Niclosamide as a Prospective Therapeutic in L-Arginine Induced Acute Pancreatitis in Rats; Concerning Autophagic p62/ NF-*k*B signaling pathway

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Abstract

Autophagic flux impairment is recently reported as a cardinal factor in acute pancreatitis (AP) pathogenesis. Niclosamide, an anthelmintic drug, has been lately proved to be a potent autophagy enhancer**.** The diminution of the various inflammatory factors unrestrained release via autophagy improvement may be helpful to improve the prognosis of AP. This study spots on investigating the potential ameliorative effect of niclosamide on autophagic flux and its consequent curative outcome on L-arginine-induced AP in rats. Thirty male wistar rats were divided into three groups. The first one is the control one, the second is L-arginine induced AP group, the third group is niclosamide treated L-arginine induced AP. Serum lipase, amylase, pancreatic tissue homogenate IL6, IL1β, TNFα, NF-kB, oxidative stress biomarkers; glutathione peroxidase activity, Hydroxy-2'-Deoxy-Guanosine and total antioxidant capacity levels were evaluated. Besides, the DNA-binding activity of nuclear erythroid related factor 2 (Nrf-2) was assessed using a pancreatic tissue nuclear extract. Both LC3-II subunit & P62 mRNA were quantified using PCR technique. Morphometric analysis of histopathological changes was done. The obtained data showed that niclosamide improved L-arginine induced AP as evidenced by significantly reduced serum lipase and amylase levels, which could be related to improvement of autophagy flux impairment as evidenced by decreased levels of LC3-II and p62 expression in pancreatic cells, in addition to anti-inflammatory effect as evidenced by decreased NF-kB and proinflammatory cytokines levels, along with improving the antioxidant capacity of the pancreatic tissue. As manifested by elevation of Nrf-2- DNA binding activity and normalization of oxidative stress biomarkers levels. These results could pave the way for niclosamide as a potential therapeutic role in acute pancreatitis.

Key words: *Acute Pancreatitis; Niclosamide; autophagy; LC3-II; P62; Nrf-2.*

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Introduction

Acutepancreatitis(AP)isapancreaticinflammatory disorder, ranging from a mild self-limited disease to a severe necrotizing inflammation in nearly 15–20% of patients with up to 30% mortality rate. No specific drugs have been presented till now for the reducing the fore-mentioned risks as AP pathophysiology is

still poorly known [1]. Experimentally induced AP by L-arginine is considered a severe necrotizing model. Twenty-four hours after intraperitoneal (I.P) injection of L-arginine, characteristic tissue inflammatory changes are verified by histological and biochemical examinations. The high reproducibility, non-invasiveness and dose-dependent acinar necrosis make this model highly suitable for exploring the AP pathogenesis as well as novel therapeutic options [2].

Several recent studies revealed that impaired autophagy is a pancreatitis key provoking event and a converging step of multiple deranged pathways, leading to disease initiation and propagation of inflammatory response that defines the severity of AP [3].

Autophagy is a sequential process via which cells degrade long-lived proteins and cytoplasmic organelles, LC3 is the most extensively used indicator of autophagosomes. LC3 is an essential protein in the process of autophagy substrate selection and autophagosome formation, serving as a recognition site for LC3-binding chaperones such as p62 that deliver their cargo to autophagosomes. Once formed, autophagosomes travel along microtubules to reach lysosomes where they fuse to generate autophagolysosomes allowing degradation of their constituents by lysosomal acid hydrolases [4].

Dysfunctional autophagy influx results in activation of NF-kB with promoting macrophages infiltration to the pancreas, leading to up-regulation of cytokines and chemokines as well as pancreatic tissue infiltration by inflammatory cells [5]. The mechanisms of these processes involve rise of reactive species of oxygen (ROS) generation due to inadequate clearance of damaged mitochondria, and accumulation of p62-containing protein aggregates; P62 accumulation causes endoplasmic reticulum stress, which sequentially leads to NF-κB activation and triggers inflammatory protein transcription [6]. This generates unrestricted release of excess pro-inflammatory factors like tumor necrosis factor- α (TNF-α), interleukin-6 (IL-6), IL-1β and IL-10, which are the cornerstone triggering components of AP. The abovementioned factors stimulate both necrotic and apoptotic pathways of pancreatic acinar cells and ultimately intensify the pancreatic tissue damage. Therefore, the diminution of the unrestrained release of several inflammatory factors via restoration of normal autophagy flux may be valuable to improve the prognosis of AP [7].

