

## Study the Effect of Apocynin on Some Renal Functions in Ischemia/Reperfusion Injury in Male Albino Rats

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### ABSTRACT

**Background:** Apocynin (4-hydroxy-3-methoxyacetophenone) which isolated from the traditional medicinal plant *Picrorhiza kurroa*, is a naturally occurring methoxy-substituted catechol, experimentally used as an inhibitor of NADPH oxidase and of the concomitant ROS production. Apocynin also proved to be a potent anti-inflammatory agent, based on the selective inhibition of the production of ROS by activated human PMNs.

**Aim of the Study:** to explore the protective effect of Apocynin and the NADPH oxidase inhibitor on kidney damage induced by ischemia/reperfusion (I/R) in a rat model. **Subjects and Methods:** Fourty male albino rats were categorized into four equal groups of 10. **Group 1:** Sham operated control group, **Group 2:** Ischemia / reperfusion (I/R) group; which underwent bilateral renal ischemia for 1 hour followed by a 23 hour reperfusion, **Group 3:** 4-week Apocynin treated group in which rats received Apocynin with a dose of 16 mg/kg /day for 4 weeks followed by bilateral renal ischemia for 1 hour then 23 hour reperfusion afterwards , and **Group 4:** 8-week Apocynin treated group in which rats received Apocynin with a dose of 16 mg/kg /day for 8 weeks then bilateral renal ischemia for 1 hour followed by 23 hour reperfusion.

After reperfusion, the animals were sacrificed, the blood samples were collected for determination of blood urea nitrogen, serum creatinine, 24 hour urine were collected for determination of creatinine clearance. Kidneys of all animals were harvested and evaluated biochemically through determination of tissue MDA, MPO, GPX and catalase level. **Results:** Kidney tissue MDA, MPO, serum BUN and Creatinine levels were found to be significantly higher in the I/R group. GPx level showed a significant decrease, while there was a slight decrease in Catalase level when compared with Sham operated group. Creatinine clearance was impaired in renal I/R group. Renal I/R injury has also induced an extensive tubular necrosis, glomerular damage, and apoptosis. Apocynin significantly reduced MDA and MPO and increased GPX and catalase in both treatment groups when compared to the I/R group ( $p < 0.001$ ). The elevated BUN and creatinine levels were significantly reduced in treatment groups, also creatinine clearance was restored to around normal value.

**Conclusion:** with accordance to the findings and outcome of the present study, Apocynin has exerted protective effects against ischemia/reperfusion injury by ameliorating the kidney damage induced by I/R injury to a significant extent. However, there was no significant difference in the outcome if the treatment was extended from 4 to 8 weeks.

**Keywords:** Apocynin, NADPH-oxidase, Ischemia, Reperfusion injury, Reperfusion injury.

### INTRODUCTION

Ischemia/ reperfusion (I/R) injury, which is a major reason for acute renal failure, occurs in many conditions, such as hypotension, sepsis, shock, open renal stone surgery, partial nephrectomy, and renal transplantation<sup>[1]</sup>. Renal I/R injury continue to be associated with significant morbidity and mortality despite advances in preventive strategies and supportive measures<sup>[2]</sup>. Although reperfusion is essential for the survival of ischemic tissue, there is good evidence for reperfusion itself causes additional cellular injury<sup>[3]</sup>. Renal I/R induces some injury in the cortical proximal tubules and a more severe generally lethal injury in the outer medullary proximal tubules , Infiltration of neutrophils and macrophages in the kidney occurs after reperfusion<sup>[4]</sup>. Production and releasing of reactive oxygen species (ROS) by

inflammatory cells, endothelial cells, platelets, injured cells, and cell debris, via many enzymatic mechanisms is one of the most important reasons for reperfusion injury<sup>[5]</sup>. NADPH-oxidase (NOX) is a major enzyme that uses NADPH to generate superoxide, initial ROS molecule, from oxygen<sup>[6]</sup>. In the kidney, NADPH oxidase components are expressed abundantly in the renal vessels and in the glomerular mesangial and podocyte cells, the macula densa, and the thick ascending limb, distal tubule, and collecting ducts<sup>[7]</sup>.

