Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

The potential antioxidant and anti-inflammatory effects of kynurenic acid on L-ornithine-induced acute pancreatitis in rats

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ARTICLE INFO

Keywords

Acute pancreatitis,
L-ornithine
Kynurenic acid
miR-216a
miR-217

Received 25/12/2023
Accepted 12/01/2024
Available On-Line 01/04/2024

ABSTRACT

Acute pancreatitis (AP) is an inflammatory disease primarily detected through the estimation of amylase and lipase as conventional serum markers that have a short half-life. MicroRNAs have emerged as biomarkers of different diseases including AP, cardiovascular disorders, and cancer. This study was designed to evaluate the possible ameliorating impact and anti-inflammatory role of kynurenic acid (KYNA) in AP-induced rats and identify the possible use of miR-216a and miR-217 as diagnostic biomarkers for AP. Forty male albino rats were divided into 4 equal groups. Group 1 (control group) rats injected (i.p) with physiological saline. Group 2 (AP group) rats were injected with a single dose of L-ornithine-HCl (ORN) (3 g/kg b.wt/i.p). Group 3 (KYNA+ORN group) rats were injected with a single dose of KYNA (300 mg/kg b.wt/i.p) 1 hour before ORN injection. Group 4 rats were injected with a single dose of KYNA (300 mg/kg b.wt/i.p). The ORN-induced AP group showed significant elevation in serum amylase and lipase activities, miR-216a and miR-217 expressions, glucose, IL-1β and IL-10 levels with a significant decrease in insulin level. Moreover, oxidative stress markers (MPO and MDA) were significantly increased, while the antioxidants SOD and GSH were markedly reduced. Interestingly, the severity of these alterations was reduced and ameliorated in KYNA+ORN group compared to AP group. Rats treated with KYNA alone showed non-significant changes. These data suggest that KYNA can alleviate the severity of ORN-induced AP in rats and serum miR-216a and miR-217 could be potential biomarkers for the diagnosis of AP and could be associated with inflammatory conditions in rats with AP.

1. INTRODUCTION

Acute pancreatitis (AP) is an acute non-infectious inflammatory disorder that ranges from mild and self-limited disease in most cases to severe necrotizing inflammation in up to 20% of the cases with a mortality rate of up to 30% (Liu et al., 2018). Amylase and lipase have been used as diagnostic markers for AP, but they have limited sensitivity and specificity and a short half-life. As a three-fold elevation of amylase or lipase is considered diagnostic (Banks et al., 2013). Therefore, in the early stage of AP when pancreatic injury isn't sufficient to cause a significant increase in enzymes, there will be no obvious increase in these enzymes' activities. Also, these enzymes can be elevated in conditions other than pancreatitis, such as other intra-abdominal inflammation, renal dysfunction, salivary gland disorders, acute liver failure and diabetic ketoacidosis (Matull et al., 2006) and (Muniraj et al., 2015).

MicroRNAs (miRNAs) are small, non-coding, single-stranded RNA molecules (~22 nucleotides) that play important regulatory roles in gene expression post-transcriptionally by base-pairing with target mRNA sequences and interfering with their translation (Holley and Topkara, 2011). MiRNAs play crucial regulatory roles in almost all physiological processes, including cellular development, proliferation, differentiation, metabolism and apoptosis (Bartel, 2004).

MiRNAs are tissue-specific and can be released into body fluids with tissue injury (Weber et al., 2010). Likewise, miRNAs are highly stable in serum, so they have shown promise as markers for diagnosis and monitoring of various diseases, including inflammatory conditions, neurological disorders, cancer, cardiovascular diseases, infectious diseases, and metabolic disorders (Wang et al., 2016). Additionally, miRNAs can be harnessed as therapeutic agents. Synthetic miRNA mimics are used to restore specific miRNA levels, which their expression decreased in certain diseases, and normalize gene expression. Meanwhile, anti-miRNA oligonucleotides can be employed to selectively inhibit the function of oncogenic miRNAs whose overexpression promote disease progression (Bonneau et al., 2019).