Noteworthy, the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a mainstay factor in the antioxidant pancreatic defense mechanism through initiating the transcription of cytoprotective genes, and its modulation may boost the prognosis of AP. Interestingly, NF-kB competes with CH1-KIX domain of CBP (cAMP-response- element-binding protein-binding protein), so it decreases the availability of CBP which is a transcriptional co-activator of Nrf2. In the meantime, it additionally promotes phosphorylation of p65 at Ser276 which in turn hinders CBP binding to Nrf2 [8].

Niclosamide is an approved anthelmintic drug with a good safety profile. Recently, niclosamide has been documented as an effective autophagy enhancer and inducer of mitochondrial fission [9]. NF-κB, ROS as well as rapamycin complex 1 (mTORC-1) are additional mechanistic targets of niclosamide [10], which suggests that this drug may be an encouraging agent for the treatment of numerous conditions including AP. So, this study was designed to discover the potential mitigating niclosamide effect on dysfunctional autophagic flux and its possible therapeutic outcome on L-arginine induced AP in rats.

Material and Methods

Animals

Thirty male wistar rats aged eight weeks old with an average weight (150-200 g) were obtained from Tanta University Animal House. All rats were housed in wire mesh cages (20 ± 2 °C, 65 ± 10% relative humidity, 12-hour light/dark cycle, standard

laboratory diet and water ad libitum) and allowed to adapt for one week before starting the experiment. All experiments carried out following the guidelines for the care and use of experimental animals in Faculty of Medicine, Tanta University, Egypt, with an approval (#48661/11/22) of the Tanta Faculty of Medicine's Animal Experiment Ethics Committee.

Experimental design

Rats were randomly equally divided into three groups (10 per each). Group 1 served as normal control group, received an intraperitoneal injection (I.P) of Dimethyl sulfoxide (DMSO) as a vehicle daily for 3 successive days. Group 2 (AP) received two I.P injections of 3.2g/kg L-arginine (Sigma, St., Louis, MO, USA), dissolved in distilled water at interval of 1 hour followed immediately by I.P injection of DMSO, the latter was administered daily for 3 days [11]. Group 3 (AP + Niclosamide) the rats were treated with L-arginine by the same regimen as group 2 followed immediately by I.P injection of 25 mg/kg/day niclosamide (Sigma, St. Louis, MO, USA) dissolved in DMSO (at a concentration of 5mg/ml), the latter was administered daily for 3 days [12].

Blood and Tissue sampling:

72 hours after the last L-arginine injection, animals were sacrificed under chloroform anesthesia. Blood was rapidly gathered in a well sterile dry centrifugation tube, standed for 30 min at room temperature to clot, followed by 20 min centrifugation (1000 x g at 4^{o}C). Sera were collected and kept at -80^{o}C for further biochemical analysis.

Pancreatic tissues were rapidly excised, washed at its place with ice-cold NaCl solution (0.9% w/v), allowed to dry on a filter paper, to be sectioned into four parts. The first one was stored in buffered paraformaldehyde (10%) for histo-pathological examination. The other three parts were stored at -80^{o}C till being used for tissue homogenate, nuclear extracts and RNA extraction.

Preparation of pancreatic tissue homogenate:

A piece of each stored pancreatic specimen was allowed to thaw then weighed and homogenized in 10 volumes of 50 mM, 7.4pH ice-cold phosphate-buffered saline (PBS) by a Potter–Elvenhjem tissue homogenizer. The previous homogenates were centrifuged (7700 x g for 30 min at 4^{o}C). The ultimate supernatant was collected and stored frozen at -80^{o}C for biochemical assay. The total protein content was determined according to the method illustrated by Lowry et al. [13]

Preparation of pancreatic nuclear extracts.

nuclear extracts of pancreatic tissue were prepared using Nuclear/Cytosol Fractionation Kit (Cat #K266-25, BioVision, Inc., CA, USA) following the manufacturer protocol.

Biochemical analysis

Serum was used for colorimetric assay of both serum lipase and amylase by using commercially available kits (Biodiagnostic., Egypt).

Nuclear factor erythroid 2-related factor 2 (Nrf-2) DNA-binding activity was estimated in pancreatic tissue nuclear extract by an enzyme-linked immunosorbent assay (ELISA) (Nrf2 Transcription FactorAssay Kit, Abcam, USA, Cat #ab207223) obeying to the manufacturer's instructions.

