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Research Article

Evaluation of Cnidoscolus Chayamansa for Antioxidant and Hepatoprotective Activity in Rats

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Abstract

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. Some selected medicinal plants have a crucial role in protecting liver from chemical injuries using in vivo and in vitro models. Herbal drugs in Sudan play a vital role in the primary health care since 90% of Sudanese people use medicinal plants to treat different diseases including liver disorders, due to high cost and inadequacy of conventional drugs used in the treatment of liver diseases. The present study was undertaken to determine the Hepatoprotective and antioxidant activity and also the evaluation of the various biochemical parameters of *Cnidoscolus Chayamansa*. 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 60-70ºc by hot continuous percolation using soxhlet apparatus. EECP at both doses possesses hepatoprotective 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 hepatoprotective and antioxidant activity, and is comparable to the standard drug Vitamin-E.

Introduction

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, CharakSamhita and Sushrut Samhita 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.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. 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). Hepatitis can be caused by drugs, viruses, bacteria, mushrooms, parasites like amoebas or giardiasis. The Indian Tradi-tional 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. Herbal drugs have become increasingly popular and their use is widespread. Licensing Regulations and pharmacovigilance regarding herbal products are still incomplete and clearcut proof of their efficacy in liver diseases is sparse. Nevertheless , a number herbals show promising activity including silymarin for antifibrotic treatment , phyllantusamarusin chronic hepatitis B, Glycyrrhizin to treat chronic viral hepatitis ,and a number of herbal combinations fromchina and Japan that deserve testing in appropriate studies. The focus of the present work is to elucidate Ayurvedic concept of liver cirrhosis, using herbal remedies.

In traditional medicine , natural, crude phytoextracts considered as alternative medicines, because some natural constituents present in them counter balance.The side- effects ofsynthetic medicines. It is therefore obvious that the therapeutic potential and risk efficacy or traditional medicinal plants is based on the direct assessment of Phytoextracts as well as effects of their purified compounds. Plants have played a significant role in maintaining human health and improving the Quality of human life for thousands of years and have served human wellasvaluable components of medicine , seasoning, beverages , cosmetics and dyes. Herbal medicines arebased on the premise that plant contains natural substances that can promote health and alleviateillness. In recent times ,focus on plants research has increased all over the world and a large body ofevidence has collected to show immense potential of medicinal plants used in various traditional systems. Today ,we are witnessing a great deal

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of public interest in the use of herbal remedies. Many Western drugs had their origin in plant extracts. There are many herbs ,which are predominantly Used to treat cardiovascular problems, cancer, central nervous system, digestive and metabolic disorders. Give their potential to produce significant therapeutic effect, they can be useful as drug or supplement in the treatment , management of various diseases. Herbal drugs or medicinal plants , their extracts and isolated compounds have demonstrated spectrum of biological activities such have been used and continued to be used as medicine in folk fore or food supplement for various disorders. Ethanopharmacological studies on such herb, medicinally important plants continue to interest investigators throughout the world.

Materials and Methods

Experimental Work

Collection and preparation of plant material

The plant leaves of *Cnidoscolus Chayamansa* were collected from available graphical sources. The plant drugs were identified, collected and stored for further use. The collected *Cnidoscolus Chayamansa* plant was washed with tap water. The leaves were cut down into small pieces, shade dried at room temperature for 1 month to avoid direct loss of phytoconstituents from sunlight. The shade dried materials were powdered to get moderately coarse powder using pulverizer, which is sieved under mesh size 80. It was then homogenized to fine powder and stored in air-tight container for further analysis.

Preparation of Plant extracts for phytochemical screening:

The plant leaves of powder of the *Cnidoscolus Chayamansa* was extracted with ethanol,chloroform and petroleum ether. About 500gm of dry powder was extracted with petroleum ether, chloroform and ethanol at 60-70^{o}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 was obtained as dark green residue. The chloroform extract was obtained as dark brown residue. The ethanolic extract was obtained as dark brown residue.

