International Journal of Pharmacognosy and Chemistry

Open Access Research Article

C. Phyllacanthus antioxidant & protective activity *in* albino wister rats

Prachetha Kolli[1], Sudhakar Kancharla[2], Dr.K.Venkata Gopaiah[3*].

1. Scientist, Microgen Health Inc, 14225, Sullyfield Cir Suite E, Chantilly, VA, USA-2015.
2. Director Clinical Laboratory, Devansh Lab Werks, 234, Aquarius Drive, Homewood, Alabama, USA-3520.
3. Associate Professor,St. Mary's College of Pharmacy,Chebrolu, Guntur-A.P-522 212-India

Article History

Received on: 01-02-2021
Revised On : 16-02-2021
Accepted on : 19-02-2021

***Keywords*:** Hepatoprotective, Antioxidant activity.

DOI:
https://doi.org/10.46796/ijpc.vi.138

Abstract

In this present study I have selected a plant, which has shown good hepato protective as well as antioxidant properties. Undoubtedly , the plant kingdom still hold many species of plant containing substances of medicinal value, which have yet to be discovered ; large numbers of plants are constantly being screened for their possible pharmacological value particularly for their hepato protective properties. A large number of plants and formulations have been claimed to have hepato protective activity. Nearly 160 phyto constituents from 101 plants belonging to 55 families have been claimed to possess liver protecting activity. In India, more than 87 plants are used in 33 patented and proprietary multi-ingredient plant formulations.The ability of EECP to enhance the levels of antioxidants along with its anti lipid per oxidative activity suggests that this compound might be potentially useful in counteracting free-radical-mediated tissue damage caused by hepato toxicity. Studies on the antioxidative potency of various flavonoids have confirmed the importance of the distribution and quantity of the hydroxyl groups. In general, the antioxidative properties of polyphenols depend on hydroxylation of ring B. The present results corroborate the protective action of EECP in D- galactosamine intoxication of rats, particularly noticeable with the high dose used by us (400 mg/kg body weight). Supplementation with this flavonoid ameliorated the hepato protective and antioxidant activity in D-galactosamine-induced hepatitis in rats.

***Corresponding Author**
Dr.K.Venkata Gopaiah
Associate Professor,
St. Mary's College of Pharmacy,
Chebrolu, Guntur-A.P-522 212-India

Inroduction

In a sense , there has always magic in plants , an unknown Genie , mysterious and omnipotent, an all pervading powerful force. From the time , man first started looking for medicine to cure illness or combination for potential products for magic remedies of unconquerable and almost fatal ailments, plants and herbs have continuously reminded mysterious to him. Plants have been utilised as a natural source of medicinal compounds since thousands of years. Human is using numerous plants and plant derived products to cures and relief from various physical and mental illness. These plants are used in traditional Chinese, Ayurveda, Siddha, Unani and Tibetan medicines. Ancient literature such as Rigveda, Yajurveda, Atharvaveda, CharakSamhita and

SushrutSamhita also describes the use of plants for the treatment of various health problems. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. 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. [1].

The liver is a vital organ of involved in the metabolism of nutrients such as carbohydrates, proteins and lipids and excretion of waste metabolites and detoxification of the exogenous and endogenous challenges like xenobiotics, drugs, viral infections and chronic alcoholism. A liver disease is a worldwide problem; conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. Herbal drugs have gained importance and popularity in recent years is numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practice as well as traditional system of medicine in India. Many naturally occurring products have been reported to contain large amount of antioxidant other than vitamin C, E and carotenoid. These antioxidant play a vital role in delaying, intercepting or preventing oxidative reactions, catalyse by free radical [2].

In this present study I have selected a plant , which has shown good hepatoprotective as well as antioxidant properties. Undoubtedly , the plant kingdom still hold many species of plant containing substances of medicinal value, which have yet to be discovered ; large numbers of plants are constantly being screened for their possible pharmacological value particularly for their hepatoprotective properties.

Free radicals

Since humans or human ancestors first evolved, adestructive class of chemical agents has assailed thehuman body. They are called “free radicals”, though theyare also termed “reactive oxygen species” andabbreviated to “ROS”. They assailed even our preprimateancestors. They assailed the dinosaurs and allother life forms that exist in the fossil record. Even thesimplest single-celled organisms that have an oxidativemetabolismare and always have been assailed by thesesame free radicals. The free radicals come from(3) oxygenand highly oxygenated molecules.

Free radicals are atoms or molecules containing unpaired electrons. Electrons normally exist in pairs in specific orbitals in atoms or molecules. Free radicals, which contain only a single electron in such any orbital, are usually unstable toward losing or picking up an extra electron, so that all electrons in the atom or molecule will be paired. Free radicals can be positively charged, negatively charged, or neutral. The presence of an unpaired electron in an atom or molecule provides great reactivity, thus shortening its half-(4) life.

