# Effect of human adrenomedullin and its binding protein on renal Ischemia/Reperfusion Injury in Rats

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**Abstract: Background:**Adrenomedullin (AM) a 52 amino acid ringed structure peptide was originally isolated from human pheochromocytoma. AM is widely distributed in various tissues and acts as a local vasoactive peptide hormone in various conditions. Acute renal failure secondary to ischemia and reperfusion carries a high morbidity and mortality rate and no specific treatment currently available. Adrenomedullin binding protein-1 augments the activity of AM and together with AM may have a beneficial effect on renal I/R injury. **Methods**: Male albino rats were subjected to renal I/R injury followed by administration of AM /AMBP-1. Rats were allowed to recover for 24 hours and blood samples were collected for measurement of AM, AMBP-1, blood urea,serum creatinine,AST,ALT and TNF-α.Renal water content was measured also. Kidney samples were taken for histopathological examination or determination of levels of renal renal malondialdehyde (MDA), an end product of lipid peroxidation; glutathione (GSH), a key antioxidant; and myeloperoxidase (MPO) activity, an index of tissue neutrophil infiltration. **Results**: AM was significantly increased while AMBP-1 was significantly decreased at 24 hrs post I/R injury. AST and ALT were significantly increased following renal I/R injury as well as blood urea, serum creatinine and TNF-α.Administration of AM /AMBP-1 reduces significantly the tissue injury parameters. Ischemia/reperfusion caused a significant decrease in tissue GSH level, which was accompanied by significant increases in MDA level and MPO activity. On the other hand, AM/AMBP-1 treatment reversed all these biochemical indices, as well as histopathological alterations that were induced by I/R. **Conclusion:** AM and AMBP-1 attenuate organ injury and inflammatory response so, they may be developed as a novel treatment for patients with acute renal I/R injury.
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**Keywords:** Ischemia and Reperfusion injury, Adrenomedullin (AM) and Adrenomedullin Binding Protein-1 (AMBP-1)

## 1. Introduction

Adrenomedullin is a peptide identified from human pheochromocytoma and initially annotated as a vasodilator peptide. AM acts a circulating hormone which elicits various biological activities in a paracrine or autocrine manner**(Ana Patricia *et al.;* 2009).**

Adrenomedullin (AM), a 52-amino acid peptide with potent vasoactive properties, was originally isolated from a human pheochromocytoma **(Kitamura *et al.*, 1993 ).** It is widely distributed in the endocrine and neuroendocrine system **(Pearson *et al.*, 2006).**, suggesting that AM plays an important role in the control of systemic and local circulation, as well as cardiovascular and fluid regulation, regulation of growth and differentiation, and secretions of other hormones. A specific binding protein to AM, adrenomedullin binding protein-1 (AMBP-1) was identified in human plasma and the purified protein was reported to be identical to human complement factor H **( Kavin *et al.*, 2010 ).**

A 120–140 kD AM-binding protein-1 (AMBP-1) was first reported in 1999 to be identical to human complement factor H **(Elsasser *et al.*, 1999).** Different Previous studies have demonstrated that human AMBP-1 synergistically enhanced rat AM-induced vascular relaxation. **(Zhang *et al.*, 2009 )**

Renal ischemia/reperfusion (I/R) injury occurs in many clinical settings including shock, vascular surgery, renal transplantation and during early allograft rejection subsequent to renal transplantation. **(Joosten *et al.*, 2005 ).** Acute renal hypoxemia and ischemia can lead to renal dysfunction in humans as well as in animal models, which have demonstrated that the tissue damage that occurs following I/R is due in part to increased oxidative stress in the tissues, especially during reperfusion. This is essential for the survival of the ischemic renal tissue ***(Rodrigo and Rivera; 2002).*** The organ dysfunction that accompanies this condition is generally associated with increased microvascular permeability, interstitial edema, impaired vasoregulation, inflammatory cell infiltration, and parenchymal cell dysfunction and necrosis. I/R elicits an acute inflammatory response characterized by activation of neutrophils. Activated neutrophils are known to induce tissue injury through the production and release of reactive oxygen metabolites and cytotoxic proteins (e.g., proteases, myeloperoxidase, lactoferrin) into extracellular fluid ***(Sener et al., 2006).***

Acute renal injury induced by ischemia and reperfusion (I/R) is a major cause of morbidity and mortality in hospitalized patients. Acute renal injury is classified according to the RIFLE (acronym indicating

Risk of renal dysfunction; Injury to the kidney; Failure of kidney function; Loss of kidney function and End-stage kidney disease) criteria **( Kavin *et al.*, 2010 ).**

There are only a few strategies implemented to prevent or limit renal injury which include fluid resuscitation,pharmacological interventions, or simply avoidance of the insulting factor. Diuretics and vasodilators are commonly used to treat ARF. However, in large randomized studies, these agents have failed to prove effective in various disease conditions. As such, there is an urgent need for developing effective strategies to combat renal I/R injury.

