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Research Article

Studies on Chemoprotective Activity of Cinnamic Acid (CA), Hexanedioic Acid (HAD) on Azaserine Induced Pancreatic Cancer in Wistar Rats

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Abstract

Aimed to investigate the effect of Cinnamic Acid (CA) and Hexanedioic Acid (HDA) on preventing pancreatic cancer in male albino rats induced by azaserine. The rats were divided into 6 groups, with rats in the first group serving as a control. The rats in groups 2-6 were injected with azaserine once a week for 3 weeks. Additionally, groups 3-4 received CA at doses of 20 mg/kg and 40 mg/kg, respectively, while groups 5-6 were given HDA at doses of 20 mg/kg and 40 mg/kg, respectively, throughout the experimental period. At the end of the 3 weeks, the animals were sacrificed and their blood parameters and liver enzymes were measured. The results showed that the treated rats had normalized blood parameters, reduced liver enzymes (SGOT, SGPT and ALP) and GGT, LDH and bilirubin, reduced digestive enzymes (amylase and lipase), and increased activities of enzymatic antioxidants (SOD, CAT, GR) and decreased MDA, PCC. Moreover, TNF alpha and IL 6 were further decreased while IL 10 was enhanced in Azaserine-induced pancreatic cancer. Our findings indicate that CA and HDA administration inhibits pancreatic cancer by modulating lipid peroxidation and antioxidant status, as well as preventing azaserine-induced histopathological changes. Furthermore, Cinnamic acid and Hexanedioic acid can act as chemopreventive agents for pancreatic cancer.

Keywords: Azaserine; Pancreatic cancer; Cinnamic acid; Hexanedioic acid

Introduction

Pancreatic cancer is a highly lethal form of cancer that affects people worldwide. There are 3 types of pancreatic cancer, the most common type being Pancreatic Ductal Adenocarcinoma (PDAC), which develops from the duct cells of exocrine tissue and accounts for over 90% of cases. Another rare subtype is adenosquamous carcinomas, which exhibit both adenocarcinoma and squamous carcinoma characteristics and account for only 1%-4% of cases. The remaining types are acinar and neuroendocrine tumours. In 2020, there were more than 495,773 new cases of pancreatic cancer and around 466,000 deaths from it according to Globocan estimates. Unfortunately, pancreatic cancer is difficult to diagnose in its early stages as it often does not cause symptoms. However, common symptoms include tummy or back pain, weight loss, indigestion, loss of appetite, Diarrhea, Constipation, Jaundice, Blood clots, and Fatigue. These symptoms can have many causes and are unlikely to be due to pancreatic cancer, but it's important to seek medical attention if you experience them.

There are several drugs that have been approved for pancreatic cancer treatment, including Fluorouracil (5-FU), Capecitabin, Gemcitabine, Erlotinib, Leucovorin, Irinotecan, Nab-paclitaxel, nanoliposomal irinotecan, and Oxaliplatin. Additionally, there are targeted treatments that focus on specific genes, proteins, or tissue environments. For instance, Erlotinib blocks the Epidermal Growth Factor Receptor (EGFR), Olaparib influences a hereditary BRCA mutation, and Larotrectinib can be used for NTRK fusion [1,2].

There are several plant-derived anti-cancer drugs that are used to treat various types of cancer, including breast cancer, stomach cancer, prostate cancer, ovarian cancer, lung cancer, Hodgkin's lymphoma, non-Hodgkin's lymphoma, brain cancer, testicular cancer, bladder cancer, melanoma, leukemia, thyroid cancer, neuroblastoma, multiple myeloma, chronic myeloid leukemia, and more. Some examples of these drugs include docetaxel, paclitaxel, vinblastine, camptothecin, vincristine, and homoharringtonine. In fact, around 60% of chemotherapeutics used to treat Pancreatic Ductal Adenocarcinoma (PDAC) are plant-derived. Paclitaxel, in

particular, is used to treat PDAC [3]. Additionally, plant products and their phytochemicals have been shown to increase drug sensitivity and combat resistance during treatment. As such, there is growing interest in exploring plant products and phytochemicals as potential solutions for PDAC. This study focuses on highlighting the use of plant products and phytochemicals in the fight against PDAC in the hopes of finding a sustainable solution to this persistent problem.

