Section: Research Paper

Protective effect of alpha-cyperone in renal ischemia-reperfusion induced acute kidney injury by modulation of metalloenzyme expression

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Abstract

Acute kidney Injury (AKI) is a common medical emergency that often occurs after an ischemia event followed by reperfusion. Matrix metalloproteinases (MMP-2) and MMP-9 are turned up in ischemia-reperfusion injury (IRI), which destroys the microvascular matrix of the kidney and causes ischemic organ damage. Further, histone deacetylase-2 (HDAC-2) is the major regulator of key signalling mechanisms in IRI. Alpha-cyperone (CYP), with its anti-inflammatory and antioxidant properties, might be a useful therapeutic strategy for kidney preservation. We employed an IRI model of the kidney to look at the expression of MMP-2, MMP-9, and HDAC-2, as well as the possible protective effect of cyperone treatment before IRI on metallozymes expression and IRI-induced renal damage. Male Wistar albino rats were selected at random and divided into three groups: sham-operated, ischemia-reperfusion, and cyperone-pretreated. The IRI model was produced by unilaterally clamping the renal artery for 45 minutes and reperfusion for 24 hours. Blood was collected for measuring serum creatinine, blood urea nitrogen (BUN), uric acid, and expression of MMPs and HDAC-2 in the renal homogenate. The effects of cyperone on the expression of MMPs and HDAC-2 in the kidney were investigated by enzyme-linked immunosorbent assay. The histopathological examinations were performed to score tubular damage and fibrosis by light microscopy. Increased expression of MMP-2, MMP-9, and HDAC-2 was seen in renal IRI. One week of pre-treatment with CYP (50 mg/kg p.o.) was sufficient to reverse these modifications. Preventive CYP treatment before renal ischemia improved renal parameters, malondialdehyde, myeloperoxidase, hydroxyproline, and pathological damage compared to control. Our results suggest that CYP pretreatment may reduce the effects of IRI induced oxidative stress, pro-inflammatory factors, and fibrosis by reducing the production of MMP-2, MMP-9, and HDAC-2. More research is needed to understand how CYP and metalloenzymes interact in AKI.

Keywords: MMPs, HDAC-2, alpha-cyperone; renal ischemia reperfusion

Introduction

Acute kidney damage is a substantial and dangerous concern in hospitalized patients globally for nephrology specialists, and it is connected with high fatality rates (Basile et al.,

2012; Ronco et al., 2019). AKI is caused by kidney ischemia/reperfusion (IR) damage, which may arise from sepsis, thromboembolic events, circulatory shock, and bypass surgery. IR damage reduces the glomerular filtration rate, which leads to renal function deterioration and an increased risk of mortality (Hoste et al., 2018). Although research into the causes of AKI has advanced, there is still a dearth of strategies and drug therapies that can successfully treat the condition.

Matrix metalloproteinases (MMPs), zinc-dependent endopeptidases, degrade and remodel the extracellular matrix (ECM) and have been associated to ischemic organ damage. (Kunugi et al., 2011). Two matrix metalloproteinases, MMP-2 (72 kD gelatinase A) and MMP-9 (92 kD gelatinase B), destroy the ECM components of basement membranes. Substrates of MMPs have been shown to cause disruption with signalling and adhesion molecules. Tissue metalloproteinase inhibitors (TIMPs) may be used as an alternate strategy to decrease MMP activity (Catania et al., 2007; Zakiyanov et al., 2019). MMP-2 and MMP-9 have been linked to IRI in a number of organs, including the kidney. (Mathalone et al., 2007; Muhs et al., 2003; Roach et al., 2002). After ischemia-reperfusion, MMP-2 and MMP-9 are increased in the kidney, and MMP activation modifies renal microvascular permeability. MMP inhibitors reduces tubular injury and improved real dysfunction (Basile et al., 2004; Catania et al., 2007; Kunugi et al., 2011; Sutton et al., 2005). MMP-2 is essential in the pathophysiology of kidney disease, where increased MMP-2 expression is seen in several experimental models of renal injury, including ischemia/reperfusion injury in the context of the kidney (Cheng et al., 2006; Dejonckheere et al., 2011; Kunugi et al., 2011). According to published evidence, IRI promotes ischemia and reperfusion induce proteolysis and ECM degradation in renal tubular epithelial cells. MMP-2 and MMP-9 both change and cleave the ECM. The acute MMP-9 buildups that neutrophils cause enhance inflammation and worsen graft degradation (Dong et al., 2021). Recent research reveals that MMPs have a role in the pathogenesis of IRI, one of the primary causes of AKI in certain clinical settings. The pathophysiology of AKI may include the vascular endothelium, inflammation, and tubular injury. Modulation of renal microvascular permeability, induction of apoptosis and necrosis, and concomitant aggravation of tubular damage during IRI are all caused by the upregulation of MMPs, which degrade the renal microvascular matrix. These findings may be traced back to an increase in the production of reactive oxygen species (ROS) in both the ischemic and reperfused areas (Cavdar et al., 2014; Ersan et al., 2017b; Kunugi et al., 2011; Novak et al., 2010). MMP inhibition has been demonstrated to attenuate IRI in a murine model of renal disease. With the help of the published data, it is now possible to comprehend how MMPs work in the kidneys and how an increase in ischemia conditions causes AKI.

