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Antioxidant Activity of The Ancient Herb, Holy Basil in CCl_4-Induced Liver Injury in Rats

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ABSTRACT

An herbal preparation called "holy basil plus herbal powder" (HBPP) containing *Ocimum santum, Withania somnifera, Pongamia pinnata, Plumbago indica, Emblica officinalis* and *Curcuma longa* was investigated as an antioxidant and hepatoprotective agent. The antioxidant activity of HBPP was investigated in rats with liver injury induced by oral administration of carbon tetrachloride:olive oil (1:1). HBPP was administered orally at 500 mg/kg daily for 7 days before. HBPP exhibited statistically significant antioxidant activity, as shown by increased levels of glutathione peroxidase (GPX), glutathione S-transferase (GST), glutathione reductase (GRD), superoxide dismutase (SOD) and catalase (CAT) and decreased level of lipid peroxidation (LPO). HBPP performed equally well as silymarin, a well-established antioxidant preparation used to protect against liver injury.

Keywords: *Holy basil plus herbal powder; carbon tetrachloride; liver enzymes; antioxidant activity, hepatoprotection*

Introduction

Reactive oxygen species produced by sunlight, ultraviolet light, ionizing radiation, chemical reactions and metabolic processes have a wide variety of pathological effects, such as DNA damage, carcinogenesis and cellular degeneration related to aging [1]. Recent studies showed that a number of plant products or extracts containing polyphenols, flavonoids and terpenes can exert an antioxidant action (2-5). Antioxidant activity could provide an important benefit in view of the free radical theory of aging and diseases associated with oxidative stress [2-4]. The potential value of antioxidants has prompted investigators to search for natural compounds with potent antioxidative activity without any intrinsic cytotoxicity.

"Holy Basil", or "Tulsi", holds an important place among the various medicinal herbs used in India (perhaps only next to Lotus). The ancient scriptures describe it as a "protector of life accompanying human beings from birth until death". There are numerous references to Tulsi in Indian mythology. The ancient Ayurvedic scriptures mention the plant in the management of several diseases. Its healing properties are so well-established that many people in India grow it in courtyards and traditionally use it daily in a ritual for the well-being of the family.

Botanists have given Tulsi (holy basil) the Latin name *Ocimum sanctum*. The species refers to both green-leafed "Rama Tulsi"

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and purple-leafed "Krishna Tulsi". More recently Tulsi has also been identified with *Ocimum tenuiflorum* meaning "basil with smaller flowers".

The seeds, leaves and roots of holy basil traditionally have been ascribed a powerful medicinal value. It is used both internally and externally. It has mild antiseptic and analgesic properties and relieves swelling. The leaves when chewed mitigate gum infections. Instillation of fresh juice of the leaves into the ears is an effective domestic remedy for earaches. A tea made with leaves of holy basil is a common remedy for cold, cough and mild indigestion.

Holy basil has diverse actions on the respiratory system. It effectively liquefies the phlegm and is effective for cough due to allergic bronchitis, asthma and eosinophilic lung disease. In a study without controls, oral administration of an aqueous extract of dried holy basil to 20 patients with asthma increased lung vital capacity and relieved labored breathing [5].

The Ayurvedic pharmacopoeia of India has a long list of herbal preparations containing holy basil as an important ingredient. In keeping with the guidelines of Ayurvedic formulations, the team of pharmacologists and phytochemists at Sewanti Ayurvedic Series at Vancouver in Canada have combined holy basil with additional key Ayurvedic herbs to enhance its bioavailability and stimulate its activity. These additional herbs include "Ashwagandha", "Karanja", "Chitraka", "Amla" and "Turmeric".

In the present study, the preparation "holy basil plus herbal powder" (HBPP), a combination of *Ocimum sanctum, Withania somnifera, Pongamia pinnata, Plumbago indica, Emblica officinalis* and *Curcuma longa* was examined. All the ingredients are described in many Pharmacopoeias and have been confirmed to possess a range of pharmacological properties including free radical scavenging. Therefore, it was worthwhile to ascertain whether the combination of all these ingredients has antioxidative activity. The present study was undertaken to investigate the antioxidant activity of HBPP in CCl_4-intoxicated liver injury in rats.