Free radicals are commonly generated via NADPH cytochrome P-450 reductase or other flavin containing reductases,although cytochrome P-450 itself may involved ,as is the case in the reduction of carbon tetrachloride to form radicals.CCl3 and .CCl2O2.Many radical can participate in recycling reaction, resulting in a sustained level of free radicle in(5)the cell, result in depletion of reduced cofactor and hypoxia

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.

Superoxide ion (or reactive oxygen species)

It is an oxygen molecule with an extra electron. This free radical can cause damage to mitochondria, DNA and other molecules. Our body can neutralize superoxide ions by producing superoxide dismutase.

Hydroxyl radical

It is formed by the reduction of an oxygen molecule in the electron transport chain. It is a neutral (not charged) form of the hydroxide ion. Hydroxyl radicals are highly reactive and form an important part of radical biochemistry. Unlike superoxide the hydroxyl radical cannot be eliminated by an enzymatic reaction. Is has a very short half- life and will only react with molecules its vicinity. Because of its high reactivity it will damage most organic molecules such as carbohydrates, DNA, lipids and proteins.

Singlet oxygen

Singlet oxygen is formed by our immune system. Singlet oxygen causes oxidation of LDL cholesterol.

Hydrogen peroxide

It is not a free radical but it is involved in the production of many reactive oxygen species. Hydrogen peroxide is

a by-product of oxygen metabolism and is neutralized by peroxidases .

Sometimes reactive nitrogen atoms are involved and these free radicals grouped under Reactive Nitrogen Species (RNS). Nitric acid is the most important RNS. Some transitional metals, such as iron and copper, have many numbers of unpaired electrons and can also act as free radicals. These metals do not have that strong electron affinity but can [6] easily accept and donate electrons.

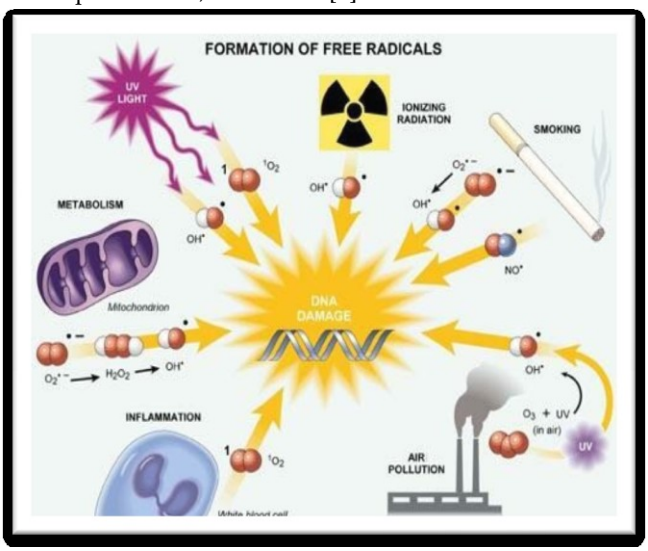
Generation of free radical

Many chemical compounds can be converted into free radicalforms. The latter species are usually quite reactive and short lived because they have a single unpaired electron in theirouter orbital. Recently, evidence has accumulated to indicatethat free radical intermediates may be important in the toxicityof a large number of substances. Free radicals can beformed by several mechanisms, including one-electron oxidation,one-electron reduction, homolytic cleavage of a C-Hbond, and in some cases by two-electron oxidation/reductionreactions. Enzyme-catalyzed oxidation also provides a mechanism by [7] which free radical intermediates can be formed.

Oxidative stress may be defined as an imbalance between pro-oxidant and antioxidant agents, in favour of the former, this imbalance may be due to an excess of pro- oxidant agents, a deficiency of antioxidant agents or both factors simultaneously. The origin of oxidative stress is an alteration of the redox status in cells, leading to a cellular response to counteract the oxidising action.

Pro-oxidant agents are all those that can directly or indirectly oxidise molecules. The most important pro-oxidant agents in biological systems are those derived from oxygen, more commonly known as reactive oxygen species (ROS), although there are also reactive species derived from nitrogen (RNS) or sulphur (RSS). Some of these molecules exhibit great reactivity, such as hydroxyl radicals (HO.), and others present mild reactivity. The biological importance of the latter relies on their capacity to be easily transformed into the hydroxyl radical, especially in the presence of iron, as in the case of superoxide radicals (O2) or hydrogen peroxide (H2O2).

The production of these reactive species occurs continuously in the organism; this production may be endogenous or exogenous. Some of these reactive species are generated as "chemical accidents", i.e. undesired secondary reactions between biomolecules or alternatively in the detoxification of xenobiotic. Other reactive species, however, are generated in vivo for a specific aim such as in the case of activated phagocytes which produce O2, and H2O2 [8].



