ECRONICON OPEN ACCESS

EC GASTROENTEROLOGY AND DIGESTIVE SYSTEM

Research Article

Anti-Inflammatory Properties of a Processed Copper Complex in L-Arginine Induced Pancreatitis - Two Experimental Studies

Vaidya Balendu Prakash[1]*, Sneha Tiwari[2], Vaidya Shikha Prakash[3] and Shakshi Sharma[4]

[1]*Director, VCPC Research Foundation, Uttarakhand, India*

[2]*Assistant Manager, Clinical Research, VCPC Research Foundation, Uttarakhand, India*

[3]*Ayurvedic Consultant, Padaav - Speciality Ayurvedic Treatment Centre, Uttarakhand, India*

[4]*Manager, Clinical Services, Padaav - Speciality Ayurvedic Treatment Centre, Uttarakhand, India*

***Corresponding Author:** Vaidya Balendu Prakash, Director, VCPC Research Foundation, Uttarakhand, India.

Received: May 20, 2019; **Published:** June 21, 2019

Abstract

Background: Recurrent Acute/Chronic Pancreatitis (RA/CP) is an inflammatory disorder of the pancreas. The disease is progressive in nature and may turn fatal in due course. The aetiology of this inflammatory condition majorly remains mysterious, especially in Indian context, where majority of the patients of RA/CP are non-alcoholics and non-tobacco users with no family history of the disease. An Ayurvedic Mineral Complex (AMC) has shown significant improvement in the clinical conditions of pancreatitis patients and significantly reduced acute exacerbations and emergency hospitalizations in a number of cases. The present set of studies was carried to understand the mechanism of AMC.

Methodology: AMC was evaluated for its pancreatitis protective properties at different doses in an existing model of L-Arginine induced pancreatitis in albino male wistar rats and compared to Methylprednisolone, a known anti-inflammatory agent. The study was carried in two phases, with three different doses of AMC used in each phase.

Results: The studies indicate that AMC was well tolerated. It did not cause mortality or any clinical signs of toxicity in male wistar rats, who were given a daily dose of AMC for twenty-one days. There was no change in body weight and food consumption pattern. It also decreased the oxidative stress, inflammatory cytokines and severity of inflammatory condition in pancreas by reducing structural changes. The best pancreatitis protective effect of AMC was observed at doses of 25 mg/kg and 19 mg/kg body weight.

Conclusion: The results of the aforesaid studies validate the stated clinical efficacy of AMC by showing its strong pancreatitis protective properties. AMC might be developed as a potential anti-inflammatory agent.

Keywords: *Pancreatitis; Ayurveda; Rasa Shastra; Chronic; Mineral Complex*

Abbreviations

RA/CP: Recurrent Acute/Chronic Pancreatitis; AMC: Ayurvedic Mineral Complex; p.o: Per Oral; TI: Test Item; RI: Reference Item

Introduction

Pancreatitis, first ascribed by Reginald Fitz in 1889, is an inflammatory disorder of the pancreas [1,2]. Pancreatitis is broadly classified as Acute and Chronic Pancreatitis. While histologic features of Acute Pancreatitis include inflammatory cell infiltrate mixed with edema and fibrinous exudates, loss of acinar cells, presence of an irregular interlobular fibrosis, infiltration of inflammatory cells and relative conservation of intralobular ducts and islets are seen in Chronic Pancreatitis [3,4]. Pancreatitis has no established cure and is largely

managed by pancreatic enzymes and vitamin supplements with intravenous fluids, antibiotics and painkillers used for resolution of acute exacerbations of the disease. Steroidal and non-steroidal anti-inflammatory drugs (NSAIDS) may also improve outcomes in patients with severe pancreatitis. However, these therapies do not bring any significant difference in the complication rates or Physiology and Chronic Health Evaluation II (APACHE II) scores in patients [5]. NSAIDS have also been found effective in preventing post ERCP Pancreatitis but certain classes such as sulindac and salicylates are also known to increase the risk of Acute Pancreatitis [6,7]. Protease, lipase and amylase are enzymes artificially given to majority of the patients suffering with Pancreatitis. While, protease helps in digestion of protein and has shown limited anti-inflammatory properties, other enzymes help in the digestion of fat and starch. [8, 9] Besides, surgical intervention is also used in some cases of Pancreatitis. However, these measures have their limitations and only certain pockets of the world can have access to these.

