The Antioxidant Impact of Supplementing High and Low doses of Ashwagandha Extract in Chronic Pancreatitis Induced Experimentally

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Abstract

The pancreas is part of the gastrointestinal system that lies in the upper abdomen behind the stomach. It is a mixed endocrine/exocrine gland enveloped by a thin layer of connective tissue. Connective tissue septa invade the gland, dividing it into lobules, serving as a support for tissue and providing a pathway for vasculature, nerve fibers, and lymphatic ducts. The pancreas lacks a complete fibrous capsule (Abozaid et al., 2020). The pancreas is composed of exocrine portion which comprises more than 95% of the pancreatic mass and it responsible for makes and secretes digestive enzymes into the intestine and endocrine portion (the islets) which makes and secretes hormones as (insulin, glucagon, somatostatin and pancreatic polypeptide) into the blood to control energy metabolism and storage throughout the body, it includes acinar and duct cells with associated connective tissue, vessels, and nerves.it comprise 1-2% of pancreatic mass Mederos et al (2021).

INTRODUCTION

The pancreas is part of the gastrointestinal system that lies in the upper abdomen behind the stomach. It is a mixed endocrine/exocrine gland enveloped by a thin layer of connective tissue. Connective tissue septa invade the gland, dividing it into lobules, serving as a support for tissue and providing a pathway for vasculature, nerve fibers, and lymphatic ducts. The pancreas lacks a complete fibrous capsule (Abozaid et al., 2020). The pancreas is composed of exocrine portion which comprises more than 95% of the pancreatic mass and it responsible for makes and secretes digestive enzymes into the intestine and endocrine portion (the islets) which makes and secretes hormones as (insulin, glucagon, somatostatin and pancreatic polypeptide) into the blood to control energy metabolism and storage throughout the body, it includes acinar and duct cells with associated connective tissue, vessels, and nerves.it comprise 1-2% of pancreatic mass Mederos et al (2021).

Acute pancreatitis (AP) is a life-threatening inflammatory disorder with a significant impact on patient health. Although its pathogenesis is not fully understood, microcirculatory disturbances, leukocyte activation, and oxidative stress are the main events in AP that is characterized by activation of digestive proteases, a widespread inflammatory cell infiltration, leukocyte activation and the release of various kinds of inflammatory mediators and reactive oxygen and nitrogen species (Akyazi et al., 2013).Repetitive attacks of acute pancreatitis have the potential to evolve into chronic disease that is characterized by fibrosis and loss of pancreatic function (Hou et al., 2019). There are no specific therapies for acute pancreatitis. Medical management is aimed at the control of symptoms with anti-inflammatory agents, steroids, and analgesics. As a result of the limitations of conventional therapy, many ethnobotanical agents are being pursued as alternative sources to develop novel and safe therapeutic agents to treat pancreatitis (Leppäniemi et al., 2019). Acute pancreatitis occurs by initial injury mechanism. It consists of three steps: (i) premature activation of trypsin in acinar cells; (ii) intrapancreatic inflammation; and (iii) extra-pancreatic inflammation. The mechanisms by which drugs initiate a cascade of damaging events remain shrouded in mystery. However, it should be borne in mind that the same is true for most responses independent of drug dose (Badraoui et al., 2020).

Oxidative stress is one of the pivotal mechanisms of AP. Excessive reactive oxygen species (ROS) provoke inflammation and development of pancreatitis through zymogen degranulation, granulocyte migration, tissue necrosis, and increased amylase and lipase activity (Ghorbani et al., 2015). In fact, ROS, as products of oxidation and peroxidation metabolism, are instantly
detoxified by natural scavengers and antioxidant agents under normal conditions (Alazragi et al., 2021). In acute pancreatitis, overproduction of ROS and the impaired neutralization ability of scavengers result in ROS accumulation in pancreatic tissue. Therefore, a great therapeutic potential can be acknowledged in enhancement of scavenger defense by utilizing antioxidant agents (Abdelgayed et al., 2021).