Pancreatic tissue homogenate IL6, IL1β, TNFα and NF-kB were evaluated using ELISA with available commercial kits of MyBiosource, Inc. Southern California, San Diego (USA) Catalog No: MBS175908, MBS355232, MBS175904 and MBS453975 respectively.

Quantitative measurement of LC3-II subunit and P62 mRNA by quantitative real-time reverse transcription PCR (rt-PCR):

RNA extraction: According to the context of the protocol from the manufacturer. Total RNA was extracted from rat pancreatic tissue using the Gene

JET RNA Purification Kit (Thermo Scientific, #K0731 USA). By a NanoDrop spectrophotometer (NanoDrop Technologies, Inc. Wilmington, USA), The total RNA concentration and purity were measured at the OD260 and OD260/280 ratios, respectively, and the RNA was then preserved at-80 ° C.

cDNA synthesis: RevertAid H Minus Reverse Transcriptase (Thermo Scientific, # EP0451,

USA) was utilized to revers transcribe the total RNA samples (5µg), producing cDNA that was stored at -20 ° C to be used for PCR.

Real-time quantitative PCR: The cDNA was used as a template for determining the relative expression of the**LC3-II and p62** genes by usage of StepOnePlus real-time PCR system (Applied Biosystem, USA). By Primer 5.0 software, The above mentioned genes primers were designed and their sequences were as follow: **LC3-II subunit (NCBI GenBank Nucleotide accession # NM_022867.2)** F: 5' -CATGCCGTCCGAGAAGACCT -3', and R: 5'-GATGAGCCGGACATCTTCCACT -3'. **P62 (NCBI GenBankNucleotideaccession#NM_175843.4)**F:5'--TCCTGCAGACCAAGAACTATGACATCG -3', andR:5'-TCTACGCAAGCTTAACACAACTATGAGACA -3'. The housekeeping gene **GAPDH** with primer sequences **(NCBI GenBank Nucleotide accession # NM_017008.4)** F: 5'-ATGTTCCAGTATGACTCCACTCACG-3' and R: 5' GAAGACACCAGTAGACTCCACGACA-3' was used as a reference for fold change in target gene expression calculation. A final volume of 25-µL PCR mix was prepared by adding 12.5 µL of 2X Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific, # K0221, USA), 2 µL of cDNA template, 1 µL forward primer, 1 µL reverse primer, and 8.5 µL of nuclease free water. Conditions of thermal cycling were started with initial denaturation at 95˚C for 10 minutes, followed by 40 cycles with denaturation (95˚C for 15 sec, annealing at 60˚C for 30 sec and extension at 72˚C for 30 sec). At the end of the last cycle, the temperature was increased from 60 to 95 °C for melting curve analysis. The relative expression level of genes was normalized to GAPDH and analyzed using the threshold cycle (Ct) $2^{-\Delta\Delta Ct}$ method [14].

Estimation of pancreatic tissue homogenates Oxidative stress Biomarkers:

Glutathione peroxidase GPx (EC.1.11.1.6) activity was assessed spectrophotometrically according to Paglia and Valentine (1967) method [15]. **8-Hydroxy-2'-Deoxy-Guanosine levels (8-OHdG)** were determined by ELISA kit (Cat# ADI-EKS-350, ENZO LIFE SCIENCES INT'L,PA 19462-1202, USA) obeying manufacturer's protocol. However **total antioxidant capacity** was assessed according to the method of Koracevic et al. [16]

Morphometric & statistical analysis for cell counting of inflammatory cells:

The software (Image J) (National Institute of Health, Bethesda, Maryland, USA) was used to measure cell counting of inflammatory cells in all groups of the present research. By which, 10 LM images at a magnification X 400 from each group were used.

Statistical Analysis

Data analysis was achieved using the GraphPad Instat software (Version 2.0 Philadelphia, 1993). Data were expressed as mean ± SD. Comparisons were done using one-way ANOVA followed by Tukey-Kramer as post ANOVA test. Criterion for significance was chosen to be at $p \leq 0.05$.