Free radical formation

Steps involving free radical generation

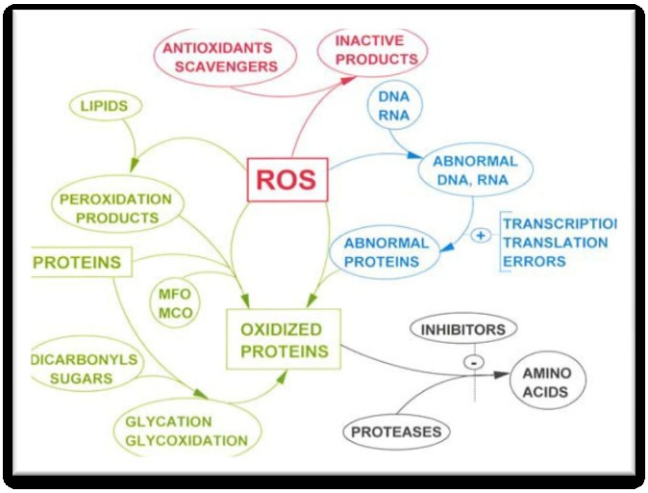
In chemistry, free radicals take part in radical addition and radical substitution as reactive intermediates. Chain reactions involving free radicals can usually be divided into three distinct processes: initiation, propagation, and termination. Initiation reactions are those, which result in a net increase in the number of free radicals. They may involve the formation of free radicals from stable species or they may involve reactions of free radicals with stable species to form more free radicals. Propagation reactions involve free radicals in which the total number of free radicals remains the same. Termination reactions are those reactions resulting in a net decrease in the number of free radicals. Typically two free radicals combine to form a more stable species, for example: $2Cl\cdot \rightarrow Cl2$

The formation of radicals may involve breaking of covalent bonds homolytically, a process that requires significant amounts of energy. The bond energy between two covalently bonded atoms is affected by the structure of the molecule. Homolytic bond cleavage most often happens between two atoms of similar electronegativity. However, propagation is a very exothermic reaction. Radicals may also be formed by single electron oxidation or reduction of an atom or molecule. An example is the production of superoxide [7] by the electron transport chain.

Reactive oxygen species (ROS)

Reactive oxygen species (ROS) are very small molecules and are highly reactive due to the presence of unpaired

valence shell electrons. ROS is formed as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling. However, during times of environmental stress ROS levels can increase dramatically, which can result in significant damage to cell structures. Platelets involved in wound repair and blood homeostasis release ROS to recruit additional platelets to sites of injury. Generally, harmful effects of reactive oxygen species on the cell are most often like - Damage of DNA, oxidations of polydesaturated fatty acids in lipids, oxidations of amino acids in proteins, oxidatively inactivates specific enzymes by oxidation of co-factors. The effect of ROS can be simply explained by the following fig.no:2 [9].



Effects of ROS

Antioxidant

Antioxidants are an inhibitor of the process of oxidation, even at relatively small concentrationand thus have diverse physiological role in the body. Antioxidant constituents of the plantmaterial act as radical scavengers, and helps in converting the radicals to less reactive species. Avariety of free radical scavenging antioxidants is found in dietary sources like fruits, vegetablesand tea, etc.Antioxidants that have traditionally been used to inhibit oxidation in foods also quench dreadedfree radicals and stop oxidation chains *in-vivo*, so they have become viewed by many as nature'sanswer to environmental and physiological stress, aging, atherosclerosis, and cancer. Thenutraceutical trend towards doubling the impact of natural antioxidants that stabilize food andmaximize health impact presents distinct challenges in evaluating antioxidant activity of purifiedindividual [26] compounds, mixed extracts, and endogenous food matrices and optimizingapplications. Broadly the possible mechanisms by which antioxidants may protect against ROS toxicity are Prevention of ROS formation 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 Facilitating the repair of damage caused by ROS Providing(e.g as a cofactor by acting to maintain a suitable redox status) a favourable [27] environment for the effective function of other antioxidant In human body, a complex combination of enzymatic and no enzymatic functions to minimize the stress induced by ROS. These antioxidants can be classified as: Endogenous antioxidant –those which are physiological in origin Exogenous antioxidant- those which cannot be produced by the human body but may [28,29] protect against pro-oxidant forces when administered as supplement

Exogenous antioxidants

For the effective protection against oxidative insults that we encounter in our daily lives regular consumption of at least some antioxidants in the diet or as supplements, appears to be very crucial. Among the exogenous antioxidantsvitC&vitE have been recognised to be especially important and deficiency of these may leads to a number of pathophysiological consequences.

Vitamin C and Citrus Bioflavonoids Vitamin C (ascorbic acid) exerts an antioxidant effect by undergoing oxidation to dehydroascorbic acid and then being regenerated. Because ascorbic acid is being constantly regenerated there is always fresh ascorbic acid available to continue the work of oxygen quenching mainly O_2 ,OH,and various lipid peroxides. The deficiency disease associated with Vitamin C is scurvy. Many of its symptoms reflect difficulty in forming new good quality connective tissues. However, it has been found consistently that Vitamin C acts best in the present of plant bioflavonoids and as a result Vitamin C is often mixed with citrus bioflavonoidspriorto being formed into supplement products. However special attention should be given in the presence of Fe^{3+} or Cu^{2+} excess. Vit C may acts as a strong pro-oxidant and may actually induce lipid peroxidation and oxidative modification of oxidative of genomic structure. Under such condition Vit C may reduce Fe^{3+} to Fe^{2+} which in turn facilitate the generation of OH [37].