Ashwagandha is a xerophytic plant that is found mostly in the drier parts of India, Sri Lanka, Afghanistan, Baluchistan and Sind, but it is also distributed in the Mediterranean regions, the Canary Islands of Spain and the Cape of Good Hope in South Africa (Deshpande et al., 2020). The major constituents of Ashwagandha are alkaloids, such as withanine alkaloids, and steroidal withanolides lactones (withaferin A and withanolide D) (Verma et al., 2021). Other components include chlorogenic acid, scopoletin, β-sitosterol, choline and amino acids (Dutta et al., 2019). The Indian species leaves of this plant are showed to contain alkaloids, chlorogenic acid, withanolides, flavonoids, glycosides, tannins and other compounds which is extracted from its leaves with cold alcohol treatment; the extract is purified and dried, and finally crystallized from aqueous alcohol (yield 0.18% on an air-dry basis) and the root is attributed to both its alkaloids and its steroidal lactones (withanolides) (Hsu et al., 2019).

This study aimed to evaluate the effect of supplementing different doses of Ashwaganda extract on modulating oxidative stress of pancreatitis rats.

**Materials and Methods**

**Materials**

Ashwaganda was obtained from Sun Potion, India while pure L-Arginine powder obtained from Science-Based Nutrition Doctors Best company, US.

**Experimental animals:**

Forty-two adult male albino rats weighing 120-130g were used in this study. Rats were supplied from the Animal House Colony of King Fahd Medical Research center, Jeddah. After the acclimatization period, rats were controlled with a 12h light/dark cycle. Animal were kept in special cages at 20-22°C and humidity (60%) at King Fahd Medical Research Center Animal Facility Breeding Colony. A commercial balanced diet and tap water were given during the experimental period ad libitum.

Rats were distributed into six groups (7/each):

G 1: Healthy control rats fed daily oral dose of dimethyl sulphoxide DMSO (1 ml) in saline.

G 2: Pancreatitis group, rats was injected with L-arginine in a dose of (350 mg/100g bw) weekly, for 4 weeks (Ali et al., 2021).

G 3: (Pancreatitis + 100 mg/kg bw (low dose aqueous extract of ashwagandha roots) Zhou et al. (2013).

G 4: (Pancreatitis +500 mg/kg bw (high dose aqueous extract of ashwagandha roots) Zhou et al.(2013)

G 5: (Healthy rats+ low dose) according to Dhuley (1998)

G 6: (Healthy rats+ high dose) according to Sabina et al., (2013)

At the end of the experimental time, rats were fasted overnight, then sacrificed under ether anesthesia. Blood was collected by retro-orbital puncture, serum was separated by allowing the blood samples to clot for 30 minutes at temperature of 25°C then centrifuged at 3000 rpm for 20 minutes. Samples were divided into frequent aliquots and stored at -20°C until analysis. Pancreas was eliminated directly and washed with saline.
Biochemical and histological analysis:

Serum lipid peroxidation marker, malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) were evaluated spectrophotometrically using Biovision Kit, CA, USA. Pancreas samples were set in 10% formalin solution. Then, sectioned and prepared using paraffin blocks. Small intestines sections were stained with hematoxylin and eosin (H&E) and evaluated by a pathologist at magnification power.

Statistical analysis:

Data was statistically analyzed using SPSS computer program. Results were presented as mean ± standard error. The differences between mean values were determined by analysis of variance (ANOVA test).

Results

Figures (1,2,3,4) represented the results of oxidative stress biomarkers in studied groups. It seems that, pancreatic rat group showed abnormal oxidative stress biomarkers as compared to control normal group. As there is a significant decrease in serum level of GSH, SOD and CAT, with a parallel increase in MDA level compared to control group. While treatment with aqueous extract of Ashwagandha roots either in low or high dose improve GSH, SOD, MDA and CAT levels, and induced a significant improvement in GSH, SOD, MAD and CAT levels as compared to pancreatitis rat group. Additionally, results showed that administering normal rats with aqueous extract of Ashwagandha roots either in high or low doses, did not induce any significant change in levels of oxidative stress biomarker CAT and SOD as compared to control normal rats, while it was noticed that,
A low dose of Ashwagandha root extract reduced GSH level and MDA level compared to control rat group. In the same way it was noticed that high doses Ashwagandha root extract increased both GSH and MDA levels as compared to normal rat group.

