International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 3 May-June 2022, pp: 1272-1278 www.ijprajournal.com ISSN: 2456-4494

Antioxidant and HepatoprotectivePotentiality of Cidoscolus Phyllacanthus and compared with marketed product

Brahmjeet, Sailesh kumar Ghatuary, Kalpana Prajapati, Satkar Prasad
RKDF School of pharmaceutical Science, Bhopal.

Submitted: 26-05-2022 Revised: 03-06-2022 Accepted: 06-06-2022

ABSTRACT:

KEYWORDS: Ayurveda , hepatoprotective, radicals, Homolytic bond, metabolism, biochemical, pharmacological value .

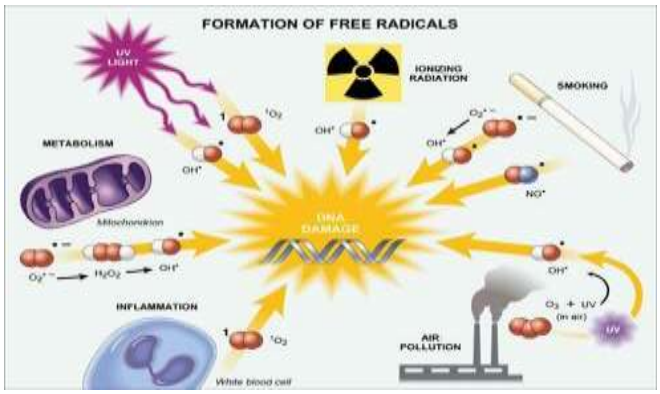
I. INTRODUCTION

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. 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.

GENERATION OF FREE RADICAL

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 (O_2) or hydrogen peroxide (H_2O_2).

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
(8) phagocytes which produce O_2, and H_2O_2.



STEPS INVOLVING FREE RADICAL GENERATION

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 3 May-June 2022, pp: 1272-1278 www.ijprajournal.com ISSN: 2456-4494

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: $2Cl\cdot \rightarrow Cl_2$

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. 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.

SOURCES OF REACTIVE FREE RADICALS

a) Mitochondrial Cytochrome Oxidases

The free radicals are formed continuously as normal by products of cellular metabolism under normal conditions, about 95% of molecular oxygen in biological system undergoes controlled reduction through the addition of four electrons in the mitochondrial cytochrome oxidase system to form water.

b) Purine Metabolism

The enzyme xanthine dehydrogenase (XDH) mainly located in the vessel walls of most tissue including cardiac and skeletal muscle, catalyses the oxidation of hypoxanthine to xanthine and xanthine to uric acid.

c) Phagocytes

As weapons for pathogen destruction and immune protection ROS have been put to good use by phagocytes NADPH oxidase located in the plasma membrane of neutrophils produce superoxide's on purpose following spontaneous dismutation superoxide's generated .

d) Drug metabolism

Microsomal and nuclear membrane electron transport system, mainly involved in drug metabolism (via cytochrome P_{450} and B_5 systems) also host ROS production NADPH oxidation both in presence and absence of mixed function oxidase substrates contribute to ROS (O_2 and H_2O_2) formation as well. Mechanism of cytochrome P450 driven reactions involves the formation of oxy and subsequently peroxyintermediates .

e) Nitric Oxide synthase

It is widely believed that endothelium derived relaxing factor (EDRF) produced by vascular endothelial cells is identical with NO (Nitric oxide) . Nitric oxide is synthesised in a wide variety of tissues and is known to be implicated in a number of crucial physiological functions eg: control of systemic blood pressure, respiration, digestion, platelet aggregation etc. The enzyme primarily responsible for the synthesis of NO is tissue specific. Nitric oxide syntheses in the endothelium and neurons is calmodulin activated. enzyme that oxidises Arginine to Citrulline in the presence of biopterin, NADPH and oxygen.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 3 May-June 2022, pp: 1272-1278 www.ijprajournal.com ISSN: 2456-4494

f) Transition Metals

Conditions (eg: Plasma pH < 6, haemolysis and ischemia- reperfusion) that lead to the release of transition metal ions (that of Iron and Copper) may remarkably amplify ROS toxicity Iron and Copper ions are capable of converting H_2O_2 to OH. In the presence of the free transition metal ions ascorbic acid, a commonly known antioxidant functions as a pro-oxidant. $^{2+}$

HEALTH AND DISEASE

Oxidative damage to DNA, proteins, and other macromolecules has been implicated in the pathogenesis of a wide variety of diseases, most notably heart disease and cancer. A growing body of animal and epidemiological studies as well as clinical intervention trials suggest that antioxidants may play a pivotal role in preventing or slowing the progression of both heart disease and some forms of cancer.