The transcriptional network can be efficiently controlled by HDAC. HDACs modulate renal signalling networks, and HDAC research has increased in recent years based on *in vitro* and *in vivo* studies. Ischemia causes damage to the brain, heart, retina, and acute kidney injury (AKI), however HDAC inhibition mitigates this effect (Aufhauser et al., 2021; Fan et al., 2013; Granger et al., 2008). Reduced ischemia-induced retinal damage can be achieved by inhibiting HDAC-2 expression (Fan et al., 2013). HDAC inhibition also accelerates recovery after AKI (Cosentino et al., 2013). The CoREST complex is stabilized in renal tubular cells by HDAC-2 targeting, which also protects against renal ischemia/reperfusion damage and suggests endothelin as a possible downstream mediator of

renal injury (Aufhauser et al., 2021). The previously reported research reaffirms the function of HDAC in kidney disease and ischemia damage, which lead to severe conditions. Valproic acid (VPA) is the main HDACi tested for renal I/R damage protection following renal artery occlusions. VPA reduces renal dysfunction and speeds recovery after I/R damage. VPA therapy lowers TNF-α and macrophage proinflammatory response following renal I/R injury in mouse models (Costalonga et al., Amirzargar et al.)

The rhizomes of Cyperus rotundus (*Cyperaceae*) have been used in Asian traditional medicine to treat a variety of clinical ailments at home, including inflammatory disease. The anti-inflammatory benefits of Cyperus rotundus main active component, α-cyperone (CYP), remain unclear (Jung et al., 2013). C. rotundus rhizomes and tubers have been used as a natural treatment to cure digestive issues and menstrual irregularities for millennia in a number of countries, including India (Peerzada et al., 2015). C. rotundus extracts have been demonstrated to have biological and pharmacological effects in a number of investigations. The essential oil from the rhizomes of C. rotundus has been analysed for its chemical make-up. Numerous studies have shown that the essential oil extracted from the rhizome of the C. rotundus plant has antioxidant, antibacterial, insecticidal, antiradical, and antimutagenic properties. (Hu et al., 2017).

However, present therapeutic options for ARI fall short of curing the condition completely, highlighting the need for a more thorough knowledge of the molecular pathways underlying ARI pathogenesis. This study set out to determine the effect of CYP pre-treatment on MMP-2, MMP-9, and HDAC-2 expression on renal IRI damage in rats, as well as examine the biochemical, oxidative stress, pro-inflammatory mediator, and pathophysiological changes associated with CYP pre-treatment in rats.

Materials and methods

Chemicals

Alpha-cyperone was generously provided by Core Analytical Services in Nashik, India. All of the other chemicals were purchased from SRL in Mumbai, India. Biochemical measurement kits were available from Erba Diagnostic of Mumbai, India. The ELISA kits for measuring MMP-2, MMP-9, and HDAC-2 were bought from Krishgen Biosystem in Mumbai, India.