**2.Material &methods**

Male Wistar Albino rats (200–270 g), purchased from the faculty of science Tanta University, were used for this study. The rats were housed in a temperature controlled room and on a 12-h light/dark cycle. The rats were fed a standard Purina rat chow diet and allowed water *ad libitum*. All protocols were approved by Tanta Faculty of medicine ethical Committee.

Rats were fasted overnight but water was given *ad libitum*. Rats were anesthetized with (100 mg/kg ketamine and 0.75 mg/kg chlorpromazine IP). Renal ischemia/ reperfusion. Briefly, a midline laparotomy incision was made to expose the abdomen. The intestines were covered in warm, moist gauze and first retracted to the right to expose the left renal pedicle. A microvascular clamp was placed around the left renal pedicle, and visual inspection of the kidney was done to confirm blanching and cessation of blood flow. The intestines were mobilized to the left to expose the right renal pedicle, and a microvascular clamp was placed in the same manner. The small intestines were then returned into the abdominal cavity. The total clamp time was 60 min, after which the clamps were removed. Restoration of blood flow into the kidneys was confirmed visually. The incision was closed in layers, and the animals were returned to their cages with food and water, and allowed to recover. At 24 h, the animals were euthanized and blood and tissue samples were harvested for analyses. (Ramirez *et al.,* 2009 ).

**Experimental Groups:**

- Group 1: 10 rats with renal I/R treated with human AM and human AMBP-1 underwent renal pedicle clamping for 60 min and immediately following removal of the microvascular clamps, received human AM (12 µg/kg BW plus human AMBP-1 (40 µg/kg BW) (Sigma Pharmaceuticals ) in 1 mL normal saline. (Kavin *et al.,* 2010 ).
- Group 2: (10 rats ) renal I/R rats treated with vehicle, underwent renal pedicle clamping for 60 min followed by removal, and received intravenous injection of human albumin (52 µg/kg BW) for a period of 30 min in 1 mL normal saline.
- Group 3;(10 rats ), sham operated animals, underwent a midline laparotomy incision and kidneys were isolated, but neither clamping nor infusion was performed.

Determination of Plasma Levels of AM: Plasma AM levels were assayed using a radioimmunoassay (RIA) kit specific for AM. Polyclonal antibodies against human AMBP-1 since the anti-human antibodies recognize the rat AMBP-1 and the anti-rat antibodies are not commercially available. The levels of AMBP-1 in band densities were determined using a method of Yang *et al.* (2009). The levels of AMBP-1 in band densities were determined using a Bio-Rad Laboratories Imaging System according the method described by Kavin *et al.,* 2010 and Yang *et al.* (2010).

Determination of Renal Water Content: The difference in water content in the kidneys was determined by the difference in the weight of the kidneys after 72 hrs of desiccation in 70°C from the initial weight, divided by the initial weight and the results are expressed as percentage ( Kavin *et al.,* 2010).

Determination of Serum Levels of Organ Injury Markers: Blood samples were centrifuged for 15 min at 2000 g to collect serum, and stored at –80°C for determination of serum levels of creatinine and blood urea nitrogen (BUN) (Young; 2001).

Aspartate aminotransferase (AST) (SGOT), and alanine aminotransferase (ALT) also, known as glutamate pyruvate tansaminase GPT. The levels were measured using commercially available assay kits (Berth and Delanghe, 2004 ).

Tumor necrosis factor-α (TNF-α) quantified with the use of specific enzyme linked immunosorbent assay (ELISA) kits according to the method of (Carrizo *et al.,* 2007).

In the renal tissue samples stored at –70 $^{0}$C,malondialdehyde (MDA) levels, an end product of lipid peroxidation, glutathione (GSH), a key antioxidant, and tissue- associated myeloperoxidase (MPO) activity, as indirect evidence of neutrophil infiltration, were measured. Additional kidney samples were placed in formaldehyde (10%) for histological evaluation.

Tissue samples were homogenized with ice-cold 150 mM KCl for the determination of MDA and GSH levels. MDA levels were assayed for products of lipid peroxidation

by monitoring thiobarbituric acid-reactive substance formation. Results were expressed as nmol MDA/g tissue. *(Beuge;1978).* GSH measurements were performed using a modification of the Ellman

procedure *(Beutler;1975).* Results were expressed in mmol GSH/g tissue.

Myeloperoxidase (MPO) is an enzyme that is found predominantly in the azurophilic granules of polymorphonuclear leukocytes (PMNL). Tissue MPO activity is frequently utilized to estimate tissue PMNL accumulation and correlates significantly with the number of PMNL determined histochemically in inflamed tissues *(Bradley et al.,1982).* MPO activity was measured in tissues in a procedure similar to that documented by *Hillegas et al.;1990*. MPO activity was expressed as U/g tissue.