Results

Niclosamide administration improved L-arginine induced acute pancreatitis in rats

I.P injection of L-arginine resulted in significant increase diagnostic markers of AP, which are serum lipase and amylase levels, compared to control group. On the other hand, Niclosamide treatment markedly reduced the serum lipase and amylase levels. Moreover,

histological investigation of L-arginine treated group **(Figure 2.B)** confirmed the development of severe necrotizing pancreatitis with focal loss of normal architecture of pancreatic acinar cells, separation of lobules, dilated blood capillaries, extravasation of red blood cells between the pancreatic acini and marked mononuclear cellular infiltration when compared to that of control group **(Figure 2.A)** (H&E, Mic. Mag. x 400). As assessed by the image analyzer, AP group showed statistically significant increase in the number of inflammatory cells when compared with control group. Data were illustrated in the histogram **(Figure 3).** Pancreatic tissue of niclosamide treated group showed regression of some of the microscopic lesions, most of pancreatic acini resembled the normal structure denoting improvement of histopathological changes **(Figure 2.C)**. Statistical results for number of inflammatory cells of niclosamide treated group showed statistically highly significant decrease of the number of inflammatory cells when compared with L-arginine induced AP group. Data were illustrated in the histogram **(Figure 3)**.

Niclosamide administration improved dysfunctional autophagic flux in of L-arginine induced acute pancreatitis in rats (Figure 1.A & B):

Intraperitoneal (I.P) injection of L-arginine resulted in elevated pancreatic LC3-II and p62 mRNA levels compared to control group (P< 0.001). However, Niclosamide treatment remarkably reduced pancreatic LC3-II and p62 mRNA levels (P< 0.001) compared to L-arginine induced AP group signifying amelioration of impaired autophagic flux in pancreatic tissues.

Niclosamide administration ameliorated pancreatic inflammation and oxidative stress status of L-arginine induced acute pancreatitis in rats (Table 1&2):

I.P injection of L-arginine resulted in significant increase in pancreatic tissue NF-kB and proinflammatory cytokines IL6, IL1β, TNF a (P< 0.001) levels compared to control group, in addition to a significant increase in hydroxy-2'-deoxyguanosine and significant decrease in GPx, total antioxidant capacity and Nrf-2 DNA binding activity compared to control group. On the other hand, Niclosamide treatment markedly reduced pancreatic tissue NF-kB and proinflammatory cytokines IL6, IL1β, TNF a (P< 0.001) levels compared to L-arginine induced AP group denoting improved pancreatic inflammation along with a significant decrease in hydroxy-2'-deoxyguanosine and significant increase in GPx, total antioxidant capacity and Nrf-2 DNA binding activity compared to L-arginine induced AP group (P< 0.001) suggesting efficient mitigation of pancreatic oxidative stress.