Materials and Methods

Medicine

Holy basil plus herbal powder (HBPP) was prepared by the in-house R&D Unit of Nagarjuna Herbal Concentrates Ltd at Kerala in India. The medicine was ground with distilled water and administered to animals orally at the dose of 250 and 500 mg/kg body weight.

Chemicals

Reduced glutathione (GSH), 1-chloro-2,4-dinitrobenzene (SRL., Mumbai), 5,5-dithio-bis-2-nitrobenzoicacid, nicotine adenine dinucleotide phosphate, epinephrine (Sigma, MO, USA), ethylenediamine-tetra-acetic acid, hydrogen peroxide (SRL, Mumbai), thiobarbituric acid (Loba Chemi, Mumbai) were used for the study. All other reagents used were of analytical grade.

Animals

Adult Wistar male rats (150–180 g) (Kerala Agriculture University, Mannuthi) were maintained in well-ventilated room temperature with natural day–night cycle in large polypropylene cages. They were fed balanced rodent pellet diet and water *ad libitum* throughout the experimental period. The animals were quarantined for one week, prior to the experiments to acclimatize to laboratory conditions. The study protocol was approved by the IAEC (Institutional animal ethics committee of CPCSEA, Govt. of India).

Antioxidant activity

The adult Wistar male rats were divided into five groups of six animals each. Group I received only distilled water (5 ml/kg per day p.o.) for seven days and served as control. Group II animals received single dose of 1:1 mixture of carbon tetrachloride and olive oil (50% v/v, 2 ml/kg p.o.) on the seventh day. Group III and IV animals were treated with HBPP suspension at a dose level 250 and 500 mg/kg per day p.o., respectively, for seven days. On the seventh day, a single dose of a 1:1 mixture of carbon tetrachloride and olive oil was given (50% v/v, 2 ml/kg p.o.). Group V animals were treated with silymarin (25 mg/kg per day p.o.) for seven days and on the seventh day, a single dose of 1:1 mixture of carbon tetrachloride and olive oil (50% v/v, 5 ml/kg i.p.) was administered. All animals were sacrificed by cervical decapitation under mild anesthesia on the eighth day. Immediately after sacrifice, the livers were dissected out, washed in ice-cold saline, and a homogenate prepared in 0.1M Tris–HCl buffer (pH 7.4). The homogenate was centrifuged and the supernatant was used for the assay of marker enzymes: glutathione peroxidase (GPX), glutathione S-transferase (GST) and glutathione reductase (GRD) by literature methods [6-8] respectively. The activities of superoxide dismutase (SOD), catalase (CAT) were determined by literature methods [9,10]. Lipid peroxidation (LPO) was estimated by the method of [11]. The total protein content was estimated by the Biuret method [12].

Statistical analysis

Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Dunnett's t-test. P-values <0.05 were considered significant.

Results

Glutathione peroxidase (GPX)

GPX activity in liver homogenates was significantly ($P < 0.001$) reduced in CCl_4-treated animals when compared to control. The HBPP treatment (500 mg/kg dose level) significantly increased ($P < 0.001$) the GPX levels when compared to CCl_4-treated animals. However, HBPP (250 mg/kg) showed a less

significant increase in GPX levels ($P < 0.02$ and $P < 0.01$, respectively) in liver homogenate when compared to CCl_4-treated animals. Silymarin (25 mg/kg p.o.)-treated animals also showed a significant ($P < 0.001$) increases of GPX level in the liver homogenate compared with CCl_4-treated animals.

Glutathione S-transferase (GST)

GST level in liver was significantly reduced ($P < 0.001$) in CCl_4-treated animals when compared with normal animals. Treatment with HBPP at 500 mg/kg dose showed a significant increase ($P < 0.001$) in GST when compared toCCl_4-treated group. HBPP (250 mg/kg) also showed a less significant ($P < 0.05$ and $P < 0.01$) increase of GST in liver homogenate. A significant ($P < 0.001$) increase of GST in the liver homogenate of silymarin-treated animals was observed.

