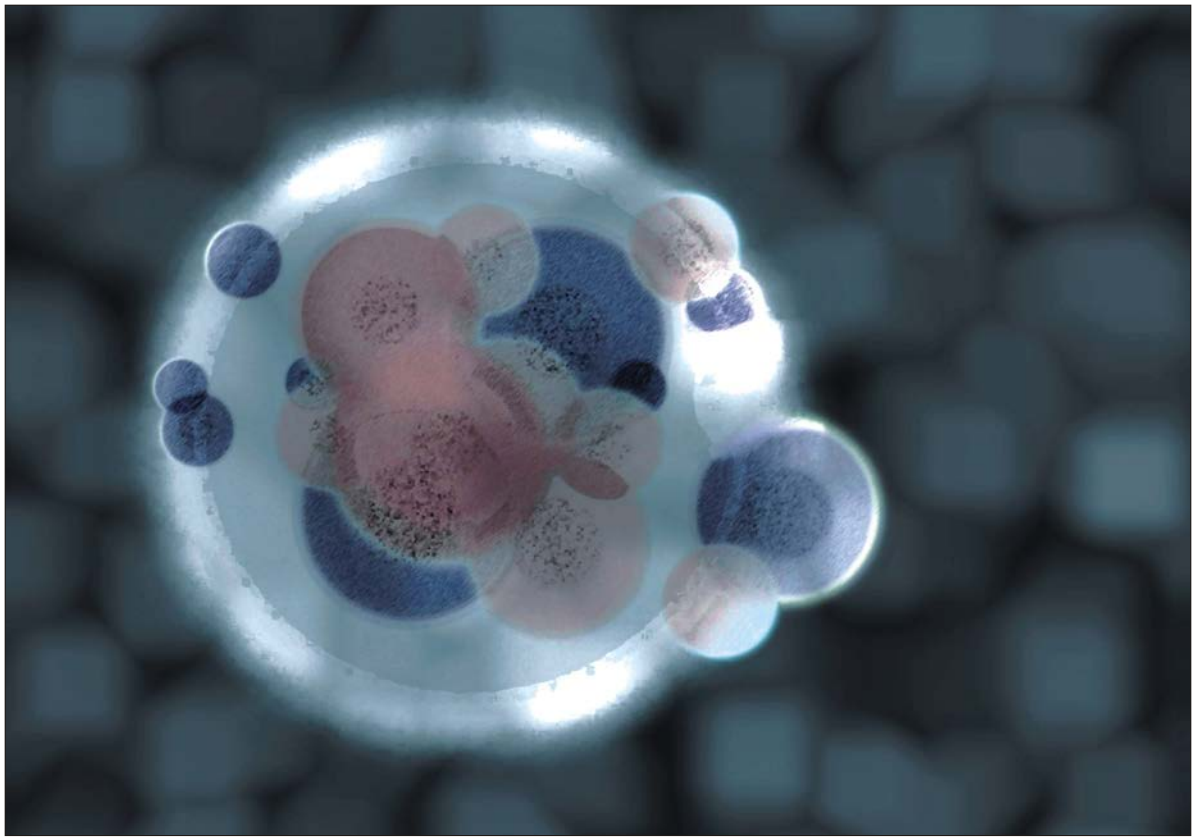




Journal of Biological Sciences

ISSN 1727-3048

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Evaluation of the Protective Effects of *Emilia sonchifolia* Linn. (DC.) on Perchlorate-Induced Oxidative Damage

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Abstract: *Emilia sonchifolia* Linn. (DC.) is a traditionally used medicinal plant seen in most tropical and subtropical regions worldwide. Various parts of the plant are used for the treatment of diseases like asthma, intermittent fevers, breast cancer, ophthalmia, nyctalopia etc. We have isolated the flavonoid fraction from *E. sonchifolia* (whole plant). Female albino rats were fed with 0.2% sodium perchlorate to induce oxidative stress. The flavonoid fraction of the plant was fed along with sodium perchlorate to another group of animals. The experiment consisted of 30 days. The antiperoxidative effect of the plant material was studied *in vivo*. The levels of lipid peroxidation products (Thiobarbituric acid reacting substances-TBARS), the activities of the antioxidant enzymes-superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), Glutathione Reductase (GR) and glutathione-S-transferase (GST) as well as the concentration of reduced glutathione (GSH) were determined. The results obtained show that the flavonoid fraction of *E. sonchifolia* is a potent inhibitor of peroxidative damage and can be used as a therapeutic agent.