Fig. 1.A: Pancreatic LC3/GADPH relative expression Fold change

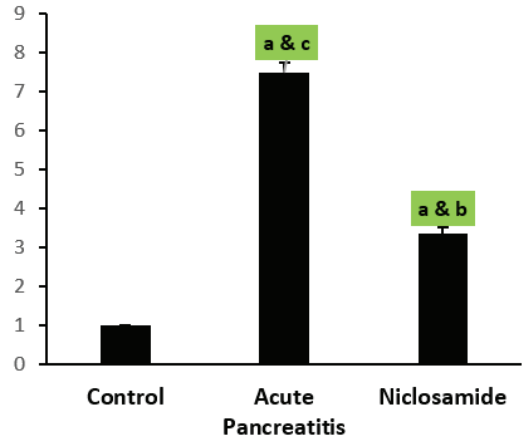

Fig. 1.B: Pancreatic P62/GADPH relative expression Fold change

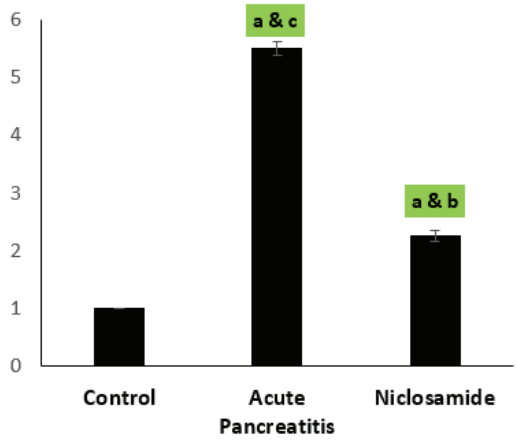

Figure (1.A&B): Quantitative real-time PCR

analysis of the mRNA expression of LC3-II subunit and P62 gene (in different groups, values expressed by fold changes ± SD. Figure 1.A shows a relative fold change of LC3-II subunit mRNA expression (7.47 ± 0.25 & 3.36 ± 0.115) for acute pancreatitis group and Niclosamide treated group respectively. However, Figure 1.B shows a relative fold change of P62 gene mRNA expression (5.50 ± 0.118 & 2.25 ± 0.095) for acute pancreatitis group and Niclosamide treated group respectively. a: $p < 0.01$ significant increase relative to control; b: $p < 0.01$ significant decrease relative to acute pancreatitis group; c: $p < 0.01$ significant increase relative to Niclosamide treated group.