Apocynin (4-hydroxy-3-methoxy-acetophenone), naturally occurring methoxy-substituted catechol, is an inhibitor of NOX and extracted from the roots of *Apocynum cannabinum* (Canadian hemp) and *Picrorhiza kurroa* (Scrophulariaceae)<sup>[8]</sup>. It was used to treat dropsy and heart troubles in India and currently

seems to be an encouraging therapy for hypertension in the light of many experimental studies<sup>[9]</sup>.

**MATERIALS AND METHODS**

The present work was carried out on 40 male albino Wistar rats ranging in weight between 150 to 200 gm. The rats were housed in isolated animal cages, in a standard animal laboratory, exposed to alternate cycles of 12h light and darkness and had free access to tap water and pelleted laboratory chow during the experimental period. All procedures were carried out according to the ethical committee of Faculty of Medicine, Tanta University.

**The animals were acclimatized for two weeks, then randomly divided into Four groups (10 rats each):**

**Group I (Sham- operated group):**

Animals of this group only underwent a midline laprotomy incision and then exposure of the two pedicles of both kidneys and then closure of incision.

**Group II (Renal I/R group):**

Animals of this group received normal diet and tap water along the period of experiment. Then both renal arteries and veins were occluded together by a traumatic clamp for 60 minutes of ischemia. Following 60 minutes of ischemia, a reperfusion period of 24 hours began by removing the clamp<sup>[7]</sup>.

**Group III (Apocynin- treated renal I /R group for 4 weeks):**

Animals of this group received normal diet, libitum and Apocynin in drinking water by a dose of 16 mg / kg / day<sup>[7]</sup> for 4 weeks then both renal arteries and veins were occluded together by a traumatic clamp for 60 minutes of ischemia. Following 60 minutes of ischemia, a reperfusion period began by removing the clamp for 24h<sup>[7]</sup>.

**Group IV (Apocynin – treated renal I / R group for 8 weeks):**

Animals of this group received normal diet, libitum and Apocynin in drinking water by a dose of 16 mg / kg / day for 8 weeks. Both renal arteries and veins

were occluded together by a traumatic clamp for 60 minutes of ischemia. Following 60 minutes of ischemia, a reperfusion period began by removing the clamp for 24h<sup>[7]</sup>.

At the end of the experimental period, all animals were anaesthetized and then sacrificed and blood sample were collected for the measurement of:

1. Serum creatinine.
2. Blood urea nitrogen (BUN).
3. Creatinine clearance
4. Kidney Catalase activity
5. Kidney Glutathione peroxidase (GPx) activity
6. Kidney malondialdehyde level (MDA)

24h urine collection was carried out for the determination of creatinine clearance levels.

Kidneys of all animals were dissected and stored in 10% formalin for estimation of tissue malondialdehyde (MDA), catalase (CAT), glutathione peroxidase (GPX), and myeloperoxidase (MPO).

**RESULTS**

The present study was performed to evaluate the protective effect of Apocynin at a dose 16mg/kg daily for 4 and 8 weeks given in drinking water, on renal functions impaired by renal ischemia-reperfusion in male albino rats.

**1. Serum creatinine level (mg/dl) in all studied groups:**

**Table 1 reveals the following:**

Renal I/R injury resulted in a significant increase in serum creatinine Level (p<0.001). However, the treated group with oral Apocynin for four weeks and eight weeks, showed a significant decrease (p <0.001) in serum creatinine versus I/R group.

Nevertheless, The comparison between the two treated group showed insignificant change.

Creatinine level in groups treated with Apocynin decreased close to the normal level but still there was significant change (p<0.001) versus sham operated group.

**Table 1: Serum creatinine level (mg/dl) in all studied groups:**

Group Number	Sham operated	Ischemia reperfusion	Apocynin treated for 4 weeks	Apocynin treated for 8 weeks
Mean ± S.D	0.459 ± 0.037	2.26 ± 0.395	1.473 ± 0.089	1.401 ± 0.134
f test	118.398			
p value	0.001*			
<b>Tukey's test</b>				
G I & G II	G I & G III	G I & G IV	G II & G III	G II & G IV
0.001*	0.001*	0.001*	0.001*	0.457

\*Denotes statistical significance at p value (p<0.001)

**2. Blood urea nitrogen level (mg/dl):**

**Table 2 reveals the following:**

- Blood urea nitrogen level showed a significant increase ( $P < 0.001$ ) in Sham operated group compared with I/R group.
- Similarly, Animals that received Apocynin for four weeks showed a significant decrease ( $p < 0.001$ ) in BUN level versus I/R group.
- However, the 8-week Apocynin treated group showed a significant decrease ( $p < 0.001$ ) in BUN level, when compared to I/R group.