On the other hand, miR-216a and miR-217 are enriched in the pancreatic acinar cells in humans and rats (Smith et al., 2016). These two miRNAs have a critical role in

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the development and progression of AP by inducing pro-inflammatory cytokines and exacerbating pancreatic injury (Zhang et al., 2015) and (Wang et al., 2022).

AP has been studied using a variety of animal models among which, is induction by intraperitoneal (i.p) injection of L-ornithine (3 g/kg), that considered a simple and non-invasive model of severe acute necrotizing pancreatitis (Rakonczay et al., 2008).

Kynurenic acid (KYNA) is an endogenous tryptophan metabolite which exerts neuroprotective, anticonvulsant (Schwarcz et al., 2012), anti-inflammatory, immune-modulatory (Wirthgen et al., 2018) and antioxidant effects (Lugo-Huitrón et al., 2011).

Therefore, the objective of this study was to investigate the anti-inflammatory effect and ameliorating role of KYNA on alterations of miRNA and other biomarkers in an experimental rat model of L-ornithine-induced acute pancreatitis and identify the potential use of miR-216a and miR-217 as diagnostic biomarkers for AP.

2. MATERIALS AND METHODS:

2.1. Experimental animals

Forty male albino rats of 8-10 weeks old and weighing 150-200 g were used in this study. The rats were obtained from the Laboratory Animals Research Center, Faculty of Veterinary Medicine, Zagazig University, Egypt. Rats were housed in stainless steel wire cages and acclimated for one week away from any stressful stimuli, at room temperature 25±5°C, humidity 60±5%, with a 12-h light/dark cycle and were given a standard rat ration and water was supplied ad-libitum. The experimental procedures were carried out by guidelines for the care and use of laboratory animals, approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, Benha University, Egypt (BUFVTM 48-09-23).

2.2. Chemicals

1-L-ornithine-HCl: L-ornithine-HCl was purchased from Alfa Aesar (Karlsruhe, Germany).

2- kynurenic acid: Kynurenic acid (KYNA) was obtained from Sigma-Aldrich (St. Louis, MO, USA).

L-ornithine-HCl (300 mg/ml) and kynurenic acid (50 mg/ml) powder were freshly prepared by dissolving in physiological saline (PS) with pH 7.4 before administration.

2.3. Design of Experimental Work

The animals were randomly divided into four groups (10 rats each) as follows: Group 1 (normal control group) rats injected (i.p) with physiological saline. Group 2 (AP group) rats were injected i.p with a single dose of L-ornithine-HCl (ORN) (3 g/kg b.wt) (Rakonczay et al., 2008). Group 3 (KYNA+ORN) rats were injected by a single dose of kynurenic acid (KYNA) (300 mg/kg b.wt/i.p) 1 hour before ORN injection (Balla et al., 2021). Group 4 rats were injected with a single dose of kynurenic acid (KYNA) (300 mg/kg b.wt/i.p).

All rats were fasted overnight before sampling at either 24 or 48 hrs. after L-ornithine injection.

2.3. Sampling

Random blood samples and pancreatic tissue specimens were collected from all animals' groups two at 24 and 48 hours after induction of acute pancreatitis by L-ornithine (3 g/kg b.wt).

2.3.1. Blood samples

Blood samples for serum separation were collected from the retro-orbital venus plexus of the rats in serum separator tubes, after 24 and 48 hrs. from the injection of ORN, then allowed to coagulate for 20 min at room temperature and centrifugated for 10 min at 2000 rpm. The serum was collected into clean, dry, labelled Eppendorf tubes. All sera were divided into two parts; 1st part was preserved at -20 °C for biochemical analysis and 2nd part was preserved at -80 °C till RNA extraction for determination of miR-216a and miR-217 gene expression.