Key words: *Emilia sonchifolia*, flavonoid fraction, sodium perchlorate, oxidative stress, lipid peroxidation, polyphenols

INTRODUCTION

Oxidative stress plays an important role in cell death associated with many diseases and leads to endogenous DNA damage (Horakova *et al.*, 2003; Dursun *et al.*, 2002; Kang, 2002; Gao *et al.*, 2001). The reactive oxygen species formed in the body of organisms will result in the peroxidation of lipids, leading to tissue damage. Certain chemicals like CCl_4 , H_2O_2 etc. are found to enhance the lipid peroxidation process. Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection against infection and degenerative diseases caused by oxidative damage. Natural plant products began to receive much attention as sources of safe antioxidants (Frankel *et al.*, 1996). Polyphenolic compounds such as flavonoids are more effective than α -tocopherol, in suppressing the peroxidation of membrane phospholipids, induced by the attack of aqueous oxygen radicals (Terao *et al.*, 1994). Dietary phytophenolics can scavenge harmful active oxygen species and thus can form an antioxidant system equivalent to that of ascorbate (Sakihama *et al.*, 2002; Moon *et al.*, 2006).

Emilia sonchifolia (Family: Compositae) is a well known annual weed found commonly in cultivated fields

and waste places throughout India. It is also seen in most tropical and subtropical regions worldwide. The plant has a height of 30-40 cm with pink or purplish flowers. It is an edible plant used in the Ayurvedic system of medicine for the treatment of gastropathy, diarrhea, ophthalmia, nyctalopia, cuts and wounds, intermittent fevers, pharyngodynia and asthma (Nair and Chopra, 1996). The crushed leaves are used externally to promote the healing of breast abscesses among tribal women. The root juice, mixed with salt and water is used to treat cataracts and redness of eyes. The leaves are rubbed on the forehead to relieve headache. Phytochemical investigations of *E. sonchifolia* had been carried out (Ram and Mehrotra, 1998; Gao *et al.*, 1993), but biochemical investigations seem to be less. The aqueous and methanolic extracts of *E. sonchifolia* leaves progressively reduced rat paw edema (Muko and Ohiri, 2000) and exhibits antitumor activities (Shylesh and Padikkala, 2000).

There is a growing interest in the pharmacological evaluation of various plants used in the Indian system of Medicine. Although the polyphenolic components of *E. sonchifolia* have been isolated, hardly any systematic work has been carried out on its *in vivo* antioxidant activity. The wound healing and other anti-inflammatory properties of the plant point to its antioxidant potency.

With these backgrounds, the present study was designed to explore the *in vivo* antioxidant nature of the flavonoid fraction of *E. sonchifolia*.

MATERIALS AND METHODS

Chemicals: All the chemicals were purchased from Sigma chemical company, USA.

Extraction of the plant material: Fresh plants were collected from Thiruvananthapuram District, Kerala, India and authenticated by an expert [Herbarium: Kerala University Botany Herbarium-voucher specimen No: 1089 (KUBH)]. The plant material was washed and dried in shade. The dried plant material (1 kg) was then crushed and taken in a round-bottomed flask. Eighty five percent ethanol was added such that it covered the material and refluxed in a water bath for 2 days at 60°C. The extract was decanted, filtered and evaporated in a rotor evaporator to remove the alcohol. The extract was cleared of low polarity contaminants such as fats, terpenes, chlorophyll, xanthophyll etc. by repeated extractions with petroleum ether (60-80°C), benzene and ethyl acetate respectively. Ethyl acetate extract contained bulk of flavonoids and this extract was evaporated in vacuum and its dried weight was 75 g. It was identified as flavonoid by the method described by (Eskin *et al.*, 1978) using Quercetin as reference. The flavonoid fraction was dissolved in distilled water and used for the *in vivo* study.

