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Antioxidant and Hepatoprotective activity of Cnidoscolus Phyllacanthus leaves against Alactosamine induced oxidative

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Abstract: *In a sense, there has always magic in plants, an unknown Genie, mysterious and omnipotent, anal 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 utilized 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, Charak Samhita and Sushrut Samhita also describe the use of plants for the treatment of various health problems. In recent times, focus on plant research has increase dall 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, hemolytic activity, larvicidal activity and anti-inflammatory activity etc.*

Keywords: *Cnidoscolus Phyllacanthus, D-Galactosamine, ECP, Silymarin, Hepatotoxicity.*

1. Introduction

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 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 antioxidants play a vital role in delaying, intercepting or preventing oxidative reactions, catalyze 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 holds many species of plant containing substances of medicinal value, which have yet to be discovered; large numbers of plants are constantly being screened for the impossible pharmacological value particularly for the hepatoprotective properties.

Since humans or human ancestors first evolved, a destructive class of chemical agents has assailed the human body. They are called "free radicals", though they are also termed "reactive oxygen species" and abbreviated to "ROS". They assailed even our pre primate ancestors. They assailed the dinosaurs and all other life forms that exist in the fossil record. Even the simplest single-celled organisms that have anoxidative metabolism are and always have been

assailed by these same free radicals. The free radicals come from oxygen and highly oxygenated molecules.(3)
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 life.(4)
Free radicals are commonly generated via NADPH cytochrome P-450 reductase or other flavin containing reductases, although cytochromeP-450itselfmayinvolved, as is the case in the reduction of carbon tetrachloride to form radicals. CCl3and.CCl2O2.Many radical can participate in recycling reaction, resulting in a sustained level of free radicle in the cell, result in depletion of reduced cofactor and hypoxia.(5)

2. Experimental design

2.1 Materials and methods

The materials required for the present work were procured from diverse sources. The Cnidoscolus phyllacanthus 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 60-70oc 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 vacuum 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.

2.2 Selection and Acclimitization of Animals

Albino rats of Wistar strains weighing between 180-220gmwereproducedfrom animal experimental laboratory, and used throughout the study. They were housed in micronyl on boxes in a control environment (temp 25+-20c) and12 hrs dark\ light cycle with standard laboratory diet and water adlib tum. The study was conducted after obtaining Institutional Animal Ethical Committee clearance. As pert he standard practice, the rats were segregated based on their gender and quarantined for 15 days before the commencement of the experiment. They were felon healthy diet and maintained in hygiene environment in our animal house.

2.3 Treatment Protocol

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

- Group1: Served as normal control and receive normal diet and water.
- Group2: Toxic control received 25mg /kg of D-galactosaminethroughI.Pfor21days.(78)
- Group3:Standardcontrolreceived25mg/kgofvitamiⁿEorallyfor21days.
- Group4: The treatment control received 200mg/kg of Ethanolic extract of Cnidoscolus Chayamansa for 21 days.
- Group5: The treatmentcontrolreceived400mg/ kg of Ethanolic extract of Cnidoscolus Chayamansa orally for 21 days.

2.4 Preparation of Drugs

- Ethanolic extract of Cnidoscolus Chayamansa was dissolved in 20ml of sterile water and was administered orally at a dose of 200mg/kg and400mg/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

3. 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 40 Cussing 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 histopathological studies (79).

3.1 Statistical Analysis

The Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Newmann Keul's multiple range tests. The values are represented as Mean + SEM. Probability value at P <0.01 was considered as statistically significant.

4. Result

4.1 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(GroupI).
Pretreatment with Ethanolic extract of Cnidoscolus Chayamansa (EECP) at a dose200mg and 400mg /kg ,orally for 21days decreased the levels of above indices like AST, ALT
,ALP,TB,GGTPandincreasedlevelsofTPandTAsignificantl y(P<0.01)ingroup 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.

4.2 Bio-Chemical 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 Chayamansa (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.

4.3 Histopathological 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 cell (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 cyto plasms. EECP pretreatment also caused marked decrease in inflammatory cells (Fig no: 11 and12).

5. 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 characteristic changes 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 bilirubin 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 heato cellular damage.
In D-galactosamine induced toxicity, increased activities of aspartate aminotransferase, alanine amino transferase, 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. The400mg/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 thio barbituric 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 thio barbituric acid reactive substances, lipid hydroperoxides and conjugated dienes in the tissues of D-galactosamine-hepatotoxic rats. Increased lipid peroxidation 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.