Vitamin E

The principal action of Vitamin E is now recognised to be the protection of the phospholipids of the cell

membranes from free radical attack. This includes not only the outer cell membrane but also the much larger area of the internal cell membranes. Vitamin E is only one of several antioxidant nutrients within the cell, but the special connection between Vitamin E and membranes is assured by the fact that Vitamin E is both fat soluble and hydrophobic and that it also readily finds a location within the membranes between the assembled phospholipid molecules there. Vitamin E is therefore the antioxidant that is in situ within the membranes ready to deal with free radicals that arise within that exact location. Vitamin E appears a key factor in our overall antioxidant defence, but also to be [38] especially significant in the nervous system .

Beta-Carotene

Beta-carotene is at the same time a Vitamin precursor thatthe body uses to make vitamin A, a carotenoid and aphytonutrient. It greatlyenhances the immune system. It is a powerful antioxidant andfree radical scavenger. Beta-carotene is the most efficientneutralizer of singlet oxygen, which has particularly high energy,and is one of the most destructive ROS molecules.

Alpha-carotene

Although, among the carotenoids, beta-carotene is afocus of attention for the supplement industry, mostresearch studies show alpha-carotene to be more potentas an antioxidant. Outstandingly, alpha-carotene wasfound to be ten times more potent as an anti-canceragent than beta-carotene and38% more potent as an antioxidant than beta- carotene. It seems wise, therefore, to include thealpha form into the best quality antioxidant formulae.

Lycopene & Lutein

Lycopene is another carotenoid antioxidant and is evenmore powerful than alpha- carotene. Lycopene and lutein in small doses may potentially preventcolon carcinogenesis. Lutein was shown to be important inprevention of lung cancer. The carotenoid antioxidants have also been found to beespecially important in the natural protection of the eyeagainst macular degeneration. Lutein, zeaxanthinandlycopene have been found to bestow a good level ofprotection.

Minerals

The minerals required for forming the superoxide dismutaseenzymes do not have to be part of the antioxidant mix. All thatis required is that good to generous dietary intakes of them bemaintained either by dietary care or by supplements. In fact,iron and copper may not need to be given and should neverbe given in excess. Excesses of either of these have beenreported to actually increase free radical generation by causingan imbalance between these minerals and Vitamin C.Magnesium is a special case. It could be included in anantioxidant strategy either alongside the antioxidants orotherwise so long as the [39] subject's intake of it is fully adequate. .

Role of herbal therapy in hepatoprotective activity

Liver diseases have become one of the major causes of morbidity and mortality in man and animals all over globe and hepatotoxicity due to drugs appears to be the most common contributing factor.Liver cell injury caused by various toxicants such as certain chemotherapeutic agents, carbon tetrachlo-ride, thioacetamide etc., chronic alcohol consumption and microbes. Among the many diseases that can affect the liver the most common is 'viral hepatitis' (Inflammation of liver caused by viral infection)[55]. Hepatitis can be caused by drugs, viruses, bacteria, mushrooms, parasites like amoebas or giardiasis. The Indian Traditional Medicine like Ayurveda, Siddha and Unani are predominantly based on the use of plant materials. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness.[56]. The association of medical plants with other plants in their habitat also influences their medicinal values in some cases. One of the important and well-documented uses of plant-products is their use as hepatoprotective agents. Hence, there is an ever in-creasing need for safe hepatoprotective agent .

Hepatoprotective herbs A large number of plants and formulations have been claimed to have hepatoprotective activity. Nearly 160 phytoconstituents from 101 plants belonging to 55 families have been claimed to possess liver protecting activity. In India, more than 87 plants are used in 33 patented and proprietary mul-ti-ingredient plant formulations[58]. Liver protective plants contain a variety of chemical constituents like phenols, Coumarins, Lignans, essential oil, monoterpenes, carotinoids, glycosides, flavonoids, organic acids, lipids, alkaloids and xanthenes. Therefore a large number of plants and formulations have been claimed to have hepatoprotectiveactivity.Inspite of the tremendous advances made, no significant and safe hepatoprotective agents is available in modern therapeutics, so the development of plant based hepatoprotective drugs has been given importance in the global market.

Solvent extraction (hot percolation method)

Preparation of petroleum ether, chloroform and ethanolic extracts of cnidosolusphyllacanthus.