In the above context, a clinic based study from North India has reported significant reduction in frequency of attacks and emergency hospitalisations with marked improvement in clinical conditions of Pancreatitis patients by using an Ayurvedic Mineral Complex (AMC) [10,11]. This formulation is a combination of Copper, Mercury and Sulphur, processed with extracts of *Luffa echinata* and *Clitorea ternatea* in lemon juice for a period of 36 months. The present study was designed to evaluate the anti-inflammatory properties of AMC (TI) in L-Arginine induced Pancreatitis in albino rats in comparison to Methylprednisolone (RI).

Materials and Methods

The study was conducted in two phases by an internationally accredited laboratory *vide* study no. VBPL-P001/18 and VBPL-P011/18 respectively (accredited by AAALAC International, recognised by Department of Scientific and Industrial Research, Government of India).

36 healthy albino male wistar rats, aged 8 - 10 weeks weighing 180 - 200 gms were chosen for each of the two phases of the study and were distributed into six equal groups. Animals were allocated to different treatment groups using randomized block design and it was ensured that there is no statistically significant difference in the body weight between the treatment groups. G1 group was considered as placebo treatment group and did not receive any treatment. G2 was disease control group, G3-G5 animals were given AMC at different doses and G6 was dosed with 6-α-methylprednisolone (Refer table 1a and 1b).

Groups	Daily dose	Duration (in days)	No. of animals
G1 - Untreated control	-	21	6
G2 - Disease control	L-Arginine 2 g/kg		6
G3 - Test group I	AMC 25 mg/kg + L-Arginine 2 g/kg		6
G4 - Test group II	AMC 50 mg/kg + L-Arginine 2 g/kg		6
G5 - Test group III	AMC 100 mg/kg + L-Arginine 2 g/kg		6
G6 - Reference group*	Methylprednisolone 30 mg/kg + L-Arginine 2 g/kg		6

Table 1a: *Details of groups and dose schedule in Phase I.*

**In G6, the treatment had to be terminated after 14 days, which was considered humane endpoint due to >20% weight loss in the animals [21].*

Groups	Daily dose	Duration (in days)	No. of animals
G1 - Untreated control	-	21	6
G2 - Disease control	L-Arginine 2 g/kg		6
G3 - Test group I	AMC 13 mg/kg + L-Arginine 2 g/kg		6
G4 - Test group II	AMC 19 mg/kg + L-Arginine 2 g/kg		6
G5 - Test group III	AMC 25 mg/kg + L-Arginine 2 g/kg		6
G6 - Reference group*	Methylprednisolone 15 mg/kg + L-Arginine 2 g/kg		6

Table 1b: *Details of groups and dose schedule in Phase II.*

**Considering the outcomes in Phase I, Methylprednisolone was dosed at 15 mg/kg in G6 Phase II. However, the treatment could be carried for 18 days only, which was considered as humane end point due to > 20% weight loss in animals [21].*

On Day 1, vehicle/TI/RI was administered to the respective groups. After 30 minutes of vehicle/TI/RI administration, L-arginine at 2.0 g/kg body weight intraperitoneal (i.p) was injected to G2 to G6, whereas vehicle of L-arginine was injected to G1. After 1 hr of vehicle/L-arginine injection, blood was drawn by retro orbital sinus and serum amylase and lipase levels were estimated. Further from Day 2, vehicle or L-Arginine 2.0 g/kg was injected intraperitoneally to G1 or G2-G6 groups after 30 minutes of vehicle/TI/RI administration.

Dose selection, route of administration, dose volume and frequency of administration

The dose levels of AMC were selected based on the therapeutic dose levels in humans. The selected highest dose 100 mg/kg of AMC is equivalent to approximately three times the human therapeutic dose. Test item was administered through oral (gavage) route as oral is the intended route of administration in humans. Each animal was dosed based on body weight. The test item/reference item was administered at the dose volume of 10 ml/kg. The animals were treated daily with vehicle or AMC for a period of 3 weeks.

The animals were observed for morbidity, mortality, clinical signs of toxicity, body weight, feed consumption, clinical pathology, chemistry and gross necropsy. All data were summarized in tabular form. Statistical analysis was performed using graph Prism program. Data for each group of animals were subjected to analysis of variance (ANOVA). All analysis and comparisons were evaluated at the 5% ($P \leq 0.05$) level.

