

HIGH PROTEIN DIET INDUCED HEPATIC OXIDATIVE STRESS AND ITS AMELIORATION BY THE HERB, *Emilia sonchifolia* (L.)DC

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Summary

Diet should be consistent with balanced composition to maintain healthy life. Unbalanced diet can cause serious adverse effects to the mankind including oxidative stress. The aim of this study is to investigate the protective effect of the medicinal herb, *Emilia sonchifolia* against the high protein diet induced oxidative stress. Twenty four male wistar rats randomly divided into 4 groups (n = 6 each) were taken. Group I (normal diet: standard pellets), Group II (high protein diet: 100% Raw Soya Flour), Group III (100% Raw Soya Flour with oral administration of 250 mg/kg b. wt. of n-hexane extract of *Emilia sonchifolia*), Group IV- n-hexane extract of *Emilia sonchifolia* alone. (250 mg/kg b.wt). The animals were sacrificed after the experimental period of 1 month. Significant (p< 0.05) increase in lipid peroxidation and decrease in antioxidant enzymes were seen in Group I rats. Treatment with n-hexane extract of *Emilia sonchifolia* (250 mg/kg) significantly (p<0.05) prevented the increase in lipid peroxidation whereas SOD, CAT, GPx, GST GSH and vitamin C were significantly (p< 0.05) increased in Group III animals. This was evident from the histopathological studies. Thus the findings suggest that *Emilia sonchifolia* is a potential therapeutic agent.

Key words: High protein diet, antioxidants, oxidative stress, *Emilia sonchifolia*.

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Introduction

The liver is the largest internal organ. It plays a role in digestion, sugar and fat metabolism, and the body's immune defense. It processes almost everything a person eats, breathes or absorbs through the skin. About 90% of the body's nutrients pass through the liver from the intestines. The liver converts food into energy, stores nutrients, and produces blood proteins. The liver also acts as a filter to remove harmful substances from the blood. In the developing fetus, blood cells are produced in the liver (1). Dietary factors plays a major role in the metabolic process and imbalance in nutritional composition can lead to liver dysfunction. Long term intake of the high protein food can cause serious malfunction in the body including liver dysfunction. The Western medicines used in the treatment of liver diseases can cause serious adverse effects. Therefore it is necessary to search for alternative drugs for the treatment of liver disease (2).

Emilia sonchifolia is a well known annual weed seen in most tropical and subtropical regions worldwide. It is used in the treatment of gastropathy, diarrhea, fevers, tumors (3). In the present study, the protective effect of n-hexane extract of *Emilia sonchifolia* against the oxidative stress caused by the raw soy flour (RSF), a high protein food in the liver of albino rats was investigated.

Materials and methods

Plant materials

Fresh plant material of *Emilia sonchifolia* was collected from Thrissur, Kerala, India. The plant was authenticated by Dr. G.V.S Moorthy, Botanical Survey of India, TNAU campus Coimbatore, with the voucher number BSI/SRC/5/23/09-10/Tech/782. The whole plant material was washed under running tap water, air dried, finely powdered and stored in airtight bottles. The powder soaked in n-hexane solvent was kept in the shaker for 48 h at room temperature. The extract was collected and concentrated at 40°C under reduced pressure using rotary evaporator. The dried extract was stored at 4°C until further use. The remaining residue was extracted again with the fresh solvent to ensure complete extraction.

Preparation of raw soy flour

Raw soya beans was purchased from the local market, Coimbatore, India and powdered and stored in an air tight container.

Animals

Male albino rats (2-3 weeks old) were used in this study. All animals were housed under a well ventilated atmosphere. Prior to the feeding experiment, they were allowed free access to water *ad libitum* and a standard pellets for 10 days to allow acclimatization to these conditions.

Experimental design

Then all animals were divided randomly into four groups, each comprising of 6 rats: Group 1 (normal diet: standard pellets), Group 2 (high protein diet: 100% RSF) Group 3 (RSF with oral administration of 250 mg/kg b. wt. of n-hexane extract of *Emilia sonchifolia*), Group IV- n-hexane extract of *Emilia sonchifolia* alone. All animals were allowed free access to the experimental diets and water *ad libitum* throughout the experimental period of 1 month. The study was approved by Institutional Animal Ethical Committee (IAEC) constituted for the purpose of CPCSEA, Govt.of.India.

At the end of the experimental period, rats were deprived of food overnight but had free access to water. The animals were sacrificed by decapitation and the liver were removed immediately, washed in ice-cold saline for antioxidant studies and preserved in 10% formalin for histopathological studies respectively.

Antioxidant studies

The tissue samples were homogenized with ice-cold saline (0.9% NaCl solution) and then were centrifuged at 4000 g for 15 min at 4°C. The supernatants were used to determine the enzymatic antioxidants like superoxide dismutase (SOD) (4), catalase (CAT) (5), Glutathione peroxidase (GPx) (6), Glutathione-S-transferase (GST) (7) and non-enzymatic antioxidants like reduced glutathione (8) and vitamin C (9).

Statistical analysis

The results obtained were expressed as mean \pm SD. The statistical comparisons among the groups were performed with Students t-test using a Statistical Package Program (SPSS 10.0 for Windows) at $p < 0.05$ level.