Materials and Methods

The study was conducted on adult healthy Wistar rats. Healthy adult Wistar rats (either sex) aged 8 weeks-12 weeks were procured from the Mahaveer enterprises, Hyderabad. All the protocols as per the Committee for Control and Supervision of Experiments on Animals (CPCSEA) guidelines on the care and use of Laboratory animals were followed and approved by the Institutional Animal Ethics Committee. Rats were kept under constant observation during the entire period of study. Cinnamic acid (W228826); Hexanedioic acid (09582) procured from Merck.

Tumour induction Azaserine was dissolved in 0.9 Nacl solutions. The rats were given a weekly intraperitoneal injection of azaserine at a dose of 5 mg/kg body weight for 3 weeks.

The rats were divided into 6 groups, with rats in the first group serving as a control. The rats in groups 2-6 were injected with azaserine once a week for 3 weeks. Additionally, groups 3-4 received CA at doses of 20 mg/kg and 40 mg/kg, respectively, while groups 5-6 were given HDA at doses of 20 mg/kg and 40 mg/kg, respectively, throughout the experimental period. At the end of the 3 weeks, the animals were sacrificed and their blood parameters and liver enzymes were measured.

Blood/serum samples

After 21 days of dosing period, on the 22nd day blood samples were collected from all the animals by retro-orbital plexuses puncture under light ether anesthesia with the help of a capillary tube. Blood samples collected in K3EDTA test tubes were utilized for hematological evaluation, whereas blood samples collected in centrifuge tubes without anticoagulant were allowed to clot at room temperature (26°C ± 2°C). Serum was harvested by centrifugation at 3000 rpm for 10 minutes, stored at -40°C for biochemical analysis and analyzed within 12 h.

Collection of tissues

After the collection of blood samples on the 22nd day, all the rats were sacrificed by cervical dislocation. All sacrificed animals were subjected to post-mortem examination to determine the presence/absence of gross and histopathological lesions. Detailed post-mortem lesions from all the animals were recorded. Tissue samples viz., pancreas and liver were collected and preserved in 10% formalin solution for histopathological examination.

Biochemical assessments

The complete blood count was analyzed using a blood analyzer. Liver enzymes, digestive enzymes, and glucose were analyzed using semi-autoanalyzer kits. To determine lipid peroxidation, the level of Thiobarbituric Acid Reactive Substances (TBARS) in tissues was measured using the method described by H. Ohkawa et al., (1979). The pink chromogen produced by the reaction of thiobarbituric acid with malondialdehyde, a secondary product of lipid peroxidation, was measured at 532 nm. The content of reduced glutathione (GSH) in tissue was determined using the Ellman method. GSH determination is based on the development of a yellow color when 5, 5′Dithio 2-Nitro Benzoic Acid (DTNB) is added to compounds containing sulfhydryl groups. A known amount of enzyme preparation was incubated with H_2O_2 in the presence of GSH for a specified time period. The amount of H_2O_2 was determined using the method described by G.L. Ellman et al., (1959). The values are expressed as µ moles of GSH utilized per minute per milligram of protein. Superoxide dismutase was assayed using the method described by P. Kakkar et al., (1984) based on the 50% inhibition of the formation of NADH-phenazine methosulfate nitroblue tetrazolium formazan at 520 nm [4-6]. One unit of the enzyme was taken as the amount required for 50% inhibition of NBT reduction per minute per milligram of protein. The activity of catalase was determined using the method described by A.K. Sinha et al., (1972). Dichromate in acetic acid was reduced to chromic acetate when heated in the presence of hydrogen peroxide (H_2O_2), with the formation of perchromic acid as an unstable catalase intermediate. The chromic acetate formed was measured at 590 nm. Catalase was allowed to split H_2O_2 for different periods of time. The reaction was stopped at different time intervals by adding a dichromate acetic acid mixture, and heating the reaction mixture. The remaining H_2O_2 was then measured colorimetrically and the values were expressed as µ moles of H_2O_2 utilized per minute per milligram of protein. TNF alpha, Il6, and Il10 were estimated using ELISA kits [7].

Histopathology Part of the tissue was immediately fixed in 10% formalin for 24 hours, then the tissue was cut open at the antimesentric border and embedded on paraffin wax 3 µm-5 µm sections, were sliced, stained with hematoxylin and eosin and the cell morphology as a whole was studied, following the standard micro technique (Figures 1 and 2).

