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Ameliorative effect of cinnamic acid against L-arginine-induced pancreatitis

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ABSTRACT

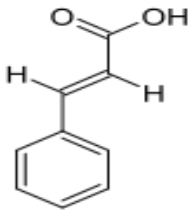
Acute pancreatitis (AP) is a localized inflammation of the pancreatic gland that often leads to local and systemic complications. Cinnamic acids have been identified as interesting compounds with antioxidant, anti-inflammatory and cytotoxic properties. A pre-clinical study using L-arginine induced AP in the rat model was attempted to evaluate the antioxidant effect of Cinnamic acid. The result confirmed that the AP condition was developed in response to the injection of L-arginine causing significant changes in different pancreatic enzymes, amylase and lipase in addition to the oxidative stress biomarkers. The treatment with Cinnamic acid caused a marked effect on these investigated parameters. The findings of the present study demonstrated that Cinnamic acid provided effective protection against AP induced by L-arginine in rats since this compound was able to ameliorate serum enzymes released from pancreas, enzymatic and non-enzymatic antioxidant defense system and to prevent the lipid peroxidation in these tissues. More detail study about Cinnamic acid treatment reversing or reducing acute pancreatitis is needed.

1. INTRODUCTION

Acute pancreatitis (AP) is a localized inflammation of the pancreatic gland that often leads to local and systemic complications, such as lung injury and renal failure. The mortality rate in patients with AP is approximately 5%, but this percentage rises to 17–20% in patients with necrotizing pancreatitis. Mortality in AP is ascribed to multiple organ failures that may be triggered by the associated systemic inflammatory response (Pandol et al., 2007).

Cinnamic acids have been identified as interesting compounds with antioxidant, anti-inflammatory and cytotoxic properties. During the last decade, natural products bearing the cinnamoyl moiety have attached much attention due to their broad spectrum of biological activities and low toxicity. Additionally, *trans*-cinnamic acid derivatives, either isolated from plant sources or synthesized, are well known for their antioxidant (Chung, H.S. and Shin, J.C. 2007), antitumor (Bezerra et al., 2006), antimicrobial (Naz et al., 2006) and anti-mycobacterial properties (Carvalho et al., 2008). Cinnamic acid derivatives, especially those combining the cinnamoyl moiety with hydroxyl groups, present strong free radical scavenging properties. Lipophilic hexyl amides and hexyl esters of cinnamic and hydrocinnamic acids, as well as the corresponding acid precursors, have been recently studied for their antioxidant profile and found to play important role in neurodegenerative diseases (ND) due to their ability to cross the blood-brain barrier (Roleira et al .,2010). Additionally, 2'-hydroxycinnamaldehyde and analog 2'-benzoyloxycinnamaldehyde induce apoptosis in cancer cells *via* the induction of cellular reactive oxygen species (ROS) (Han et al., 2004).

Previous studies have shown that cytotoxic and anti-inflammatory effects of antioxidant cinnamic acids are associated with their pro-oxidant effects (Pontiki et al., 2011). The antioxidant, anti-inflammatory and anticancer properties of cinnamic acids are known to be influenced to a great extent by the substitutions of the aryl ring and the double bond. To enhance the anticancer and anti-inflammatory activity of these derivatives (Pontiki et al., 2009) a series of new cinnamic acids with the appropriate substituents have been synthesized. These series have been evaluated for their: (a) antioxidant activity in different assays (b) anticancer activity in different cell lines and (c) ability to inhibit soybean lipoxygenase. Hence, the present study was conducted to evaluate the efficiency of cinnamic acid nanoparticles to reduce the incidence of acute pancreatitis.



Structure of Cinnamic acid

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2. MATERIAL AND METHODS

2.1. Chemicals

L-arginine and Cinnamic acid were obtained from Sigma Aldrich Chemical Co., St. Louis, Mo. The USA. All other chemicals and reagents were used for an analytical grade.

2.2. Animals

A total number of 60 male Swiss albino rats weighing 130 - 150 g (135 ± 10) g were purchased from the Egyptian National Authority for Drug Research and Control, Ministry of Health was used in this study. The animals were kept under environmentally controlled conditions (constant temperature 25 – 27 °C with a 12 h light/dark cycles) for one week before starting the experiment for laboratory acclimatization. All rats were kept in plastic cages and they were provided with commercial pellet diet (standard laboratory chow) and tap water ***ad libtium***. All animals were observed daily for abnormal signs. In each experimental group, 15 rats were used.

All animal procedures were conducted in compliance with the Ethics committee of National Research Center conformed to the "Guide for the Care and Use of Laboratory Animals" published by the US National Institute of Health (NIH publication, No.85-23, 1996).