2.3.2. Tissue specimens

After blood sampling, rats were euthanized according to the Animal Ethics Committee, the pancreas was quickly removed, washed with normal saline to remove blood and frozen at -20 °C until further use for determination of oxidative/antioxidant parameters.

2.4. Biochemical analysis:

Serum amylase, lipase, and blood glucose were determined using colorimetric assay kits supplied y Spinreact (Girona, Spain), according to the method described by (Foo and Bais, 1998, Young, 1995 and Young, 2001), respectively. Serum insulin was evaluated by ELISA kit (BioVendor, Heidelberg, Germany, Catalog No. RTC018R) according to the manufacturer's instructions. Serum IL-1β and IL-10 were also evaluated by ELISA kits (Boster Biological Technology, Wuhan, China, Catalog. No: EK0393 and MyBioSource, CA, USA, Catalog. No: MBS269138) respectively according to the manufacturer instructions. Moreover, the oxidative stress/antioxidant markers levels were estimated in pancreatic homogenate using ELISA kits according to the manufacturer's instructions. Malondialdehyde (MDA) (My BioSource, CA, USA, Catalog No: MBS268427). Myeloperoxidase (MPO), superoxide dismutase (SOD) and glutathione (GSH) (CUSABIO, Wuhan, China, Catalog NO: MPO: CSB-E08722r, SOD: CSB-E08555r and GSH: CSB-E12144r).

2.5. Molecular analysis for MicroRNA:
Extraction of RNA

Frozen serum samples (200 µl) from each rat were allowed to thaw and used for RNA extraction. RNeasy Mini Kit (Qiagen, Germany, GmbH) was used to extract total RNA as described by the manufacturer's protocol. Primers used were supplied by BioBasic (Markham Ontario, Canada) and are listed in Table (1).

Table (1): Primer's sequence of miR-216a and miR-217 used in qPCR.

Gene	Primer sequence (5'-3')	Reference
U6	GCTTCGGCAGCACATATACTAAAAT	(Chen et al., 2013)
	CGCTTCACGAATTTGCGTGTCAT	
miR-216a	TAATCTCAGCTGGCAACTGTG	(Qian et al., 2021)
	GAACATGTCTGCGTATCTC	
miR-217	CATGCTCGAGCTTATCAAGGATAAAATACCATG	(Yang et al., 2019)
	GTTACGGCCGCTTGAGATCTACTCTAATTTCTTT TTTAAC	

SYBR green rt-PCR

25µl reaction consisting of 12.5µl of the 2x QuantiTect SYBR Green PCR Master Mix (Qiagen, Germany, GmbH), 0.25µl of Reverse transcriptase enzymes (Thermo Fisher, USA), 0.5µl of Forward primer (20 pmol), 0.5µl Reverse primer (20 pmol), 8.25µl of RNase Free Water, 3µl of Template RNA. The reaction was carried out in real real-time PCR machine (Stratagene MX3005P).

Cycling conditions for SYBR green rt-PCR

Reverse transcription was done at 50°C /30 min. In addition, Primary denaturation at 94°C/15 min. meanwhile, Amplification (40 cycles) includes Secondary denaturation at 94°C /15 sec, Annealing at 60°°C/30 sec and extension at 72°C /30 sec.

2.6. Statistical analysis

Statistical analysis was carried out using SPSS version 27.0. Data were treated as a complete randomization design according to Steel et al. (1997). Differences between groups were analyzed using Two-way ANOVA and a post hoc Duncun test.P< 0.05 values are considered significant.

3. RESULTS

Serum amylase and lipase activities

Serum amylase and lipase activities showed significant increases in the ORN-treated group when compared to the control group. In the KYNA+ORN group, pre-treatment with KYNA significantly decreased serum amylase and lipase activities by (44.66%, 53.5%) at 24 hr and (14.69%, 25.91%) at 48 hr compared to the ORN-treated group and their activities returned to control levels at 48 hr. Meanwhile, non-significant changes were observed in the activity of serum amylase and lipase in the KYNA-treated group compared to the control group (table 2).