Animals

Male Wistar rats weighing 200–250 g were purchased from the National Institute of Biosciences in Pune and acclimated for two weeks before to the trials. During the experiment, the animals were kept in a room with adequate ventilation (16-18 air changes per hour), controlled temperature (20-24^{o}C), relative humidity (45-65%), and a 12-hour light/dark cycle. The animal had complete access to feed pellets as well as clean water. All procedures were carried out in accordance with the requirements of the Committee for the Purpose of Regulation and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The Institutional Animal Ethics Committee of the Dr. M. S. Gosavi College of Pharmaceutical Education and Research, Nashik (Ref. No. MSGCOPER/IAEC/01/2016) approved the animal use and care investigations.

Experimental Protocol

Using the outlined model, the renal IRI was constructed (Banaei, 2016; Cavdar et al., 2014; Ersan et al., 2017b, 2017a; Kennedy and Erlich, 2008; Kunugi et al., 2011). Male Wistar rats were divided into three groups of six. For the control groups, sodium carboxymethyl cellulose (Na-CMC) in water (0.5% w/v) is used as the vehicle, whereas the treatment group uses medicines suspended in Na-CMC in water. Following groups were pre-treated for 7 days and one hour before ischemia. Group I: Sham group received Na-CMC in water; Group II: non-diabetic control group rats received water and ischemia-reperfusion (I/R); Group III: non-diabetic rats + alpha-cyperone (50 mg/kg p.o.) in water + I/R. In group II and III, the left renal artery was isolated and clamped using a rat bulldog clamp for 45 minutes on the operating day following sodium pentobarbitone (50 mg/kg i.p.). Following the removal of the clamp, the incision was stitched, and the stitches were then covered with povidone-iodine ointment. Although the renal artery was not constricted, the method was the same in Group I. The animals were kept in the cage for 24 hours after reperfusion before being put to euthanasia. Prior to and during the experiment, the rats body weight was measured. After drawing blood from the retroorbital plexus, centrifuging at 7000 rpm for 15 min at 4°C separated the serum. Serum creatinine, BUN, and uric acid were measured in the collected serum. The rats were then euthanized, and the kidneys were removed and stored for biochemical research. A part of the kidney was preserved separately in 10% buffered formalin, hematoxylin and eosin (H&E), and Masson trichome (MT) staining for histological study.

Measurement of biochemical parameters

Serum creatinine, BUN, and uric acid were all measured using Erba Diagnostic Kits.

Renal tissue homogenate preparation

Kidneys were homogenised, chopped, and centrifuged at 7000 rpm for 20 minutes at 4°C in 10% w/v phosphate buffer (20 mM, pH 7.4). Supernatants were collected and analysed for their effects on tissue parameters.

Estimation of antioxidative parameters

Malondialdehyde (MDA) levels were estimated using the thiobarbituric acid reaction technique of Okhawa et al., 1978 to evaluate lipid peroxidation. Tissue homogenate (0.2 ml), 8.1% sodium dodecyl sulphate (0.2 ml), 30% acetic acid (pH 3.5) (1.5 ml), and 0.8% thiobarbituric acid (1.5 ml) were mixed together in this technique. After 60 minutes of heating at 95^{o}C, the reaction mixture was cooled on ice. The mixture was centrifuged at 5000 rpm for 20 minutes after adding 5.0 ml of a 15:1 (v/v) n-butanol: pyridine solution. Malondialdehyde (MDA) concentrations were reported as units per mg of protein, and the absorbance of the resulting pink colour in the organic layer was determined to be at 532 nm (Ohkawa et al., 1979)

Determination of Myeloperoxidase (MPO) activity and Hydroxyproline (HP) level in the kidney tissue.

MPO activity was assessed using the Suzuki et al. (1983) technique. In brief, the experimental samples (10 ul) were treated for 5 minutes at 37 °C with 80 ul of 0.75 mM H_2O_2 and 110 ul of a 3,3,5,5-tetramethylbenzidine (TMB) solution. After stopping the reaction with

50 uL of 2 M H_2SO_4, the absorbance at 450 nm were measured spectrophotometrically (in mM H_2O_2 consumed per minute per mg protein) (Suzuki,' et al., 1983). The Woessners (1961) approach was used to determine the hydroxyproline content (Woessner).

MMP-2, MMP-9, and HDAC-2 Estimation

MMP-2, MMP-9, and HDAC-2 expressions in kidney homogenate were determined using a commercially available enzyme-linked immunosorbent assay (GENLISATM ELISA kits, Rat MMP2/gelatinase A, Rat MMP-9/gelatinase B, and Rat HDAC-2).