AIM OF WORK:

The present study was undertaken to determine the Hepatoprotectiveand antioxidant activity and also the evaluation of the various biochemical parameters of CnidoscolusPhyllacanthus.

HEPATOPROTECTIVE AND ANTIOXIDANT WORK IS PLANNED TO CARRY OUT AS OUTLINEDBELOW

Cnidoscolus Phyllacanthus

SOLVENT EXTRACTION (HOT PERCOLATION METHOD)

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

Extraction of whole plant of CnidoscolusPhyllacanthususing Soxhlet apparatus using hydro alcohol as solvent.

In-vivo evaluation of the plant extract of CnidoscolusPhyllacanthus

Selection, grouping and acclimatization of the animals.

I. Induction of hepatotoxicity by D-galactosamine.
II. Treatment protocol with the extract.
III. Study of serum parameters analysis.
IV. Bio-chemical analysis viz- serum analysis and histopathological studies.
V. Statistical analysis.

DESCRIPTION

It is large, leafy, throny shrub. It is aarboreal plant that has short trunk, cylindrical and branched from the base.

The extracts of cnidoscolus have high inhibition percentage of cell growth. These species are commonly used to treat tumors and inflammation. The roots, bark and latex are used for the treatment of inflammatory processes, genitourinary and in general as antiseptic, dermatologic and ophthalmic. It is also used to treat kidney diseases, urinary infections, contusions, fractures, wounds, warts and hemorrhoids. It is also a good sourse of protiens, vitamins and minerals.

METHOD

The Cnidoscolusphyllacanthus plant was collected and identified. The leaf was cut down

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 3 May-June 2022, pp: 1272-1278 www.ijprajournal.com ISSN: 2456-4494

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^{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(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 Effect of Cnidoscolusphyllanthusand VitaminE pre-treatment on biochemical parameters of the rats intoxicated with D-Galactosamine.

Effect of Cnidoscolusphyllanthusand VitaminE pre-treatment on biochemical parameters of the rats intoxicated with D-Galactosamine.

Group. No.	TREATMENT DOSE (mg/Kg)	AST (IU/mL)	ALT (IU/mL)	ALP (IU/mL)	TP (gm/dl)	TB (mg/dl)	GGTP (mg/dl)	Total Albumin (g/dl)
I	Normal control 10ml/kg normal saline	44.40± 1.52	30.09± 1.49	23.68± 1.30	5.15± 0.08	1.92± 0.08	96.90± 2.75	3.80± 0.16
II	Toxic control 25mg/kg D-galactosamine	*a 105.90 ± 2.40	*a 94.49± 1.05	*a 144.10± 2.35	*a 3.16± 0.22	*a 4.40± 0.26	*a 173.42± 2.90	*a 2.20± 0.07
III	Standard control Vitamin E 25mg/kg	*b 60.10± 1.20	*b 40.56± 1.06	*b 56.4± 1.70	*b 3.90± 0.08	*b 2.8± 0.15	*b 122.20± 1.95	*b 2.90± 0.05

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 3 May-June 2022, pp: 1272-1278 www.ijprajournal.com ISSN: 2456-4494

IV	Treatment control EECP 200mg/kg	*b 68.65± 1.46	*b 54.82± 2.72	*b 65.86± 2.30	*b 4.60± 0.25	*b 3.30± 0.20	*b 136.30± 3.04	*b 2.54± 0.04
V	Treatment control EECP 400mg/kg	*b 62.45± 1.15	*b 47.94± 0.97	*b 58.50± 1.95	*b 4.05± 0.26	*b 2.95± 0.18	*b 130.94± 1.23	*b 2.30± 0.09

Valuesare expressed as Mean ± SEM.
Values are found out by using one way ANOVA followed by Newmannkeul's multiple range tests.
*a – values are significantly different from Normal control at P< 0.01.
*b – values are significantly different from Toxic control(G2) at p< 0.01.