Serum insulin and blood glucose levels

The ORN-treated group revealed a significant increase in serum fasting blood glucose level along with a significant reduction in serum insulin level compared to the control group. However, KYNA+ORN-treated rats revealed a significant decrease in serum fasting blood glucose level whereas serum insulin level showed significant elevation compared to ORN-treated rats. KYNA+ORN-treated rats showed significant enhancement in glucose levels while a significant reduction in insulin compared to those of the control group.
Meanwhile, non-significant changes were observed in glucose and insulin levels in KYNA-treated rats compared to rats in the control group (table 2).

Serum inflammatory marker levels

The ORN-treated group exhibited significant elevation in serum IL-1β levels (at 24 and 48 hr) with a significant increase in IL-10 level (at 48 hr) compared to the control group. Meanwhile, the KYNA+ORN group exhibited a significant decline in serum IL-1β level (39.63% at 24 hr, 48,15% at 48 hr) along with a significant enhancement in IL-10 level compared to the ORN-treated group. Serum IL-1β and IL-10 were significantly increased in the KYNA+ORN group compared to the control group. On the other hand, non-significant alteration was observed in IL-1β and IL-10 levels in KYNA-treated group compared to the control group (table 2).

Table (2): Protective effect of Kynurenic acid (KYNA) on serum parameters in L-ornithine (ORN)induced acute pancreatitis in rats.

Parameters	Animal groups	Time /hour	
		24 hour	48 hour
Amylase (U/L)	Control	654.91 ± 25.27^{cA}	640.30 ± 13.59^{bA}
	ORN	1780.60 ± 15.42^{aA}	734.83 ± 20.39^{aB}
	KYNA+ORN	985.33 ± 10.73^{bA}	626.92 ± 12.21^{bB}
	KYNA	650.33 ± 11.02^{cA}	639.90 ± 17.76^{bA}
Lipase (U/L)	Control	22.71 ± 0.63^{cA}	21.13 ± 1.13^{bA}
	ORN	130.86 ± 9.39^{aA}	29.45 ± 1.16^{aB}
	KYNA+ORN	61.44 ± 3.32^{bA}	21.82 ± 1.02^{bB}
	KYNA	20.95 ± 1.71^{cA}	21.31 ± 0.69^{bA}
Insulin (ng/mL)	Control	3.28 ± 0.32^{aA}	3.47 ± 0.28^{aA}
	ORN	0.90 ± 0.02^{cA}	0.75 ± 0.04^{cA}
	KYNA+ORN	1.81 ± 0.19^{bA}	2.00 ± 0.06^{bA}
	KYNA	3.36 ± 0.24^{aA}	3.45 ± 0.23^{aA}
Glucose (mg/dl)	Control	106.19 ± 6.18^{cA}	107.39 ± 9.11^{cA}
	ORN	202.71 ± 7.57^{aB}	234.38 ± 7.31^{aA}
	KYNA+ORN	147.81 ± 6.04^{bA}	136.99 ± 2.59^{bB}
	KYNA	108.53 ± 6.80^{cA}	106.41 ± 5.57^{cA}
IL-1B (pg/ml)	Control	33.89 ± 1.38^{cA}	30.63 ± 0.32^{cA}
	ORN	83.77 ± 2.71^{aA}	76.30 ± 2.44^{aB}
	KYNA+ORN	50.57 ± 4.04^{bA}	39.56 ± 0.34^{bB}
	KYNA	30.02 ± 2.16^{cA}	30.18 ± 1.26^{cA}
IL-10 (pg/ml)	Control	24.54 ± 1.61^{bA}	24.72 ± 1.95^{cA}
	ORN	27.26 ± 3.13^{bB}	36.87 ± 2.15^{bA}
	KYNA+ORN	69.19 ± 3.98^{aB}	88.84 ± 1.46^{aA}
	KYNA	28.49 ± 1.21^{bA}	27.07 ± 2.54^{cA}

a, b and c: Different superscript letters within the same column showed significant differences (P<0.05)
A, B and C: Different superscript letters within the same row showed significant differences (P<0.05).