Histopathological study

The kidneys were extracted and placed in 10% buffered formalin before being cut in half parallel to the main axis. They were then rinsed and soaked for 24 hours in isopropyl alcohol, xylene, and paraffin for microscopic analysis. To analyse the gross morphology and detect fibrosis, 5 m thick paraffin-embedded tissue slices were cut and stained with hematoxylin and eosin (H&E) following deparaffination and Masson's trichome (MT) staining respectively. The collagen distribution was determined using Masson's trichome staining technique. Each specimen was analysed for histopathologic abnormalities in the glomeruli, tubules, interstitium, and blood arteries and categorised as (−) none, (+) mild, (+++) moderate, and (+++) severe damage (Cavdar et al., 2014).

Statistical analysis

All values were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism (Graph Pad Software Inc., version 6.0 for Windows, San Diego, California, USA). One-way analysis of variance (ANOVA) followed by Dunnett's test was used for data analysis; $P<0.01$ was considered statistically significant.

Results

Effect of CYP on MMP-2, MMP-9, and HDAC-2 expression in renal homogenate after renal ischemia reperfusion injury in rats

In contrast to the sham group, the control group had substantially greater expressions of MMP-2, MMP-9, and HDAC-2 ($P< 0.01$). Pretreatment with alpha-cyperone inhibited the expression of MMP-2, MMP-9, and HDAC-2 in the kidney tissue of non-diabetic rats subjected to renal IRI, compared to the control group ($P < 0.01$) (Fig 1)