**Statistical analysis**

All data are expressed as means _ SE and compared by one-way analysis of variance (ANOVA). When the ANOVA was significant, post hoc testing of differences between groups was performed using Student–Newman–Keuls' method. The survival rate was estimated by Kaplan–Meier method and compared by the log-rank test. A *P* value <0.05 was considered statistically significant. Statistical presentation and analysis of the present study was conducted, using the mean, standard deviation, SPSS V.16.

## 3. Results

Serum samples from sham and renal I/R rats were examined for AM and AMBP-1. AM shows significant increase in I/R group compared with sham operated group (Tab. 1; Fig. 1). Rats subjected to renal I/R had a significant decrease in AMBP-1 (Tab. 2; Fig. 2).

Renal water content was significantly reduced by administration of AM/AMBP-1 after renal I/R injury (Tab. 3; Fig. 3).

Human AM/AMBP-1 improved renal functions after I/R injury where blood urea and serum creatinine were significantly elevated after I/R vehicle treated group with significant decrease after AM/AMBP-1 administration. Whereas no significant difference between sham operated and AM/AMBP-1 operated group.Tabs (4 &5) Figs (4 &5).

Serum levels of AST and ALT were significantly increased after I/R injury. Administration of AM/AMBP-1 attenuated the AST and ALT levels.Tabs 6, 7; Figs 6, 7).

Pro inflammatory cytokines especially TNF-α generally increased after I/R injury. Serum TNF- α was increased significantly after renal I/R injury and significantly reduced after administration of AM/AMBP-1 (Tab 8; Fig 8).

The renal tissue MDA content in the sham group (18.5 ± 1.9 nmol/g) was elevated by I/R injury (45.0 ± 4.2 nmol/ g ); however, AM /AMBP-1 treatment significantly decreased the I/R-induced elevation in renal MDA level (22.8 ± 3.1 nmol/g ).Tab 9. Fig. 9

In accordance with that, ischemia and reperfusion caused a significant decrease in renal GSH level (0.5 ± 0.07 mmol/g) when compared to control group (1.5 ± 0.12 mmol/g), whereas in the AM /AMBP-1 group, renal GSH content was found to be preserved (1.3 ± 0.14 mmol/g), without significant difference from that of the control group (Tab. 9,Fig. 10). MPO activity, which is accepted as an indicator of neutrophil infiltration, was significantly higher in the kidney tissue of the I/R group (14.4± 2.0 U/g) than that of the control group (5.9± 0.8 U/g ). On the other hand, AM /AMBP-1 treatment in the I/R group significantly decreased renal tissue MPO level (7.2 ± 0.7), which was not different from that of the control group. (Tab. 9, Fig. 11)

Table (1): Comparing adrenomedullin level (pg/ml) in sham and renal I/R rats at 24 hours post injury.

| Case number | Sham Group | I/R Group |
|---|---|---|
| Range | 49 -76 | 60 - 95 |
| Mean | 61 | 80.30 |
| ±SD | 9.74 | 10.43 |
| t. test | 4.275 | |
| *p*. value | 0.001* | |

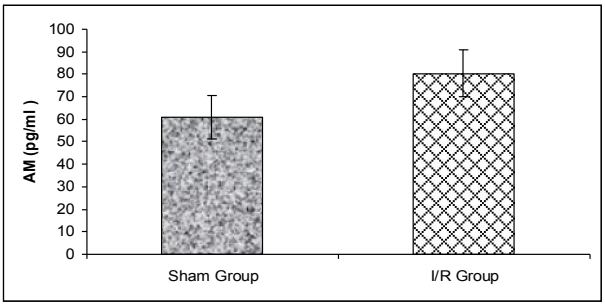


**Fig (1):** Comparing adrenomedullin level (pg/ml ) in sham and renal I/R rats at 24 hours post injury

**Table (2):** Comparing serum adrenomedullin binding protein -1 (AMBP-1) pg/ml level in sham and renal I/R rats at 24 hours post injury.

| Case number | Sham Group | I/R Group |
|---|---|---|
| Range | 3.8 – 6.20 | 1.5 - 3 |
| Mean | 5.02 | 2.28 |
| ±SD | 0.84 | 0.51 |
| t. test | 8.757 | |
| *p*. value | 0.001* | |

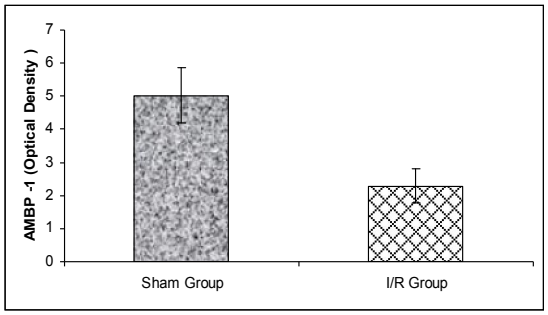


**Fig (2):** Comparing serum adrenomedullin binding protein -1 (AMBP-1) level in sham and renal I/R rats at 24 hours post injury.