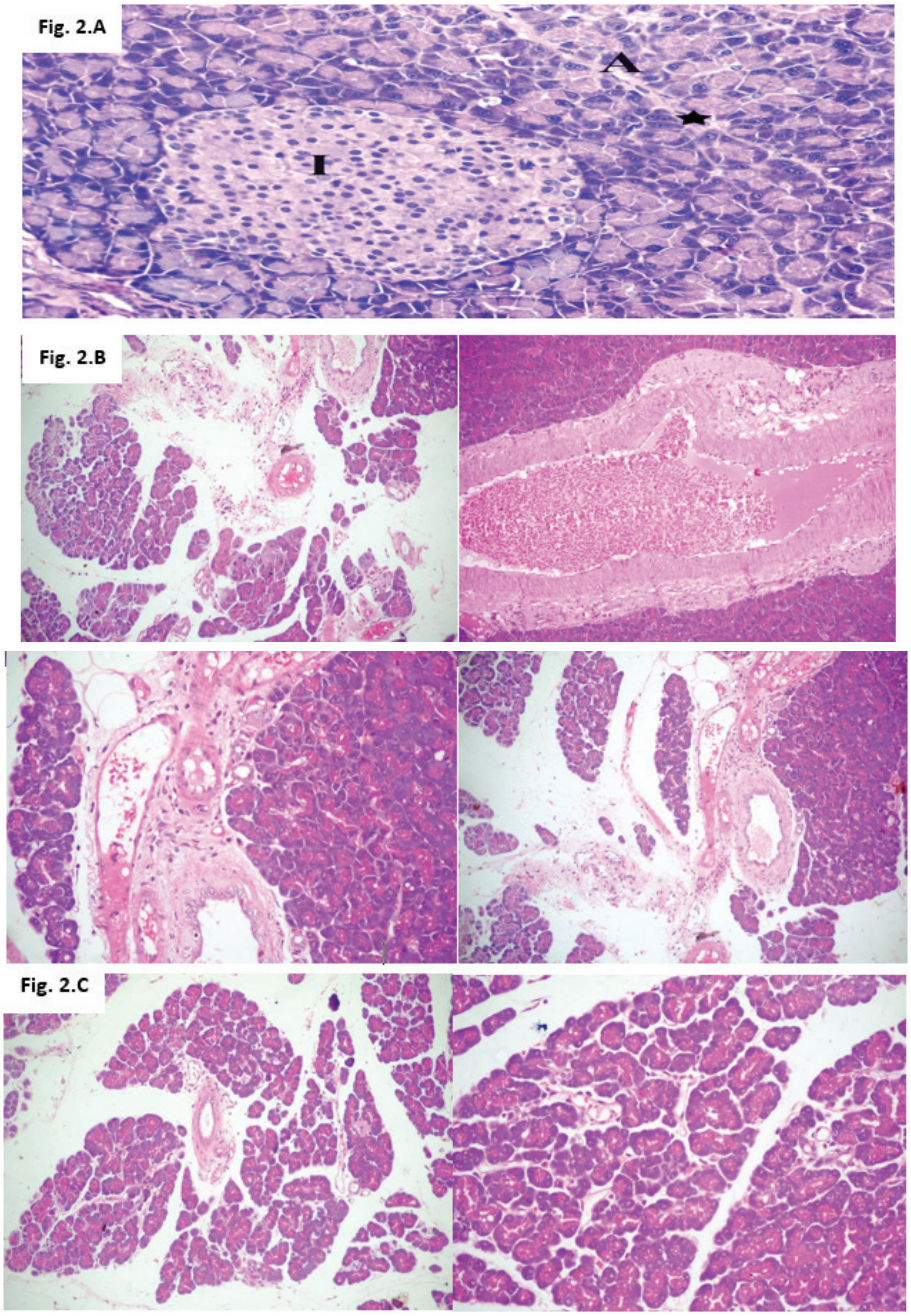

Figure 2: A photomicrograph of a pancreatic section from different studied groups (H&E, Mic. Mag. x 400). Fig. 1.A showing part of pancreatic lobules of control group, with closely packed exocrine acini (A) and a pale stained islet of Langerhans (I) is seen in-between the acini. Notice the thin connective tissue septae (asterisks) in-between the pancreatic lobules. An intralobular duct lined with cubical cells is seen in-between the acini. Notice the normal blood vessel. Fig. 2.B: pancreatic sections from acute pancreatitis group, showing focal loss of normal architecture of pancreatic acinar cells and separation of lobules, dilated blood capillaries with extravasation of red blood cells between the pancreatic acini and marked mononuclear cellular infiltration. Fig. 2.C: pancreatic sections from niclosamide treated group, regression of some of the microscopic lesions, most of pancreatic acini resembled the normal structure. (H&E, Mic. Mag. x 400).

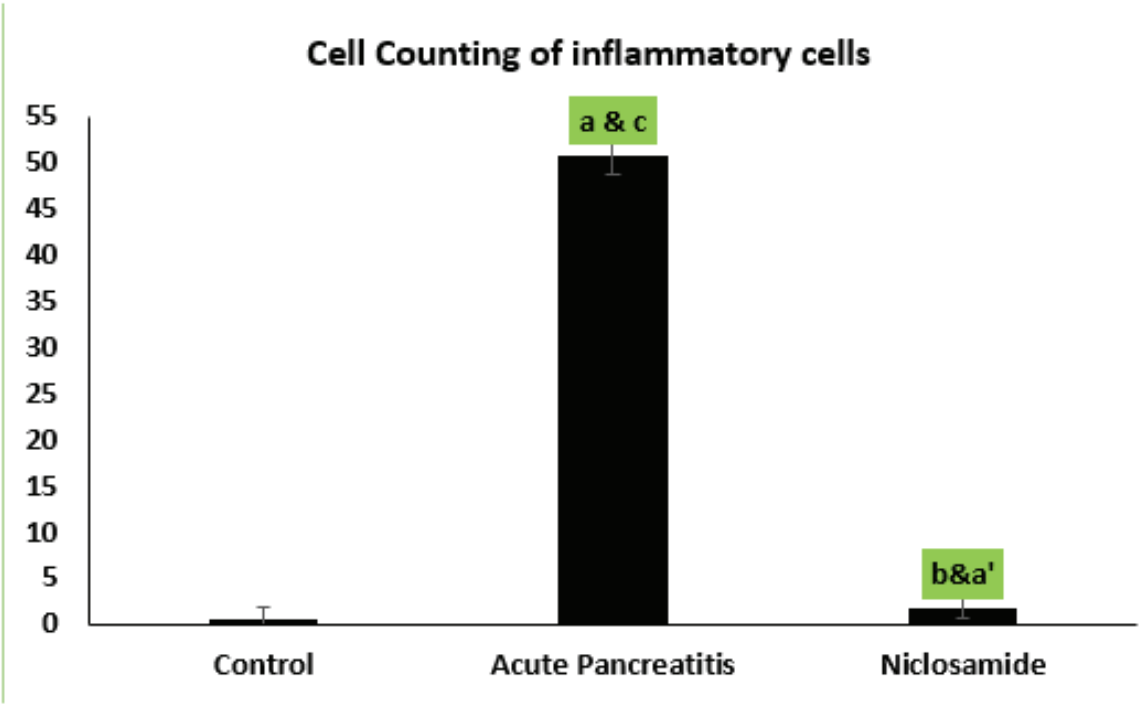

Figure 3: The number of inflammatory cells in different studied groups expressed as mean ± SD. Figure 3 shows a cell counting (0.600 ± 0.843, 50.800 ± 2.044 & 1.700 ± 1.160) for control group, acute pancreatitis group and Niclosamide treated group respectively.

a: $p < 0.01$ highly significant increase relative to control; a': $p > 0.05$ non-significant relative to control; b: $p < 0.05$ significant decrease relative to acute pancreatitis diseased group. c: $p < 0.01$ significant increase relative to Niclosamide treated group.