Equipment Used

Soxhlet apparatus

Materials used

Petroleum ether Chloroform Ethanol

Shade dried Cnidoscolusphyllacanthus

Method

The Cnidoscolusphyllacanthus plant was collected and identified. The leaf was cut down into small pieces, shade dried and powdered to get moderately coarse powder, which is sieved under mesh. About 500gm of dry powder was extracted with petroleum ether, chloroform and ethanol at 60-70°c by hot continuous percolation using soxhlet apparatus. The extraction was continued for 72hrs. the petroleum ether, chloroform and ethanolic extract was filtered and concentrated to a dry mass by using vaccum distillation the petroleum ether extract(4gms) was obtained as dark green residue. The chloroform extract (5gms) was obtained as dark brown residue. The ethanolic extract (7.2gms) was obtained as dark brown residue.

Experimental models

For the study of hepatoprotective and antioxidant activity an animal model was added that 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. The drug tested should be administered orally

Drug dosage approximate the optimum therapeutic range for human, scaled the test animal weight.

Laboratory animal models

Induction of hepato toxicity and free radicals in animal model experimental pharmacological studies in animal liver

To investigate and evaluate hepatoprotective substance, it is customary to subject animals to a range of toxic agents. 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. Excessive alcohol intake results indisequilibriumin iron homeostasis and iron overload which further enhance oxidative stress by catalyzing the formation of 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 . 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.

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. Which in turn is converted to Peroxy 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 leads to cellular damage as a result of lipid per oxidation. 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 polymorphonuclear 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.

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. 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.

Materials and methods

ANIMALS : Albino wistar rats (180-220gm)

CHEMICALS : D- galactosamine

: Vitamin E

:Ethanolic extract of Cnidoscolusphyllanthus

Selection And Acclimitization Of Animals

Albino rats of wistar strains weighing between 180-220gm were produced from animal experimental laboratory, and used throughout the study. They were housed in micronylon boxes in a control environment(temp 25+-2^{0}c) and 12 hrs dark\ light cycle with standard laboratory diet and water ad libitum . The study was conducted after obtaining Institutional Animal Ethical Committee clearance. As per the standard practice, the rats were segregated based on their gender and quarantined for 15 days before the commencement of the experiment. They were fed on healthy diet and maintained in hygiene environment in our animal house.

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 for 21 days.[78]

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 *Cnidoscolusphyllanthus*orally for 21 days.

Group 5 :The treatment control received 400mg/kg of Ethanolic extract of *Cnidoscolusphyllanthus* orally for 21 days.

Preperation of drugs

Ethanolic extract of *Cnidoscolusphyllanthus*was dissolved in 20ml of sterile water and was administered orally at a dose of 200mg/kg and 400mg/kg/rat.

D-Galactosamine was diluted in sterile water and administered I.P at a dose of 25mg/kg/rat.

Vitamin E was diluted in sterile water and administered orally at a dose of 25mg/kg

Methodology

On day 22 after 24 hrs of Galactosamine administration animals in all the groups were humanely sacrificed using Ketamine HCl and 4ml of blood was withdrawn by cardiac puncture and allowed to clot for 30mins at room temperature. The serum was separated by using cooling centrifuge and used for the assay of marker enzymes viz AST, ALT, ALP, TP, TB,GGPT and total albumin. The livers were dissected out immediately, washed with ice- cold saline and 10% homogenates in phosphate buffer solution (PH 7.4) were prepared Liver homogenate was used for the assay of Lipid peroxidation (Lpo) while some fraction of homogenates were centrifuged at 7000rpm for 10 min at 4^0 C using refrigerated centrifuge, and the supernatants were used for the assay of Superoxide dismutase (SOD), catalase(CAT), Glutathione peroxidase(GPx). Some portion of liver from each group was aseptically excused and stored in 10% formalin for histopathologicalstudies(.

Result& discussion

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).

Pretreatment with Ethanolic extract of *Cnidoscolus phyllanthus* (EECP) 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.

Biochemical observation in liver homogenate tissue

In liver homogenate, there was significant decrease in SOD, CAT and GPx levels and increase in LPO levels were observed in animals treated with galactosamine 25mg/kg (group II) as compared to normal control group (Group I). Pretreatment with Ethanolic extract of *Cnidoscolus phyllanthus* (EECP) at a dose of 200mg/kg and 400mg/kg orally for 21 days increase the levels of above indices like SOD,CAT and GPx levels and decrease levels of LPO significantly (P<0.01) in group IV and V. Vitamin-E pretreatment produced significant increase in (P< 0.01) Liver homogenate enzyme such as SOD, CAT, GPx levels and decrease the levels of LPO significantly (P<0.01) in group III. Table no shows the levels of non-enzymatic antioxidants such as reduced glutathione, Vitamin C and Vitamin E in the tissues (liver) of D-galactosamine hepatotoxic and control rats. The levels of non-enzymatic antioxidants in D-galactosamine hepatotoxic rats significantly decreased. EECP both doses administered rats showed significantly increased levels of these non-enzymic antioxidants as compared with untreated hepatotoxic rats.

