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Protective effect of *Emilia sonchifolia* on azaserine-induced pancreatic dysplasia

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Abstract

Aim: The present study was undertaken to investigate the effect of *Emilia sonchifolia* on azaserine (O-diazoacetyl-L-serine)-induced pancreatic dysplasia in Wistar albino rats.
Methods: Administration of azaserine [30 mg/kg body weight intraperitoneal (i.p.) weekly for 1 month] to male Wistar albino rats resulted in pancreatic dysplasia, which was evident from the histopathological studies.
Results: A significant decrease of pancreatic and hepatic enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), and non-enzymatic antioxidants like vitamin C, glutathione (GSH) content, and a significant increase in pancreatic serum amylase and lipase, and hepatic marker enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were found. Treatment of rats with the n-hexane extract of *Emilia sonchifolia* for 16 weeks resulted in a concomitant reduction in pancreatic and hepatic damage.
Conclusion: The results suggest that *Emilia sonchifolia* can be used as a therapeutic agent against precancerous lesions which could prevent pancreatic dysplasia.

Keywords: azaserine; dysplasia; *Emilia sonchifolia*; pancreatic cancer

1. Introduction

Pancreatic cancer is one of the most lethal cancers, with most patients dying of their disease within 1 year; it is the fourth leading cause of cancer deaths in the United States. Pancreatic cancer accounts for about 220,000 deaths each year. Smoking, obesity, and diabetes are important and are potentially modifiable risk factors for pancreatic cancer in populations of the Asia-Pacific region. Activities to prevent them can be expected to lead to a major reduction in the number of deaths from this cancer, particularly in Asia with its enormous population.[1]

The only US Food and Drug administration approved therapies for pancreatic cancer, gemcitabine and erlotinib, produce objective responses in <10% of patients. The increased oxidative stress is also highly associated with the induction of pancreatic cancer. Reactive oxygen species (ROS) and other free radicals may contribute to cancer initiation through oxidative damage and nitration of DNA, thus inducing mutations.[2] The enforced expression of antioxidant enzymes has profound effects in altering the malignant phenotype of pancreatic cancer both *in vitro* and *in vivo*.[3]

Azaserine (O-diazoacetyl-L-serine) is a direct-acting bacterial mutagen and a pancreatic carcinogen in Wistar rats. Previous studies have demonstrated that azaserine is

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carcinogenic for the pancreas, liver, and kidney and causes major abnormalities of growth and differentiation of the exocrine pancreas, including adenocarcinoma in some rats.[4]

Lifestyle and/or environmental factors, especially dietary factors, play an important role in influencing cancer risk. A large number of medicinal plants and their active constituents have shown beneficial therapeutic potential against the risk of cancer. For centuries, people have been using herbs for medicinal purposes. In fact, the use of herbal medicine can be traced back at least 5000 years.

Recently, a greater emphasis has been given towards research on complementary and alternative medicine that deals with cancer management. Plants have a long history of use in the treatment of cancer and have been traditionally used as medicines in many countries. Even in the USA, the use of plants and phytomedicine has increased dramatically in the past 2 decades.[5] Medicinal plants are promising sources of potential antioxidants.[6] There is a well-documented association between increased consumption of antioxidants and decreased incidence of cancer.[7] Antioxidants have received increased attention by nutritionists and medical researchers for their potential effects in the prevention of cancer. Therefore, there is an ongoing search for better controlling and preventive methods in order to reduce cancer mortality and related side effects.[8]

Emilia sonchifolia (Asteraceae) is a well-known annual weed seen in most tropical and subtropical regions worldwide. It is an edible plant used in the Ayurvedic system of medicine for the treatment of tumors, inflammation, cough, rheumatism, and wounds.[9] Fresh juice and the methanolic extract of *E. sonchifolia* leaves were found to be potent inhibitors of hydroxyl radical formation and superoxide radical generation *in vitro*.[10] The aqueous and methanol extracts of *E. sonchifolia* leaves progressively reduced rat paw edema.[11] The n-hexane extract of *E. sonchifolia* possesses a good antioxidant activity and the high performance thin layer chromatography determination showed the presence of terpenoids.[12] The present study was undertaken to evaluate the protective effect of *E. sonchifolia* on azaserine-induced pancreatic dysplasia.