Glutathione reductase (GRD)

GRD activity was significantly decreased ($P < 0.001$) in CCl_4-treated animals when compared to controls. A significant increase ($P < 0.001$) in the level of GRD was observed in HBPP (500 mg/kg) treated rats when compared withCCl_4-treated animals. HBPP (250 mg/kg) also showed less significant ($P < 0.02$ and $P < 0.01$) increase in the GRD when compared withCCl_4-treated animals. The silymarin-treated group also showed significant ($P < 0.001$) increase in the level of GRD when compared toCCl_4-treated animals.

Superoxide dismutase (SOD)

SOD level was significantly reduced ($P < 0.001$) inCCl_4-treated animals when compared with normal animals. The HBPP (500 mg/kg) showed significant increase ($P < 0.001$) in SOD when compared to CCl_4-treated animals; HBPP at 250 mg/kg showed a less significant increase ($P < 0.01$) in SOD in liver homogenate compared withCCl_4-treated animals. Silymarin treated animals also showed a significant ($P < 0.001$) increase in SOD when compared toCCl_4-treated animals.

Catalase (CAT)

CAT activity was significantly ($P < 0.001$) reduced in CCl_4-treatment when compared to control. HBPP at 500 mg/kg dose significantly increased ($P < 0.001$) CAT in liver homogenate when compared toCCl_4-treated animals. Treatment with HBPP at 250 mg/kg dose also showed a less significant ($P < 0.02$ and $P < 0.01$) increase of CAT when compared with CCl_4-challenged animals. The silymarin-treated group also showed significant ($P < 0.001$) increase in CAT when compared toCCl_4-treated animals.

Lipid peroxidation (LPO)

The LPO level of liver homogenates significantly increased ($P < 0.001$) in CCl_4-challenged rats when compared to control rats. Treatment with HBPP at 500 mg/kg dose showed significant ($P < 0.001$) decrease in LPO when compared with CCl_4-treated animals. HBPP at 250 mg/kg dose also showed less significant ($P < 0.01$) decrease in LPO in liver homogenate when compared with CCl_4-treated animals. The silymarin-treated group showed a significant ($P < 0.001$) decline in the LPO when compared to CCl_4-treated animals.

Discussion

GPX plays a pivotal role in H_2O_2 catabolism [13] and aids the detoxification of endogenous metabolic peroxides and hydroperoxides with the help of GSH [14]. GPX activity was significantly reduced after CCl_4 treatment when compared to

Table 1: Antioxidant activity of HBPP against CCl_4-induced liver damage

Parameter	Normal control	CCl4 control	HBPP suspension + CCl4		Silymarin (25 mg/kg) +CCl4
			250 mg/kg	500 mg/kg	
GPX (nmol of GSH oxidized/min/mg protein)	310.6 ± 21.7	169.1±21.5a,b	269.9±12.7c,d	291.4±13.1b,c	301.4 ± 9.6b,c
GST (nmol of CDNB conjugate formed/min/mg protein)	282.4 ± 25.8	152.9±16.9a,b	217.7 ± 5.2c,f	260.4±10.9b,c	279.1 ± 12b,c
GRD (nmol of GSSG utilized/min/mg protein)	20.3 ± 1.0	11.8 ± 0.9a,b	16.6 ± 1.2c,d	19.2 ± 0.9b,c	21.8 ± 0.8b,c
SOD (Katal/g protein)	80.6 ± 3.4	38.2 ± 2.3a,b	59.9 ± 3.1c,e	69.8 ± 3.5b,c	77.2 ± 3.5b,c
CAT (nmol of H2O2 decomposed/min/mg protein)	178 ± 6.7	45 ± 4.8a,b	74.7 ± 2.1c,f	160 ± 9.9b,c	175 ± 4.7b,c
LPO (nmol of MDA/mg protein)	6.2 ± 0.8	15.5 ± 1.3a,b	8.1 ± 0.6c,e	7.4 ± 1.5b,c	5.8 ± 0.7b,c

a Control compared with CCl_4-treated animals.
b$P < 0.001$.
c CCl4-treated animals compared with HBPP.
d$P < 0.02$.
e$P < 0.01$.
f $P < 0.05$.

controls. The restoration of the GPX activity to nearly normal levels when CCl_4 was administered after pretreatment with HBPP, is proposed to be due to more effective antioxidant activity involving scavenging and/or detoxifying the endogenous metabolic peroxides generated after CCl_4 injury in the liver tissue.