Results

In phase I of the study, one animal of the disease control group with severe pancreatitis died on day 11 and two animals in the reference item treated group with moderate pancreatitis died on day 13 and 14, due to unknown reasons. Weight loss (more than 20%) was noted in animals of G6 and the treatment could be continued for 14 days only. Significant pancreatitis protective properties were noted in G3 (AMC 25 mg/kg). In phase II of the study, one animal of G6 died on day 18. Histopathology could not be carried for this animal due to autolysis. Weight loss (more than 20%) was noted in G6 even after reduction of doses to half in the second phase and this group could be treated for 18 days only. G4 (AMC 19 mg/kg) and G6 (AMC 25 mg/kg) showed similar results with significant pancreatitis protective properties.

There was no significant change in clinical pathology except in Myeloperoxidase levels that reduced in test item group compared to disease control group in both pancreas and lungs. Levels of inflammatory cytokines also reduced in groups that were treated with AMC.

AMC caused no gross pathological changes in any of the organs. Figure 1a and 1b show histopathological grading in the different groups [12]. Histological changes observed in the groups are depicted in figure 2a and 2b. No changes were observed in histopathology of lungs, liver, kidneys, stomach and intestine.

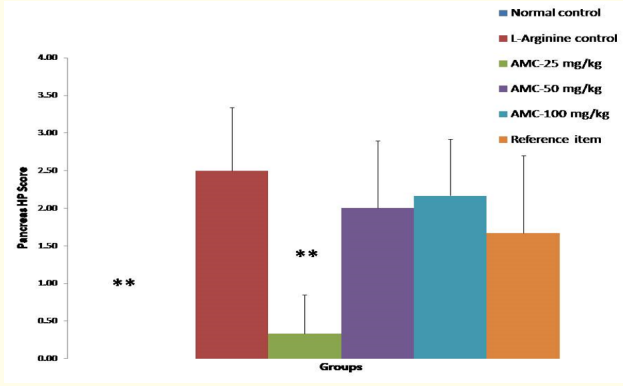

Figure 1a: *Graphical representation of results of histopathology reports in the groups - Phase I.*

*** Statistically significant (p-value < 0.001); analysis done using Dunn's Multiple comparison test (ANOVA non-parametric test).*

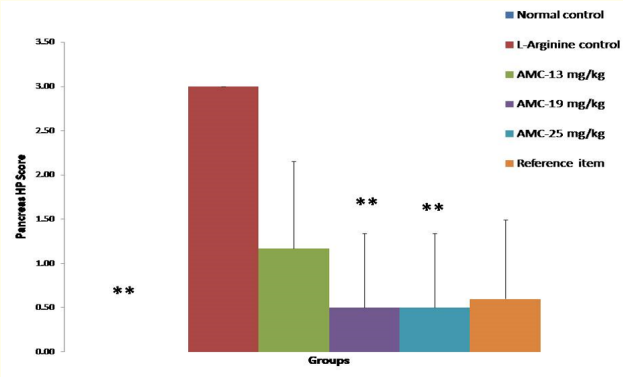

Figure 1b: *Graphical representation of results of histopathology reports in the groups - Phase II.*

*** Statistically significant (p-value <0.0001); analysis done using Dunn's Multiple comparison test (ANOVA non-parametric test).*

