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Effect of *Emilia sonchifolia* (Linn.)DC on alcohol- induced oxidative stress in pancreas of male albino rats

Dominic Sophia, Murugesan Gomathy, Thomas Shebin, Paramasivam Ragavendran, Chinthamony Arulraj, Velliyur Kanniapan Gopalakrishnan*

Department of Biochemistry, Karpagam University, Coimbatore- 641 021 (T.N.), India

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ABSTRACT

Objective: To explore the efficacy of *n*-hexane extract of *Emilia sonchifolia (E. sonchifobia)* against ethanol induced pancreatic dysfunction in the young Wistar albino rats. **Methods:** The rats were divided into four groups. Control rats in group Ⅰ received distilled water orally, group Ⅱ received oral administration of 20% (w/v) ethanol dissolved in drinking water, group Ⅲ received oral administration of 20% (w/v) ethanol in distilled water+*n*-hexane extract of *E. sonchifolia* (250 mg/kg body weight), and group Ⅳ received oral administration of *n*-hexane extract of *E. sonchifolia* (250 mg/kg body weight) alone. Liver marker enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), pancreatic enzymatic antioxidants superoxide dismutase, lipid peroxidation, catalase, glutathione peroxidase, non-enzymatic antioxidants glutathione and vitamin C were measured and compared. **Results:** Administration of 20% ethanol for 16 weeks significantly increased the liver marker enzymes AST, ALT($P<0.05$), reduced the pancreatic enzymatic antioxidants superoxide dismutase, lipid peroxidation, catalase, glutathione peroxidase, glutathione and vitamin C($P<0.05$). Histopathological examination showed that the ethanol provoked the oxidative stress which was demonstrated as pancreatic necrosis and oedema. Simultaneous administration of *n*-hexane extract of *E. sonchifolia* (250 mg/kg body weight) protected the pancreas against the damage induced by ethanol which was confirmed by the histopathological studies and the normalization of biochemical parameters. **Conclusions:** Thus *n*-hexane extract of *E. sonchifolia* shows a promise in therapeutic use in alcohol induced oxidative stress.

1. Introduction

Alcoholism is a major health problem throughout the world, regardless of racial, ethnic, and socioeconomic factors[1]. Consumption of alcohol (ethanol) is a leading cause of diseases and death worldwide. Ethanol exerts profound effects on the endocrine and exocrine pancreas[2]. It has been reported that the pancreatic acinar cell can metabolize ethanol as effectively as the liver[3]. The pancreas can metabolize ethanol by means of oxidative and nonoxidative pathways to generate metabolites, such as acetaldehyde and fatty acid ethyl esters (FAEEs)[4]. The major oxidative enzyme system uses either alcohol dehydrogenase or the cytochrome p450 system, whereas the nonoxidative pathway uses the FAEE synthase enzyme pathway. The metabolites generated by both oxidative and nonoxidative pathways are injurious to both exocrine and endocrine pancreas.

Alcohol has been linked to the causation of many human disease states. One of the most appreciated and investigated diseases is alcohol-induced pancreatitis[1]. Heavy alcohol consumption is known to be a major cause of chronic pancreatitis, which has been linked to malabsorption, diabetes, and pancreatic cancer[5]. There is now compelling experimental evidence *in vitro* and *in vivo* that FAEE are toxic mediators in ethanol-induced organ injury[6].

Oxidative stress may result from exposure to a variety of agents present in the environment. External sources of reactive oxygen species (ROS) include radiation, UV light,

*Corresponding author: Velliyur Kanniapan Gopalakrishnan, Department of Biochemistry, Karpagam University, Coimbatore- 641 021 (T.N.), India.
Tel: 91-0422-6453777
Fax: 91-0422-2611043
E-mail: vkgopalakrishnan@gmail.com

chemical reagents, pollution, cigarette smoke, drugs of abuse, and ethanol[7]. Ethanol intake causes accumulation of ROS. There are a number of findings associated with the ethanol induce toxicity in which oxidative stress and proinflammatory cytokine production thought to be the leading putative etiological factor[8]. Oxidative stress can act through a number of important mediators to produce cell injury and death[9]. Ethanol can lead to an increase in the production of ROS and/or a decrease in the levels of antioxidant defenses causing a redox imbalance and resulting in the oxidative damage of lipids, proteins, and DNA. A complex system of antioxidant defenses has evolved that generally holds the oxidative attack from ROS. On occasions, however, this balance can be perturbed, leading to oxidative stress[10]. Therefore, oxidative stress can contribute, at least in part, to the damage observed in pancreas[11].

