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HEPATOPROTECTIVE AND ANTIOXIDANT ACTIVITY OF STANDARDIZED HERBAL EXTRACTS IN ALBINO (WISTAR) MALE RATS

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Abstract

Herbal medicine has its origins in ancient cultures. It involves the medicinal use of plants to treat disease and enhance general health and wellbeing. More recently, herbs and spices have been identified as sources of various phytochemicals, many of which possess powerful antioxidant activity. Antioxidant factors found in plants are based upon constituent nutrients with demonstrated radical-scavenging capacities as well as upon non-vitamin or mineral substances. The Liver care extract contains active components from various parts of *Boerhavia diffusa L, Eclipta alba, Phyllanthus niruri, Tinospora cordifolia and Trikatu (Piper longum L. , Piper nigrum and Zingiber officinale*) supplied bt Auropharma, Puducherry, were investigated for its hepatoprotective effect on paracetamol (2g/kg/b.wt/p.o) induced acute liver damage in Wistar albino rats. Hepatoprotection activity was measured by diagnostic marker enzymes such as AST, ALT ALP, total bilirubin and direct bilirubin; tissue enzyme parameters such as GSH, SOD and CAT. The Liver care extract at the dose of (100 mg/kg/p.o) produced significant hepatoprotective effect in albino (Wistar) male rats and the effects of Liver care extract were comparable to that of standard drug Silymarin. These results suggest that Liver care extract exhibits a very significant hepatoprotective effect in albino (Wistar) male rats.

Keywords: Liver care extract, hepatoprotective activity, antioxidant activity, *Boerhavia diffusa L, Eclipta alba, Phyllanthus niruri, Tinospora cordifolia and Trikatu.*

Introduction:

The aim of the present context is to evaluate the hepatoprotective potential of Liver care extract gift sample from Auropharma, Puducherry in conventional animal model induced

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hepatotoxicity. The liver is the key organ regulating homeostasis in the body, it is involved with almost all the biochemical pathways related to growth, fight against disease, nutrient supply, energy provision and reproduction. (1) The liver is expected not only to perform physiological functions but also to protect against the hazards of harmful drugs and chemicals. In spite of tremendous scientific advancement in the field of hepatology in recent years, liver problems are on the rise. Liver diseases are mainly caused by toxic chemicals, excessive consumption of alcohol, infections and autoimmune disorders. Now a day's drug-induced liver toxicity is a common cause of liver injury drugs like antibiotics, Nonsteroidal anti-inflammatory drugs, herbal and dietary supplements, Cardiovascular drugs (10%), Central nervous system agents and antineoplastic drug. (2) It accounts for approximately one-half of the cases of acute liver failure and mimics all forms of acute and chronic liver disease. Plants are a source of a wide range of natural products that possess various therapeutic properties and are continuously explored to develop novel drugs.(3.4)

There are numerous herbal formulations (Liv 42, Liv-52, Liver cure, Livol, Livomyn, Livfit, Livogen and Livactine) claimed to have hepatoprotective activities. (5) The Liver care extract was a gift sample and a project wok at Auropharma, Puducherry which contains active components from various parts of *Boerhavia diffusa L, Eclipta alba, Phyllanthus niruri, Tinospora cordifolia and Trikatu (Piper longum L., Piper nigrum and Zingiber officinale*) and the present investigation was undertaken to evaluate the hepatoprotective activity of Liver care extract in paracetamol induced hepatotoxicity in rat model.

Liver care extract:

Boerhavia diffusa L its synonym *Boerhavia africana Lour*. commonly known as red hogweed / Punarnava belongs to *Nyctaginaceae* family it is *Boerhaavia diffusa* is widely dispersed, occurring throughout India, the Pacific, and southern United States. Plant parts are applied as a stomachic, cardiotonic, hepatoprotective, laxative, diuretic, anthelmintic, febrifuge, expectorant and, in higher doses, as an emetic and purgative.(6,7)

Eclipta alba its synonym *Eclipta prostrata* Roxb commonly known as Bhringraja belongs to family *Asteraceae* are widely an erect or prostrate, branched annual herb upto 30-40 cm high. Fresh juice of the leaves is given in the treatment of oedema, fevers, liver disorders, and rheumatic joint pains; it is also used to improve the appetite and to stimulate digestion. *Eclipta alba* widely dispersed, occurring throughout Central America and South-East Asia.(8,9)

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Phyllanthus niruri its synonym *Niruris annua Raf* commonly known as gale of the wind / stonebreaker belongs to family *Phyllanthaceae*. *Phyllanthus* has been used in Ayurvedic medicine for over 2,000 years and has a wide number of traditional uses including internal use for jaundice, gonorrhoea, frequent menstruation, and diabetes and topical use as a poultice for skin ulcers, sores, swelling, and itchiness. *Phyllanthus niruri* is native to Argentina Northeast, Argentina Northwest, Belize, Bolivia, Brazil.(10,11)