2.3. Experimental design

The animals were divided at random into four groups; each group is composed of ten rats according to the following scheme:

Group I (Control): Normal healthy were orally administrated with 0.5 ml olive oil

Group II (AP): Rats were injected intraperitoneally with L-arginine (250 mg/ 100g three times per 10 days (Alison et al., 2010).

Group III (cinnamic): Rats will be administered orally with Cinnamic acid (60 mg/kg body weight dissolved in olive oil daily for 21 days).

Group IV (AP+cinnamic): Rats will be injected intraperitoneally with L- arginine 250 mg/100g three times per 10 days then rats will be administered orally with Cinnamic acid (60 mg/kg body weight).

At the end of the experiment, mice were anesthetized using diethyl ether. The blood was collected by heart puncture into a plain tube and left to coagulate at 37 ºC for 15 min. The clotted blood was centrifuged, and the serum was separated and stored at -20 ºC. The Pancreas was dissected, rinsed in ice cold isotonic saline, blotted dry with a filter paper and preserved at -20 ºC for further biochemical analysis. Portions of the pancreas were collected on 10% neutralized formalin for further processes for histopathological analysis.

2.4. Determination of the biochemical parameters

Determination of lipase and amylase activities in serum

Activities of lipase and amylase were determined using a diagnostic kit purchased from (Salucea Company Italian medical company).

Determination of oxidative stress markers in pancreatic tissue homogenate

Lipid peroxidative products were measured using the thiobarbituric acid test for malondialdehyde (MDA), as described by Yoshioka et al. (1979). Reduced glutathione (GSH) content was performed in the pancreatic tissue homogenate according to Beutler *et al.,* (1963). Measurement of oxidized glutathione (GSSG) assay according to Srivastava and Beutler (1967)

2.6. Statistical analyses.

The SPSS (version 20) was used in data analyses. Data were analyzed with a one-way analysis of variance (ANOVA) followed by a post hoc test (LSD) for multiple comparisons. The data were expressed as mean ± standard error (SE). P values < 0.05 were considered statistically significant.

3. RESULTS

The data represented in table (1) indicated that pancreatic enzymes (amylase and lipase) activities were significantly increased in pancreatic tissue of L-arginine injected rats than control animals. Cinnamic acid treatment showed a significant decrease in pancreatic enzyme levels compared with the AP group.

Table 1 Statistical analysis for pancreatic enzymes tests in the different groups

	Control	AP	cinnamic	AP + cinnamic
Amylase (U/L)	322.1±2.4^{C}	829.1±107.3^{ab}	311.8±4.6^{C}	422.4±3.7^{c}
Lipase (U/L)	163.6±8.8^{C}	319.4±22.1^{ab}	141.3±12.6^{C}	189.5±4.3^{c}

Each value is the mean ± SD. Data with different superscript are significantly different at p≤0.05. a, b and c denote significant change versus control, cinnamic and AP groups, respectively.

Injection of L-arginine resulted in a significant increase in pancreatic content of MDA the index of high lipid peroxidation and an indicator of ROS production, as well as GSSG and GSSG/GSH ratio while GSH level decreased significantly in pancreatic tissue of L-arginine injected rats than control animals. The oral administration of cinnamic acid showed a significant decrease in MDA, GSSG and GSSG/GSH ratio levels as well as a significant increase in the level of GSH compared with controls.

Table 2 Statistical analysis for pancreatic antioxidants and MDA levels in the different groups

	Control	AP	cinnamic	AP + cinnamic
GSSG (µmol /g tissue)	47.0±1.2^{c}	99.15± 5.3^{ab}	41.6±1.3^{c}	60.3±6.9^{c}
GSH (µmol /g tissue)	66.9±2.2^{c}	29.4±1.2^{ab}	83.0±0.35^{c}	48.5±8.7^{c}
GSSG/GSH ratio	0.70±0.04^{c}	3.37± 0.04^{ab}	0.50±0.01^{c}	1.25±0.08^{abc}
MDA (nmol/g)	20.9±3.7^{c}	98.2±14.3^{ab}	10.8±2.3^{c}	40.1±2.3^{c}

Each value is the mean ± SD. Data with different superscript are significantly different at p≤0.05. a, b and c denote significant change versus control, cinnamic and AP groups, respectively.

4. DISCUSSION

The present study aimed to evaluate the effect of cinnamic acid as an antioxidant against the overdosage of arginine on certain biological functions in male albino rats.

This study was concerned with the analysis of certain biochemical parameters including determination of the serum enzymes (lipase and amylase) as well as the changes in the activity of the concentration of MDA, GSSG, GSH, and GSSG /GSH ratio.