2. Methods

2.1. Collection of plant material

E. sonchifolia was collected in September 2009 from Thrissur, Kerala, India. The plant was authenticated by Dr. G.V.S Moorthy, Botanical Survey of India, Tamil Nadu Agricultural University (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.

2.2. Extraction of plant material

Plant powder (500 g) was soaked in n-hexane solvent and kept in the shaker for 48 hours at room temperature. The extract was collected and concentrated at 40°C under reduced pressure using a 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.

2.3. Animals

The male Wistar rats (2–3 weeks old) were procured from the animal house of Karpagam University, Coimbatore. The animals were caged in well ventilated hygienic conditions and were given food and water *ad libitum* during the course of the experiment. The study was approved by the Institutional Animal Ethical Committee (IAEC) constituted for the purpose of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

2.4. Experimental design

The animals were divided into four groups of six rats each: Group I – control, Group II – animals administered with intraperitoneal (i.p.) injection azaserine at a dose of 30 mg/kg body weight, Group III – azaserine + treatment with the n-hexane extract of *E. sonchifolia* (250 mg/kg body weight) continued for a period of 16 weeks, Group IV– the n-hexane extract of *E. sonchifolia* alone (250 mg/kg body weight) for 16 weeks.

2.5. Induction of carcinogenesis in rats

Azaserine (Sigma Chemical Co., St. Louis, MO, USA) dissolved in 0.9% NaCl solution was injected i.p. at a dose of 30 mg/kg body weight once weekly for 4 successive weeks and sacrificed after 16 weeks.

2.6. Determination of serum biochemical parameters

After the experimental period of 16 weeks, the animals were sacrificed under light chloroform anesthesia. Blood was drawn from the paraorbital venous complexes and serum separation tubes, allowed to clot for 30 minutes at room temperature, and then centrifuged at 1000*g* for 10 minutes and stored at 4°C. Biochemical parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), amylase, and lipase were estimated within 6 hours of animal sacrifice. The liver and 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 tissue lipid peroxidation

Lipid peroxidation (LPO)[13] 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. Estimation of antioxidant assays

The enzymatic antioxidants such as superoxide dismutase (SOD),[14] catalase (CAT),[15] glutathione peroxidase (GPx),[16]

glutathione-S-transferase (GST),[17] and the non-enzymatic antioxidants such as reduced glutathione (GSH)[18] and vitamin C were evaluated in the tissue homogenates of the liver and pancreas.

2.9. Statistical analysis

The results obtained were expressed as mean ± standard deviation (SD). The statistical comparisons among the groups were performed using one-way analysis [ANOVA; SPSS version 10.0 (SPSS Inc., Chicago, IL, USA)] at $p < 0.05$ level.

3. Results

3.1. Effects of the n-hexane extract of E. sonchifolia on the serum biochemical parameters of azaserine- induced experimental animals

The effects of the n-hexane extract of *E. sonchifolia* on serum of control and experimental animals are shown in Tables 1 and 2. The serum liver maker enzymes AST, ALT, ALP, and pancreatic enzymes amylase and lipase were found to be significantly ($p < 0.05$) increased in Group II azaserine-induced animals when compared with Group I control animals. Treatment with the n-hexane extract of *E. sonchifolia* (Group III) showed a significant ($p < 0.05$) decrease in serum AST, ALT, ALP, amylase, and lipase levels. There was no significant change between the control Group I and the n-hexane extract of *E. sonchifolia* alone treated Group IV animals.

3.2. Effects of the n-hexane extract of E. sonchifolia in the liver of control and pancreatic cancer induced experimental animals

Pancreatic cancer induction in rats caused a significant ($p < 0.05$) increase in hepatic LPO level and decreased the activities of SOD, CAT, GPx, GST, GSH, and vitamin C compared to control group animals. Administration of the n-hexane extract of *E. sonchifolia* significantly ($p < 0.05$) reduced the LPO reaction in Group III animals and significantly enhanced the enzymatic and non-enzymatic antioxidants (Tables 3 and 4). There was no significant change between the control Group I and the n-hexane extract of *E. sonchifolia* alone treated Group IV animals.

Table 1
Effects of the n-hexane extract of *Emilia sonchifolia* on serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) in control and experimental animals.