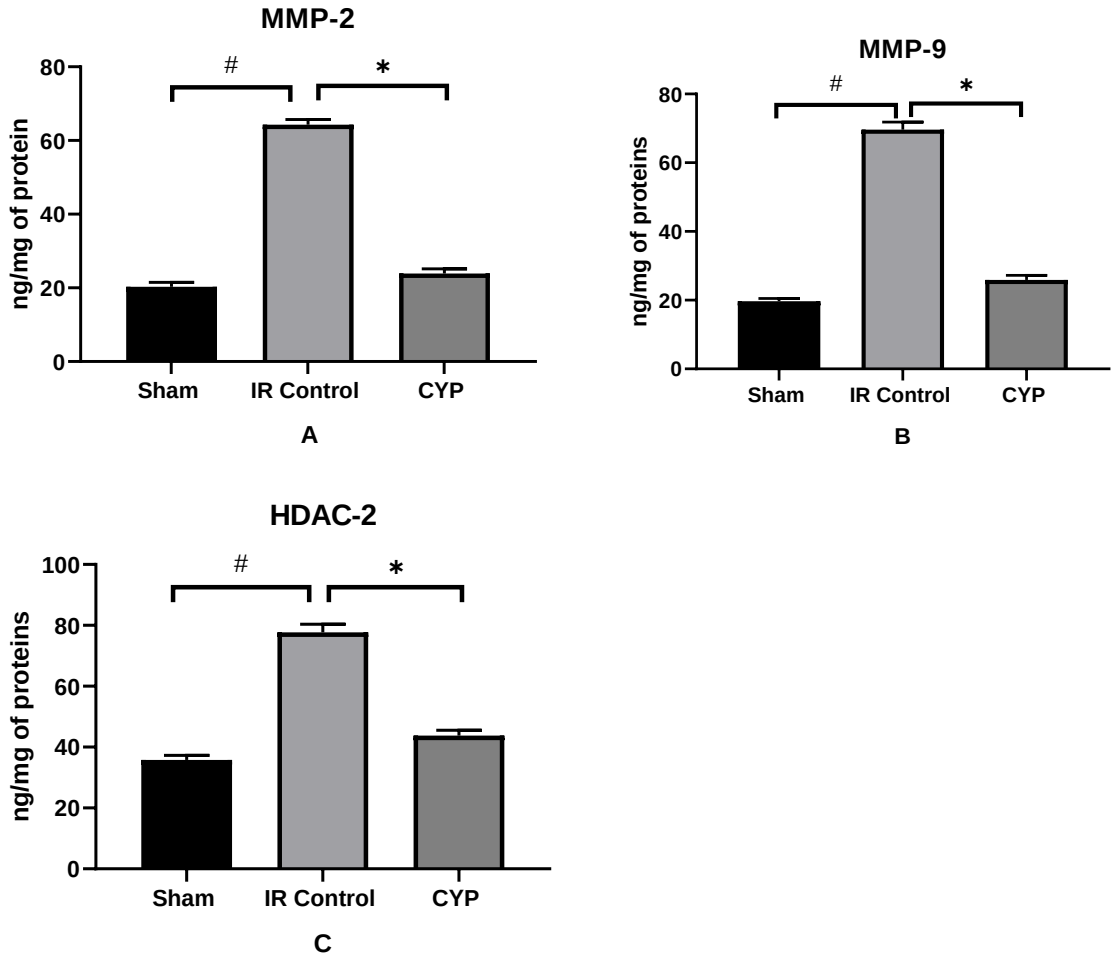

Fig: 1 Effect of CYP on **A.** MMP-2 expression **B.** MMP-9 expression **C.** HDAC-2 expression in renal IRI in non-diabetic rats. Values are expressed as mean ± S.E.M. (n=6), using one-way ANOVA followed by Dunnett's test. #*P*<0.01 when IR control group compared with sham group, **P*<0.01 CYP pre-treated group compared with IR control group

Effect of α-cyperone on renal function in renal ischemia reperfusion injury in rats

Serum Creatinine, BUN, and uric acid levels in non-diabetic rats were significantly higher than in the sham group (*P* <0.01), suggesting a decline in renal function. When compared to the IR control group, the aforesaid renal indices were significantly reduced (*P* <0.01) after pretreatment with 50 mg/kg of CYP for one weeks prior to ischemia. (Table 1).

Table: 1 Effect of α-cyperone on renal function in rats with renal ischemia reperfusion injury

Groups	Serum Creatinine mg/dl	BUN mg/dl	Uric acid mg/dl
IR Control (Vehicle)	1.5±0.04#	43.85±1.65#	2.81±0.11#
Sham group (Vehicle)	0.86±0.01	31.95±0.95	2.04±0.05
CYP 50 mg/kg p.o + IR	0.81±0.03*	27.25±1.14*	1.15±0.03*

Note: Values are expressed as mean ± S.E.M. (n=6), using one-way ANOVA followed by Dunnett's test. #*P*<0.01 when IR control group compared with sham group, **P*<0.01 CYP pre-treated group compared with IR control group.

Effect of α-cyperone on MDA, MPO, and Hydroxyproline content in renal homogenate

Renal ischemia damage (IR control) in rats showed significantly (*P*<0.01) higher levels of MDA, a diagnostic marker of oxidative stress, MPO, a marker of inflammation, and hydroxyproline, a signal of fibrosis, compared to the sham group. Treatment with CYP (50 mg/kg) one week before ischemia resulted in a substantial (*P*<0.01) decrease in MDA content, MPO activity, and hydroxyproline levels in renal homogenate as compared to the IR control group. (Table 2).

Table:2 Effect of α-cyperone on MDA, MPO and Hydroxyproline (HP) content in renal homogenate

Groups and Treatment	MDA nmol/mg of protein	MPO uM/mg of protein	HP ug/mg of protein
IR Control (Vehicle)	78.52±3.5#	56.9±2.2#	8.6±0.37#
Sham group (Vehicle)	62.8±1.2	42.2±1.1	7.2±0.28
CYP 50 mg/kg p.o + IR	18.2±1.5*	24.4±1.1*	4.5±0.29*

Note: Values are expressed as mean ± S.E.M. (n=6), using one-way ANOVA followed by Dunnett's test. #*P*<0.01 when IR control group compared with sham group, **P*<0.01 CYP pre-treated group compared with IR control group.