Histological assessment

**Figure 5 (A).** Photomicrographs of a section in the pancreas of a control rat (group 1) showing: exocrine pancreas reveal normal architecture. Pancreatic lobules are separated by thin connective tissue septa (Arrows) containing the interlobular ducts (D). The lobules contained acini of different sizes and shapes. Islets of Langerhans are irregularly shaped patches of endocrine tissue located within the pancreas;

**Figure 5 (B).** Photomicrographs of a section in the pancreas of a rat in group 2 (Chronic pancreatitis group) showing: Marked infiltration of inflammatory cells is observed mainly in the interlobular area (Arrows). A picture of focal fat necrosis. The lobules show prominence of ductules (*). Islets of Langerhans appeared normal. (H&E A × 100, B × 200)

**Figure 5 (C).** Photomicrographs of a section in the pancreas of a rat in group 3 (Pancreatitis + low dose ashwagandha) showing: large areas of disturbance in acinar pattern and a picture of focal fat necrosis (F) are found. Cellular infiltrations are diffusely distributed in between acini and around blood vessels (*). Widening of interstitial spaces, which might suggest interstitial oedema, are also detected (arrows). Many acini are largely vacuolated with destruction of their cytoplasm. Cellular infiltration between acini is also noted (*). (H&E: A × 100, B × 200).
Discussion

Chronic pancreatitis as common chronic disorders worldwide with rising incidences. Death may result from systemic inflammatory response or pancreatic dysfunction syndromes, leading chronically to loss of pancreatic function and fibrosis.
Prevention of oxidative stress and acinar cell injury throughout the initial stage of pancreatitis prevent the pathological development to acute pancreatitis (Allawadhi et al., 2018).

L-arginine, was proven as one of the most common animals’ models in inducing chronic pancreatitis Biczo et al (2018). L-arginine triggers permanent destruction of pancreatic mitochondria and facilitates many pathologic reactions as hyperamylasemia, trypsinogen activation, necrosis, vacuolization, and inflammation. This agreed with Mirmalek et al (2016) and Aziz et al (2017) who showed the role of L-arginine in inducing oxidative stress and chronic pancreatitis.

Mizunuma et al (1984) were first to report that intraperitoneal (i.p.) administration of excessive doses of L-arginine (500 mg/100 g body weight) in rats selectively damage pancreatic acinar cells without any morphological change in islets of Langerhans or other organs. Based upon this observation, Tani et al (1990) reported an L-arginine induced rat model of necrotizing acute pancreatitis.

In the rat model of L-arginine induced pancreatitis, partial inhibition of Larginase activity, the enzyme which converts Larginine to L-ornithine and urea, ameliorates pancreatitis indicating a role of one of its metabolites in induction of pancreatitis (Blafo et al., 2020). Further at high dose, L-ornithine has also been reported to cause acute pancreatitis (Beczo et al., 2018). L-arginine is also a substrate for nitric oxide synthase and is known to induce nitrostatic and oxidative stress. There is some evidence to support that these pathways might also contribute to pancreatic injury in this model of pancreatitis.

Our results showed that L-Arginine induced pancreatitis that manifested by blood lipid parameters which is early was described by Yadav and Pitchumoni (2003) who linked an association between hyperlipidemia (HLP) and chronic pancreatitis. Nevertheless, primary lipid disorders, especially hypertriglyceridemia or chylomicronemia, may independently induce CP and are responsible for up to 7% of cases (De Pretis et al., 2018).