Experimental design: Female Albino rats (Sprague Dawley strain) weighing 80-100 g were used for the study. The animals were housed in polypropylene cages in rooms maintained at 25±1°C. Drinking water was given *ad libitum*. Rats were fed with standard laboratory diet supplied by Lipton India Ltd. For the maintenance of the experimental animals, the Institutional Ethical guidelines were absolutely followed as per CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) rules.

Grouping of animals: The animals were divided into four groups of six each as:

Group I: Control-Normal lab diet

Group II: Normal lab diet + Sod.perchlorate (0.2% in diet).

Group III: Normal lab diet + Sod. perchlorate+ flavonoid fraction of *E. sonchifolia* (1 mg/100 g body wt.)

Group IV: Normal lab diet + Sod.perchlorate + flavonoid fraction of *E. sonchifolia* (2.5 mg/100 g body wt.)

The doses of the flavonoid fraction were fixed according to the results of a previous dose-dependent study which included the doses: 0.5, 1, 2 and 2.5 mg/100 g body weight, of which we have selected 1 and 2.5 mg for the experiment.

The plant material was administered orally by gastric intubation. Sodium perchlorate (0.2% in diet) was mixed with the normal lab diet and fed to the animals daily, except to those in the control group.

The duration of the experiment was 30 days. Diet intake had been monitored daily and the body weight of animals recorded in every two weeks. At the end of the experimental period, rats were deprived of food overnight and subjected to euthanasia. The tissues were collected for various biochemical estimations.

Biochemical estimations: TCA-TBA-HCl reagent was used for the estimation of TBARS in the tissue homogenates by the method of (Niehaus and Samuelsson, 1968). The activity of SOD was determined by the method of (Kakkar *et al.*, 1984), for which, the tissue homogenates were prepared in 0.25 M sucrose buffer. The method of (Maehly and Chance, 1954) was followed for the assay of Catalase using H₂O₂ as the substrate. GSH was estimated by the method of (Benke *et al.*, 1974). Protein estimation was done according to the procedure of (Lowry *et al.*, 1951). Glutathione peroxidase activity was estimated by the Dithio bisnitro benzoic acid (DTNB) method as explained by (Brien and Little, 1969). The activity of Glutathione reductase was determined by the method described by (Bergmeyer, 1965). Assay of Glutathione-S-transferase was done according to the procedure of (Habig *et al.*, 1974).

Statistical analysis: Statistical significance was calculated using Student's t-test. The data given in the figures are the average values indicated in each case ±SEM. The analysis was done by the method described by (Bennett and Franklin, 1967).

RESULTS

The daily diet intake of the rats did not differ among the groups during the feeding period. Also, there was no significant difference on the body weight of animals among the different groups compared to the control.

It is observed that the levels of peroxidation products (TBARS) were significantly enhanced by perchlorate

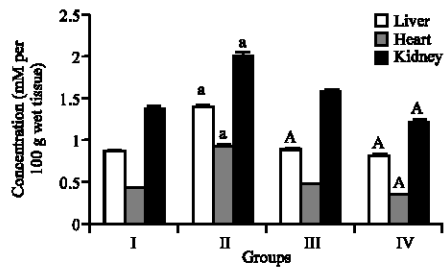


Fig. 1: Concentration of TBARS. Unit: mM per 100 g wet tissue. Foot Note: Values are mean of six estimations per group $\pm$ SEM. Group II is compared with Group I. a: $p < 0.01$, b: $0.01 < p < 0.05$. Group III and IV are compared with Group II. A: $p < 0.01$, B: $0.01 < p < 0.05$. No symbol: Not significant