HISTOPATHOLOGICAL STUDIES OF LIVER TISSUE

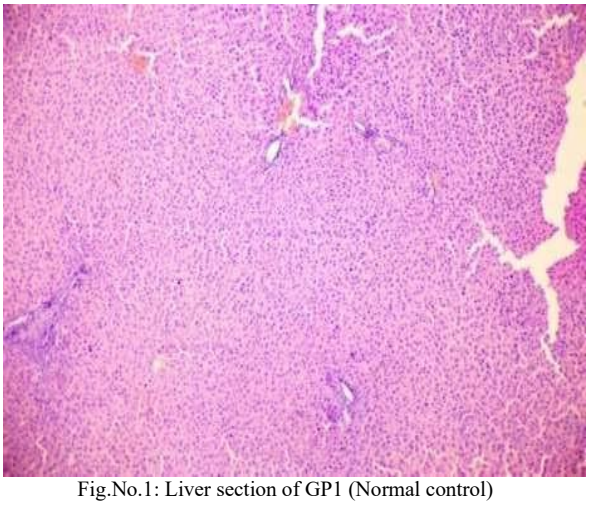

Fig.No.1: Liver section of GP1 (Normal control)

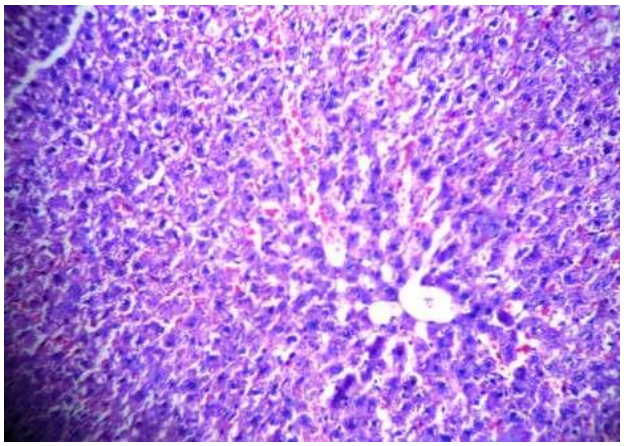

Fig.No.2: Liver section of GP2(toxic control)

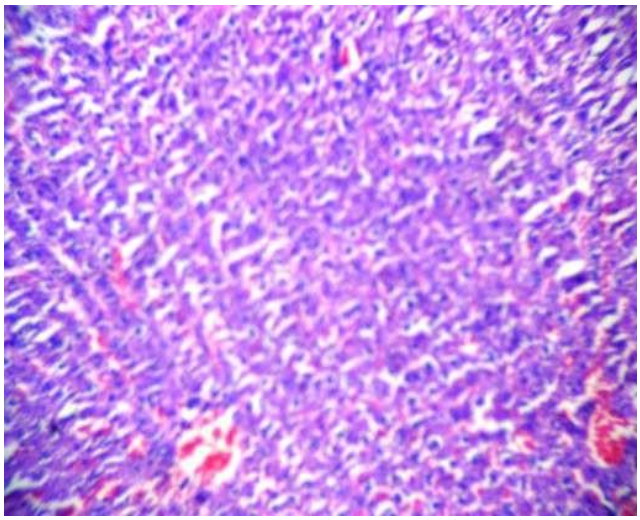

Fig.no.3: Liver section of GP3 (standard control)

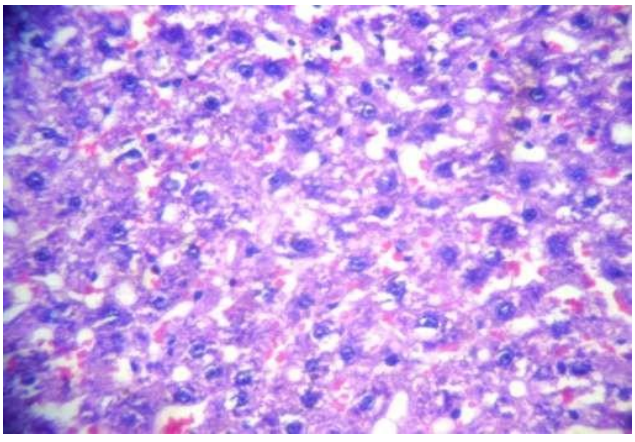

Fig.no.4: Liver section of GP4(Cnidoscolus Chayamansa200 mg/kg/rat)

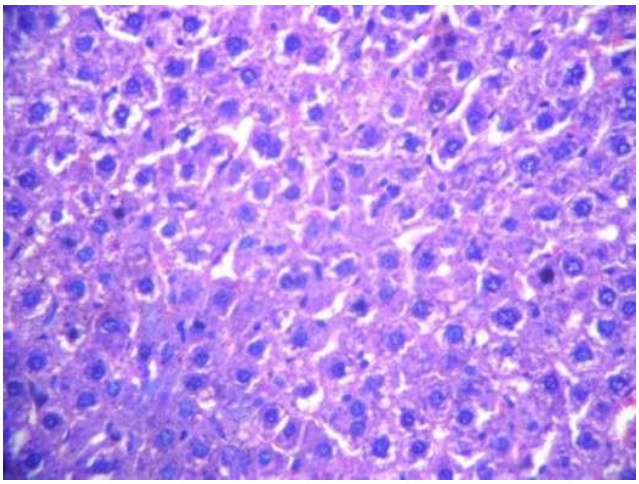

Fig.no.5: Liver section of GP5(Cnidoscolus Chayamansa400 mg/kg/rat)

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