Effect of α-cyperone on morphological changes in kidney of in rats with renal ischemia reperfusion injury

HE and MT staining were used to examine the fibrosis and gross pathologic abnormalities in the kidney tissue, respectively. Each kidney slide had at least 10 areas that were analyzed, and scores were used to determine the degree of alterations. Figure 2 depicts the impact of medications on rats with ischemic kidney injury before a one-week drug pre-treatment on kidney tissue stained with HE and MT. A: renal I/R control (vehicle); B: sham group (vehicle); C: CYP 50 mg/kg p.o. + I/R in HE staining: Changes in vascular abnormalities (red arrow), tubular necrosis and degeneration (black arrow), and neutrophil infiltration (blue arrow) in the kidneys, D: renal I/R control (vehicle); E: sham group (vehicle); F: CYP 50 mg/kg p.o. + I/R in MT staining: alterations in collagen deposition (white arrow) and tubular degeneration (yellow arrow) in the kidneys. Kidneys exposed to IR had observable kidney damage. Compared to the sham group, the renal I/R group exhibited moderate to severe significant modifications in the histological examination of necrosis and congestion in the glomeruli, inflammatory cell infiltration, glomerular hyperplasia, and tubular edema of renal tissue (P <0.01). One week before ischemia, the pre-treated group received CYP (50 mg/kg), which substantially reduced the differences in HE-staining (P< 0.01). In the CYP group, congestion is mild. On MT staining, the control group exhibited moderate to severe collagen

deposition and tubular degeneration compared to the sham group, which showed modest alterations, but the pre-treated CYP group showed mild to rare collagen deposition and tubular degeneration. ($P< 0.01$).

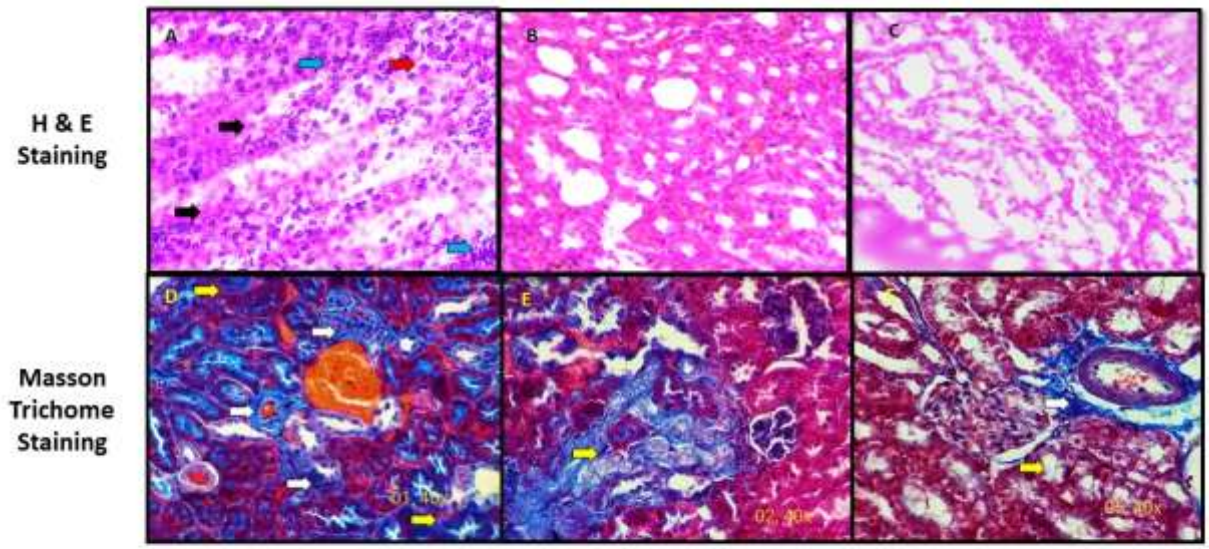

Figure 2 The effect of CYP on HE and MT stained kidney tissue. A: I/R control; B: sham group; C: CYP 50 mg/kg p.o. + I/R in HE staining: to study changes in vascular anomalies (red arrow), tubular necrosis and degeneration (black arrow), and neutrophil infiltration (blue arrow) in the kidneys. D: I/R control; E: sham group; F: CYP 50 mg/kg p.o. + I/R in MT staining: to study changes in collagen deposition (white arrow) and tubular degeneration (yellow arrow) in the kidneys. (Magnification 40x)

Discussion

The aim of the current investigation was to ascertain whether or not MMP-2, MMP-9, and HDAC-2 expression levels were elevated throughout the progression of IRI-induced AKI. We looked into how α-cyperone (CYP), a naturally occurring compound found in the rhizomes of *Cyperus rotundus*, affected the expression of HDAC-2, MMP-2, and MMP-9 because these enzymes are intricate for the progression of renal ischemia damage. We performed a variety of assays evaluating biochemical parameters, oxidative profiles, pro-inflammatory mediators, and histological examinations to assess whether CYP treatment improved kidney function and thus alleviated ischemic AKI. Our results show that CYP has an influence on MMP-2, MMP-9, and HDAC-2 in an experimental model of renal IRI, and to the best of our knowledge, this is the first research of its sort.