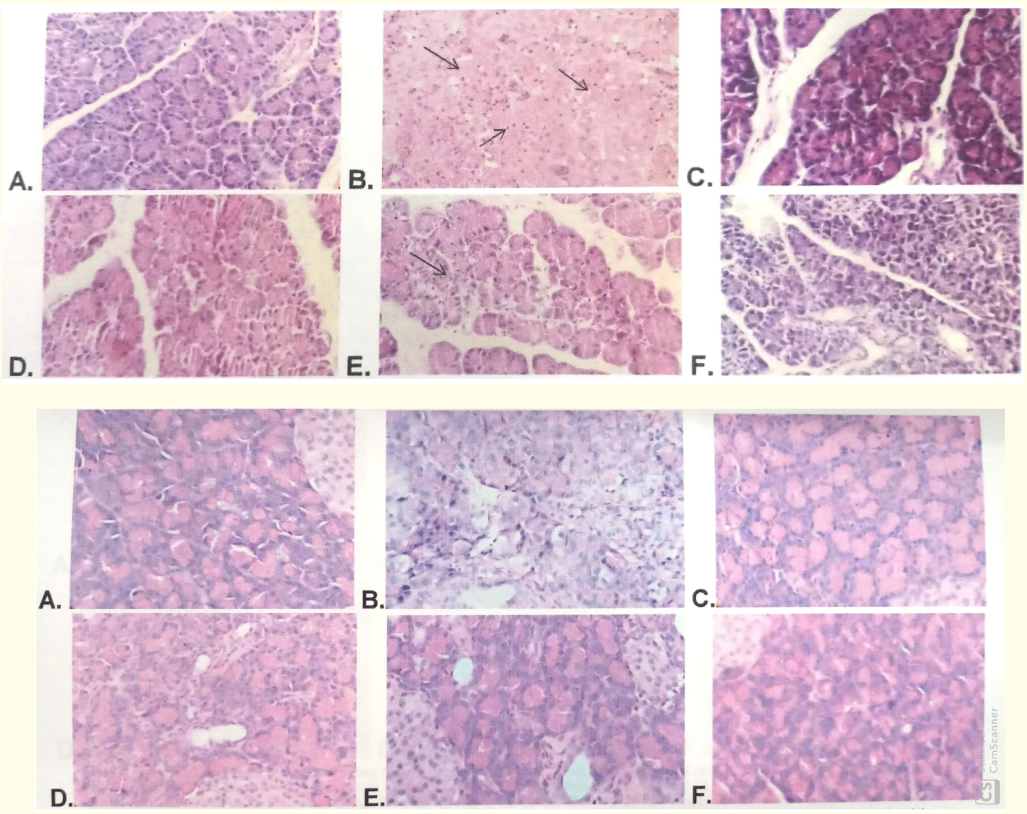

Figure 2a and 2b: *A. G1 (x400) Showing normal tissue architecture; B. G2 (x400) Showing severe pancreatitis involving severe cellular degradation, necrosis, inflammatory cell infiltration and microcytic vacuolation; C. G3 (x400) Showing normal tissue architecture; D. G4 (x400) Showing normal tissue architecture; E. G5 (x400) Showing moderate pancreatitic features; F. G6 (x400) Showing almost normal tissue architecture.*

Discussion

Chronic pancreatitis is a progressive inflammatory disease of the pancreas and is marked by irreversible morphologic changes and fibrotic replacement of the gland [13]. Several noninvasive severe basic amino acid-induced pancreatitis models are appreciated animal model, where model reproduce in most laboratory and is similar to morphological features of human pancreatitis. Consequently, the investigation of basic amino acid-induced pancreatitis may offer us a better understanding of the pathogenesis and possible treatment options of the human disease [14].

In this study, pancreatitis was induced by L-Arginine and the protective effect of AMC was evaluated in male Wistar rats and compared with reference item, methylprednisolone [14,15]. Parameters such as morbidity, mortality, clinical signs of toxicity, changes in body weight, food consumption, clinical pathology parameters, myeloperoxidase level, levels of inflammatory cytokines, serum amylase and lipase, gross pathological and histopathological changes were observed. The results of the study clearly demonstrates that AMC decreased the severity of inflammatory condition in pancreas by reducing structural changes and also decreased inflammatory cytokines and oxidative stress in pathological findings.

AMC is derived from *Rasa Shastra* in *Ayurveda*, which deals with the therapeutics of processed metals and minerals in combination with substances of plant and animal origin. Most of the raw materials used in *Rasa Shastra* are moderately to severely toxic in their raw forms [16]. However, traditional methodologies convert these toxic metals into non-toxic and therapeutic mineral forms [11].

Mercury, Copper and Sulphur are toxic in raw forms [17,18]. However, traditional literature and some studies also describe Copper to have strong anti-inflammatory properties (*shoth nashak*) [19,20]. AMC has been used in clinical practice in India for the treatment of patients of different variants of Pancreatitis and could bring complete and sustainable relief in a number of patients. The present study is first of its kind which was aimed to understand the mechanism behind the observed clinical efficacy of AMC in Pancreatitis patients.

Methylprednisolone is a known anti-inflammatory agent. In this study, AMC has shown significant reduction in inflammation in L-Arginine induced Pancreatitis in comparison to Methylprednisolone. Thus, the study was able to produce interesting findings and shows the need for further exploration of traditional knowledge of *Rasa Shastra* for its hidden therapeutics. Also, AMC should be studied further to develop it as a potential anti-inflammatory agent by using more scientific tools in its processing from raw material to finished state.