Results and Discussion

In this research, scientists for the first time discovered azaserine (O-diazoacetyl-L-serine) from fungal cultures of Streptomyces species. Azaserine is a well-known inhibitor of purine ribonucleotide biosynthesis and is used as a reliable model for carcinogenesis. This toxic, mutagenic, and carcinogenic compound is believed to cause cancer by inhibiting enzymes that are involved in DNA synthesis. Researchers use the azaserine-rat model to study neoplastic developments from the early stages [8]. In this study, the induction of pancreatic cancer by azaserine resulted in

exocrine pancreatic damage and hepatic lesions.

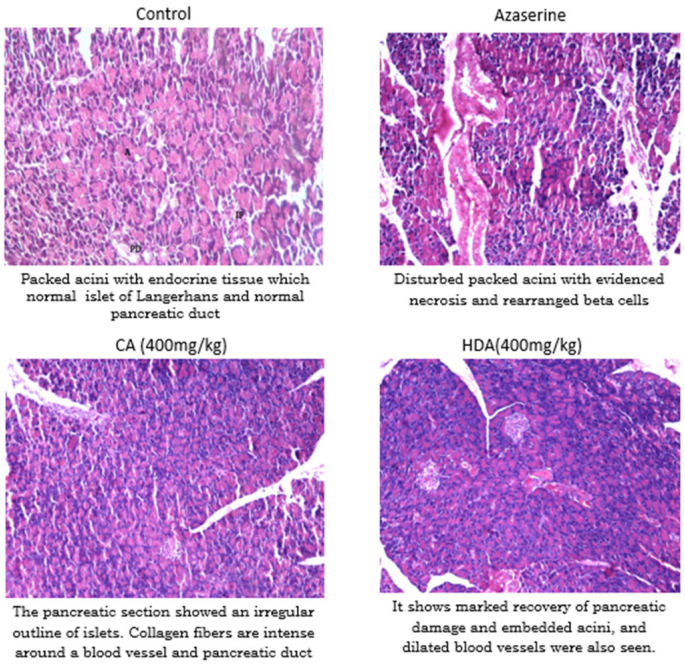

Figure 1: Histopathology of pancreas in animals treated with CA and HDA in azaserine induced pancreatic cancer

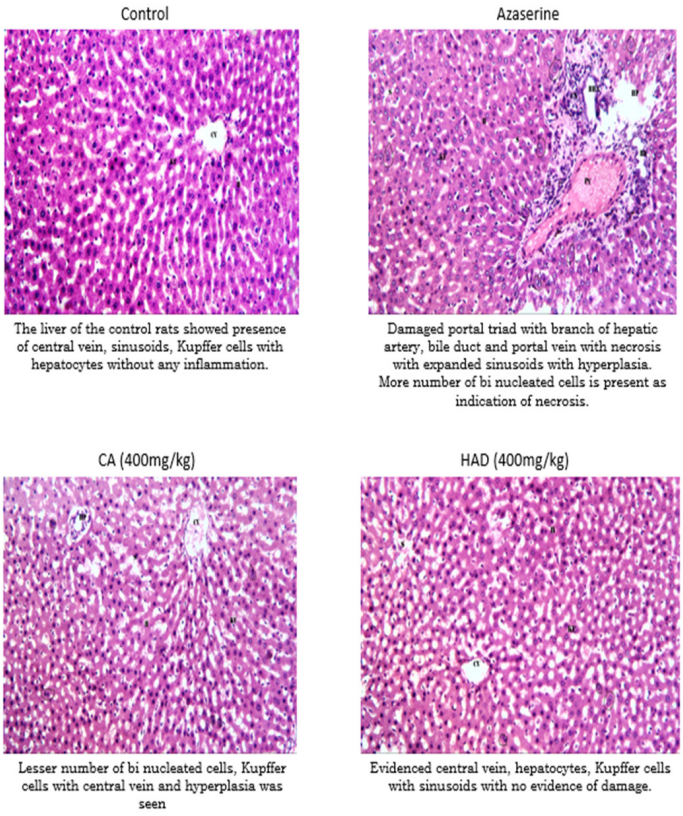

Figure 2: Histopathology of liver in animals treated with CA and HDA in azaserine induced pancreatic cancer

The Complete Blood Count (CBC) is a test that measures the levels of 3 types of blood cells: Red blood cells, white blood cells, and platelets. Chemotherapy and radiation exposure can significantly reduce blood cell levels, increasing the risk of infection [9]. The CBC test, particularly the lymphocyte count, can reflect the response of cellular immunity in cancer patients, and changes in hematological parameters can influence disease progression. Hemoglobin (Hb) and Packed Cell Volume (PCV) are indirectly associated with an increased risk of cardiac failure in cancer patients, as

well as fatigue and bleeding. An elevated Total Leukocyte Count (TLC) predicts a poorer prognosis, while the white blood cell count (total and differentials) and platelet count can predict disease severity and mortality risk.