Oxidative stress/antioxidant markers in the pancreatic tissue

Pancreatic MPO activity and MDA level were significantly increased, whereas pancreatic SOD activity and GSH level showed a significant decreased in the ORN-treated group compared to the control group. Conversely, KYNA+ORN-treated group revealed a significant reduction in MPO activity and MDA level with significant elevations in SOD activity and GSH level compared to the ORN-treated group. MPO and MDA revealed significant elevation along with a significant decline in SOD and GSH compared to the control group. Meanwhile, non-significant alterations were detected in pancreatic MPO, MDA, SOD, and GSH in the KYNA-treated group compared to the control (table 3).

Table (3): Protective effect of Kynurenic acid (KYNA) on pancreatic tissue parameters in L- ornithine (ORN) induced acute pancreatitis in rats.

Parameters	Animal groups	Time /hour	
		24 hour	48 hour
MPO (ng/mg)	Control	1.04 ± 0.10^{cA}	1.07 ± 0.05^{cA}
	ORN	7.88 ± 0.15^{aB}	14.08 ± 2.03^{aA}
	KYNA+ORN	4.89 ± 0.18^{bA}	2.96 ± 0.23^{bB}
	KYNA	0.90 ± 0.10^{cA}	0.94 ± 0.12^{cA}
MDA (nmol/g)	Control	1.02 ± 0.06^{cA}	1.07 ± 0.05^{cA}
	ORN	7.94 ± 0.56^{aB}	13.44 ± 1.34^{aA}
	KYNA+ORN	4.73 ± 0.34^{bA}	3.35 ± 0.42^{bB}
	KYNA	1.00 ± 0.11^{cA}	1.01 ± 0.13^{cA}
GSH (ng/mg)	Control	159.68 ± 6.12^{aA}	158.02 ± 4.43^{aA}
	ORN	37.24 ± 4.19^{cA}	45.81 ± 2.63^{cA}
	KYNA+ORN	85.31 ± 3.73^{bB}	107.26 ± 5.32^{bA}
	KYNA	170.46 ± 15.6^{aA}	166.19 ± 13.67^{aA}
SOD (U/mg)	Control	159.78 ± 4.10^{aA}	170.66 ± 7.21^{aA}
	ORN	41.07 ± 7.05^{cA}	55.41 ± 2.45^{cA}
	KYNA+ORN	95.46 ± 3.08^{bB}	128.87 ± 9.34^{bA}
	KYNA	171.97 ± 8.89^{aA}	180.34 ± 21.37^{aA}

a, b and c: Different superscript letters within the same column showed significant differences (P<0.05)
A, B and C: Different superscript letters within the same row showed significant differences (P<0.05).

Serum MicroRNA expression

ORN-treated rats showed overexpression in miR-216a and miR-217 compared to control group. KYNA+ORN-treated rats showed significant decreases in serum miR-216a (by 51.31%, 63.25%) and miR-217 (by 54.95%, 57.37%) expression at 24 hrs. and 48 hrs. compared to ORN-treated rats while they showed significant increases compared to control. Meanwhile, there were non-significant changes in both miRNA expressions in the KYNA-treated group compared to the control group (table 4).