Several kidney pathologies, such as IRI-induced acute kidney injury, renal hypertension, glomerulosclerosis, tubulointerstitial fibrosis, nephropathy due to transplantation, diabetic nephropathy, and polycystic kidney disease, have been linked to changes in MMP expression and activity (Catania et al., 2007). Increased expression of MMPs, which are intrinsic to the inflammatory response, causes IRI and exacerbates damage (Dong et al., 2021; Novak et al., 2010). The expressions of MMP-2 and MMP-9 in the glomeruli, tubules, and interstitium rise as a consequence of IRI, as shown by several investigations (Caron et al., 2005a; Cavdar et al., 2014; Dejonckheere et al., 2011; Ersan et al., 2017b, 2017a)

Proteinuria is caused by structural alterations in the glomeruli, and collagen-IV, the predominant type of collagen in the ECM, is degraded by MMP, resulting to aberrant ECM

deposition and breakdown (Naylor et al., 2021). CYP pre-treatment restores the natural architecture of glomeruli and tubules by decreasing collagen invasion is consistent with our results and suggests a participation of metallozymes in this degradation. We found that in the renal IRI, the expressions of MMP-2, MMP-9, and HDAC-2 were considerably higher in the rats that had renal ischemia. MMP-2, MMP-9, and HDAC-2 expressions were decreased when rats were treated with CYP for a week prior to ischemia reperfusion damage. This result strongly suggests that CYP protects against AKI by modulating metalloenzymes.

Studies looking into the advantages of MMP inhibition were driven by the theory that MMPs control AKI and are responsible for alterations in the glomeruli, tubular epithelial cells, and vascular endothelium. This was corroborated by the finding that the MMP-2 and MMP-9 selective inhibitor minocycline reduced enhanced vascular permeability after IRI. MMP inhibitor have so far proved successful in reducing renal damage and have been considered therapeutic targets in IRI. (Dejonckheere et al., 2011; Sutton et al., 2005). Ischemic damage increases MMP activity, which degrades zonula occludens-1 in the glomerulus, occludin in endothelial cells, and cadherin in tubular epithelial cells (Caron et al., 2005a, 2005b). According to a recent study, oxidative stress damages the endothelium glycocalyx by increasing HDAC activity, which increases the production of MMP and decreases TIMP by cleaving syndecan and proteoglycans (Ali et al., 2019).

This research aimed to determine whether or not pretreatment with CYP reduced morphological and functional impairment of the renal system in AKI due to IRI. In the renal IRI group, we noticed a significant increase in serum creatinine and BUN levels post-IRI, comparable with prior studies. Pre-treatment with CYP significantly restored these levels. (Aufhauser et al., 2021; Cavdar et al., 2017, 2014; Ersan et al., 2017b, 2017a). The effect may be due to inhibition of MMPs and HDAC-2 by CYP pretreatment.

IRI is worsened by uric acid through the ROS and inflammatory cascade in the heart (Shen et al., 2021). Due to the presence of oxygen during reperfusion, hypoxanthine is converted to xanthine and uric acid, leading to the production of superoxide and free radicals that further harm the organ (Chan, 2002). Our results demonstrate that renal I/R damage is associated with elevated blood uric acid levels, which can be mitigated by CYP pre-treatment in the renal IRI group. This may be the result of CYP's antioxidant activity.