Groups	AST[d]	ALT[d]	ALP[d]
Normal control	125.59 ± 0.24[a]	81.71 ± 0.36[a]	158.62 ± 0.26[a]
Azaserine control	256.38 ± 0.34[b]	134.67 ± 0.34[b]	278.58 ± 0.31[b]
Azaserine + plant extract (250 mg/kg)	143.39 ± 0.28[c]	96.45 ± 0.31[c]	178.47 ± 0.41[c]
Plant extract alone (250 mg/kg)	125.43 ± 0.38[a]	81.86 ± 0.16[a]	158.54 ± 0.46[a]

Values are expressed as mean ± standard deviation for six animals.
Units: ALP = μmoles of phenol liberated, ALT, AST = μmoles of pyruvate liberated/L.
[a] Groups I and IV sharing common superscript "a" do not differ significantly at $p < 0.05$ (Duncan Multiple Range Test).
[b,c] Groups II and III with the superscripts "b" and "c", respectively, differ significantly at $p < 0.05$ (Duncan Multiple Range Test).
[d] Values not sharing common superscript letters differ significantly at $p < 0.05$ (Duncan Multiple Range Test).

Table 2
Effects of the n-hexane extract of *Emilia sonchifolia* on the activities of serum amylase and lipase.

Groups	Amylase (U/L)[d]	Lipase (U/L)[d]
Normal control	157.88 ± 0.32[a]	11.35 ± 0.20[a]
Azaserine control	366.24 ± 0.98[b]	45.39 ± 0.45[b]
Azaserine + plant extract (250 mg/kg)	178.27 ± 0.71[c]	20.70 ± 0.50[c]
Plant extract alone (250 mg/kg)	157.69 ± 0.42[a]	11.29 ± 0.32[a]

Values are expressed as mean ± standard deviation for six animals.
Units: amylase = amount of starch in mg hydrolyzed in 20 minutes; lipase = the acid released to neutralize 1 mL of N/10 NaOH.
[a] Groups I and IV sharing common superscript "a" do not differ significantly at $p < 0.05$ (Duncan Multiple Range Test).
[b,c] Groups II and III with the superscripts "b" and "c", respectively, differ significantly at $p < 0.05$ (Duncan Multiple Range Test).
[d] Values not sharing common superscript letters differ significantly at $p < 0.05$ (Duncan Multiple Range Test).

3.3. Effects of the n-hexane extract of E. sonchifolia in the pancreas of control and pancreatic cancer induced experimental animals

The induction of pancreatic dysplasia by azaserine (Group II) caused a significant ($p < 0.05$) decrease in pancreatic antioxidant levels like SOD, CAT, GPx, GST, GSH, and vitamin C compared to control Group I animals. Administration of the n-hexane extract of *E. sonchifolia* (Group III) showed a significant ($p < 0.05$) increase in the pancreatic enzymatic antioxidants SOD, CAT, GPx, GST, and non-enzymatic antioxidants GSH and vitamin C (Tables 5 and 6). However, there was no significant change between the control Group I and the n-hexane extract of *E. sonchifolia* alone treated Group IV animals.

3.4. Histopathological studies

Histopathological examination of the pancreatic section of rats treated with azaserine (Group II) showed atypical acini in increased numbers (2–5 per acini). These acini revealed enlargement of cells, nuclear enlargement with nuclear pleomorphism, and hyperchromasia. Moderate to severe dysplasia was found in scattered acini. Group III rats treated with the n-hexane extract of *E. sonchifolia* showed a lower number of atypical acini. Groups I and IV showed normal pancreatic acinar cells (Fig. 1).

Histopathological examination of the liver section of the rats treated with azaserine (Group II) showed pleomorphic hepatocytes having larger nuclei. The cells were arranged in a trabecular pattern in some places. Stroma showed extensive

Table 3
Effects of the n-hexane extract of *Emilia sonchifolia* on the activities of enzymatic antioxidants in liver of control and experimental animals.

Particulars	LPO	SOD	CAT	GPx	GST
Normal control	1.31 ± 0.05[a]	8.84 ± 0.06[a]	72.55 ± 0.34[a]	7.46 ± 0.26[a]	9.83 ± 0.12[a]
Azaserine control	4.16 ± 0.26[b]	3.27 ± 0.11[b]	34.53 ± 0.46[b]	2.45 ± 0.26[b]	3.76 ± 0.21[b]
Azaserine + plant extract (250 mg/kg)	1.75 ± 0.23[c]	7.61 ± 0.32[c]	69.21 ± 0.23[c]	6.42 ± 0.30[c]	8.21 ± 0.05[c]
Plant extract alone (250 mg/kg)	1.30 ± 0.10[a]	8.92 ± 0.03[a]	72.74 ± 0.32[a]	7.41 ± 0.27[a]	9.81 ± 0.25[a]

