysfunction of enzymatic system and structural and metabolic disarray further more depletion of intracellular GSH renders the hepatocytes highly susceptible to oxidative stress and apoptosis.[24]

Induced by CCl_4

CCl_4 induce liver damage by producing free radical intermediates. CCL_4 is converted to trichloromethyl radical (CCl_3) by the P-450 system[25]. Which in turn isconverted toPeroxy radical (CCl_3O_2) which causes the damage.

Induced by D- galactosamine

Galactosamine is a hexosamine derived from galactose. It causes liver injury via the generation of free radicals and depletion of UTP nucleotides. Galactosamine produces the hepatotoxic effect by selectively reducing the uridine pool in hepatocytes. This intern inhibits mRNA and protein synthesis, alters the composition of cellular membranes and finally leadsto cellular damage as a result of lipid per oxidation[26]. The hepatocyte death is represented as apoptosis and subsequently necrosis. Other mechanism of galactosamine hepatotoxicity stated that galactosamine increases intestinal permeability and subsequently facilitates bacterial translocation to the liver. Lipo polysaccharides activate kupffer cells to secrete tumor necrosis factor-α ,which raises expression of intercellular adhesion molecule 1 in endothelial cells and this promotes the adhesion of polymorphonuclear cells to vascular and hepatic endothelial cells , leading to polymorpho nuclear infiltration and hepatocyte damage. Galactosamine induces rise in SGOT, SGPT and total bilirubin where as decrease in total protein. Galactosamine shows pathological changes like moderate degeneration and necrosis of hepatocyte.[8]

Induced by INH+RIF

During metabolism of INH, Hydrazine can be produced by both directly (From INH) and indirectly (from acetyl hydrazine). The direct pathway involves hydrolysis of the amide bond of INH to produce Iso nicotinic acid and hydrazine[27]. The indirect pathway involves acetylation of INH to acetyl-INH by N- acetyl transferase hydrolysis of acetyl INH to Isonicotinic acid and acetyl hydrazine, and hydrolysis deacetylation to hydrazine. Hydrazine is a known

hepatotoxin.[9]

STATISTICAL ANALYSIS

The Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by NewmannKeul's multiple range tests[28]. The values are represented asMean ± SEM. Probability value at P <0.01 was considered as statistically significant increase.[10]

RESULTS

Table no 1: Extractive values of powdered *Aerva lanata.*

S.No.	Extract	%w/w yields
1.	Ethanolic extract of *Aerva lanata*	16%

Table no 2: phytochemical analysis of *Aerva lanata*

S.No.	*Test*	*Methanolic fraction of herbal extract*	*Results*
1.	*Test for Alkaloids*		
	Mayer's test	*Yellow coloured precipitate*	+
	Hager's test	*Yellow precipitate*	+
	Dragendrof's test	*Orange colour*	+
	Wagner's test	*Reddishbrown precipitate*	+
2.	*Test for flavonoids*	*Dark yellow colour*	
	a) Shinoda test		–
3.	*Test for proteins*		
	a) Biuret test	*Pink or purple violet*	+
4.	*Test for Carbohydrates*		
	Benedicts test	*Characteristic colour Orange*	+
	Fehling's test	*Red precipitate*	–
5.	*Test for Terpenoids*		
	a) Salkowski test	*Reddish brown colour*	+

Table no 3: EFFECT OF EEAL ON THE LEVELS OF NON ENZYMATICANTIOXIDANTS IN THE LIVER TISSUE OF D-GALACTOSAMINE – HEPATOTOXIC AND CONTROLRATS

GROUPS	GLUTATHIONE MG/100G TISSUE	VITAMIN-C MG/100G TISSUE	VITAMIN-E MG/100G TISSUE
Normal control 10ml/kg normal saline	132.60±3.45	0.82±0.08	5.92±0.60
Toxic control 25mg/kg D-galactosamine	73.55±1.70*a	0.30±0.02*a	2.40±0.30*a
Standard control Vitamin E 25mg/kg	110.32±2.70*b	0.74±0.07*b	5.60±0.55*b
Treatment control EEAL 200mg/kg	98.05±2.16*b	0.60±0.04*b	4.92±0.50*b
Treatment control EEAL 400mg/kg	91.90±1.95*b	0.69±0.06*b	5.02±0.48*b

BIOCHEMICAL OBSERVATIONS

Significant increase in (P< 0 .01) Serum Aspartate Transaminase (AST) , Alanine Transaminase (ALT), Alkaline phosphatase (ALP) , Total bilirubin (TB) and Gamma- glutamyl transpeptidase(GGTP) and significant decrease in (P< 0.01) Total protein(TP) and Total albumin(TA) levels were observed in animals treated with galactosamine 25mg/kg (Group II) as compared to normal control group(Group I)[30].

Pretreatment with Ethanolic extract of *Aerva lanata* (EEAL) at a dose 200mg and 400mg /kg ,orally for 21days decreased the levels of above indices like AST, ALT , ALP, TB, GGTP and increased levels of TP and TA significantly(P <0.01)in group IV and V.

Vitamin-E pretreatment produced significant decrease in (P< 0.01) serum AST, ALT, ALP, TB,GGTP and significant increase in TP and TA at (P< 0.01) in group III.[34]

DISCUSSION

Antioxidant is a general term for any compound that can counteract unstable molecules called freeradicals

that damage DNA, cell membranes, and other parts of cells. Because free radicals lack a full complement of electrons, they steal electrons from other molecules and damage those molecules in the process. Antioxidants neutralize free radicals by giving up some of their own electrons. In making this sacrifice, they act as a natural "off" switch for the free radicals[42]. This helps break a chain reaction that can affect other molecules in the cell and other cells in the body. But it is important to recognize that the term "antioxidant" reflects a chemical property rather than a specific nutritional property[36].

While free radicals are damaging by their very nature, they are an inescapable part of life. The body generates free radicals in response to environmental insults, such as tobacco smoke, ultraviolet rays, and air pollution, but they are also a natural byproduct of normal processes in cells. When the immune system musters to fight intruders, for example, the oxygen it uses spins off an army of free radicals that destroy viruses, bacteria, and damaged body cells in an oxidative burst[37]. Some normal production of free radicals also occurs during exercise. This appears to be necessary in order to induce some of the beneficial effects of regular physical activity, such as sensitizing your muscle cells to insulin.[44]

CONCLUSION

Oxidative stress occurs when there are too many free radicals in the body. This 14mbalance can occur due to increased production of free radicals or decreased antioxidant defenses.

Free radicals play an important role in the normal physiological functioning of the body and contributeto a person's health. However, when the body produces an excess of free radicals, it can increase a person's disease risk.[41]

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