The Mean Corpuscular Volume (MCV) is a laboratory measurement that determines the average size and volume of red blood cells. It can be useful in identifying the cause of anaemia. To calculate the MCV value, you multiply the haematocrit percentage by ten and divide that number by the erythrocyte count. An increased MCV value is linked to factors such as aging, nutrition, and alcohol abuse. It is also recognized as a predictor of survival in chronically ill patients [10]. A high MCV indicates that the red blood cells are larger than normal. Although there are many common causes for a high MCV, it can also be a warning sign of myelodysplastic syndromes, a group of blood cancers. The levels of CBC were observed to be affected in pancreatic cancer induced by Azaserine. This resulted in decreased levels of Hb, TLC, TRC, and PCV, and increased levels of MCV and MCHC. However, with the use of CA and HDA in varying doses, the levels were significantly restored to normal (Table 1).

Table 1: Effect of Cinnamic Acid (CA), Hexanedioic acid (HDA) on hematological parameters in Azaserine-induced pancreatic cancer

Groups/Parameter	Haemoglobin (g/dL)	Total Leukocyte Count (109/L)	Total Erythrocyte Count (1012/L)
Vehicle Control	13.47 ± 0.22***	7.27 ± 0.23***	8.01 ± 0.09***
Azaserine Control	10.68 ± 0.35	9.44 ± 0.36	7.24 ± 0.08
CA (20mg/kg) bd.wt+Azaserine	12.09 ± 0.42*	9.23 ± 0.16ns	7.52 ± 0.07*
CA (40mg/kg) bd.wt+Azaserine	14.23 ± 0.39***	7.62 ± 0.25***	7.92 ± 0.03***
HDA (20mg/kg) bd.wt+Azaserine	11.42 ± 0.37ns	8.47 ± 0.11*	7.46 ± 0.05ns
HDA (40mg/kg) bd.wt+Azaserine	12.78 ± 0.41**	7.91 ± 0.19**	7.77 ± 0.07***
All values are expressed as Mean ± S.E.M. Statistical comparisons were made by using One way ANOVA followed by Dunnett's multiple comparison test using Graph pad prism version 8 and found significantly different at p>0.05ns, *p<0.05, **p<0.01, ***p<0.001 when compared to disease control			

Asynchronous liver metastasis occurs when liver metastases are detected at the same time as the initial diagnosis of PDAC, while metachronous liver metastasis occurs when metastases are found during follow-up imaging exams, regardless of the time after the initial diagnosis of PDAC. Elevated levels of AST and ALT, as well as increased CA19-9 levels, may increase the risk of synchronous liver metastasis in PDAC patients [11]. The AST/ALT ratio has been identified as an adverse prognostic marker in PDAC. Doctors may measure liver enzymes and bilirubin levels to monitor liver function in patients with pancreatic cancer, as elevated bilirubin levels are common in this disease. If it is possible for a solid malignant tumor to cause an increased level of ALP among pancreatic cancer patients, especially those who have undergone surgical removal of the tumor. This increased level of ALP can be associated with lymph node involvement, leading to a higher risk of rapid relapse and progression of the disease [12]. One way to detect pancreatic cancer is by measuring a specific protein in the blood, which can also be present in non-cancerous conditions. This protein is different from CA19-9 and is often associated with pancreatic cancer. The induction of Azaserine results in significant damage to the liver cells, as evidenced by increased levels of liver enzymes (SGOT, SGPT, and AKP) in the bloodstream. However, when rats were treated with CA and HDA after induction, there was a significant decrease in their circulatory liver enzyme levels (Table 2).