Table (4): Protective effect of Kynurenic acid (KYNA) on serum miR-216a and miR-217 gene expression levels in L- ornithine (ORN) induced acute pancreatitis in rats:

Animal groups	miR-216a		miR-217	
	24 hours	48 hours	24 hours	48 hours
Control	1.00 ± 0.00^{cA}	1.00 ± 0.00^{cA}	1.00 ± 0.00^{cA}	1.00 ± 0.00^{cA}
ORN	7.25 ± 0.24^{aA}	5.17 ± 0.24^{aB}	10.01 ± 0.15^{aA}	8.14 ± 0.21^{aB}
KYNA+ORN	3.53 ± 0.22^{bA}	1.90 ± 0.06^{bB}	4.51 ± 0.33^{bA}	3.47 ± 0.23^{bB}
KYNA	0.95 ± 0.02^{cA}	0.83 ± 0.03^{cA}	0.97 ± 0.02^{cA}	0.93 ± 0.02^{cA}

a, b and c: Different superscript letters within the same column showed significant differences (P<0.05)
A, B and C: Different superscript letters within the same row showed significant differences (P<0.05).

4. DISCUSSION

Acute pancreatitis (AP) is considered a serious gastrointestinal inflammatory disorder with a high mortality rate particularly in severe cases (Carnovale et al., 2005). Administration of ORN caused impairment of the whole pancreatic functions in the current study. Concerning tothe exocrine pancreatic function in the AP group, serum amylase and lipase activities revealed significant increases compared to a control group that agreed with Qiu et al., (2015) and Shahin et al., (2022) who stated that these increases in enzymes activities in AP correlated with the pancreatic injury, acinar cell destruction and releasing of pancreatic enzymes into bloodstream. Pretreatment with KYNA significantly decreased amylase and lipase activities compared to the AP group. Our results partially agreed with Balla et al., (2021) who reported that KYNA significantly decreased amylase activity because it has anti-inflammatory properties and significantly reduced pancreatic injury observed in AP.

Regarding to insulin and glucose levels, there were significant reductions in insulin levels along with a significant elevation in glucose concentration in the AP group. Our findings were in harmony with Abdel-Hakeem et al., (2020) who said that decreased insulin level and increased glucose concentration are a result of the destruction of β-cells and reduction in islets cellularity. Furthermore, it is believed that AP is associated with pancreatic exocrine and endocrine damage because of the intimate link between the two portions of the pancreas. Moreover, it was demonstrated that oxidative stress and proteolytic enzymes released during AP caused diabetes by necrosis of β-cells, thus decreasing insulin levels but increasing glucose levels in blood (Butler et al., 2017). On the other hand, KYN administration reversed the effect of ORN resulting in a significant elevation of insulin level and a significant decline in glucose level. This effect owed to the antioxidant properties of KYNA by Reactive oxygen species (ROS) scavenging and oxidative stress reduction (Lugo-Huitrón et al., 2011) and its anti-inflammatory effect so it reduced pancreatic tissue damage during AP (Balla et al., 2021).

In the current study, serum IL-1β levels showed a significant increase in the AP group compared to the control. The obtained results coincided with Rakonczay et al., (2008). IL-1β is one of several cytokines and inflammatory mediators synthesized by inflammatory cells attracted to the injured area in the early phases of AP and involved in the complex pathophysiology of AP as it promotes the releasing of other pro-inflammatory cytokines, inducing tissue damage and development of systemic inflammatory response syndrome (SIRS) (Pereda et al., 2006). On the other hand, serum IL-10 exhibited a significant increase in the AP group by 48 hours compared to the control group. This increase was in accordance with Chen et al., (2015) and Pancreas et al., (2017) who stated that IL-10 increased in pancreatitis as a defence mechanism to inhibit the

synthesis of the pro-inflammatory cytokine and attenuate the disease severity.

KYNA pretreatment significantly decreased IL-1β levels whereas IL-10 levels significantly increased compared to the AP group. This elevation of IL-10 went in harmony with Marciniak et al., (2018) who found that KYNA enhanced IL-10 levels in hepatic Injury while the decline in IL-1β concurred with Sun et al., (2022) who stated that KYNA exerts its anti-inflammatory effects in inflammatory diseases via activating G-protein-coupled receptor 35 (GPR35) and inhibiting NLRP3 inflammasome activation. Also, KYNA inhibits PI3K/Akt and mitogen-activated protein kinase (MAPK) pathways resulting in the suppression of inflammation (Wirthgen et al., 2018).