Herbal alternatives are one of the best ways to minimize these disease conditions[12]. The protective effect of fruit and vegetable intake can provide certain phytonutrients, such as isothiocyanates, polyphenols and flavonoids, to act as antioxidants to counteract the by-products of the oxidative metabolites from alcohol[13]. *Emilia sonchifolia* (Family: Asteraceae) (*E. sonchifolia*) commonly known as lilac tassel flower is a traditionally used medicinal plant found in most tropical and subtropical regions worldwide. Various parts of the plant are used for the treatment of diseases[14]. This weed is used in ethnomedicine against inflammation, eye sores, convulsion, cuts, wounds, rheumatism and insect bites[15,16]. In addition, anticonvulsant activity of aqueous extract has previously been reported[17]. Phytochemical studies indicated that the aerials parts of *E. sonchifolia* contain alkaloids and flavonoids and terpernes[18]. The present study is to evaluate the potentiality of *E. sonchifolia* on ethanol induced oxidative stress and to assess its effect on pancreatic antioxidant status using Wistar rats as an experimental model.

2. Materials and methods

2.1. Plant collection

E. sonchifolia was collected from Thrissur, Kerala, India. The plant was authenticated by Dr. G.V.S Moorthy, Botanical survey of India, TNAU Campus, Coimbatore. The voucher number is BSI/SRC/5/23/09-10/Tech/782. The whole fresh plant material was washed under running tap water, air dried and powdered.

2.2. Preparation of extract

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 ℃ under reduced pressure using rotary evaporator. The dried extract was stored at 4 ℃ until further use. The remaining residue was extracted again with the fresh solvent to ensure complete extraction.

2.3. Animals

The male Wistar rats weighing between 50-55 g were obtained from Animal house of Karpagam University, Coimbatore. The animals were caged in well ventilated hygienic conditions. They were divided randomly into four groups each containing six. The study was approved by Institutional Animal Ethical Committee constituted for the purpose of CPCSEA, Government of India.

2.4. Experimental design

Group Ⅰ: Control rats; Group Ⅱ: Rats administered with 5 mL of 20% (w/v) ethanol; Group Ⅲ: Rats administered with 5 mL of 20% (w/v) ethanol and were treated with *n*-hexane extract of *E. sonchifolia* (250 mg/kg body weight); Group Ⅳ: Rats administered with *n*-hexane extract of *Emilia sonchifolia* alone (250 mg/kg body weight).

2.5. Liver function assays

After the experimental period the animals were sacrificed under light chloroform anesthesia. The blood was drawn from the *p*-orbital venous complexes and serum separation tubes, allowed to clot for 30 min at room temperature and then centrifuged at 1 000 × *g* for 10 min and stored at 4 ℃. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT)[19] were estimated within 6 h of animal sacrifice.

2.6. Lipid peroxidation and antioxidant enzyme assays

The pancreas were excised immediately, cleaned free of extraneous material and perfused with ice cold saline (0.9%) and stored in 10% formalin, which are used for the antioxidant and histopathological studies respectively.

2.7. Estimation of pancreatic lipid peroxidation

Lipid peroxidation (LPO)[20] was calculated on the basis of the molar extinction coefficient of malondialdehyde (MDA) and expressed in terms of nanomoles of MDA/mg protein.

2.8. Antioxidant assays

The enzymatic antioxidants such as superoxide dismutase(SOD)[21], catalase(CAT)[22], glutathione peroxidase(GPx)[23], and the non-enzymatic antioxidants such as reduced glutathione[24], and vitamin C[25] were evaluated in the tissue homogenates.

2.9. Statistical analysis

The results obtained were expressed as mean±SD. The statistical comparisons among the groups were performed

using one way analysis (ANOVA) (SPSS 10.0) at $P<0.05$ level.

3. Results

3.1. Liver marker enzymes

In the present study, serum AST and ALT levels has increased significantly in group Ⅱ rats after the ethanol treatment($P < 0.05$). Treatment with the *n*-hexane extract of *E. sonchifolia* (group Ⅲ) was found significantly effective in the normalization of these markers when compared to ethanol treated group Ⅱ rats($P < 0.05$). The *n*-hexane extract of *E. sonchifolia* alone (group Ⅳ) did not show any significant difference as compared to control group rats (Table 1).

3.2. Lipid peroxidation assay

The ethanol treated group II showed a significant increase in pancreatic LPO level ($P < 0.05$). Treatment of rats (group Ⅲ) with the *n*-hexane extract of *E. sonchifolia* showed significant protective activity against the ethanol treated group rats($P < 0.05$) (Table 2). No significant difference was found in the MDA level between control and only *n*-hexane extract of *E. sonchifolia*.