The effects of oxidative stress and inflammation on chronic kidney disease (CKD) are reciprocal; oxidative stress induces inflammation via multiple mechanisms, which in turn generates ROS and activates leukocytes and resident cells, which in turn causes further oxidative stress. (Ruiz et al., 2013) Ischemia generates free radicals that cause damage, while neutrophil infiltration, cytokine activation, cellular apoptosis, necrosis, and microvascular damage augment the damage from reperfusion (Chan, 2002). Many disease-related processes are initiated and exacerbated by the onset of oxidative stress and the uncontrolled formation of ROS. In the present study, renal I/R damage in rats led to an increase in the levels of renal MDA, MPO, and hydroxyproline in the kidney tissue. The rats treated with CYP for one week before I/R in rats showed significant restoration of renal MDA, MPO, and

hydroxyproline (HP) levels. MDA is considered a critical marker for lipid peroxidation and oxidative stress. CYP significantly reduces oxidative stress, the inflammatory index (MPO), and restores HP in the kidney tissues of rats, indicating that the antioxidant and anti-inflammatory properties of CYP may be contributing to its renoprotective effect. Our findings suggest that CYP has antioxidant, anti-inflammatory, and anti-fibrotic traits, and that it may inhibit metalloenzymes and halt the progression of AKI by lowering kidney MDA, MPO, and HP levels.

In accordance with previous studies, we found that histopathology scores in IRI-treated rats were similar in our study. GBM is mostly composed of collagen IV, which gives podocytes structure and form. Ischemia-induced acute kidney injury in rats was related with enhanced collagen staining, highlighting the fibrotic process in this model. The results of Masson's trichrome assay, which showed a rise in total collagen volume in the kidney IR group, corroborated these observations (Cavdar et al., 2014; Naylor et al., 2021). In rats, ischemia injury caused kidney cell destruction. More than 50% of the morphological changes are related to vascular changes, tubular necrosis and degeneration, and neutrophilic infiltration, which was revealed under light microscopic examination in HE staining to assess morphological changes and collagen deposition and tubular degeneration in MT staining to assess fibrosis. MMP-2, MMP-9, and HDAC-2 expression elevated in rats following renal I/R damage, consistent with prior research(Aufhauser et al., 2021; Banaei, 2016; Cavdar et al., 2014; Kunugi et al., 2011). Increased levels of MDA are associated with the generation of oxidative stress, which corresponds with histological damage. Additionally, an increase in MPO in rat renal homogenate causes an increase in pro-inflammatory mediators. Serum creatinine and BUN levels, which are indicators of glomerular and tubular health, are known to rise in patients with renal IRI-induced AKI. Pretreatment with CYP enhanced renal function by lowering high Serum Cr and BUN in I/R. The kidneys of renal IRI rats pretreated with CYP (50 mg/kg p.o.) for a week exhibited no or very minimal alterations to the glomerular architecture. The structural evidence for the nephroprotective action of CYP is supported by the finding that pre-treatment with CYP before renal ischemia reduces fibrosis in rats, may be due to antioxidant and anti-inflammatory action of CYP.

In conclusion, our work is one of just a few to show that CYP has a protective function in renal IRI by decreasing MMP-2, MMP-9, and HDAC-2 expression. In rats with renal ischemia, our study demonstrates that targeting of MMP-2, MMP-9, and HDAC-2 with α-cyperone may maintain glomerular and tubular architecture and thereby stop the onset of disease. A pre-treatment of α-cyperone (50 mg/kg) for one week resulted in a reduction in the levels of serum creatinine, BUN, and uric acid. In addition to this, it restored levels of MPO and hydroxyproline, which led to a considerable reduction in MDA levels, which is a by-product of lipid peroxidation that was caused by the oxidative damage. Therefore, α-cyperone is a treatment alternative that is both safe and effective for treating acute renal failure. It also has the potential to help slow the course of kidney damage in scenarios involving renal ischemia.

5. Conclusion

The present investigation showed that α-cyperone pre-treatment gives renoprotection by enabling targeting of metalloenzymes that destroy key structural components of the kidneys. However, our results show that pre-treatment with α-cyperone improves renal function by decreasing oxidative stress, pro-inflammatory mediators, fibrosis, and renal biochemical markers. Pre-treatment with α-cyperone in a rat model of renal IR may prevent further kidney damage from occurring, and it is a cost-effective and safe alternative for treating such conditions in humans. To what extent α-cyperone modulates certain signalling pathways in renal IRI remains to be investigated.

Declarations:

Conflict of interest:

The Authors declare that they have no conflicts of interest to disclose

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Author contribution:

Rakesh Daude carried out experiments, gathered and assessed the literature, and wrote the article. Dr. Jigna Shah was in charge of supervising and revising the text. The final manuscript was reviewed and approved by all authors.

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