Tinospora cordifolia synonym *Tinospora sinensis* (Lour.) commonly known as gurjo belongs to family *Menispermaceae.* It is a large, deciduous, extensively-spreading, climbing vine with several elongated twining branches. *Tinospora cordifolia* has an importance in traditional ayurvedic medicine used for ages in the treatment of fever, jaundice, chronic diarrhoea, cancer, dysentery, bone fracture, pain, asthma, skin disease, poisonous insect, snake bite, eye disorders. *Tinospora cordifolia* indigenous to tropical regions of the Indian subcontinent.(12,13)

Piper longum L. synonym *Chavica roxburghii* Miq its common name long Pepper family *Piperaceae.* A perennial slender, aromatic climber, glabrous with branches soft angular and grooved when dry, perennial woody roots, creeping and jointed stems. Treat chronic bronchitis, asthma, constipation, gonorrhea, paralysis of the tongue, diarrhoea, cholera, chronic malaria, viral hepatitis, respiratory infections, stomach-ache, bronchitis, diseases of the spleen, cough, and tumors. *Piper longum L* native to India, Malaysia, Nepal, Sri Lanka and Vietnam.(14,15)

Piper nigrum synonym *Piper colonum C.Presl* common name black pepper. The black pepper plant is a woody climber and may reach heights of 10 metres (33 feet) by means of its aerial roots. Used traditionally for the treatment of various diseases including; cough, cold, dyspnoea, throat diseases, intermittent fever, dysentery, stomach-ache, worms and piles. the production of black pepper occurs in India, Malaysia, Indonesia, China, Thailand, Sri Lanka, Vietnam, Brazil and Madagascar. (16)

Zingiber officinale, synonym *Amomum zingiber L*. common name *Ginger.* It possesses multiple biological activities, including antioxidant, anti-inflammatory, antimicrobial, anticancer, neuroprotective, cardiovascular protective, respiratory protective, antiobesity, antidiabetic, antinausea, and antiemetic activities. *Zingiber officinale* native to Southeast Asia but widely cultivated throughout the tropics since antiquity. (17)

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Experimental animal:

The institutional animal ethics committee (Register No.160/1999/CPCSEA), proposal no: 831; dated 20.04.2011, Annamalai University, Annamalai Nagar, India; approved the experimental design. Albino (Wistar) male rats of 150-200g (weight) were used for the study. Animals were housed in well ventilated room (temperature 23±2^{o}C, humidity 55-60% and 12h light/dark cycle) at Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University. Animals were fed with standard pellet diet and water *ad libitum*. All studies were conducted in accordance with Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) norms and the National Institute of Health guidelines "Guide for the Care and use of Laboratory Animals"(18)

Drugs and Chemicals

Silymarin, was purchased Chemosyn Ltd.,Vapi,India: Paracetamol from Micro labs Ltd., Bangalore, India:, Carboxy Methyl Cellulose was purchased from S.D. Fine Chemicals Ltd., Mumbai, India and Liver care extract gift sample from Auropharma, Puducherry and other solvents/reagents were analytical grade.

Experimental design

Four groups (I - IV) each comprising of six albino (Wistar) male rats of 150-200g were selected. Group I served as control and received orally 0.5% Sodium CMC (1 ml each) for seven days. Groups III rats received oral dose 100 mg/Kg body wt of Liver care extract. Group III rats received oral dose of Silymarin (25mg/Kg body wt). Paracetamol at a dose of 2gm/KG body wt p.o were administered on the 15th day to all animals in groups of II, III, and IV. After 48 hrs administration of paracetamol dosing the rats were sacrificed cervical decapitation under Xylazine + Ketamine (16 + 100 mg/kg i.m.), blood samples were collected via abdominal aorta puncture using sodium citrate (3.8%w/v) as anticoagulant and the serum separated were used for the determination of diagnostic marker enzymes levels were analyzed in Secomam semi auto analyzer. The ventral side of the rat was cut and the liver samples were removed and weighed. The ventral side of the rat was cut and liver samples were removed and weighed. The tissues were then transferred to ice cold saline container. It was homogenised and the levels of antioxidants enzymes estimated.

Results:

Table: 1. Effect of Liver care extract on biological parameters.