**Table (3):** Comparing renal water content after renal I/R.

| Case number | Sham Group | Vehicle treated animals | AM/AMBP-1 |
|---|---|---|---|
| Range | 50-84 | 65-95 | 53-85 |
| Mean | 72 | 92.1 | 65.2 |
| ±SD | 10.13 | 9.38 | 9.63 |
| F. test | 8.636 | | |
| *p*. value | 0.001* | | |
| Tukey's test | | | |
| Sham Group & Vehicle treated animals | Sham Group & AM/AMBP-1 | Vehicle treated animals& AM/AMBP-1 | |
| 0.017* | 0.130 | 0.001* | |

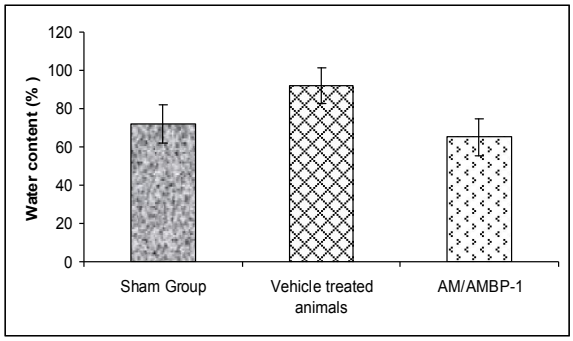


**Figure (3):** Comparing renal water content after renal I/R.

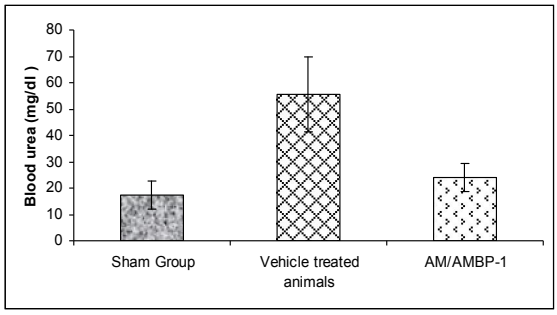


**Fig. (4):** Comparing blood urea (mg/dl )

**Table (4):** Comparing blood urea (mg/dl )

| Case number | Sham Group | Vehicle treated animals | AM/AMBP-1 |
|---|---|---|---|
| Range | 10-25 | 32-70 | 18-33 |
| Mean | 17.20 | 55.60 | 24.10 |
| ±SD | 5.32 | 14.31 | 5.40 |
| F. test | 12.365 | | |
| *p*. value | 0.001* | | |
| Tukey's test | | | |
| Sham Group & Vehicle treated animals | Sham Group & AM/AMBP-1 | Vehicle treated animals& AM/AMBP-1 | |
| 0.001* | 0.111 | 0.001* | |

**Table (5):** Comparing serum creatinine (mg/dl )

| Case number | Sham Group | Vehicle treated animals | AM/AMBP-1 |
|---|---|---|---|
| Range | 0.7-1.1 | 1.8 -3.40 | 0.7-1.30 |
| Mean | 0.88 | 2.76 | 1.004 |
| ±SD | 0.129 | 0.470 | 0.175 |
| F. test | 16.325 | | |
| *p*. value | 0.001* | | |
| Tukey's test | | | |
| Sham Group & Vehicle treated animals | Sham Group & AM/AMBP-1 | Vehicle treated animals& AM/AMBP-1 | |
| 0.001* | 0.367 | 0.001* | |