3.3. Pancreatic enzymatic antioxidant assays

The levels of pancreatic enzymatic antioxidants SOD, CAT, GPx were depleted significantlyin ethanol treated group rats as compared to control group($P < 0.05$). Administration of *n*-hexane extract of *Emilia sonchifolia* increased the levels of enzymatic antioxidants SOD, CAT, GPx significantly as compared to ethanol treated group. The *n*-hexane extract of *Emilia sonchifolia* alone treated group rats exhibited no significant changes in SOD, CAT, GPx levels as compared to control group (Table 2).

3.4. Pancreatic non- enzymatic antioxidant assays

Table 3 shows that the ethanol treatment significantly increased the levels of pancreatic non-enzymatic antioxidants glutathione (GSH) and vitamin C($P < 0.05$). After the treatment of *n*-hexane extract of *Emilia sonchifolia*, GSH and vitamin C levels were increased significantly ($P < 0.05$) in compare to the ethanol treated group. There was no significant change between the *n*-hexane extract of *Emilia sonchifolia* alone treated group and control group rats.

3.5. Histopathological analysis

Histopathological study of the pancreas in the experimental group Ⅱ animals showed the congestion of cells and mild

Table 1
Effect of *n*-hexane extract of *E. sonchifolia* on serum biochemical parameters against oxidative stress in albino rats (IU/L) (mean±SD, *n*=6).

Group	ALT	AST
I-Control	80.75±1.07	126.56±0.47
II-Ethanol induced	140.71±0.76$^{\Delta}$	262.33±0.82$^{\Delta}$
III-Ethanol induced+ *n*-hexane extract treated	92.30±0.37*	135.68±1.16*
IV-*n*-hexane extract alone treated	80.70±0.59	126.47±0.75

$^{\Delta}P < 0.05$ *vs*. control group; *$P < 0.05$ *vs*. ethanol induced group.

Table 2
Effect of *n*-hexane extract of *E. sonchifolia* on enzymatic antioxidants against oxidative stress in albino rats(mean±SD, *n*=6).

Group	LPO	SOD	CAT	GPx
I-Control	1.31±0.02	6.29±0.09	15.43±0.24	7.39±0.30
II-Ethanol induced	4.03±0.10$^{\Delta}$	3.56±0.29$^{\Delta}$	8.57±0.33$^{\Delta}$	4.03±0.06$^{\Delta}$
III-Ethanol induced+ *n*-hexane extract treated	1.97±0.03*	5.26±0.06*	13.44±0.32*	6.28±0.11*
IV-*n*-hexane extract alone treated	1.30±0.02	6.29±0.10	15.58±0.21	7.45±0.31

$^{\Delta}P < 0.05$ *vs*. control group; *$P < 0.05$ *vs*. ethanol induced group.
Units- LPO-nM/mg protein; SOD-Inhibition of 50% nitrite formation/min/mg protein; CAT- μ moles of H_2O_2 consumed/mg protein; GPx- μ moles of glutathione utilized/min/mg protein.

Table 3
Effect of *n*-hexane extract of *E. sonchifolia* on non-enzymatic antioxidants against oxidative stress in albino rats (mean±SD, *n*=6).

Group	GSH	Vitamin C
I-Control	12.46±0.24	1.41±0.06
II-Ethanol induced	7.61±0.28$^{\Delta}$	0.66±0.06$^{\Delta}$
III-Ethanol induced+ *n*-hexane extract treated	11.17±0.17*	1.17±0.04*
IV-*n*-hexane extract alone treated	12.35±0.26	1.46±0.07

$^{\Delta}P < 0.05$ *vs*. control group; *$P < 0.05$ *vs*. ethanol induced group. Units: GSH, Vitamin C- μ g/mg protein.

lymphocytic infiltration. Treatment with *n*-hexane extract of *E. sonchifolia* restored the damaged tissue to its normalcy (Figure 1).