Values are expressed as mean ± SD for six animals.
CAT = catalase; GPx = glutathione peroxidase; GST = glutathione-S-transferase; LPO = lipid peroxidation; SOD = superoxide dismutase.
Units: CAT = μmol of H_2O_2 consumed/minute/mg protein; GPx = μmol of glutathione oxidized/minute/mg protein; GST = μmole of glutathione utilized/minute/mg protein; LPO = nmoles/mg protein; SOD = inhibition of 50% nitrite formation/minute/mg protein.
[a] Groups I and IV sharing common superscript "a" do not differ significantly at $p < 0.05$ (Duncan Multiple Range Test).
[b,c] Groups II and III with the superscripts "b" and "c", respectively, differ significantly at $p < 0.05$ (Duncan Multiple Range Test).

areas of necrosis. Sinusoidal spaces were congested and central veins dilated. Group III rats treated with the n-hexane extract of *E. sonchifolia* showed normal triads and a central venous system. Hepatocytes appeared normal. Sinusoidal spaces also appeared normal. A few foci of necrosis were seen in the parenchyma. Groups I and IV showed normal liver sections (Fig. 2).

4. Discussion

The major finding of the present study was the demonstration for the first time that the n-hexane extract of *E. sonchifolia* significantly reduced the extent of pancreatic dysplasia induced by azaserine. Azaserine is a carcinogen in rats and causes major abnormalities of growth and differentiation of the exocrine pancreas, including adenocarcinoma in some rats.[4] In the present study, induction of pancreatic cancer by azaserine caused exocrine pancreatic damage and hepatic lesions.

The liver and pancreas are intimately associated with the proper functioning of the digestive tract. The liver plays an important role in the modulation of the process of carcinogenesis, as it is the primary site for the biotransformation of xenobiotics including carcinogens, as well as anticancer drugs.[19] Furthermore, a statistically significant appearance of pancreatic and liver damage was observed in pancreatic cancer patients. The pancreas produces amylase and lipase in large quantities and secretes them into the small intestine. Diseases of the pancreas most commonly cause elevated amylase and lipase. The increase of serum amylase and lipase were found in pancreas abnormalities.[20] An increase in serum pancreatic enzymes may also be directly associated with hepatic damage and a reduced clearance of pancreatic enzymes by the liver reticuloendothelial system, as postulated for advanced liver diseases. Liver damage is also suspected to play a role in inducing pancreatic hyperenzymemia. It is thought that circulating pancreatic enzymes are removed by the reticuloendothelial system in the body, and the liver is suspected to be a major organ for amylase removal.[21] Administration of the n-hexane extract of *E. sonchifolia* significantly reduced the activity of liver and pancreatic enzymes in azaserine-induced rats.

A marked elevation in the levels of serum AST, ALT, and ALP, which is indicative of hepatocellular damage, was found in Group II rats. This elevation could potentially be attributed to the release of these enzymes from the cytoplasm into the blood circulation after rupture of the plasma membrane and cellular damage of the liver.[22] The increase in ALT and AST activity suggests a hepatic origin. They are liberated only when hepatocytes are destroyed, thus reflecting more serious hepatic disease. By contrast, the highest concentration of ALP is found in the intestine, liver, spleen, bone, and kidney. Thus, the significant levels of AST, ALT, and ALP levels confirmed the liver injury. Impaired hepatocytes were thought to be the main reason for the increased ALT, AST, and ALP activity.[23]

ROS and other free radicals may contribute to cancer initiation through oxidative damage and nitration of DNA, thus inducing mutations.[2] In areas with tissue damage, inflammatory responses could send survival and proliferative signals to cells repopulating the damaged area, leading to the promotion of the growth of healthy tissues, as well as tumors.[24] There are data suggesting that the acinar cell damage is followed within minutes by the production of ROS. The primary free radical production may exacerbate the cell damage, causing lesions of cell membranes and cytoskeleton, impairing functions of intracellular proteins, damaging DNA, evoking LPO, and decreasing the level of antioxidants.[25]

Table 4
Effects of the n-hexane extract of *Emilia sonchifolia* on the activities of non-enzymatic antioxidants in liver of control and experimental animals.