Table 2: Effect of Cinnamic Acid (CA), Hexanedioic Acid (HDA) on haematological parameters in Azaserine-induced pancreatic cancer

Groups/Parameter	MCV (fL)	MCHC (g/dL)	Packed Cell Volume (%)
Vehicle control	56.71 ± 0.05**	30.33 ± 0.06***	43.12 ± 0.09***
Azaserine control	57.98 ± 0.06	32.87 ± 0.02	38.87 ± 0.05
CA (20 mg/kg) bd.wt+Azaserine	57.89 ± 0.04ns	31.86 ± 0.09**	40.85 ± 0.08**
CA (40 mg/kg) bd.wt+Azaserine	55.72 ± 0.05***	30.42 ± 0.05***	42.99 ± 0.04***
HDA (20 mg/kg) bd.wt+Azaserine	56.76 ± 0.01**	31.87 ± 0.08**	40.88 ± 0.06**
HDA (40 mg/kg) bd.wt+Azaserine	56.98 ± 0.09**	31.12 ± 0.07**	43.35 ± 0.07***
All values are expressed as Mean ± S.E.M. Statistical comparisons were made by using One way ANOVA followed by Dunnett's multiple comparison test using Graph pad prism version 8 and found significantly different at p>0.05ns, *p< 0.05, **p< 0.01, ***p<0.001 when compared to disease control			

Cinnamic acid has various mechanisms of action, such as stimulating insulin secretion, improving pancreatic β-cell functionality, inhibiting hepatic gluconeogenesis, enhancing glucose uptake, increasing insulin signalling pathway, delaying carbohydrate digestion and glucose absorption, and inhibiting protein glycation and insulin fibrillation [13]. In HepG2 cells, CA treatment significantly reduced lipid accumulation induced by OA and in db/db mice, it significantly reduced TG content in a dose-dependent manner. In rats with gentamycin-induced liver dysfunction, CA treatment significantly reduced the serum activities of SGOT, SGPT, and AKP levels, and

attenuated oxidative stress by decreasing MDA and oxide and increasing CAT and GPX [14].

The elevated GGT levels have been linked to decreased survival in unresectable PDAC patients. It is worth noting that inflammation-related oxidative stress and inflammatory cytokines like tumor necrosis factor-alpha can cause elevated serum GGT levels [15]. Furthermore, GGT may directly contribute to cancer progression and metastasis, as studies on melanoma cells have shown that increased GGT activity results in growth advantages both *in vitro* and *in vivo* [16]. The LDHA plays a crucial role in the last step of aerobic glycolysis and is connected to the advancement of tumors. When LDHA is overexpressed, it boosts the growth of pancreatic cancer cells. However, reducing the expression of LDHA can significantly hinder cell growth [17]. Cancer patients with high levels of LDH in their serum are more likely to have metastases and a worse prognosis. Elevated serum LDH concentrations are linked to a shorter survival rate in patients with several solid tumors, including gastrointestinal and lung cancers. The CA and HDA on LDH, GGT, and bilirubin levels, which were observed to decrease significantly in rats with pancreatic cancer induced by Azaserine.

Pancreatic amylase plays a crucial role in digesting carbohydrates, converting them into glucose-a small molecule that provides energy to the body. Meanwhile, pancreatic lipase breaks down fat globules, producing fatty acids and glycerol which are energy-dense molecules that are utilized by all cells. Fatty acids and glycerol are transported throughout the body *via* blood and lymph vessels [18]. For some people, the first indication of pancreatic cancer is diabetes. About 1 in 4 people with pancreatic cancer develop diabetes within 6 months to 36 months before being diagnosed with pancreatic cancer [19]. The high blood sugar caused by insulin resistance and the inability to suppress liver glucose release can boost proliferation and promote epithelial-mesenchymal transition, cancer stem cell properties, and metastatic potential in pancreatic cancer [20]. Pancreatic cancer induction agents, such as Azaserine, affect both the exocrine and endocrine portions of the pancreas. The use of CA and HDA treatment protected the pancreas of rats induced with pancreatic cancer, as evidenced by the enhanced serum levels of amylase, lipase, and glucose presented in Tables 3-8.