Many investigators have suggested that GST offers protection against LPO by promoting the conjugation of toxic electrophilic reactive species with GSH and thus inactivating them [15]. GST plays a physiological role in the detoxification of potential alkylating agents. Chemicals like chloroform and CCl_4 alter hepatic GST activity [16]. The GST levels were significantly reduced in CCl_4-treated animals and restoration of levels was observed after the treatment with HBPP. This may be attributed to the direct action of the HBPP on the hepatic GST activation, the mechanism of which is not presently known. An increase in GRD activity implies that HBPP can protect the liver tissue from oxidative damage by GSH regenerated from its oxidized form (GSSG).

In the present study, the SOD activity was significantly reduced in CCl_4-intoxicated rats. The SOD activity was brought back to near normal levels after treatment with the HBPP in CCl_4-intoxicated rats. Decreased activity of CAT was observed in animals treated with CCl_4. Presumably, a decrease in CAT activity could be attributed to cross-linking and inactivation of the enzyme protein by the lipid peroxides. Decreased CAT activity is linked to destruction of the enzyme as a result of oxidative stress caused by CCl_4. The fact that CAT activity was restored to normal after treatment with HBPP further suggests the antioxidant property of this extract against oxygen free radicals (OFRs).

The level of LPO in tissue is related to oxidative membrane damage and alterations in structure and function of cellular membranes. The level of thiobarbituric acid reactive substance (TBARS) is an indirect measurement of lipid peroxidation [2]. The lipid peroxide levels in tissue were found to be significantly elevated in CCl_4-challenged rats. This oxidative effect is a consequence of CCl_4 activation by cytochrome P450 to trichloromethyl radical (CCl_3·) which readily reacts with oxygen to form the trichloromethyl peroxyl (CCl_3O_2·) radical [17]. These free radicals trigger cell damage through two mechanisms, (a) covalent binding to cellular macromolecules and (b) lipid peroxidation which affects the ionic permeability of the membrane causing disintegration and solubilization of the membrane structure. The diminished LPO activity after treatment with the HBPP extracts may be attributed to the antioxidant activity scavenging the CCl_3· free radicals generated due to the metabolic transformation of CCl_4 in the liver. Free radical reactions are implicated in the progression of cancer, inflammation, atherosclerosis, hepatocellular damage and the biological process of aging. Compounds with hepatoprotective activity combined with antioxidant activity have a synergistic effect in preventing the initiation and progression of hepatocellular diseases [18].

Silymarin is a standardized extract of milk thistle seeds (*Silybum marianum*), containing a mixture of flavonolignans consisting of silibinin, isosilibinin, silicristin, silidianin and others. Both in vitro and animal research suggests that silymarin has hepatoprotective (antihepatotoxic) properties that protect liver cells against toxins [19].

Chemically modified silibinin, silibinin dihydrogen disuccinate disodium (trade name Legalon SIL), a solution for injection, is currently being tested as a treatment of severe intoxication with hepatotoxic substances, such as poisoning by death cap mushrooms (*Amanita phalloides*) [20]. The observation that HBPP performed equally as well as silymarin in reducing oxidative stress after CCl_4-intoxication, suggests that in the future HBPP could be tested in similar cases of hepatotoxicity.

Although the precise mechanism of action of HBPP has not been elucidated, it can be safely assumed that the lowering of enzyme levels that are responsible for combating oxidative stress is responsible for cell injury caused by CCl_4, and that enhancing these enzyme levels is responsible for the antioxidant activity of HBPP. It can be concluded that the HBPP preparation possesses definite antioxidant activity in the liver.

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