Induction of Hepato-toxicity and free radicals in animal model:

Selection And Acclimitization Of Animals

Albino rats of wistar strains weighing between 180-220gm were 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

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. Group 3: Standard control received 25mg/kg of vitamin E orally for 21 days. Group 4: The treatment control received200mg/kg of Ethanolic extract of Cnidoscolus Chayamansa for 21 days.Group 5 :The treatment control received 400mg/kg of Ethanolic extract of Cnidoscolus Chayamansa orally for 21 days.

Preperation of Drugs

Ethanolic extract of *Cnidoscolus Chayamansa* 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 of25mg/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 icecold 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 histopathological studies.

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 $\pm$ SEM. Probability value at P <0.01 was considered as statistically significant.

Results and Discussions

There is a 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 Chayamansa (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. There is no changes in 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.

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: 1). 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:2).Vitamin-E (Group-III) exhibited protection from galactosamine induced changes in the liver (Fig no:3). 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: 4 and 5).

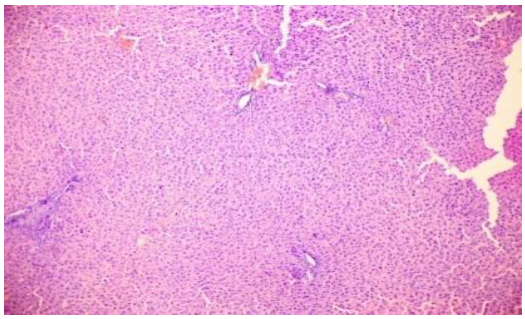

Fig No. 1 : Liver Section (normal /Control

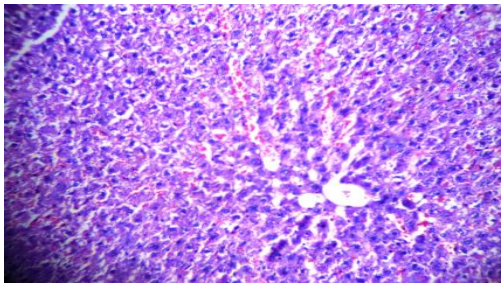

Fig No. 2: Liver section of GP_2 (toxic control

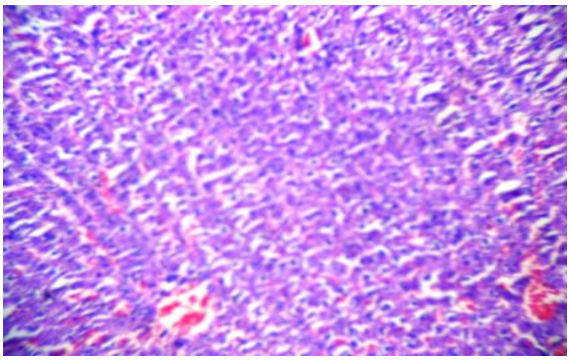

Fig No. 3: Liver section of GP_3 (standard control)

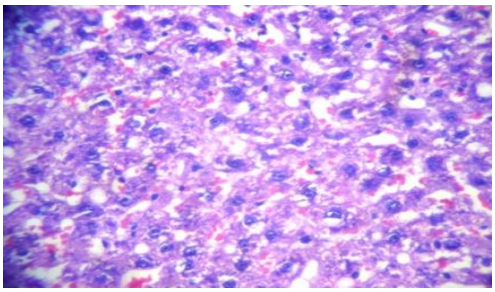

Fig No. 4: Liver section of GP_4 (*Cnidoscolus Chayamansa* 200 mg/kg/rat)

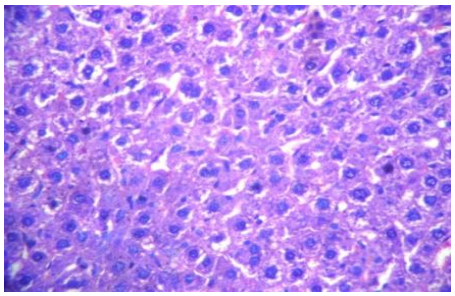

Fig No. 5: Liver section of GP_5 (*Cnidoscolus Chayamansa* 400 mg/kg/rat)

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