Table 3: Effect of Cinnamic Acid (CA), Hexanedioic Acid (HDA) on liver biochemical parameters in Azaserine-induced pancreatic cancer

Groups/Parameter	MCV (fL)	MCHC (g/dL)	Packed Cell Volume (%)
Vehicle control	56.71 ± 0.05**	30.33 ± 0.06***	43.12 ± 0.09***
Azaserine control	57.98 ± 0.06	32.87 ± 0.02	38.87 ± 0.05
CA (20 mg/kg) bd.wt+Azaserine	57.89 ± 0.04ns	31.86 ± 0.09**	40.85 ± 0.08**
CA (40 mg/kg) bd.wt+Azaserine	55.72 ± 0.05***	30.42 ± 0.05***	42.99 ± 0.04***
HDA (20 mg/kg) bd.wt+Azaserine	56.76 ± 0.01**	31.87 ± 0.08**	40.88 ± 0.06**
HDA (40 mg/kg) bd.wt+Azaserine	56.98 ± 0.09**	31.12 ± 0.07**	43.35 ± 0.07***
All values are expressed as Mean ± S.E.M. Statistical comparisons were made by using One way ANOVA followed by Dunnett's multiple comparison test using Graph pad prism version 8 and found significantly different at p>0.05ns, *p<0.05, **p<0.01, ***p<0.001 when compared to disease control			

Table 4: Effect of Cinnamic Acid (CA), Hexanedioic Acid (HDA) on biochemical analysis in Azaserine-induced pancreatic cancer

Groups/Parameter	GGT	LDH	Bilirubin
Vehicle control	2.45 ± 0.08***	342.78 ± 4.23***	0.38 ± 0.02***
Azaserine control	5.01 ± 0.04	477.12 ± 3.76	0.52 ± 0.04
CA (20 mg/kg) bd.wt+Azaserine	4.23 ± 0.09***	421.09 ± 5.12***	0.46 ± 0.05ns
CA (40 mg/kg) bd.wt+Azaserine	2.56 ± 0.12***	367.98 ± 3.8***	0.38 ± 0.03***
HDA (20 mg/kg) bd.wt+Azaserine	4.47 ± 0.14**	433.09 ± 4.44***	0.48 ± 0.04ns
HDA (40 mg/kg) bd.wt+Azaserine	2.72 ± 0.14**	381.09 ± 4.12***	0.42 ± 0.06**
All values are expressed as Mean ± S.E.M. Statistical comparisons were made by using One way ANOVA followed by Dunnett's multiple comparison test using Graph pad prism version 8 and found significantly different at p>0.05ns, *p<0.05, **p<0.01, ***p<0.001 when compared to disease control			

Table 5: Effect of Cinnamic Acid (CA), and Hexanedioic 0041cid (HDA) on tissue biochemical analysis in Azaserine-induced pancreatic cancer

Groups/Parameter	Amylase	lipase	Glucose
Vehicle Control	2351 ± 89.45*	43.65 ± 3.87***	78.09 ± 1.54***
Azaserine Control	2673 ± 76.09	74.19 ± 4.52	109.98 ± 2.26
CA (20 mg/kg) bd.wt+Azaserine	2456 ± 56.96ns	56.98 ± 4.78*	88.92 ± 1.23***
CA (40 mg/kg) bd.wt+Azaserine	2378 ± 77.71*	45.23 ± 3.99***	72.09 ± 2.01***
HDA (20 mg/kg) bd.wt+Azaserine	2533 ± 67.12ns	63.61 ± 5.82ns	89.09 ± 1.04***

HDA (40 mg/kg) bd.wt+Azaserine	2419 ± 88.03ns	51.98 ± 4.12**	81.09 ± 2.17***
All values are expressed as Mean ± S.E.M. Statistical comparisons were made by using One way ANOVA followed by Dunnett's multiple comparison test using Graph pad prism version 8 and found significantly different at p>0.05ns, *p<0.05, **p<0.01, ***p<0.001 when compared to disease control			

Table 6: Effect of Cinnamic Acid (CA), Hexanedioic Acid (HDA) on tissue biochemical analysis in Azaserine-induced pancreatic cancer

Groups/Parameter	SOD	CAT	GR
Vehicle Control	2.12 ± 0.12***	0.97 ± 0.12***	5.12 ± 0.16***
Azaserine Control	1.47 ± 0.04	0.45 ± 0.06	3.49 ± 0.12
CA (20 mg/kg) bd.wt+Azaserine	1.87 ± 0.08*	0.68 ± 0.03ns	4.58 ± 0.09***
CA (40 mg/kg) bd.wt+Azaserine	2.02 ± 0.11***	0.86 ± 0.03**	5.17 ± 0.11***
HDA (20 mg/kg) bd.wt+Azaserine	1.68 ± 0.04ns	0.59 ± 0.04ns	4.33 ± 0.21**
HDA (40 mg/kg) bd.wt+Azaserine	1.96 ± 0.09**	0.74 ± 0.05*	4.87 ± 0.17***
All values are expressed as Mean ± S.E.M. Statistical comparisons were made by using One way ANOVA followed by Dunnett's multiple comparison test using Graph pad prism version 8 and found significantly different at p>0.05ns, *p<0.05, **p<0.01, ***p<0.001 when compared to disease control			