Concerning to pancreatic tissue oxidative stress/antioxidant biomarkers, there were significant increases in MPO activity and MDA levels along with a significant decline in SOD activity and GSH level in the AP group compared to the control group. This elevation in MPO activity was consistent with Biczó et al., (2010) who reported that ORN increased pancreatic MPO activity which is a marker indicating tissue leukocyte infiltration. MPO is an enzyme released by activated neutrophils and contributes to tissue damage and oxidative stress in the pancreas, exacerbating the inflammatory process (Simsek et al., 2018). The elevation in MDA level agreed with Czakó et al., (2000) and Shahin et al., (2022). The latter explained MDA is a byproduct of lipid peroxidation and its increase in AP indicates elevated lipid peroxidation and oxidative stress.

Meanwhile, the reduction in the pancreatic activity of SOD and GSH levels in the AP group was in harmony with Mirmalek et al., (2016) and Shahin et al., (2022). This decline contributed to the production of excess ROS that depletes the endogenous antioxidants leading to increased lipid peroxidation and development of pancreatic injury (Czakó et al., 1998).

Conversely, rats co-exposed to KYNA+ORN showed a significant decline in pancreatic MPO activity and MDA level along with a significant elevation in pancreatic SOD activity and GSH level compared to ORN group. This decrease in MPO activity concurred with Poles et al., (2021), and decreased MDA was in agreement with Reyes Ocampo et al., (2014). Meanwhile, elevations in SOD activity agreed with Ferreira et al., (2018) and GSH levels were consistent with Bratek-gerej et al., (2021). The latter stated that KYNA exerts its antioxidant effect by ROS scavenging resulting in a reduction of oxidative stress.

Regarding to miRNAs, rats in the AP group showed overexpression in miR-216a and miR-217 compared to the control group. This findings concurred with Goodwin et al., (2014) and Wang et al., (2017) who established that these miRNAs were overexpressed in serum during pancreatic injury and were highly correlated with AP severity. On the other hand, KYNA+ORN group exhibited significant decline in miR-216a and miR-217 expressions compared to the AP group. Balla et al., (2021) demonstrated that KYNA significantly alleviates the pancreatic damage observed in AP via its anti-inflammatory and antioxidant properties. Therefore, it can decrease miRNA expressions.

It was observed in the KYNA+ORN group that amylase and lipase activities returned to control levels by 48 hours, while both miR-216a and miR-217 expressions were still significantly increased compared to the control group. It proved that this increase in miRNA expressions may not be resulted only by escaping from damaged pancreatic tissue, but also other factors could be involved (Lee et al., 2023). Recent research have provided evidence to support that miR-216a and miR-217 in serum are involved in the development and progression of AP through several mechanisms. Transforming growth factor-beta (TGF-β) upregulation during AP induced miR-216a production from acinar cells, which inhibits target genes phosphatase and tensin homolog (PTEN) and mothers against decapentaplegic homolog 7 (Smad7) expression, and activates phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) signaling pathway resulting in aggravation of damage of pancreatic tissue and subsequently systemic inflammation (Zhang et al., 2015). Moreover, it was demonstrated that the overexpression of miR-217 downregulated YY1-associated factor 2 (YAF2) genes, enhanced the production of pro-inflammatory cytokines and exacerbated cell injury (Wang et al., 2022).

5. CONCLUSIONS

Based on the present findings, we can conclude that pretreatment with KYNA significantly ameliorates the severity of ORN-induced acute pancreatitis in rats by inflammatory mediators and oxidative stress markers and this effect may be due to the strong antioxidant and anti-inflammatory properties of KYNA. Moreover, serum miR-216a and miR-217 could be potential biomarkers for the diagnosis and evaluation of AP in rats as they provide clear advantages over other conventional serum biomarkers (amylase and lipase) in detecting pancreatic exocrine injury and inflammatory response associated with AP.

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