

Silver nanoparticles combined with chitosan demonstrate strong and targeted efficacy against pancreatitis

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Abstract

Background: Pancreatitis, a prevalent inflammatory condition affecting the pancreas, presents with symptoms such as abdominal pain, nausea, and vomiting. The quest for innovative therapeutic strategies to address this condition has led to growing interest in silver nanoparticles (AgNPs) doped with chitosan due to their antimicrobial and anti-inflammatory properties. Chitosan, a natural polysaccharide derived from crustacean exoskeletons, exhibits biocompatibility and various biological activities, including antimicrobial and anti-inflammatory effects. This study investigates the efficacy of AgNPs-Chito in alleviating pancreatitis in an animal model induced by oral administration of ethyl alcohol. Methods: Adult male Swiss albino rats were divided into control, induced (pancreatitis induced but not treated), and treated with AgNPs-Chito groups. Parameters including liver function markers, blood glucose levels, pancreatic enzymes, and oxidative markers were evaluated. Results indicate that AgNPs-Chito treatment significantly improved liver function markers, reduced blood glucose levels, and decreased levels of pancreatic enzymes compared to the induced group. Furthermore, AgNPs-Chito exhibited antioxidant properties by reducing reactive oxygen species levels and enhancing glutathione S-transferase activity. Histopathological examination revealed protective effects on pancreatic tissue integrity. Conclusion: These findings suggest the potential therapeutic benefits of AgNPs-Chito in mitigating alcohol-induced pancreatitis through its multifaceted antimicrobial, anti-inflammatory, and antioxidant actions. Further research is warranted to elucidate the mechanisms underlying these effects and to explore the translational potential of AgNPs-Chito as a novel therapy for pancreatitis.

Keywords: Chronic pancreatitis, pancreatic fibrosis, Silver nanoparticle, chitosan nanoparticle, therapeutic strategies

1. Introduction

Pancreatitis is a common inflammatory disease that affects the pancreas, an organ responsible for producing enzymes and hormones involved in digestion. It is often characterized by abdominal pain, nausea, and vomiting. In recent years, there has been a growing interest in developing novel therapeutic approaches for this condition [1].

Silver nanoparticles (AgNPs) have gained attention for their antimicrobial and anti-inflammatory properties. They exhibit unique properties and have been utilised in various fields, including medicine and healthcare [2].

AgNPs were found to possess potent antimicrobial activity against a wide range of pathogens, including bacteria, viruses, and fungi. Chitosan is a natural polysaccharide derived from the exoskeleton of crustaceans, such as shrimp, lobsters, and crabs. It is known for its biocompatibility, low toxicity, and biodegradability [3]. Chitosan has been shown

to possess various biological activities, including antimicrobial, anti-inflammatory, and wound-healing properties [4].

AgNPs doped with chitosan have gained popularity due to their unique properties and potential applications in medicinal fields. The combination of AgNPs with chitosan (AgNPschito) enhances the antimicrobial and anti-inflammatory properties of AgNPs. The potent and selective anti-pancreatitis properties of AgNPs-Chito have been extensively studied [5]. The nanoparticles exhibit excellent penetration into the tissues, enabling them to reach the affected area more effectively [6]. The antimicrobial properties of AgNPs are well known. They can disrupt microbial cell membranes, leading to cell death. AgNPsChito exhibits enhanced antimicrobial activity against pancreatic pathogens, such as bacteria, viruses, and fungi [7].

Chitosan also has anti-inflammatory properties. It has been shown to reduce inflammation by inhibiting the release of

proinflammatory mediators and scavenging reactive oxygen species. When AgNPs-chito, their anti-inflammatory properties are further enhanced, providing additional benefits in the treatment of pancreatitis [8]. Therefore, In an animal model of pancreatitis induced by alcohol, the purpose of this study is to determine if AgNPs-Chito improves its efficacy for alleviating pancreatitis.

2. Materials and Methods

Forty adult male Swiss albino rats weighing between 110 and 130g and aged between 4 and 4.6 months were used in this study. To induce pancreatitis in these rats, we gave them oral feeding with 10% ethyl alcohol for 28 days. Following that, a total of three groups of rats were used: Group I was the control group, which did not receive any therapy; Group II was the induced group, which did not receive any treatment; and Group III was the pancreatic group that received AgNPs-Chito (20 mg/kg) for 21 days. A syringe with a capacity of 200 μ L AgNPs-Chito was used orally. Rats were housed in the Animal House in accordance with the ethical criteria of the Benha University Ethical Committee. Rats were handled and grown humanely. Rats with fasting glucose levels higher than 95 U/L were classified as pancreatitis after receiving alcohol for 30 days.

Preparation of Biological Samples

Blood and pancreas tissue samples were collected after the sacrifice using isoflurane. To collect serum samples, a centrifuge at 1000g was used, and for tissue samples, a centrifuge at 3000g was used [9]. Following the analysis, the samples were stored at -80 °C until further analysis could be conducted.