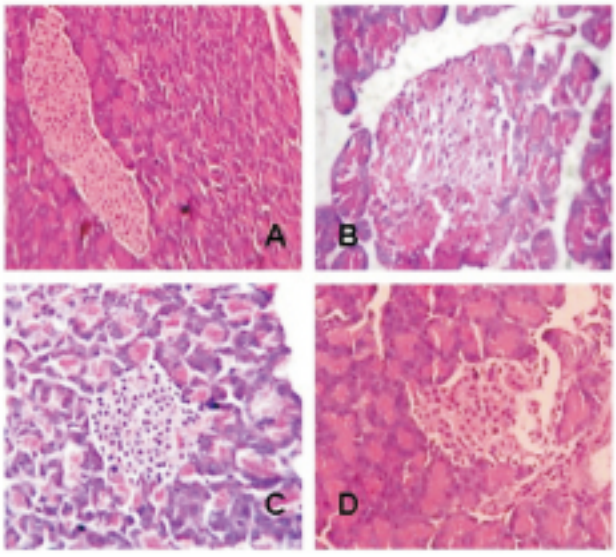

Figure 1. Histopathology of pancreas.
A – Pancreas section of the normal rats (group Ⅰ) showing the normal exocrine acini and endocrine islets;
B – Pancreas section of the ethanol-induced rats (group Ⅱ) showing the congestion of cells and mild lymphocytic infiltration;
C – Pancreas section of the ethanol-induced rats treated with the *n*-hexane extract of *E. soncifolia* (group Ⅲ) showing the absence of congestion and cellularity- reversal of toxic changes;
D – Pancreas section of the *n*-hexane extract of *E. soncifolia* alone (group Ⅳ) treated rats showing the normal exocrine acini and endocrine islets.

4. Discussion

Liver damage is a common problem due to alcohol consumption and ethanol may be responsible for the increment of AST and ALT, important marker enzyme of the liver function test[12]. Various experimental and clinical studies suggest that ethanol produces pancreatic injury in which oxidative stress may play a crucial role in the pathogenesis. Ethanol intake causes accumulation of ROS, like superoxide, hydroxyl radical, and hydrogen peroxide[26] or a decrease in the levels of antioxidant defenses causing a redox imbalance and resulting in the oxidative damage of lipids, proteins, and DNA. These reactive moieties cause lipid peroxidation. Ethanol treatment has been reported to activate ERK1/2, increase COX-2 immunoreactivity, enhance activation of transcription factors like NF-κB or AP-1, and induce rapid lipid peroxidation[4,27]. Alcohol toxicity is caused not only directly by ethanol but also by its metabolic products, including the ROS produced during its biotransformation.

In agreement with the previous reports, the present study also revealed that the administration of ethanol significantly increased the lipid peroxidation, reduced the antioxidant levels and provoked damage to the organs. Biological systems show different mechanisms to protect themselves from the damaging effects caused by activated species. These mechanisms include free radical scavenging, termination of chain reactions and activities of antioxidant enzymes such as SOD, CAT and GPx[28].

SOD, a metalloprotein, is involved in the antioxidant defense mechanism as the first enzyme by lowering the amount of O_2^-. CAT is a hemoprotein, localized in the peroxisomes and induces the decomposition of H_2O_2 to water and oxygen[29]. GPx reduces hydrogen peroxide and other organic peroxides at the expense of GSH, which is in turn oxidized to form glutathione disulfide (GSSG). GSH is regenerated by GR with the consumption of NADPH[30]. A number of research studies have shown evidence that acute ethanol administration decreases hepatic GSH content[31,32]. GSH is the major antioxidative tripeptide in the cell and plays pivotal role in the detoxification of toxicants, metabolism of nutrients and regulation of various pathways to maintain cellular homeostasis[33]. Different enzymatic and non enzymatic reactions could be involved in GSH mediated scavenging of free radicals and other oxygen species. It has been observed that acute and chronic intake of ethanol causes GSH depletion both in time as well as dose dependent fashion[34,35]. Vitamin C may effectively protect against the deleterious effects of ethanol-induced abnormalities. It may also interact with ethanol extracellularly and hence alleviate the overall teratogenic effect of ethanol[36].

Several studies have shown that the activity of antioxidant enzymes is significantly reduced in the animals when exposed to ethanol. All these biochemical alterations are likely to be responsible for ethanol-induced multi-organ damages including brain, liver, esophagus, urinary bladder and pancreas as well as stomach and the persistence or perpetuation of these damages are related to the carcinogenesis of each organ. Previous studies in the rat enteral ethanol model have also shown that alcohol increases free radical formation and oxidative stress in pancreas[37,38-41]. Several *in vivo* studies have indicated that antioxidant treatment by using herbs can prevent or reduce growth retardation and/or the occurrence of malformations in the organs upon ethanol exposure[12,42,43]. Similarly, in the present study treatment with the *n*-hexane extract of *E. sonchifolia* significantly ameliorated the damage caused by ethanol by preventing the elevation of serum AST and ALT and restored its antioxidant levels which may be due to its antioxidant capacity. This is confirmed by the histopathological studies also. The congestion of cells and mild lymphocytic infiltration in the pancreas of the ethanol induced rats were restored to its normalcy when treated with *n*-hexane extract of *E. sonchifolia*. Thus the *n*-hexane extract of *E. sonchifolia* can be effectively used as the therapeutic drug for the management of alcohol-induced oxidative stress.

Conflict of interest statement

We declare that we have no conflict of interest.

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