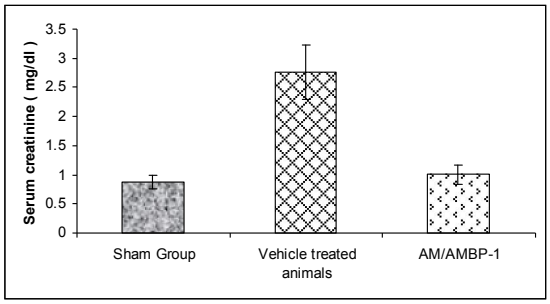


**Fig. (5):** Comparing serum creatinine (mg/dl )

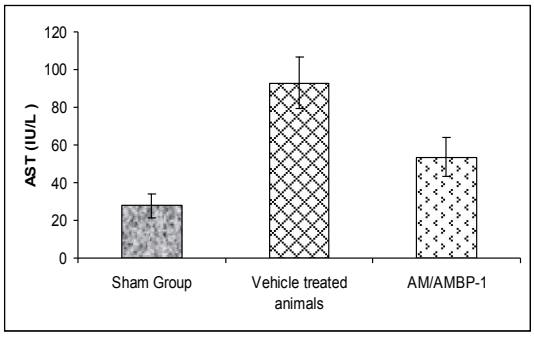


**Fig. (6):** Comparing serum AST ( IU/L ) sham, vehicle treated and AM/AMBP-1 groups.

**Table (6):** Comparing serum AST (IU/L) sham, vehicle treated and AM/AMBP-1 groups.

| Case number | Sham Group | Vehicle treated animals | AM/AMBP-1 |
|---|---|---|---|
| Range | 20 -40 | 75-115 | 40 -70 |
| Mean | 27.70 | 93.00 | 53.50 |
| ±SD | 6.429 | 13.759 | 10.124 |
| F. test | 21.352 | | |
| *p*. value | 0.001* | | |
| Tukey's test | | | |
| Sham Group & Vehicle treated animals | Sham Group & AM/AMBP-1 | Vehicle treated animals& AM/AMBP-1 | |
| 0.001* | 0.001* | 0.001* | |

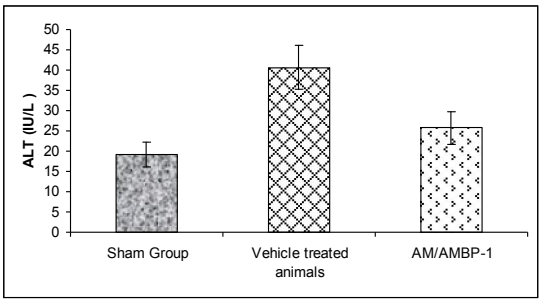


**Fig (7):** Comparing serum ALT ( IU/L ) sham, vehicle treated and AM/AMBP-1 groups

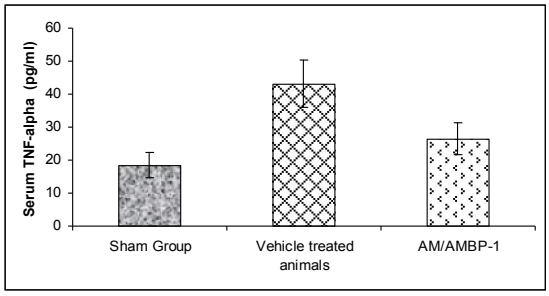


**Fig (8):** Comparing serum TNF- α ( pg /mL ) sham, vehicle treated and AM/AMBP-1 groups.

**Table (7):** Comparing serum ALT ( IU/L ) sham, vehicle treated and AM/AMBP-1 groups.

| Case number | Sham Group | Vehicle treated animals | AM/AMBP-1 |
|---|---|---|---|
| Range | 15-26 | 30-47 | 19-31 |
| Mean | 19.10 | 40.60 | 25.70 |
| ±SD | 3.071 | 5.440 | 4.001 |
| F. test | 11.639 | | |
| *p*. value | 0.002* | | |
| Tukey's test | | | |
| Sham Group & Vehicle treated animals | Sham Group & AM/AMBP-1 | Vehicle treated animals& AM/AMBP-1 | |
| 0.001* | 0.002* | 0.001* | |

**Table (8):** Comparing serum TNF- α ( pg /mL ) sham, vehicle treated and AM/AMBP-1 groups.

| Case number | Sham Group | Vehicle treated animals | AM/AMBP-1 |
|---|---|---|---|
| Range | 13 -24 | 30 -52 | 20 -35 |
| Mean | 18.50 | 43.10 | 26.50 |
| ±SD | 3.865 | 7.030 | 4.836 |
| F. test | 9.325 | | |
| *p*. value | 0.001* | | |
| Tukey's test | | | |
| Sham Group & Vehicle treated animals | Sham Group & AM/AMBP-1 | Vehicle treated animals& AM/AMBP-1 | |
| 0.001* | 0.003* | 0.001* | |

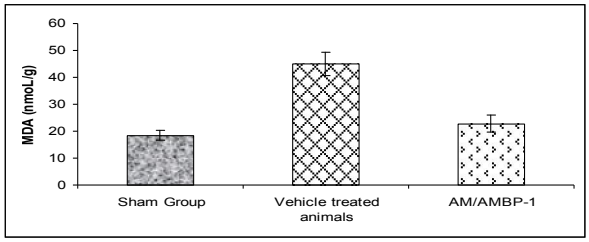


Fig. (9): Comparing renal malondialdehyde MDA (nmoL/g), sham, vehicle treated and AM/AMBP-1 groups.