Particulars	Glutathione (GSH)[d]	Vitamin C[d]
Normal control	48.41 ± 0.19[a]	1.56 ± 0.03[a]
Azaserine control	21.52 ± 0.31[b]	0.72 ± 0.02[b]
Azaserine + plant extract (250 mg/kg)	39.48 ± 0.40[c]	1.38 ± 0.007[c]
Plant extract alone (250 mg/kg)	48.57 ± 0.43[a]	1.58 ± 0.02[a]

Values are expressed as mean ± SD for six animals.
Units: GSH, vitamin C = μg/mg protein.
[a] Groups I and IV sharing common superscript "a" do not differ significantly at $p < 0.05$ (Duncan Multiple Range Test).
[b,c] Groups II and III with the superscripts "b" and "c", respectively, differ significantly at $p < 0.05$ (Duncan Multiple Range Test).
[d] Values not sharing common superscript letters differ significantly at $p < 0.05$ (Duncan Multiple Range Test).

Table 5
Effects of the n-hexane extract of *Emilia sonchifolia* on the activities of enzymatic antioxidants in pancreas of control and experimental animals.

Particulars	LPO	SOD	CAT	GPx	GST
Normal control	1.35 ± 0.02[a]	6.47 ± 0.28[a]	15.57 ± 0.36[a]	7.59 ± 0.37[a]	6.44 ± 0.34[a]
Azaserine control	4.18 ± 0.07[b]	2.51 ± 0.26[b]	6.37 ± 0.34[b]	3.46 ± 0.32[b]	3.91 ± 0.06[b]
Azaserine + plant extract (250 mg/kg)	1.81 ± 0.07[c]	5.91 ± 0.05[c]	13.68 ± 0.27[c]	6.61 ± 0.33[c]	5.92 ± 0.10[c]
Plant extract alone (250 mg/kg)	1.34 ± 0.02[a]	6.63 ± 0.31[a]	15.59 ± 0.25[a]	7.62 ± 0.33[a]	6.53 ± 0.40[a]

Values are expressed as mean ± SD for six animals.
CAT = catalase; GPx = glutathione peroxidase; GST = glutathione-S-transferase; LPO = lipid peroxidation; SOD = superoxide dismutase.
Units: CAT = μmol of H_2O_2 consumed/minute/mg protein; GPx = μmol of glutathione oxidized/minute/mg protein; GST = μmole of glutathione utilized/minute/mg protein; LPO = nmoles/mg protein; SOD = inhibition of 50% nitrite formation/minute/mg protein.
[a] Groups I and IV sharing common superscript "a" do not differ significantly at $p < 0.05$ (Duncan Multiple Range Test).
[b,c] Groups II and III with the superscripts "b" and "c", respectively, differ significantly at $p < 0.05$ (Duncan Multiple Range Test).

LPO may lead to the formation of several toxic products such as 4-hydroxynoneal and MDA, which can attack cellular targets including DNA, inducing mutagenicity and carcinogenicity.[26] Thus, enhanced LPO and failure of antioxidant defense mechanisms lead to tissue damage. The increased levels of LPO in the pancreas and liver of Group II cancer-bearing animals may be due to the formation of free radicals generated by azaserine. The observed significant reduction in LPO in the n-hexane extract of *E. sonchifolia* was presumably due to its antioxidant capacity, which has the ability to scavenge the free radicals.

SOD plays an important role in protecting the cells from oxidative damage, by converting superoxide radicals into hydrogen peroxide, which is further metabolized by CAT to molecular oxygen and water. The increased superoxide radical levels in tumor cells, as compared with normal cells, may explain the decrease of the enzyme activity in malignant tissues compared to normal tissues.[27] Meanwhile, superoxide radicals can increase LPO, thus increasing the generation of reactive oxygen intermediates and MDA.[28] The superoxide radical has been shown to directly inhibit the activity of CAT. Likewise, singlet oxygen and peroxyl radicals have been shown to inhibit CAT activity.[29] In the present study, decreased activity of SOD and CAT was observed in the pancreas and liver of cancer-bearing animals, and this decrease was antagonized when the n-hexane extract of *E. sonchifolia* was administered.