Table 1: A comparison of serum amylase, lipase and pancreatic tissue homogenate inflammatory and oxidative stress biomarkers among the studied groups using ANOVA test.

	Parameters	Control n=10	Acute pancreatitis n=10	Nilosamide treated n=10	ANOVA test	
		Mean ± SD	Mean ± SD	Mean ± SD	F value	P value
Direct indicators of AP	Serum Amylase U/L	876 ± 135b	1908 ± 255 a, c	1090 ± 171 b	79.36	<0.001**
	Serum Lipase U/L	946 ± 112 b	2701 ± 293 a, c	1166 ± 155 b	223.50	<0.001**
Inflammatory markers	IL6 pg/mg.tissue ptn	421 ± 87 b, c	861 ± 94 a, c	525 ± 96 a, b	61.70	<0.001**
	IL1β pg/mg.tissue ptn	332 ± 101 b, c	1064 ± 122 a, c	445 ± 72.50 a, b	153.80	<0.001**
	TNF α pg/mg.tissue ptn	26.90 ± 9.98 b	193 ± 52.70 a, c	35.10 ± 10.90 b	88.31	<0.001**
	NFKB ng/mg.tissue ptn	2.17 ± 0.44 b	4.77 ± 0.94 a, c	2.72 ± 0.38 b	45.85	<0.001**
Oxidative stress markers	Nfr-2 DNA binding activity	1.97 ±0.37 b	0.66 ±0.57 a, c	1.53 ±0.23 b	26.19	<0.001**
	GPx U/mg.tissue ptn	6.82 ± 2.10 b	1.24 ± 0.31 a, c	5.24 ± 1.63 b	34.70	<0.001**
	8-hydroxy-2'-deoxyguanosine ng/ mg.tissue ptn	4.85 ± 0.96 b	9.08 ± 0.71 a, c	5.82 ± 1.10 b	56.27	<0.001**
	Total AntiOxidant Capacity nmol/ mg.tissue ptn	2.84 ± 0.26 b	1.36 ± 0.16 a, c	2.55 ± 0.31 b	96.46	<0.001**

a= significant with control, b= significant with Acute pancreatitis, c= significant with niclosamide treated group, *= significant p< 0.05

Table 2: Pearson's Correlation between serum amylase, lipase and pancreatic tissue homogenate inflammatory and oxidative stress biomarkers in niclosamide treated group

	Serum Amylase U/L	Serum Lipase U/L	IL6 pg/ mg.tissue ptn	IL1 β pg/ mg.tissue ptn	TNF α pg/ mg.tissue ptn	NFKB ng/ mg.tissue ptn	Nfr-2 DNA binding activity	GPx U/ mg.tissue ptn	8-hydroxy-2'-deoxyguanosine ng/mg.tissue ptn
Serum Lipase U/L	0.790*								
IL6 pg/mg.tissue ptn	0.870*	0.733*							
IL1 β pg/mg.tissue ptn	0.637*	0.770*	0.530						
TNF α pg/ mg.tissue ptn	0.774*	0.815*	0.703*	0.786*					
NFKB ng/ mg.tissue ptn	0.679*	0.520*	0.729*	0.515	0.613*				
Nfr-2 DNA binding activity	-0.883*	-0.804*	-0.753*	-0.664*	-0.823*	-0.563*			
GPx U/mg.tissue ptn	-0.900*	-0.795*	-0.956*	-0.564*	-0.814*	-0.752*	0.823*		
8-hydroxy-2'-deoxyguanosine ng/mg.tissue ptn	0.920*	0.769*	0.947*	0.464	0.664*	0.716*	-0.831*	-0.931*	
Total Anti-Oxidant Capacity nmol/ mg.tissue ptn	-0.914*	-0.814*	-0.928*	-0.649*	-0.702*	-0.728*	0.883*	0.903*	-0.954*

***= significant p< 0.05**

Discussion

A potentially fatal inflammatory disease of the pancreas is acute pancreatitis (AP). Its pathogenesis remains mysterious, and it has not established clear therapies [17]. Exocrine pancreas cells are one of the highest protein synthesis and trafficking rates that make pancreatic acinar cells more in need of removing defective cytoplasmic organelles. Manifest acute pancreatitis occurs when autophagy deficiency overwhelms the unfolded protein response ability to remove protein aggregates [18]. AP is documented to have remarked early large vacuoles accumulation of in acinar cells with intra-acinar cell trypsinogen activation; the majority of these vacuoles are autophagic with reduced rates of long-lived proteins degradation along with LC3 as well as p62 accumulation that suggest impaired autophagic flux [19].