**Table (9):** Comparing renal malondialdehyde MDA (nmoL/g ),glutathione GSH (umoL/g ) and myeloperoxidase MPO activity (U/g ) sham, vehicle treated and AM/AMBP-1 groups.

| | Sham Group | Vehicle treated animals | AM/AMBP-1 |
|---|---|---|---|
| MDA (nmoL/g) | | | |
| Mean | 18.5 | 45.0 | 22.8 |
| ±SD | 1.9 | 4.2 | 3.1 |
| Significance | Sham Group & Vehicle treated animals | Sham Group & AM/AMBP-1 | Vehicle treated animals& AM/AMBP-1 |
| | *P*<0.001* | Insignificant | *P*< 0.001* |
| GSH (umoL/g ) | | | |
| Mean | 1.5 | 0.5 | 1.3 |
| ±SD | 0.12 | 0.07 | 0.14 |
| Significance | Sham Group & Vehicle treated animals | Sham Group & AM/AMBP-1 | Vehicle treated animals& AM/AMBP-1 |

| | | | |
|---|---|---|---|
| | ***P*< 0.001*** | **Insignificant** | ***P*< 0.01*** |
| **MPO activity (U/g)** | | | |
| **Mean** | 5.9 | 14.4 | 7.2 |
| **±SD** | 0.8 | 2 | 0.7 |
| **Significance** | **Sham Group & Vehicle treated animals** | **Sham Group & AM/AMBP-1** | **Vehicle treated animals& AM/AMBP-1** |
| | ***P*< 0.001*** | **Insignificant** | ***P*< 0.01*** |

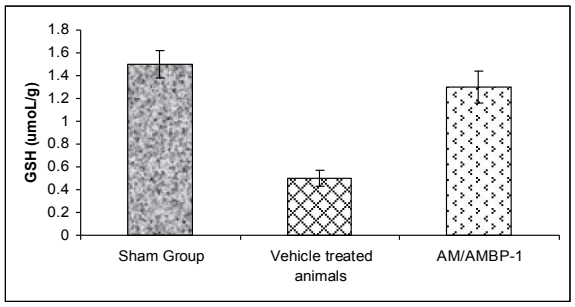


Fig. (10): Comparing renal glutathione GSH (umoL/g ) sham, vehicle treated and AM/AMBP-1 groups.

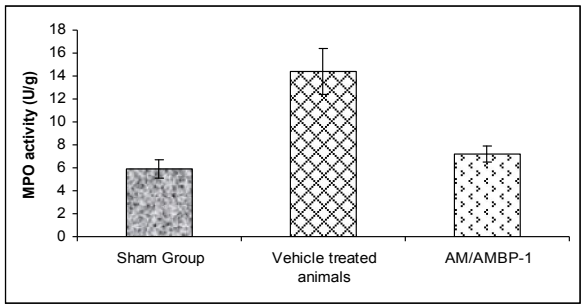


Fig. (11): Comparing renal myeloperoxidase MPO activity (U/g ) sham, vehicle treated and AM/AMBP-1 groups.

Renal Histopathology

**The histopathological findings of this study revealed the following:**

***Group I:*** Sham operated group,a regular renal tissue with glomeruli and tubuli and interstitial tissues as shown in (Fig. 12).

***Group II:*** Ischemia/ Reperfusion vehicle treated animals showed mild tubular necrosis in six out of ten rats (Fig.13) while other four showed very mild tubular necrosis, slight proteinaceous casts and mild medullary congestion (Fig.14).

***Group III*:** In AM/AMBP-1 treated group,only minor morphological changes in the form of mild hydropic degeneration,reduced tubular dilatation and regenerated glomeruli (Fig.15).

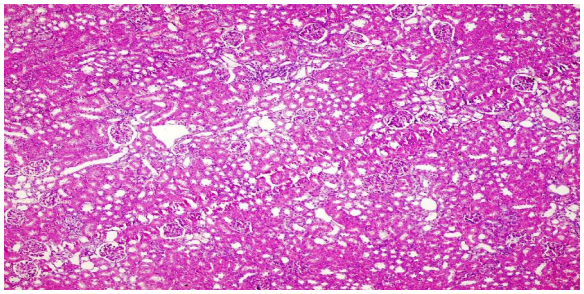

**Fig. (12):** Section from sham operated group revealed normal appearance of the glomeruli and tubules.

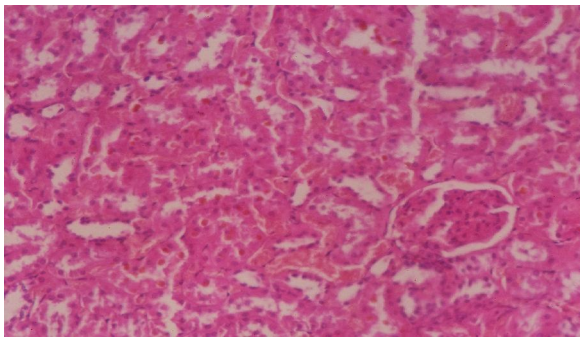

**Fig. (13):** Section from the kidney of Ischemia/ Reperfusion vehicle treated revealed preserved near-normal morphology a mild tubular necrosis