Table 7: Effect of Cinnamic Acid (CA), Hexanedioic acid (HDA) on tissue biochemical analysis in Azaserine-induced pancreatic cancer

Groups/Parameter	GSH	PCC	MDA
Vehicle Control	20.24 ± 1.23**	3.32 ± 0.37***	2.23 ± 0.75***
Azaserine Control	14.23 ± 0.96	6.22 ± 0.13	7.12 ± 0.64
CA (20 mg/kg) bd.wt+Azaserine	19.19 ± 1.1**	4.72 ± 0.32**	5.34 ± 0.76ns
CA (40 mg/kg) bd.wt+Azaserine	19.44 ± 0.84**	3.01 ± 0.33***	2.21 ± 0.88***
HDA (20 mg/kg) bd.wt+Azaserine	16.75 ± 1.09ns	5.21 ± 0.34ns	4.44 ± 0.64ns
HDA (40 mg/kg) bd.wt+Azaserine	18.6 ± 0.87*	3.29 ± 0.26***	2.45 ± 0.9***
All values are expressed as Mean ± S.E.M. Statistical comparisons were made by using One way ANOVA followed by Dunnett's multiple comparison test using Graph pad prism version 8 and found significantly different at p>0.05ns, *p<0.05, **p<0.01, ***p<0.001 when compared to disease control			

Table 8: Effect of Cinnamic Acid (CA), Hexanedioic Acid (HDA) on tissue biochemical analysis in Azaserine-induced pancreatic cancer

Groups/Parameter	TNF alpha	IL6	IL10
Vehicle Control	18.23 ± 0.89***	27.8 ± 0.98***	128 ± 1.23***
Azaserine Control	29.1 ± 0.94	38.12 ± 0.81	72 ± 1.45
CA (20 mg/kg) bd.wt+ Azaserine	22.76 ± 0.65***	31.09 ± 1.23***	96 ± 1.12***
CA (40 mg/kg) bd.wt+Azaserine	19.56 ± 0.73***	28.44 ± 1.08***	112 ± 2.05***
HDA (20 mg/kg) bd.wt+Azaserine	25.76 ± 0.66*	34.09 ± 1.22*	91 ± 1.94***
HDA (40 mg/kg) bd.wt+Azaserine	20.96 ± 0.48***	29.08 ± 1.4***	101 ± 1.52***
All values are expressed as Mean ± S.E.M. Statistical comparisons were made by using One way ANOVA followed by Dunnett's multiple comparison test using Graph pad prism version 8 and found significantly different at p>0.05ns, *p<0.05, **p<0.01, ***p<0.001 when compared to disease control			

Based on docking analysis by D. Modak et al., (2021) n-Hexadecanoic acid was found to have strong inhibition of Cyclooxygenase-2 (COX-2), Tumor Necrosis Factor (TNF-α), and Interleukin (IL-6) [21]. Lupin species have been reported to contain Hexadecanoic acid, which has antioxidant properties and maintains the levels of SOD, CAT, and reduced LPO and MDA in CCl4-intoxicated rats, according to K. Mazumder et al., (2020) [22]. B. Bharath et al., (2021) found that HA can result in 77.83% apoptotic cells and inhibit cells in the G0/G1 phase, as evidenced by increased fluorescence intensity and cell cycle analysis [23].