In the tissues, GPx is a major enzymatic component for the disposal of peroxides, and a prolonged depression in the activity of this enzyme may lead to intracellular peroxide accumulation. GST maximizes the conjugation of free radicals and various lipid hydroperoxides to GSH to form water-soluble products that can be easily excreted out. Depletion in GSH levels to the extent observed in this study could further lead to a drastic decrease in the total antioxidant status of the body of the animals. This is because GSH helps to recycle vitamins C and E (cellular antioxidants), blocks free radical damage, enhances the antioxidant activity of vitamin C, and plays a critical role in the detoxification of harmful compounds.[30] Vitamin C is water-soluble and also has an additional role in protecting or regenerating oxidized carotenoids or tocopherols.[31] The decreased activity of GPx, GST, GSH, and vitamin C in the pancreas and liver of cancer-bearing animals may be due to excessive oxidative damage. Oxidative stress-induced tissue damage can be prevented or ameliorated by favoring the balance towards a lower oxidative stress status. These alterations were significantly reversed towards normal levels in the animals treated with the n-hexane extract of *E. sonchifolia*. The biochemical findings were also supported by histopathological studies, which showed the pancreatic and hepatic damage. Treatment with the n-hexane extract of *E. sonchifolia* restored the damaged tissue to its normalcy.

Antioxidants are potent scavengers of free radicals and serve as inhibitors of neoplastic processes.[32] Cancer chemoprevention using antioxidant approaches has been suggested to offer a good potential in providing important fundamental benefits to public health, and is now considered by many clinicians and researchers as a key strategy for inhibiting, delaying, or even reversal of the process of carcinogenesis.[33] Therefore, the anticancer activity of the n-hexane extract of *E. sonchifolia* might be due to its active components present in it which can enhance the antioxidant activity of *E. sonchifolia*.

In conclusion, from these observations, it was suggested that suppression of azaserine-induced pancreatic dysplasia and liver damage in rats may be due to the antioxidant activity of *E. sonchifolia*. Further, the potential ability of the active constituents of *E. sonchifolia* against pancreatic dysplasia is under progress in our laboratory.

Table 6
Effects of the n-hexane extract of *Emilia sonchifolia* on the activities of non-enzymatic antioxidants in pancreas of control and experimental animals.

Particulars	Glutathione (GSH)[d]	Vitamin C[d]
Normal control	12.58 ± 0.22[a]	1.44 ± 0.02[a]
Azaserine control	7.47 ± 0.37[b]	0.65 ± 0.15[b]
Azaserine + plant extract (250 mg/kg)	11.55 ± 0.24[c]	1.23 ± 0.02[c]
Plant extract alone (250 mg/kg)	12.70 ± 0.11[a]	1.45 ± 0.03[a]

Values are expressed as mean ± SD for six animals.
Units: GSH, vitamin C = μg/mg protein.
[a] Groups I and IV sharing common superscript "a" do not differ significantly at $p < 0.05$ (Duncan Multiple Range Test).
[b,c] Groups II and III with the superscripts "b" and "c", respectively, differ significantly at $p < 0.05$ (Duncan Multiple Range Test).
[d] Values not sharing common superscript letters differ significantly at $p < 0.05$ (Duncan Multiple Range Test).