Histo pathological observations

Histology of liver sections of normal control animals (Group I) showed normal liver architecture with were brought out central vein, were preserved cytoplasm and prominent nucleus and nucleolus (Fig no:8). The liver sections of galactosamine treated animals (Group II) showed hepatic cells with serum toxicity characterized by inflammatory cell collection, scattered inflammation across liver parenchyma, focal necrosis and swelling up of vascular endothelial cells (Fig no:9). Vitamin-E (Group-III) exhibited protection from galactosamine induced changes in the liver (Fig no:10). Ethanolic extract of *Cnidoscolus phyllanthus* (EECP) pretreatment at a dose of 200mg and 400mg/kg (group IV and V) appeared to significantly prevent the galactosamine toxicity as revealed by the hepatic cells with were preserved cytoplasms. EECP pretreatment also caused marked decrease in inflammatory cells (Fig no: 11 and 12).

Discussion

D-galactosamine is a well-established hepatotoxicant that induces a diffuse type of liver injury closely resembling human viral hepatitis[80]. Liver damage induced by D- galactosamine, reflects disturbances of liver cell metabolism, which lead to characteristicchanges in the serum enzyme activities. Elevated serum enzymes are indicative of cellular leakage and loss of functional integrity of the hepatocyte[81].When the liver cell plasma membrane is damaged, a variety of enzymes such as aspartate aminotransferase,alanine aminotransferase, alkaline phosphatase ,total bilurubin and gamma- glutamyl transpeptidase are released into the blood stream. Their estimation in the serum is useful as a quantitative marker of the extent and type of hepatocellular damage. In D-galactosamine induced toxicity, increased activities of aspartate aminotransferase, alanine aminotransferase,alkaline phosphatase,total biluribin and gamma- glutamyl transpeptidase and decrease activities of total protein and total albumin were observed in serum. EECP seems to preserve the structural integrity of the hepatocyte membrane as evidenced from the significant reduction in the activities of these enzymes. The 400mg/kg dose had a better effect than the low dose of EECP (200mg/kg). The higher concentration might have resulted in the production of more by products that would have interfered with the activity. Treatment with EECP significantly decreased these enzyme activities, indicating that EECP has a hepatoprotective effect against a D-galactosamine-induced liver injury. D-galactosamine-induced oxidative damage is generally attributed to the formation of the highly reactive hydroxyl radical (OH·), the stimulator of lipid peroxidation and the source of destruction and damage to the cell membrane[82]. D-galactosamine toxicity enhanced lipid peroxidation and reduced antioxidants were reported in the kidney.[83] The previous studies show that D-galactosamine-induced

rats significantly increased thiobarbituric acid reactive substances, lipid hydroperoxides and conjugated dienes in liver and kidney[84,85]. In the present study, we observed an increase in the levels of thiobarbituric acid reactive substances, lipid hydroperoxides and conjugated dienes in the tissues of D-galactosamine-hepatotoxic rats. Increased lipidperoxidation in various tissues has long been known to cause functional degradation; thus, the degradation of vital tissue leading to complications may be indirectly due to increased oxidative stress. Treatment with EECP and Vitamin-E showed a significant reduction which might be due to the antioxidant ability of these compounds and the consequent reduction in lipid peroxidation. EECP possesses antioxidative and free-radical scavenging effects. Oxidative stress is an imbalance between reactive oxygen species and the antioxidant defense mechanisms of a cell or tissue, which leads to lipid peroxidation, DNA damage, and the inactivation of many enzymes[86]. The enzymatic antioxidant defense system is the natural protector against lipid peroxidation that includes superoxide dismutase, catalase and glutathione peroxidase.Reduced activities of these enzymes in the tissue of D-galactosamine-hepatotoxic rats were observed in our study. Superoxide dismutase protects against the superoxide radical ($O_2^{\cdot-}$), which damages the membrane and its biological structure. Catalaseprimarily decomposes hydrogen peroxide to H_2O at a much faster rate, sharing this function with glutathione peroxidase. Glutathione peroxidase may play an important role in the removal of lipid hydroperoxides. The balance between these enzymes is important for the efficient removal of oxygen radicals from tissues [87]. Therefore, reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and H_2O_2. Significant increases in the activities of these enzymes were observed on EECP administration. The second line of defense consists of the non-enzymic scavengers glutathione, ascorbic acid, and α-tocopherol, which scavenge residual free radicals escaping from decomposition by the antioxidant enzymes. Moreover, enzymic antioxidants are inactivated by the excessive levels of free radicals and hence the presence of non-enzymic antioxidants is presumably essential for the removal of these radicals [88].Glutathione a major non-protein thiol in living organisms plays a central role in coordinating the antioxidant defense process. Glutathione reacts directly with reactive oxygen species and electrophilic metabolites, protects the essential thiol group from oxidation, and serves as a substrate for several enzymes including glutathione peroxidase. The lowered glutathione in D-galactosamine induced rats represents the increased utilization of glutathione as a result of oxidative stress.Perturbation in the redox status of glutathione not only impairs cellular defense against toxic compounds but also results in enhanced oxidative stress and oxidative injury [89]. Apart from glutathione, α-tocopherol and ascorbic acids are important free-radical scavengers which protect cell membrane against toxic agents. Both vitamins C and E have a synergistic action in scavenging oxygen-derived free radicals[90].Vitamin C functions as a free-radical scavenger of oxygen radicals and successfully prevents detectable oxidative damage under all types of oxidative stress. Ascorbic acid appears to trap the peroxyl radical in the aqueous phase with a rate large enough to lipids and dehydroascorbate is produced in this reaction. A thiol cycle converts the dehydroascorbate into ascorbate. The thiol cycle consists of a GSSG/GSH couple[91]. Thus glutathione in blood keeps up the cellular levels of the active form of vitamin C. When there is a reduction in glutathione, the cellular level of ascorbic acid is also lowered. The observed decrease in the levels of α-btocopherol and ascorbic acid in the D-galactosamine rats might be due to an antioxidant defense against increased ROS or due to a decrease in glutathione levels in D-galactosamine-hepatotoxic rats[92]. In this respect, reported that ascorbic acid and α-tocopherol decreased in liver diseases, particularly in D-galactosamine- hepatotoxic rats. Our study observed increase the levels of these antioxidants in EECP and Vitamin-E administered rats. The ability of EECP to enhance the levels of antioxidants along with its antilipid peroxidativeactivity suggests that this compound might be potentially useful in counteracting free-radical-mediated tissue damage caused by hepatotoxicity. Studies on the antioxidative potency of various flavonoids have confirmed the importance of the distribution and quantity of the hydroxyl groups. In general, the antioxidative properties of polyphenols depend on hydroxylation of ring B. The present results corroborate the protective action of EECP in D-galactosamine intoxication of rats, particularly noticeable with the high dose used by us (400 mg/kg body weight). Supplementation with this flavonoid ameliorated thehepatoprotective and antioxidant activity in D-galactosamine-induced hepatitis in rats.