Pancreatic enzymes and blood sugar estimation

COBAS Integra (USA) was used to test the rats' blood amylase and lipase activity. Rats' tail veins were used to collect blood samples [10]. A blood glucose monitoring device was used to test the rats' blood glucose levels at 7-8 a.m. after they went without meals from 5 p.m. to 7 a.m. [11]. Rats' tail veins were used to collect blood samples [12]. Diabetic rats were defined as those whose fasting glucose levels were more than 180 mg/100 mL. By verifying the animals' glucose readings twice, the diabetic state was established.

Measurement of Redox Parameters

To assess their function in controlling oxidative stress in vivo, two redox parameters—reactive oxygen species (ROS) [13] and glutathione-S-transferase (GST) [14]—were chosen. Using the sulfosalicylic acid and DTNB

techniques, the absorbance of the reaction mixture was measured at 412 nm to quantify the amounts of GST [15,16].

Analysis of Liver Function Markers

The serum samples were used to measure liver function indicators such as GGT and alkaline phosphatase (ALK). Serum indicators were measured using commercial kits according to the manufacturer's recommendations [9], [17].

Histopathological Analysis

The dissected pancreas tissues were stored in a 10% formalin solution. After that, they were cut into pieces with thicknesses between 5 and 7 μ m. Subsequently, the slices underwent H&E staining [18], [18], [19,20].

3. Results Effect of AgNPs-Chito on liver, blood glucose levels, and pancreatic enzymes in different rat groups

Table (1) reveals the results of the study conducted to evaluate the effect of AgNPs-Chito on liver and blood glucose levels in different rat groups. The variables measured were FBS, PPBS, GGT, Lipase, and Amylase. In the control group (Cnt), the FBS level was 50.25 ± 2.15 , while in the model group, the FBS level was significantly higher at 179.9 ± 0.48 ($p < 0.01$). The administration of AgNPs-Chito in the experimental group resulted in a decrease in the FBS level to 88.10 ± 0.35 ($p < 0.01$). Similarly, the PPBS level was significantly higher in the model group (264.5 ± 1.78) compared to the control group (114.5 ± 0.84) ($p < 0.01$). However, the administration of AgNPs-Chito significantly decreased the PPBS level to 135.18 ± 0.86 ($p < 0.01$). The GGT level was also significantly higher in the model group (134.15 ± 1.57) compared to the control group (16.25 ± 0.24) ($p < 0.01$). The administration of AgNPs-Chito significantly decreased the GGT level to 55.15 ± 2.25 ($p < 0.01$). The Lipase level was significantly higher in the model group (118.27 ± 3.50) compared to the control group (42.38 ± 2.14) ($p < 0.01$). However, the administration of AgNPs-Chito significantly decreased the Lipase level to 66.12 ± 1.63 ($p < 0.01$). Finally, the Amylase level was also significantly higher in the model group (204.89 ± 2.09) compared to the control group (123.52 ± 2.25) ($p < 0.01$). The administration of AgNPs-Chito significantly decreased the Amylase level to 118.25 ± 2.59 ($p < 0.01$). Overall, the results suggest that the administration of AgNPs-Chito can help in controlling blood glucose levels and improving liver function in rats.

Table (1) Effect of AgNPs-Chito on liver and blood glucose levels in different rat groups.

Variable	Cnt	Model	AgNPs-Chito
FBS	50.25±2.15 ^a	179.9±0.48 ^b	88.10±0.35 ^c
PPBS	114.5±0.84 ^a	264.5±1.78 ^b	135.18±0.86 ^c
GGT	16.25±0.24 ^a	134.15±1.57 ^b	55.15±2.25 ^c
Lipase	42.38±2.14 ^a	118.27±3.50 ^b	66.12±1.63 ^c
Amylase	123.52±2.25	204.89±2.09	118.25±2.59

In each group results for 6 rats are expressed by means ± SE. Small (a-c) letters showing the marked change at $P \leq 0.05$. The same letters show (non-significant) and the significance is expressed by dissimilar letters. **Cnt**: control group.

Table (2) Effect of AgNPs-Chito on oxidative and antioxidant markers in different rat groups.

Variable	Cnt	Model	AgNPs-Chito
ROS	0.99±0.15 ^a	3.15±1.08 ^b	1.42±0.31 ^c
GST	18.15±0.47 ^a	6.37±1.17 ^b	10.15±0.77 ^c

In each group results for 6 rats are expressed by means ± SE. Small (a-c) letters showing the marked change at $P \leq 0.05$. The same letters show (non-significant) and the significance is expressed by dissimilar letters. **Cnt**: control group.