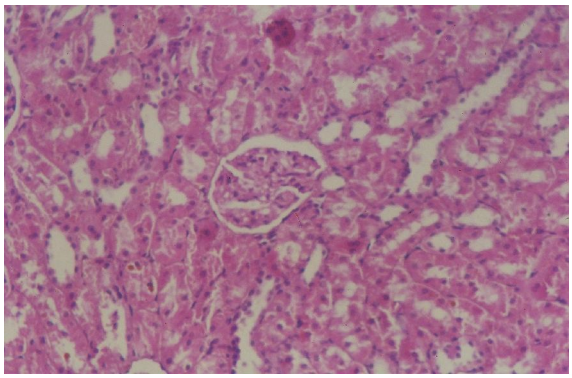

**Fig. (14):** Section from the kidney of Ischemia/ Reperfusion vehicle treated group revealed very mild tubular necrosis, slight proteinaceous casts and mild medullary congestion

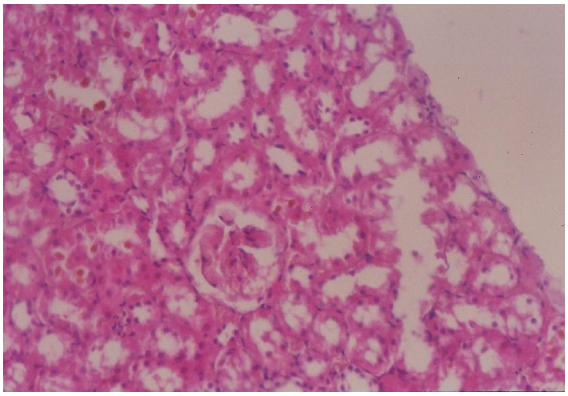

**Fig. (15):** Section from the kidney of AM/AMBP-1 treated group revealed minor morphological changes in the form of mild hydropic degeneration.

**4.Discussion**

Since its discovery in 1993*(Kitamura et al.,1993)*, AM has attracted the interest in the cardiovascular field because of its potent and long lasting vasoprotective prosperities (*Carrizo et al., 2007).*

In our study Adrenomedullin level was significantly increased after renal I/R compared with sham operated group and significant decrease of AMBP-1 in the I/R group compared with the sham operated group. Decrease of AMBP-1 which comprises the bioactivity of AM and provides the basis for a combined intervention with AM/AMBP-1. Treatment the renal I/R injured rats with AM/AMBP-1 reduced significantly renal edema,organ injury and inflammatory responses as evidenced in this study by significant reduction of the renal water content that is well recognized parameter to assess organ injury. So, AM/AMBP-1 may be of benefit in renal I/R injury.

Previous studies showed that administration of AM or AMBP-1 alone failed to produce a significant protection *(Wu et al.,2005 )*

Elevated AM level after I/R injury may be protective mechanism to counteract cardiovascular disorder in this I/R situation to restore the vascular responsiveness. However reduced AMBP-1 appears to be responsible for reduced vascular responsiveness to AM. The vascular responsiveness is very essential for renal perfusion so, combined administration of AM/AMBP-1 is required. *(Young et al., 2010 ).*

AM/AMBP-1 beneficial effect came from their vasoactive and anti-inflammatory prosperities as evidenced in this study by significant reduction of TNF-α. Previous studies have shown that AM or AMBP-1 alone only moderately reduces TNF-α production in Kupffer cells, while AM and AMBP-1 in combination dramatically downregulate TNF- α production *(Wu et al.,2003 )*. The direct anti-inflammatory effect of AM/AMBP-1 is mediated through both cAMP- dependant pathway and proline –rich tyrosine kinase 2 (Pyk-2)- ERK1/2-dependant induction of peroxisome proliferator- activated receptor γ (PPAR- γ. *(Miksa et al.,2007).*

Moreover, AM has been shown to downregulate chemokine levels both *in vivo* and *in vitro (Iwamoto et al.,2003 and Gonzalez-Rey et al.,2006)* [11,17]. It can inhibit neutrophil activation and migration to inflammatory sites like the liver by suppressing upregulation of the adhesion molecule CD11 *(Saito et al., 2001).*

Acute renal failure produced by ischemia and reperfusion is a clinical and experimental syndrome characterized by a major reduction in glomerular filtration rate, extensive tubular damage, tubular cell necrosis, glomerular injury, and signs of tubular obstruction with cellular debris Much of tubular and glomerular dysfunction has been postulated to occur during the reperfusion period following ischemia, during which the generation of oxygen free radicals contributes to reperfusion injury.*(Donnahoo et al.,1999).*

In the present study, renal I/R caused an increase in the renal MDA level, an indicator of lipid peroxidation, and depleted the antioxidant pool as evident from the declined levels of reduced glutathione. Moreover, oxidative injury of the tissues was accompanied by neutrophil infiltration, as evidenced by high tissue MPO levels. Both the oxidative renal damage and tissue neutrophil accumulation due to I/R were totally abolished by AM/AMBP-1.