Conclusion

In conclusion, our findings demonstrated that EECP at both doses possesses the patho protective and antioxidant activity, which is evidenced by lowered serum hepatic marker enzyme activities. Among the two dosages tested, 400 mg/kg/body weight showed more promising the patho protective and antioxidant activity, and is comparable to the standard drug Vitamin-E.

References

1. Vijayalakshmi, R., Ranganathan,R. Ethnobotanical Studies on Some Traditional Medicinal Plants in Cuddalore District. *Drug Invention Today*.2011, *3(7)*: 160- 164.
2. Mohamed, S.T.S., Madhusudhana C.C., Ramkanth,S., Rajan, V.S.T., Mahesh,K.K., Gauthaman, K. Hepatoprotective Herbs – A Review.*Int. J. Res. Pharm. Sci.*,2010. *1(1).* 1-5http://www.phytochemicals.info/free-radicals.php
3. Harmon,D., Gerontol,J., 1956,11:298-300
4. Kappus,H.Arch.Toxicology.1987,60:144
5. Harsh, M.Cellinjuary and cellular adaptation. *Text book of pathology* .,2005,*5:* 38-40.
6. Abheri, D. S., Anisur, R. M., and Ghosh,A.K. Free Radicals and Their Role in Different Clinical Conditions: An Overview. *International Journal of Pharma Sciences and Research*,.2010,*1(3)*: 185-192.
7. http://www.uv.es/frag/oxidative_stress.htm
8. Marian,V.,Dieter, L., Jan, M.,Mark, T.D., Cronin. Free radicals and antioxidants in normal physiological functions and human disease.*The International Journal of Biochemistry & Cell Biology*.,2007,*39:* 44–84.
9. Dipak,D.,Ranjith, K.,Banerjee.Reactive oxygen species :Oxydative Damage and Pathogenesis.*Current science.*1999,*3(5)*:77.
10. Hellsten,Y.The role of Xanthine oxidase in exercise.*Elesevier Science publisher,*1994:221-234.
11. Edmonds,S.E.,Blake,D.R.Hypoxia,oxidative stress and exercise in rheumatoid arthritis.*Elesevier science publishers B.V.,Amstradam*,1994:389-422.
12. Lilus,E.M.,Manila,P.Photon emission of phagocytes in relation to sterss and disease.*Experientia*.,1992,*48*:1082-1091.
13. Otta,D.M.E.,Lindstrom.Seppa,p.,Sen,C.K.Cytoc hrome P_{450} dependent enzyme and oxidant-mediated responses in rainbow trout exposed to condaminated sediments. *Ecotoxic Environ Safe.,* 1994,*27*:265-280.
14. Karuzine ,Archkov,A.L.The oxidative inactivation of cytochrome P_{450} in monooxigenasereaction.*Free Rad Biol Med*.,1994,*16*:74-97.
15. Beckman,J.S.,Crow,J.P.Pathological implication of nitric oxide ,superoxide and peroxynitrite formation.BiochemSoc Trans.,1993,21:330-334.
16. Hogg,N.,Darley.,UsmarV.M.,Wilson ,M.T.,Moncada,S.The oxidation of alpha
17. tocopherol in human low density lipoprotien by the simultanious generation of super oxide and nitric oxide.FEBS Lett.1993,326:199-203.
18. Jenkins,R.R.,Halliwell,B.Metal binding agents possible role in exercise. In exercise and oxygen toxicity. Elsevier science publishers., Amstradam,B.V.,1994:59-76.
19. Buettner,G.R.Ascorbate autoxidation in the presence of is iron and copper chelates.
20. Free Radical Res Commun.,1986,1:349-353.
21. Sies,H.Biochemistry of the peroxysomes in the liver cells. AngewChemInt Ed Engl.1974,13:706-718.
22. Kukreja,R.C.,Kontos,H.A.,Hess,M.L.,Ellis,E.F. PGH synthase and lipoxigenase generate superoxide in the presence of NADH of NADPH.Circ Res,1986,59:612- 619.
23. Arduini,A.,Eddy,L.,Hochestein,P. Detection of ferryl myoglobin in the isolated ischemic rat heart.Free Red Biol Med.,1990,9:511-513.
24. Uday, B., Dipak, D.,Ranajit, K. B. Reactive oxygen species: Oxidative damage And pathogenesis.CURRENT SCIENCE,1999,77(5):658-666.
25. Carwin,H.The rational design,mechanistic study and therapeutic application of chemical compounds.Comprehensive medicinal chemistry,1990,2(3):734-738.
26. Gutteridge,J.M.C.,Halliwell,B.Oxygen and suphur radicals in chemistry and Med.1986,22:47.
27. MARK ,P,.Antioxidants.CLINICAL NUTRITION INSIGHTS.,1998,1:1-4.
28. Ohno,H.S.,Fujii,K.,Yamashita,J.H.,Kizaki,T.,Oh-ishi,S.,Tanaguchi,N.Superoxide dismutases in exercise and disease.Elesivier science publishers,1994.,127-162.