An expanding role of autophagy in controlling NF-kB signaling pathway has been lately explicated;

autophagy evidently inhibits expression of inflammatory cytokines, in particular by eradicating p62, the adaptor protein that usually triggers activation of NF-kB [20, 21]. Oppositely, failure of autophagic flux affects cellular clearance, causing pancreatic acinar cells cytokine release via activating the p62- Tumor necrosis factor receptor-associated factor 6 (TRAF6)-NF-kB pathway that sparks the inflammatory response through up-regulation of expression of inflammatory cytokines [22, 23], this finding comes in agreement with our results herein as L-arginine induced AP group displayed significant increase in pancreatic levels of LC3 and p62 signifying impaired autophagy flux in addition to upregulation of the level of the key transcription factor NF-kB with consequent increase in proinflammatory cytokines, TNF-α, IL-6 and IL1β levels.

A recently recognized interplay between autophagy, oxidative stress and inflammation plays a critical role in AP pathogenesis; Piplani et al., 2019 demonstrated that impaired autophagic flux resulted in downregulation of lysosomal-associated membrane protein-1 (LAMP-1) with consequent accumulation of dysfunctional mitochondria that excessively generate ROS, resulting in tissue damage [24].

Mounting proofs have demonstrated that Niclosamide amends diverse biochemical intracellular signaling pathways and biological processes, including uncoupling of oxidative phosphorylation, autophagy, and the Wnt/βcatenin signaling pathway [25], designating that beyond utilization in parasitic infection treatment, it can also be applicated in other diseases. The remarkable effect of niclosamide as an enhancer of autophagic flux, has been consistently reported in recent studies [26, 27] and attributed to its ability to inhibit the mammalian target of rapamycin complex 1 (mTORC1) via cytoplasmic acidification by releasing protons from lysosomes leading to prevention of ubiquitin- containing aggregates [28]. These findings are consistent with our results that showed that Niclosamide significantly decreased the expression of LC3-II, p62 indicating recovery of AP associated retarded autophagy flux process.

Notably, Zhang et al., 2017 demonstrated that Niclosamide treatment significantly increased levels of anti-inflammatory cytokines and decreased levels of pro-inflammatory cytokines, exerting a substantial protective role against renal ischemia perfusion injury in rats (12). Moreover, Jin et al. documented that niclosamide blocked (TNF-α)-induced IκBα phosphorylation, translocation of NF-κB p65 subunit, and expression of NF-κB-regulated genes in acute myelogenous leukemia cells. In addition, niclosamide prevented the DNA binding of NF-κB to the promoter of its target genes [29]. Furthermore, Cerles et al, 2016 reported that Niclosamide exerted a manifest neuroprotective effect in experimental oxaliplatin-induced neurotoxicity through downregulation of oxaliplatin-mediated H2O2 production in neuron-like cells, in addition to reducing levels of IL6, TNFα, and advanced oxidized protein products resulting in prevention of cell death and preserving the neuronal integrity [30], these results came in consonance with our findings which proved that niclosamide resulted in significant reduction of the level of the key transcription factor NF-kB with consequent declining of levels of proinflammatory cytokines, TNF-α, IL-6, IL-1β along with enhancing the antioxidant capacity of the pancreatic tissue as evidenced by upregulation of Nrf-2 DNA binding activity, Glutathione peroxidase activity as well as total antioxidant capacity in addition to decreasing Hydroxy-2'-Deoxy-Guanosine levels (8-OHdG). Interestingly, Liu et al., 2019 reported that Niclosamide could initiate non-canonical LC3 lipidation, indicating that Niclosamide may additionally induce Beclin 1-independent non-canonical autophagy [31].

In conclusion: based on our data herein, restoring normal autophagy flux process with reduced accumulation of p62 and consequent NF-kB downregulation, culminating into Nrf2 enhancement could be the underlying mechanism of Niclosamide-induced improvement of L-arginine induced AP. This finding paves the way for Niclosamide as potential

therapy of AP.

Conflict of Interests: The authors declare that they have no conflict of interests.

Funding: This research did not receive any specific grant from funding agencies in the public, Commercial or not for profit sectors.

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