Conclusion

AMC might be a potent anti-inflammatory test material and needs to be studied and scientifically developed.

Acknowledgement

We are grateful to Vipragen Biosciences Pvt Ltd, Karnataka, India for carrying the study systematically within the stipulated time frame. We also appreciate the efforts of Mr Devendu Prakash, CEO, Shashi Chandra Rasa Shala, Uttar Pradesh, India for preparing test material.

Financial Support

We are thankful to VCPC Research Foundation, Uttarakhand, India for sponsoring the project.

Conflict of Interest

Authors declare no conflict of interest.

Bibliography

1. Busnardo AC., *et al*. "History of the pancreas". *American Journal of Surgery* 146.5 (1983): 539-550.
2. Torpy JM., *et al*. "Pancreatitis". *Journal of the American Medical Association* 307.14 (2012): 1542.
3. Jain D. "Acute pancreatitis". PathologyOutlines.com (2019).
4. Iglesias-García J., *et al*. "Histological evaluation of chronic pancreatitis by endoscopic ultrasound-guided fine needle biopsy". *Gut* 55.11 (2006): 1661-1662.

5. Dong LH., *et al*. "Corticosteroid therapy for severe acute pancreatitis: a meta-analysis of randomized, controlled trials". *International Journal of Clinical and Experimental Pathology* 8.7 (2015): 7654-7660.

6. Pezzilli R., *et al*. "NSAIDs and Acute Pancreatitis: A Systematic Review". *Pharmaceuticals (Basel)* 3.3 (2010): 558-571.

7. Moslim MA., *et al*. "A Case of Suggested Ibuprofen-Induced Acute Pancreatitis". *American Journal of Therapeutics* 23.6 (2016): e1918-e1921.

8. Craik CS., *et al*. "Proteases as therapeutics". *Biochemical Journal* 435.1 (2011): 1-16.

9. Ianiro G., *et al*. "Digestive Enzyme Supplementation in Gastrointestinal Diseases". *Current Drug Metabolism* 17.2 (2016): 187-193.

10. Prakash VB., *et al*. "Relevance of metal based Ayurvedic formulations in the management of recurrent acute/ chronic pancreatitis". *TANG [Humanitas Medicine]* 7.2 (2017): e9.

11. Prakash VB., *et al*. "Impact evaluation of Ayurvedic Treatment Protocol on three hundred nineteen cases of different variants of Pancreatitis". *Pancreatic Disorders and Therapy* 8 (2018): 2.

12. Sharma S., *et al*. "Severe Chronic Pancreatitis due to Recurrent Acute Injury: NonInvasive Chronic Pancreatitis Model of Rat". *JOP: Journal of the Pancreas* 18.2 (2017): 107-120.

13. Otsuki M., *et al*. "Animal models of chronic pancreatitis". *Gastroenterology Research and Practice* (2010): 403295.

14. Kui B., *et al*. "Recent advances in the investigation of pancreatic inflammation induced by large doses of basic amino acids in rodents". *Laboratory Investigation* 94.2 (2014): 138-149.

15. Divya SK., *et al*. "Protective Effect of Silymarin on L-Arginine Induced Acute Pancreatitis in Rats". *International Journal for Pharmaceutical Research Scholars (IJPRS)* 2.4 (2013).

16. Savrikar SS and Ravishankar B. "Introduction to 'Rasashaastra' the Iatrochemistry of Ayurveda". *African Journal of Traditional, Complementary and Alternative Medicines* 8.5 (2011): 66-82.

17. Tchounwou PB., *et al*. "Heavy metal toxicity and the environment". *Experientia Supplementum* 101 (2012): 133-164.

18. Badiye A., *et al*. "Copper Toxicity: A Comprehensive Study". *Research Journal of Recent Sciences* 2 (2013): 58-67.

19. Jackson GE., *et al*. "Design of copper-based anti-inflammatory drugs". *Journal of Inorganic Biochemistry* 79.1-4 (2000): 147-152.

20. Thunus L., *et al*. "Anti-inflammatory properties of copper, gold and silver, individually and as mixtures". *Analyst* 120.3 (1995): 967-973.

21. ENV/JM/MONO(2000)7. Guidance document on the recognition, assessment, and use - OECD (2000).

Volume 6 Issue 7 July 2019