Results and Discussion

In the present study RSF was selected because of its high protein content. High-protein diets are not recommended because they restrict healthful foods that provide essential nutrients and do not provide the variety of foods needed to adequately meet nutritional needs. Individuals who follow these diets are therefore at risk for compromised vitamin and mineral intake, as well as potential cardiac, renal, bone, and liver abnormalities overall (10). In this study, there was significant increase in lipid peroxidation and reduction in SOD, CAT, GPx, GST, GSH and vitamin C in Group II animals fed with RSF and treatment with n-hexane extract of *Emilia sonchifolia* (250 mg/kg) significantly ($p < 0.05$) prevented the increase in lipid peroxidation and brought them near to normal levels, whereas SOD, CAT, GPx, GST (Table 1), GSH and vitamin C (Table 2) were significantly ($p < 0.05$) increased in Group III animals. There was no significant change in Group I and IV animals.

Table 1: Effect of n-hexane extract of *Emilia sonchifolia* on the activities of enzymatic antioxidants in liver of control and experimental animals

Groups	LPO	SOD	CAT	GPx	GST
Group I- Normal control	1.25±0.03 ^a	8.13±0.03 ^a	70.32±0.20 ^a	7.32±0.02 ^a	9.07±0.03 ^a
Group II- RSF	2.87±0.09 ^b	4.48±0.11 ^b	45.26±0.24 ^b	4.08±0.01 ^b	4.96±0.03 ^b
Group III- RSF + HEES (250 mg/kg)	1.70±0.01 ^c	7.48±0.09 ^c	65.23±0.14 ^c	6.96±0.03 ^c	8.62±0.03 ^c
Group IV- HEES alone (250mg/kg)	1.27±0.02 ^a	8.15±0.03 ^a	70.34±0.17 ^a	7.33±0.03 ^a	9.06±0.04 ^d

Values are expressed as mean ± SD for six animals.

Values not sharing common superscript letters differ significantly at $p < 0.05$ (DMRT).

Units: LPO - nM/mg protein.; SOD - inhibition of 50% nitrite formation/min/mg protein;

CAT - μmol of H_2O_2 consumed/min/mg protein; GPx - μmol of glutathione oxidized/min/mg protein;

GST- μmole of glutathione utilized/min/ mg protein.

Table 2: Effect of n-hexane extract of *Emilia sonchifolia* on the activities of non-enzymatic antioxidants in liver of control and experimental animals.

Groups	GSH	Vit C
Group I- Normal control	47.08±0.04 ^a	1.59±0.007 ^a
Group II- RSF	26.13±0.52 ^b	0.81±0.003 ^b
Group III- RSF + HEES (250 mg/kg)	41.59±0.78 ^c	1.33±0.009 ^c
Group IV- HEES alone (250mg/kg)	47.07±0.04 ^a	1.58±0.01 ^a

Values are expressed as mean ± SD for six animals.

Values not sharing common superscript letters differ significantly at $p < 0.05$ (DMRT).

Units: GSH, vitamin C ($\mu\text{g}/\text{mg}$ protein).

High-protein, low-carbohydrate diets have untoward clinical consequences for patients with coronary artery disease, including progression of diabetic nephropathy, exacerbation of gouty diathesis, increases in circulating free fatty acids, and increases in low density lipoprotein cholesterol levels (11). Nutrients may be one of the causative factors of oxidative stress. They cause redox imbalance and further lead to a number of diseases produced through accumulating reactive oxygen species (ROS) *in vivo* (12).

The oxidation induced by ROS can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases, such as cancer, liver injury and cardiovascular disease (13). The ROS generation in tissues is efficiently scavenged by the enzymatic and non-enzymatic antioxidants. The decrease in the activities of antioxidant enzymes is in close relationship with the induction of lipid peroxidation (14). Hence, antioxidants are important and play a major role in the prevention and treatment of diseases associated with oxidants or free radicals. In the present study, it was observed that treatment with the n-hexane extract have brought down the elevated level of LPO and also significantly enhanced the reduced levels of SOD, CAT, GPX, GST and GSH which may be due to its increased antioxidant content which has the capacity to scavenge the free radicals induced by the intake of high protein food.

Histopathological examination of the liver section of the Group II animals fed with RSF showed larger and pleomorphic hepatocytes, cytoplasmic vacuolization and compressed sinusoidal spaces. The Group III animals treated with n-hexane extract of *Emilia sonchifolia* showed normal central venous system and most of the hepatocytes appear normal and sinusoidal spaces are also found to be normal. Group I and IV animals showed normal liver sections (Figure1).

Fig.1 Histopathology of Liver

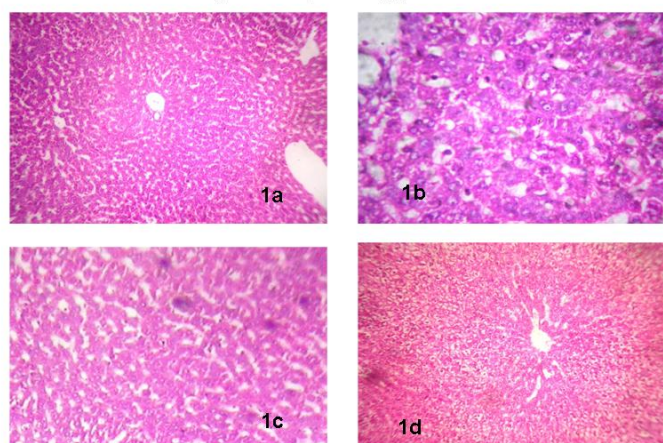


Fig 1a- Group I (control) showing normal liver section

Fig 1b -Group II (RSF) showing showed larger and pleomorphic hepatocytes, cytoplasmic vacuolization and compressed sinusoidal spaces.

Fig 1c - Group III showing normal central venous system and most of the hepatocytes appear normal and sinusoidal spaces are also found to be normal

Fig 1d - Group IV showing normal liver section

Thus it was concluded that the hepatoprotective effect of *Emilia sonchifolia* may be due to the active constituents present in the plant.

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