Pancreas is prone to oxidative stress than other organs, ROS produced during acute pancreatitis was the major source of pancreatitis acinar cell damage (Abdelzaher et al., 2021). The present study revealed that, induction of chronic pancreatitis by L-arginine reduced the antioxidant defense capacity. Conversion of L-arginine to L-ornithine and urea by arginase enzyme is a critical stage in the initiation of CP by L-arginine (Shosha et al., 2020). Results agreed with earlier findings on different models of CP which show that, ROS induced lipid peroxidation reduced serum CAT level, and depletion in GSH content at an initial phase of CP (Chvanov et al., 2015). Additionally, Perez et al. (2015) showed elevated MDA levels together with GSH reduction, thus indicates pancreatic injury and progression from mild to severe.

Different plant constituents were noticed to have activity against inflammatory factors and oxidative stress biomarkers which are the key event in the chronic pancreatitis (Rajendran et al., 2020). In the present study, an improvement was noticed in oxidative stress biomarkers after administering either low or high dose of Ashwagandha root extract, these results were like a study by Dar et al (2019) which revealed a reduction in MDA level in blood and heart tissues after supplementing Ashwagandha root extract. Additionally, the in vitro studies apparently demonstrated antioxidant activity of Ashwagandha by its potential role in scavenging hydroxyl radicals and 1,1-diphenyl-2-picrylhydrazyl DPPH radicals (Devendiran et al., 2020). A study by Choudhary et al., (2017) revealed that, Ashwagandha root extract improved GSH levels in a dose dependent manner. Accordingly, Ashwagandha inhibited MDA through different mechanisms such as free radical scavenging without modifying the glutathione system, one of the main physiological antioxidant systems (Elhadidy et al., 2018). Similar results were obtained by (Sengupta et al., 2018) similar results were obtained by Roma and Jonas, 2020).

Moreover, Malik & Soni, (2019) recorded the protective effect of Ashwagandha extract against Cerulein induced acute pancreatitis and a reduction in elevated MDA levels which add evidence to its antioxidant in acute pancreatitis. Meanwhile, Lim et al., (2018) have determined that Ashwagandha extract potentiates the antioxidative system and enhanced GSH-dependent enzymes, it also caused a considerable elevation SOD and CAT levels in cardiac tissue of rats with type 2 diabetes. Additionally, a previous study by RajaSankar et al., (2009) revealed that treatment with Ashwagandha extract improved physiological abnormalities in mouse model of Parkinson disease PD. These data suggests that Ashwagandha extract is a potential drug in treating oxidative damage and physiological abnormalities seen in the PD mouse. The study results were similar to those obtained by Sudha and Reni (2016). The anti-peroxidative effect observed in stress, may be attributed to the presence of steroidal lactones withaferin and withanolides, which are the main active components of ashwagandha (Sengupta et al., 2018). Similar results were recorded by khan et al (2019) and Langade et al (2019).

Previous studies by Anwer et al (2012) and Joshi and Joshi (2021) reported activities of both enzymatic and non-enzymatic histopathological and antioxidants examination of pancreas in type II diabetic rats. Where administration of Ashwagandha
extract to type II diabetic rats caused a significant decrease in blood glucose and tissue LPO levels with significant increase in GSH contents, when compared with the type II diabetic control rats. In addition, WS treated rats also showed a significant increase in the activities of antioxidant enzymes namely GPx, GR, GST, SOD and CAT when compared with type 2 diabetic control rats. Significant reduction in the number and size of pancreatic cells were preserved to near normal morphology by the administration of Ashwagandha extract in type 2 diabetic rats as evident from histopathological examination. These results indicate that Ashwagandha extract has shown strong free radical scavenging activity and helped in improving the non-enzymatic and enzymatic antioxidants in type II diabetic rats.

Conclusion

The study showed that applying L-arginine-induced triggers chronic pancreatitis, though administering aqueous extract of Ashwagandha roots either high or low dose would positively diminish biomarkers of oxidative stress and restore histological features.

REFERENCES