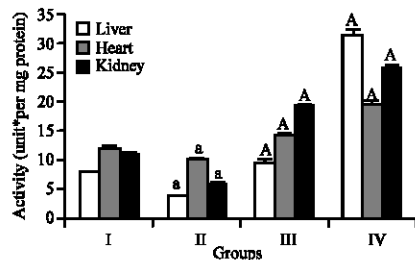


Fig. 2: Activity of SOD. Unit: Unit* per mg protein. (Unit*-Enzyme concentration required to inhibit optical density at 560 nm of chromogen production by 50% in 1 minute). Foot Note: Same as that of Fig. 1

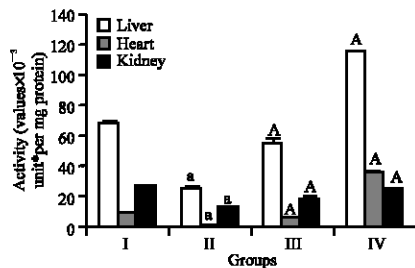


Fig. 3: Activity of Catalase. Unit: Values $\times 10^{-3}$ Unit* per mg protein (Unit*-Velocity constant per sec). Foot Note: Same as that of Fig. 1

administration (Fig. 1). But the flavonoid fraction of the plant, when treated orally reduced their concentrations significantly. The effect is more in group received 25 mg plant extract.

Due to the perchlorate treatment, activities of the antioxidant enzymes-SOD (Fig. 2) and catalase (Fig. 3) were found to be lowered. The level of GSH (Fig. 4) also showed a reduction. The enzymes of the glutathione

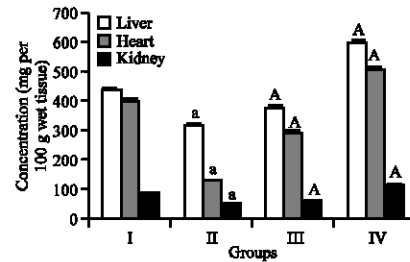


Fig. 4: Concentration of Reduced Glutathione. Unit: mg per 100 g wet tissue. Foot Note: Same as that of Fig. 1

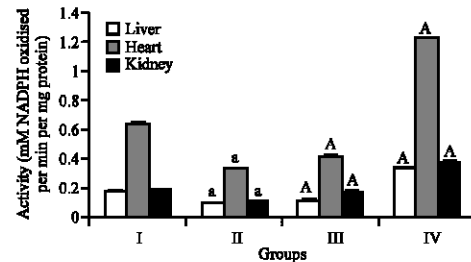


Fig. 5: Activity of Gpx. Unit: μ M NADPH oxidized per min per mg protein. Foot Note: Same as that of Fig. 1

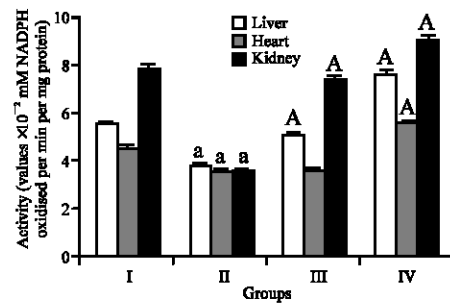


Fig. 6: Activity of GR. Unit: Values $\times 10^{-2}$ μ M NADPH oxidized per min per mg protein. Foot Note: Same as that of Fig. 1

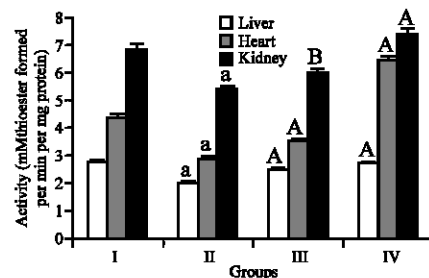


Fig. 7: Activity of GST. Unit: μ M thioester formed per min per mg protein. Foot Note: Same as that of Fig. 1

system- Gpx (Fig. 5), GR (Fig. 6) and GST (Fig. 7) also showed diminished activities due to perchlorate administration. But the administration of the plant material considerably enhanced the above parameters.