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	Total bilirubin (mg/100ml)	Direct bilirubin (mg/100ml)
Group I (Normal control)	54.30± 0.315	25.21± 0.496	96.10± 0.194	0.88± 0.008	0.25± 0.004
Group II (Paracetamol control)	140.02± 1.278***	62.11± 2.287***	392.13± 4.221***	2.16± 0.0225***	0.69± 0.023***
Group III (Test; paracetamol 100mg/kg/day of Liver care extract received rats)	65.43± 1.304***/ a	27.36± 0.045***/ a	141.26± 6.353***/ b	0.95± 0.01**/a	0.36± 0.005**/a
Group IV (Reference ; paracetamol + Silymarin treated Rat)	52.14± 1.254	24.73± 0.547	93.42± 4.354	0.87± 0.346	0.21± 0.048

Values are mean ± S.E.M. of animals in each group. Comparisons: a. group II Compared with group I; b. group III Compared with group II & V c. group IV compared with group II &V; ***=P<0.001 highly significant, **=P,0.01 moderately significant.

Table: 2. Effect of Liver care extract tissue enzyme parameters

Groups	GSH (µmol)	SOD (nmol)	CAT(µmol)
Group I (Normal control)	11.01±0.11	5.89±0.21	9.4±0.12
Group II(paracetamol control)	5.31±0.12***	1.02±0.12***	2.9±0.44***
Group III (test ; paracetamol+100 mg/kg/day of liver care Extract)	6.96±0.17**/b	3.64±0.1**/b	6.78±0.01**/ b
Group IV (Reference ; paracetamol Silymarin treated rats)	10.02±0.14	5.2±0.15	8.92±0.24

Comparisons: a. group II Compared with group I; b. group III Compared with group II & V c. group IV compared with group II &V; ***=P<0.001 highly significant, **=P,0.01 moderately significant.

Table: 3. Effect of Liver care extract on **wet weight of the liver**

Groups	Wet weight of the liver (g)
Group I (Normal control)	3.77±0.070
Group II (Paracetamol control)	6.43±0.022***
Group III (Test; paracetamol 100mg/kg/day of Liver care extract received rats)	5.76±0.035**/b
Group IV (Reference ; paracetamol + silymarin treated Rat)	4.32±0.024

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Discussion:

Drug induced hepatotoxicity, with paracetamol as an inducing agent is well documented. This well-known antipyretic and analgesic agent, is safe in therapeutic doses, but can produce fatal hepatic at toxic doses inn humans, rats and mice. Paracetamol is metabolized in the liver via three pathways 1) glucuronidation, 2) sulfation (both account for 95% of metabolism) or 3) via the cytochrome P450 enzyme system (5%). (19) In this pathway, paracetamol is converted to a toxic metabolite, NAPQI. Glutathione (a tripeptide) then binds to toxic metabolite forming a non-toxic compound. Hepatotoxicity occurs when there is a rapid depletion of glutathione leading to the accumulation of the toxic metabolite in the liver.

This toxicity occurs because of its reactive metabolite, N-acetyl-P-benzoquinoneimine (NAPQI). NAPQI exerts its toxicity primarily *via* its oxidative effect on cellular proteins. Sulfhydryl compounds are among the most important endogenous antioxidants. Glutathione (GSH) is the main intracellular non protein sulfhydryl compound which plays an important role in the maintenance of cellular proteins and lipids in their functional states. NAPQI binds to GSH, forming a conjugate which results in conversion of GSH to an oxidized form of glutathione. When GSH levels are lowered, the toxic effects of oxidative insult are exacerbated, resulting in increased membrane and cellular damage. At this point, other protein and non-protein Sulfhydryl groups present in the cell provide an important alternate protection.(19)

The liver predisposes to oxidative stress presumably by amplifying the capacity of free radical chain reaction. An obvious sign of hepatic injury is the leakage of cellular enzymes into the plasma due to the disturbance caused in the transport functions of hepatocytes. When liver cell membrane is damaged, a variety of enzymes located normally in cytosol is released into the blood; thereby causing increased enzyme levels is the serum such as AST, ALT, ALP and TB. The parameters such as AST, ALT, ALP and TB in blood and GSH, SOD, CAT enzymes in liver tissue have been found to be of great importance in the assessment of liver damage.

The abnormally high levels of serum ALT, AST, ALP and TB observed in our study are the consequence of paracetamol induced liver dysfunction which denotes damage to the hepatic cells. Analysis of the rat liver enzymes at the dose of 100 mg/kg/day of Liver Care Extract revealed a statistically significant lowering of serum liver enzymes (*i.e*) AST, ALT,

with ales marked effect in the levels of ALP. These effects were comparable to the enzyme levels in the Silymarin treated group, which is suggestive of protective action in the liver.

Conclusion:

From the results it was concluded that the Liver care extract supplied from Auropharma, Puducherry has significant action on paracetamol induced hepato-toxicity. And proved to be the hepatoprotective and antioxidant activity of standardized in Liver care extract herbal extracts against albino (wistar) male rats.

Conflicts of interest: None declared.

Ethical approval: Register No.160/1999/CPCSEA, proposal no: 831; dated 20.04.2011, Annamalai University, Annamalai Nagar,

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