In accordance with these biochemical changes, the morphologic evaluation of the tissues revealed that AM/AMBP-1 was also effective in protecting the kidney against I/R-induced degenerative changes. In addition to its beneficial effects at tissue level, AM/AMBP-1 treatment caused a dramatic reduction in the serum levels of TNF-α level, known as indicators of systemic inflammatory response.

Because free radicals play an important role in the physiopathological situations involving lipid peroxidation reactions, cellular oxidative defense mechanisms are therefore necessary to prevent the oxidative stress-induced peroxidation of membrane lipids, as well as other target macromolecules. By protecting cell membranes, AM/AMBP-1 probably reduces the deleterious effects of oxidative stress in living cells. *(Yang et al.,2002 ).*

In the present study, MDA, an index of lipid peroxidation, was increased in the kidney tissues indicating the presence of I/R-induced oxidative damage. In accordance with the aforementioned observations, the findings of the present study show that AM/AMBP-1 reduces the I/R-induced renal damage and dysfunction by reducing lipid

peroxidation. Because AM/AMBP-1 treatment prevented elevations in tissue MDA, it seems likely that a AM/AMBP-1 meliorates I/R-induced oxidative injury, in part by scavenging mainly superoxide radicals*(Kavin et al.,2010 ).* GSH is an important constituent of intracellular protective mechanisms against various noxious stimuli including oxidative stress *(Sener et al.,2006 ).*

However, reduced GSH as the main component of endogenous non-protein sulfhydryl pool is known to be a major low molecular weight scavenger of free radicals in the cytoplasm. The results of the present study support the notion that depletion of tissue GSH is one of the major factors that permits lipid peroxidation and subsequent tissue damage. Renal GSH levels were decreased significantly following I/R injury. However, administration of AM/AMBP-1 maintained GSH levels and thus protected the renal tissue against oxidative stress. The replenishment of GSH level in the AM/AMBP-1 -treated rats may be related to the antioxidant and free-radical scavenging effects of AM/AMBP-1.

During ischemia and, in particular, the reperfusion phase, tissue injury involves the participation of several potential sources of toxic oxygen species, including mitochondrial electron transport systems, purine catabolism by xanthine oxidase and infiltration of phagocytes (neutrophils and monocytes). *(Young et al., 2010).* The importance of circulating PMNL as mediators in I/R has also been widely investigated. ROS can generate hypocholorus acid (HOCl) in the presence of neutrophil-derived MPO and initiate the deactivation of antiproteases and activation of latent proteases, which lead to tissue damage *(Reiter et al.,2001)* Several methods have been used to define the role of neutrophils in reperfusion tissue injury. One of them is neutrophil-specific enzyme, MPO activity. On the other hand, oxidative stress could also be involved in inflammatory glomerular lesions caused by series of mediators including cytokines and chemokines that lead to leukocyte activation, production of ROS and increased glomerular damage *(Rodrigo and Rivvera;2002).* Peripheral monocytes infiltrating the kidney have traditionally been considered the primary source of renal TNF. On the other hand, oxidants released during the reperfusion of ischemic tissue stimulate transcription factors involved in TNF expression *(Li et al., 1999).* It has been shown that exogenous TNF induces renal cell apoptosis, glomerular endothelial damage, fibrin deposition, cellular infiltration and renal failure. In the present study, the elevated tissue MPO activity and the increased serum TNF-a level indicate the contribution of neutrophil infiltration and the involvement of pro-inflammatory cytokine TNF-a in I/R-induced renal injury. Reversal of these parameters by AM/AMBP-1 treatment suggests that the mechanism of the protective effect of AM/AMBP-1 involves inhibition of inflammatory cell infiltration and release of TNF-a. In accordance with these findings, we have previously shown that RVT reduced sepsis-induced injury by inhibiting neutrophil infiltration and thereby regulating the release of inflammatory mediators *(Kolgazi et al., 2006).*

**Conclusions**

Due to the complexity and severity of renal I/R injury, there is an obvious need for the development of novel treatments to prevent and/or minimize the injury. Since the pathophysiology of renal I/R injury constitutes oxidative stress, inflammation, and apoptosis, therapy should be directed against all aspects of the pathology. In conclusion, this study demonstrates that AM/AMBP-1, reduces I/R-induced renal injury and that the protective effect of AM/AMBP-1 can be attributed, at least in part, to its ability to balance oxidant_antioxidant status, to inhibit neutrophil infiltration and to regulate the inflammatory mediators, suggesting a future role in the treatment of oxidative renal injury and subsequent organ failure due to ischemia/reperfusion

Future studies will determine whether AM/AMBP-1 is able to protect against oxygen radical production and apoptosis in renal I/R injury.

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