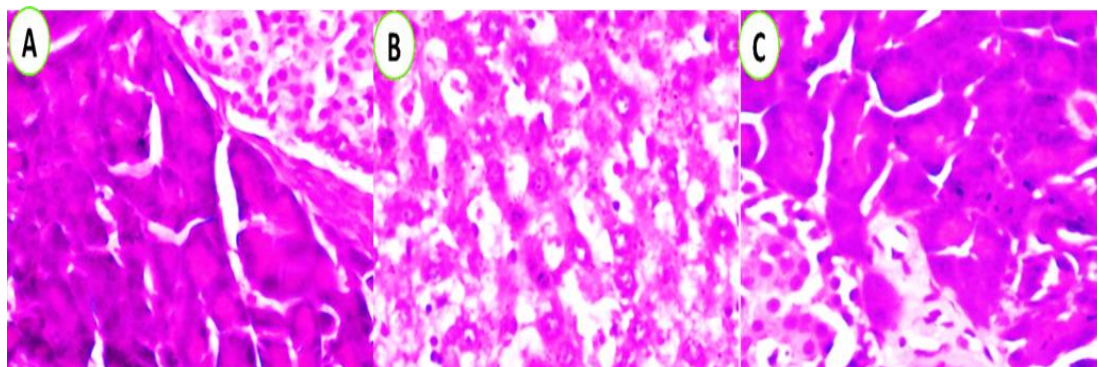


Fig. (1) Histopathology of the pancreas. The scale bar is 50 μ m

Effect of AgNPs-Chito on oxidative markers in different rat groups

The results of Table (2) show that the level of ROS (reactive oxygen species) was significantly higher in the Model group (3.15±1.08) as compared to the Control (Cnt) group (0.99±0.15). However, treatment with AgNPs-Chito resulted in a significant reduction in ROS levels (1.42±0.31) as compared to the Model group. Moreover, the level of GST (glutathione S-transferase) was significantly lower in the Model group (6.37±1.17) as compared to the Control group (18.15±0.47). However, treatment with AgNPs-Chito resulted in a significant increase in the level of GST (10.15±0.77) as compared to the Model group. These results suggest that AgNPs-Chito may have antioxidant properties and can help in reducing oxidative stress in rats.

Histopathology examination

There was no histopathological alteration and the normal histological structure of the islands of Langerhans (Figure 1a). Focal inflammatory cell infiltration was noticed in between the tubules at the cortex. The corticomedullary portion showed tubular cystic dilatation (Figure 1b). Mild atrophy was detected in the islands of Langerhans (Figure 1c).

4. Discussion

Chronic pancreatitis is largely caused by excessive alcohol use. It is characterized by a progressive loss of pancreatic structures and a significant fibrosis of the pancreatic tissue. In the current experiment, group II's blood lipase and alpha-amylase levels were elevated by alcohol delivery [20]. This corroborated the results of Kiru and Umar, who hypothesized that alcohol's pro-inflammatory metabolic effects

and hyperplasia might be the causes of the higher blood lipase and amylase activity seen in rats administered alcohol [21]. Both oxidative and non-oxidative pancreatic damage brought on by alcohol metabolism have the potential to trigger fibrotic and inflammatory reactions. They continued by saying that alcohol use increases the risk of pancreatitis by starting inflammatory cascades that lead to chronic pancreatitis [22].

In the current investigation, group AgNPschito histopathology revealed a loss of pancreatic acini architecture along with an expansion of gaps. Similar results were discovered by Lee et al., who connected ethanol-induced oxidative stress to acinar damage. This stress results in the generation of free radicals, which in turn causes the lipid bilayer of the cell membrane to peroxide and ultimately disintegrate [23]. Chitosan, a cationic polysaccharide, exhibits protective and antioxidant activity evidenced by a reduction in blood biochemical disturbances and histological alterations, a decrease in oxidative stress marker activity, and an improvement in the functional and structural pancreatic injuries caused by ethyl alcohol in rats in grades III and IV. Chitosan's solubility in gastric acid also greatly reduces its bioavailability and, consequently, its pharmacodynamic effects [18].

Researchers have reported AgNPs (AgNPs) as an efficient antibacterial agent in a number of trials. AgNPs are primarily utilized in the treatment of burns and other open wounds in order to prevent nosocomial pathogens and wound infections. AgNPs' advantageous physicochemical characteristics play a major role in research and medicine. Antifungal, anti-inflammatory, antiviral, antibacterial, antiangiogenic, and antiplatelet characteristics are known to be present in AgNPs [24].

5. Conclusion

In conclusion, this study highlights the promising therapeutic potential of AgNPsChito in the treatment of pancreatitis induced by alcohol. The administration of AgNPsChito resulted in significant improvements in liver function, blood glucose levels, pancreatic enzymes, and oxidative stress markers in an animal model of pancreatitis. These findings suggest that AgNPs-Chito possesses multifaceted therapeutic properties, including antimicrobial, anti-inflammatory, and antioxidant effects, which contribute to its efficacy in alleviating pancreatitis.

Conflict of interest : NA

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