It is well known that neutrophils and macrophages produce ROS to kill tumor cells. These cells produce a burst of superoxide, which is primarily mediated by nicotinamide adenine dinucleotide phosphate oxidase, leading to the production of hydrogen peroxide. Additionally, during inflammation, activated macrophages produce nitric oxide, which reacts with superoxide to produce peroxynitrite radicals. These radicals are similar in their activity to hydroxyl radicals and contribute to tumor cell apoptosis. In pancreatic tissue, injured acinar cells and activated immune cells release a large amount of oxygen free radicals, which results in increased Malondialdehyde (MDA) and decreased Superoxide Dismutase (SOD)

and total glutathione (GSH) levels. Treatment with antioxidants has been shown to reduce acinar cell necrosis and alleviate the severity of pancreatic tissue injury [24]. Proteins undergoing carbonyl modification due to oxidative stress can lead to various negative effects such as loss of protein function, abnormal clearance, disrupted cellular redox balance, interference with cell cycle, and cancer progression. The increased levels of oxidative stress observed in cancerous cells have been linked to higher overall protein carbonylation in multiple cancer types [25]. However, supplementation with suitable antioxidants CA and HDA contributes to the antioxidant defense system, enhancing the levels of SOD, CAT, and GR in the pancreas.

In various tests, cinnamic acid was found to be the most potent antioxidant and an effective inhibitor of several enzymes, including α-glucosidase, α-amylase, lipase, ACE, renin, iNOS, and XO. It also showed a high affinity for the active sites of α-glucosidase and ACE, with high docking scores. Cinnamic acid derivatives are particularly good at inhibiting lactate dehydrogenase with a low Km, as they compete with NAD+/NADH for binding to the enzyme. While they are less effective against other NAD (+)-dependent dehydrogenases. Cinnamic acid and its derivatives are also able to reduce the production of fructosamine and Nε-(Carboxymethyl) Lysine (CML), as well as preventing oxidative protein damage, such as protein carbonyl formation and thiol oxidation of BSA. In addition, cinnamic acid has been shown to enhance the levels of SOD, CAT, and GSH in the brain homogenate of STZ-induced diabetic rats, which indicates significant antioxidant activity.

Chronic inflammation, both systemic and local, can increase the likelihood of developing Pancreatic Ductal Adenocarcinoma (PDAC). The presence of inflammatory cells in the tumor microenvironment also contributes to the growth and spread of PDAC. Inflammation is closely linked to the immune system, with the same types of immune cells involved in both inflammation and immune response. Recent studies suggest that interleukin expression changes may indicate poor prognosis in advanced pancreatic cancer, as per S.T. Chari et al., (2015). In particular, Tumor Necrosis Factor-alpha (TNFα) and IL6 are known to increase cancer risk, growth, and cachexia. Cytokines, produced by both tumor and inflammatory cells, play a crucial role in this inflammatory and immunomodulatory scenario. Among them are pro-inflammatory cytokines like IL-1β, IL-6, and TNFα, anti-inflammatory cytokine IL-10, and the dual-face cytokine transforming growth factor beta (TGFβ), which has opposing effects depending on the context. These treatments helped to reduce the levels of pancreatic TNF alpha and IL6 and increase the levels of IL10 in pancreatic homogenate [26].

Based on a study by S. Adisakwattana et al., (2008) it was found that cinnamic acid (100 μM) does not activate insulin secretion in INS-1 pancreatic β-cells [27]. Another study conducted, observed an increase in caspase-3-dependent beta-cell apoptosis and downregulation of the anti-apoptotic gene Bax (belonging to the Bcl-2 family) after exposure to 25 mM glucose for 72 hours According to a recent study by K. Sangpairoj et al., (2022) the presence of hexadecenoic acid at a cytotoxic concentration resulted in the apoptotic death of MDA-MB-231 cells [28].

Conclusion

Inflammation can cause cellular reprogramming that leads to Acinar-to-Ductal Metaplasia (ADM). ADM is a reversible process where pancreatic acinar cells take on characteristics of ductal cells. This process typically occurs in response to pancreatic damage and is believed to be a precursor to pancreatic cancer. Acinar cells create and release digestive enzymes, while ductal cells transport those enzymes to the small intestine. The Azaserine induction tends the pancreas to oxidative stress and inflammation with evidenced histology of damaged acini, intra and interlobular pancreatic ducts, and islets of the pancreas. Pancreatic cancer can often spread to the liver. Similar results were obtained in the present study i.e., partial damage to the liver in terms of necrosis, binucleated cells, and disrupted portal triad. Treatment with Cinnamic Acid (CA) and Hexadecenoic Acid (HDA) showed significant degeneration pancreas and liver in a dose-dependent manner.

Further studies are required to identify the exact mechanism of pancreas protective activity and anticancer activity using suitable biomarkers of pancreatic cancer.

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Conflict of Interest

Authors have no conflict of interest to declare.

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