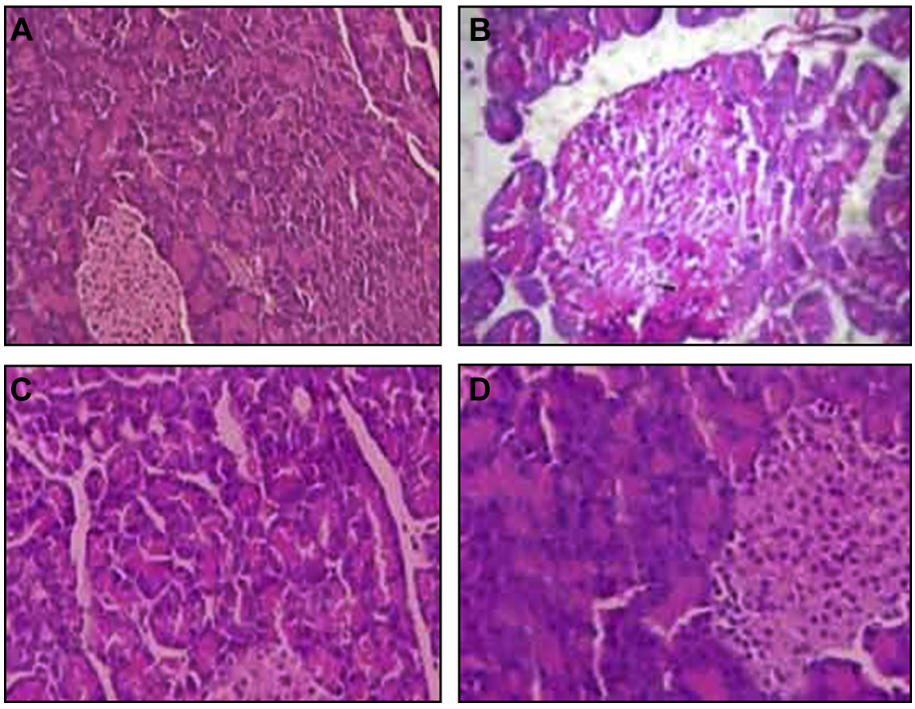

Fig. 1. Histopathology of the pancreas. (A) Control rats (Group I) shows selection of normal pancreas acini. (B) Azaserine-injected rats (Group II) shows atypical acini in increased numbers. The acini reveal enlargement of cells, nuclear enlargement with nuclear pleomorphism and hyperchromasia. Moderate to severe dysplasia is seen. (C) Azaserine-injected rats treated with *n*-hexane extract of *Emilia sonchifolia* (Group III) show atypical acini in increased numbers. (D) *n*-hexane extract of *Emilia sonchifolia*-only-treated rats (Group IV) shows a section of normal pancreas.

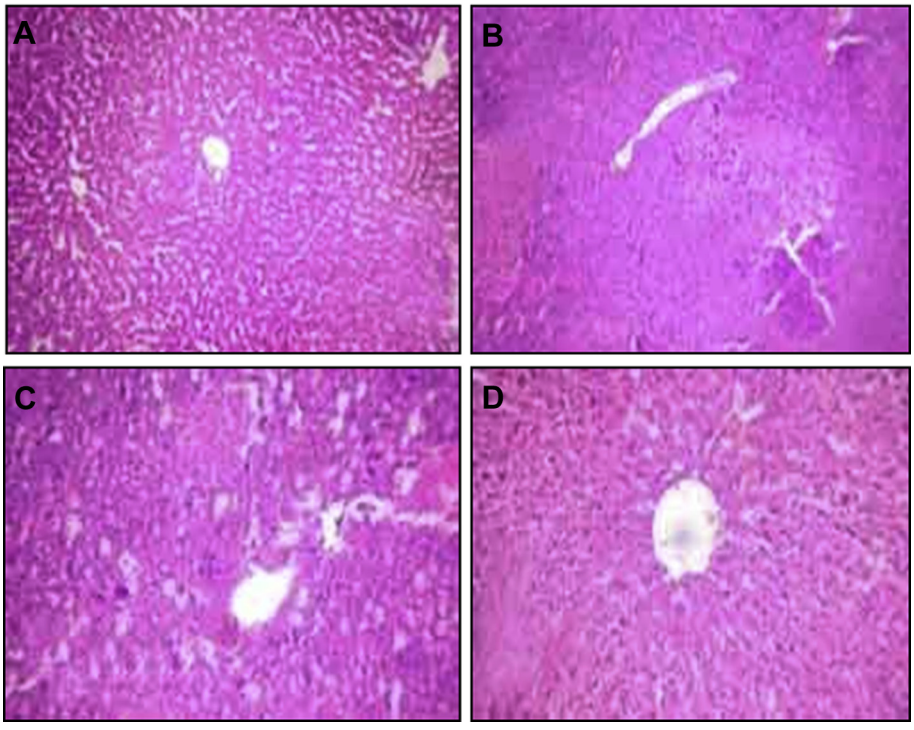

Fig. 2. Histopathology of the liver. Control rats (Group I) shows normal liver sections. (B) Azaserine-injected rats (Group II) shows pleomorphic hepatocytes having larger nuclei. The cells are arranged in a trabecular pattern in some places. Stroma show extensive areas of necrosis. Sinusoidal spaces are congested and central veins are dilated. (C) Azaserine-injected rats treated with *n*-hexane extract of *Emilia sonchifolia* (Group III) show normal triads and central venous system. Hepatocytes and sinusoidal spaces appear normal. Only a few foci of necrosis are seen in the parenchyma. (D) *n*-hexane extract of *Emilia sonchifolia*-only-treated rats (Group IV) shows a normal liver section.

Conflicts of interest

All authors declare no conflicts of interest.

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