29. Sulekha, M., Satish, Y.,Sunita, Y.,Rajesh,K.N.Antioxidants: A Review. Journal of Chemical and Pharmaceutical Research,2009., 1 (1):102-104.
30. Shalini,S.International Journal of Physiotherapy.2012.2(1):7-15.
31. Hercılia ,M. H.,Jofre ,J.S. F., Rui ,C.,Irineu, T. V., Bonfim, A.S., Junior.International Journal of Physiotherapy.2006.,3(2):144-153.
32. Halliwel,B.In oxygen radicles and disease process.Hardwardaccademic publisher.,Netherlands,1997:1-14.
33. Eqbal M.A.D.,Aminah ,A.,Halimah,A.S. Natural Antioxidants, Lipid Profile, Lipid Peroxidation, Antioxidant Enzymes of Different Vegetable Oils., Advance Journal of Food Science and Technology., 2011.,3(4): 308-316.
34. Ralf, D. Metabolism and functions of glutathione inbrain.,Progress in Neurobiology.,2000.,62 :649-671.
35. Chih ,c.L., Wu-hsiung, H.,Augusta, A., Leslie, M. K., Ted ,H. C. Expression of glutathione peroxidase and catalase in copper-deficient rat liver and heart., Nutritional Biochemistry.,1995.,6:256-262.
36. Anna P., Giorgio ,F., Enrico, B., Fiorella ,P. Analysis of glutathione: implication in redox and detoxification. Clinica ChimicaActa 2003,333:19–39.
37. Noeleen, B. L., Brendan O'C., Ciarán, Ó.'Fi.,Mary.,J .O The phylogeny of the mammalian heme peroxidases and the evolution of their diverse functions.BMCEvol Biol. 2008.,8: 101.
38. Dominique ,P., Pascal, C., Fernand, L., Catherine. R. Antioxidant activity of some ascorbic and cinnamic acids derivatives. IL Farmaco.,1998.,53 :85-88
39. Estany S., J.R. Palacio, J.R.,Barnadas, R.,Sabes, miborra,A., Mart´ınez ,P. Antioxidant activity of N-acetylcysteine, flavonoids and -tocopherol on endometrial cells in culture.Journal of Reproductive Immunology,2007.,75: 1–10.
40. Lawrence,G.,Plaskett,B,A., Antioxidants our indispensible protection. Biomedical information services.,2003:1-11.
41. Pascoe,G.A.,Redd,D.J.Cell calcium,vitaminE,andthol redox system in cytotoxicity.,
42. Free radical Bio Med.1989,6:209-224.
43. Sen,C.K,Agarwal,Y.P.,Hanninen,O.Changes in intracellular calcium and membrane disentegration following exposure of myogenic cells to oxidative stress.XXXII Congress of the international union of physiological Sciences.1993.,214:345-356.
44. Orolv,S.N.,Sen,C.K.,Kolosova,I.A.Evidence for the involvement of G protien in the modulation of sodium and calcium cluxes in myogenic L6 and arotic smooth musclecells.BiochemBiophys Res Commun.1993,191:802-810.