DISCUSSION

Increased production of TBARS is claimed as an evidence for enhanced lipid peroxidation. In the present study, the levels of these lipid peroxidation products are highly elevated in rats received sodium perchlorate. This shows that sodium perchlorate acts as a potent inducer of oxidative damage. Such an overproduction of the lipid peroxides under similar conditions has already been reported (Marnett, 2002). The administration of flavonoids from *E. sonchifolia* significantly reversed the effect of sodium perchlorate, which was evidenced by the low levels of peroxidation products in the animals of the plant product treated groups. Similar results were reported by several scientists (Marnett, 2002; Mojzisova and Kuchta, 2001).

SOD and catalase are the major antioxidant enzymes present in the system. SOD is a defensive oxido-reductase capable of removing superoxide (O_2^-) radicals by catalyzing its dismutation to O_2 and H_2O_2 (Fridovich, 1983; Misra and Fridovich, 1972). Catalase is a heme protein which functions to destruct H_2O_2 . In the experiment, the lowered activity of SOD and catalase in the perchlorate treated animals show the effectiveness of perchlorate as an agent to enhance peroxidation. These results are in agreement with the reports of (Venukumar and Latha, 2002) and (Horakova *et al.*, 2002). There are several reports on the ability of plant products in enhancing the activity of the antioxidant enzymes (Aviram *et al.*, 2002; Borek, 2001; Soares De Moura *et al.*, 2002; Yagi *et al.*, 2002). The results obtained in the present study also show that the flavonoids from *E. sonchifolia* significantly enhance the activity of SOD and catalase.

The normal system contains a very high concentration of reduced glutathione (GSH). It is a major antioxidant present in our body. It is a scavenger of singlet oxygen and hydroxyl radicals. In the experiment, the concentration of reduced glutathione was considerably diminished in the perchlorate treated group. Supplementing the flavonoid fraction significantly increased the content of GSH. The maintenance of the GSH level depends on the activities of various enzymes, namely GSH reductase, GSH peroxidase and GSH-S-transferase. In the experiment, all these components of the glutathione system namely GSH, GR, GPx and GST were considerably diminished in perchlorate treated group. Supplementation of the plant material significantly elevates all these parameters. Similar results have been reported by a number of investigators (Jadhav and Bhutani, 2002; Roig *et al.*, 2002; Sushmakumari *et al.*, 1989).

Thus, on the lights of the above observations, it can be stated that the flavonoid fraction from *E. sonchifolia* can act as potent therapeutic agent against various conditions of oxidative stress. All its beneficial effects are maximal when it is supplemented at a dose of 1 mg/100 g body wt./day.

Many plant polyphenols such as flavonoids are reported to be potent antioxidants (Jadhav and Bhutani, 2002; Barth *et al.*, 2002; O'Byrne *et al.*, 2002; Roig *et al.*, 2002). Their antioxidant activity is mainly related to the structural characteristics and is proportional to the number of free phenolic hydroxyl groups (Joyeux *et al.*, 1995). Free radicals are highly unstable chemical compounds, which have been implicated in mediating vascular and tissue damage associated with several diseases (Kumar *et al.*, 2001). The radical scavenging activity of flavonoids makes them effective antioxidants. They act as chain breaking antioxidants by donating a hydrogen atom to a peroxy or alkoxy radical, thus interfering with the propagation of lipid peroxidation. The results obtained in the study suggest that the flavonoid fraction isolated from *E. sonchifolia* possesses significant antioxidant activity and hence its inclusion in the diet is highly beneficial in protecting the body against oxidative damage.

Thus the usage of the plant material can be recommended due to its edible nature, easy availability, cost effectiveness compared to other leafy vegetables and above all, due to its anti-oxidative property.

ACKNOWLEDGMENTS

We thank Dr. Valsala Kumari, Curator, Department of Botany, University of Kerala for authenticating the plant material. The financial assistance as a research grant to Dr. Annie Abraham (SARD, Order No: (T) 17/R&D augmentation/04/KSCSTE, dated 20-2-2004) from KSCSTE, Government of Kerala, India is greatly acknowledged.

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