The biomarkers implicated by the obtained data presented in this study include increased serum amylase and lipase. Also, MDA, GSSG, and GSSG/GSH ratio were increased while GSH was decreased in pancreatic tissue.

Data from this study demonstrated an elevation in lipase and amylase activities at 1day post-injection of 250 mg/100g rat three times per 10 days with arginine were agreed with (Liu

et al., 2017), which significantly decreased after 21 days. That progressive decrease in pancreatic secretory capacity might be due to the destruction of up to 90% of acinar cells. We found that L-Arginine could successfully induce AP as evidenced by serum amylase elevation in agreement with (Prasong et al., 2019). Our data were also supported by findings of (Matalka *et al*., 2013) who revealed that L-arginine-induced acute pancreatitis as evidenced by a dramatic increase in plasma lipase levels, and to a lesser degree in amylase levels in L-arginine-treated group, suggesting that lipase might be more specific biochemical marker than amylase for diagnosis of acute pancreatitis, in agreement with (Lankisch *et al*., 2015) and (Mirmalek et al., 2016) who stated that plasma lipase or amylase activities, most commonly used biomarkers in acute pancreatitis, are at least tripled compared to upper limit of normal; however the plasma levels may not be dependent on pancreatitis severity, besides, amylase rise is detected up to 3–5 days, while lipase level stays high for 8–14 days and is, therefore, more sensitive for diagnosis in delayed presentation.

Supplementation of cinnamic acid succeeded in decreasing the level of amylase and lipase at 1 and 21 days. Oxidative stress is known to have a crucial role in the development of pancreatic acinar damage Ateyya *et al*., (2016). To assess pancreatic damage, levels of malondialdehyde (MDA), an end-product of lipid peroxidation, was measured following pancreatitis, lipids are one of the main targets for free radicals' damage.

Using MDA as a marker of oxidative stress, there has been a growing interest in studying the role played by lipid peroxidation in cancer progression. MDA is a low-molecular-weight aldehyde that can be produced from free radical attacks on polyunsaturated fatty acids. Increased plasma MDA level has been reported in breast cancer (Omayma et al., 2018).

The later will induce lipid peroxidation by removing one hydrogen atom from polyunsaturated fatty acids and form hydroperoxides. As a result, perturbations in cellular fluidity and membrane integrity lead to the disintegration of cells and necrotic cell death. Consequently, subcellular structures released into the extracellular media will induce several inflammatory events and further worsen the ongoing damage (Matalka *et al*., 2013).

The results of the present study showed that intraperitoneal administration of an overdose of arginine increased MDA level at both 1 and 21 days. This reflects the fact that it evoked MDA and induced oxidative stress. Such increase of MDA was in agreement with the previous studies of Hegyi *et al*., (2004); Bülbüller *et al*., (2005); (Matalka et al., (2013); Ateyya *et al*.,(2016) and Mirmalek et al., (2016) Who reported that high MDA level was an indication of oxidative stress induced by arginine, that Peroxidation of membrane lipids by ROS releases toxic byproducts such as MDA, which in turn leads to activation of complement cascade, other cytokines as a final consequence. MDA is directly associated with tissue injury and organ failure in pancreatitis. For this reason and also for its early peak after 3–5 hours going back to normal after 12 hours, MDA is considered as a marker of AP severity in early stages (Mirmalek et al., 2016).

On the other hand, administration of cinnamic acid demonstrated a magnificent recovery in MDA level at 1 and 21 days, this reflects that cinnamic acid had a curative effect and inhibited the peroxidation of cell membrane lipids and kept its integrity due to its potent antioxidative activity as reported by (Shon *et al*., 2002); (Liu *et al*., 2009); (Anraku *et al*., 2009); (Park and Kim, 2010); (Mohamed, 2011);(Luo and Wang, 2013) and (Wu *et al*., 2015).

In this study, arginine administration resulted in a significant decrease in the level of GSH as well as a significant increase in GSSG levels and GSSG/GSH ratio. Cinnamic acid improved GSH level due to its ability for decreasing ROS levels and improving the GSH/GSSG ratio and counteracts increased ROS levels, which is attributed rather to an increase in total glutathione pool and enhanced reduction of this molecule then to substantial changes in mitochondrial membranes or to the hypoglycemic effect of the cinnamic acid (Ortiz-Avila et al., 2015).

5. CONCLUSION

The findings of the present study demonstrated that Cinnamic acid provided effective protection against acute pancreatitis induced by L-arginine in rats since this compound was able to ameliorate serum biochemical parameters, enzymatic and non-enzymatic antioxidant defense system and to prevent the lipid peroxidation in these tissues.

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