Effect ofCnidoscolusphyllanthusand Vitamin E pre-treatment on biochemical liver parameter in D-Galactosamine induced hepatotoxicity.

Group. No.	TREATMENT DOSE (mg/Kg)	SOD (U/mg) Protein	CATALASE (U/mg) Protein	GPX (U/mg) Protein	MOA (U/mg) Protein
I	Normal control 10ml/kg Normal saline	132.25± 2.40	290.40± 2.40	1.10± 0.05	3.90± 0.17
II	Toxic control 25mg/kg D-galactosamine	*a 68.20± 1.65	*a 190.75± 2.70	*a 0.40± 0.02	*a 7.40± 0.12
III	Standard control Vitamin E 25mg/kg	*b 118.05± 2.80	*b 260.45± 1.92	*b 0.85± 0.02	*b 4.50± 0.14
IV	Treatment control 200mg/kg EEPL	*b 96.50± 1.60	*b 230.05± 1.80	*b 0.55± 0.02	*b 5.60± 0.28
V	Treatment control 400mg/kg EEPL	*b 105.65± 2.62	*b 240.75± 2.65	*b 0.74± 0.02	*b 4.80± 0.08

Values are expressed as Mean ± SEM.
Values are finding out by using one way ANOVA followed by Newmannkeul's multiple range tests.
*a – values are significantly different from Normal control at P< 0.01.
*b – values are significantly different from Toxic control(G2) at p< 0.01.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 3 May-June 2022, pp: 1272-1278 www.ijprajournal.com ISSN: 2456-4494

EFFECT OF EECP ON THE LEVELS OF NON ENZYMATIC ANTIOXIDANTS IN THE LIVER TISSUE OF D-GALACTOSAMINEHEPATOTOXIC AND CONTROL RATS

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 EECP 200mg/kg	98.05±2.16*b	0.60±0.04*b	4.92±0.50*b
Treatment control EECP 400mg/kg	91.90±1.95*b	0.69±0.06*b	5.02±0.48*b

Values are expressed as Mean ± SEM.
Values are found out by using one way ANOVA followed by Newmannkeul's multiple range tests.
*a – values are significantly different from Normal control at P< 0.01.
*b – values are significantly different from Toxic control (G2) at p< 0.01.

II. RESULT :

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. CONCLUSION

In conclusion, our findings demonstrated that EECP at both doses possesseshepatoprotective 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 promisinghepatoprotective 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). 1-5
[3]. http://www.phytochemicals.info/free-radicals.php
[4]. Harmon,D.,Gerontol,J.,1956,11:298-300
[5]. Kappus,H.Arch.Toxicology.1987,60:144
[6]. Harsh, M.Cellinjuary and cellular adaptation. Text book of pathology .,**2005**,5: 38-40.
[7]. 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.

[8]. http://www.uv.es/frag/oxidative_stress.htm

[9]. 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.

[10]. Dipak,D.,Ranjith, K.,Banerjee.Reactive oxygen species :Oxydative Damage and athogenesis.Current science.**1999**,3(5):77.

[11]. Hellsten,Y.The role of Xanthine oxidase in exercise.Elesevier Science publisher,**1994**:221-234.

[12]. Edmonds,S.E.,Blake,D.R.Hypoxia,oxidative stress and exercise in rheumatoid arthritis.Elesevier science publishers B.V.,Amstradam,**1994**:389-422.

[13]. Lilus,E.M.,Manila,P.Photon emission of phagocytes in relation to sterss and disease.Experientia.,**1992**,48:1082-1091.

[14]. Otta,D.M.E.,Lindstrom.Seppa,p.,Sen,C.K.C ytochrome P_{450} dependent enzyme and oxidant-mediated responses in rainbow trout exposed to condaminated sediments. Ecotoxic Environ Safe., **1994,**27:265-280.