- But, there was an insignificant change ( $P > 0.05$ ) between the 4-week Apocynin treated group versus the 8-week Apocynin treated group.
- BUN level in both treated groups decreased in comparison with sham operated group but still there was a significant change ( $p < 0.001$ ) when compared to Sham operated group.

• **Table (2): Blood urea nitrogen level (mg/dl) in all studied groups:**

Groups Number	Sham operated	Ischemia reperfusion	Apocynin treated for 4 weeks	Apocynin treated for 8 weeks	
Mean ± S.D	65.11 ± 11.48	291.58 ± 45.25	123.63 ± 3.99	110.39 ± 7.67	
f test	174.448				
p value	0.001*				
<b>Tukey's test</b>					
G I & G II	G I & G III	G I & G IV	G II & G III	G II & G IV	G III & G IV
0.001*	0.001*	0.001*	0.001*	0.001*	0.221

\*Denotes statistical significance at p value ( $p < 0.001$ )

**3. Creatinine clearance (ml/min)**

**Table 3 reveals the following:**

- **Sham group versus I/R group:** a significant increase ( $P < 0.001$ ) in Creatinine clearance level was observed.
- **4-week Apocynin treated group compared to I/R group :** a significant decrease ( $p < 0.001$ ) in Creatinine clearance level resulted.

- **8-week Apocynin treated group compared to I/R group:** showed a significant decrease ( $p < 0.001$ ) in Creatinine clearance level.
- **4-week Apocynin treated group compared to 8-week Apocynin treated group:** insignificant change ( $P > 0.05$ ).
- **Sham operated group versus both treated groups:** insignificant change ( $p > 0.05$ ) was reported.

**Table (3): Creatinine clearance value (ml/min) in all studied groups:**

Groups Number	Sham operated	Ischemia reperfusion	Apocynin treated for 4 weeks	Apocynin treated for 8 weeks	
Mean ± S.D	1.10 ± 0.17	0.53 ± 0.07	0.96 ± 0.18	1.04 ± 0.19	
f test	26.209				
p value	0.001*				
<b>Tukey's test</b>					
G I & G II	G I & G III	G I & G IV	G II & G III	G II & G IV	G III & G IV
0.001*	0.058	0.383	0.001*	0.001*	0.288

\*Denotes statistical significance at p value ( $p < 0.001$ )

**4. Kidney Catalase activity (U/g) in all studied groups**

Table 4 reveals the following:

- **Sham group versus I/R group:** a significant decrease ( $p < 0.05$ ) in Renal catalase level showed was observed.
- **4-week Apocynin treated group compared to I/R group:** a significant increase ( $p < 0.05$ ) in Renal catalase level resulted.
- **8-week Apocynin treated group compared to I/R group:** showed a significant decrease ( $p < 0.001$ ) in Renal catalase level.
- **4week Apocynin treated group compared to 8-week Apocynin treated group:** insignificant change ( $P > 0.05$ ).
- **Sham operated group versus both treated groups:** insignificant change ( $p > 0.05$ ) was reported.

Table (4): Kidney MPO (U/gm tissue) in all studied groups:

Groups Number	Sham operated	Ischemia reperfusion	Apocynin treated for 4 weeks	Apocynin treated for 8 weeks	
Mean ± S.D	10.57 ± 2.67	26.49 ± 3.32	11.34 ± 2.45	11.65 ± 3.93	
f test	59.315				
p value	0.001*				
<b>Tukey's test</b>					
G I & G II	G I & G III	G I & G IV	G II & G III	G II & G IV	G III & G IV
0.001*	0.587	0.446	0.001*	0.001*	0.825

\*Denotes statistical significance at p value ( $p < 0.001$ )

**5. Kidney Glutathione peroxidase (GPx) activity (U/mg ptn) in all studied groups**

Table 5 reveals the following:

- **I/R group versus Sham group:** a significant decrease ( $p > 0.001$ ) in GPx level was observed.
- **4-week Apocynin treated group compared to I/R group:** a significant increase ( $p < 0.001$ ) in GPx level resulted.
- **8-week Apocynin treated group compared to I/R group:** showed a significant decrease ( $p < 0.001$ ) in GPx level.
- **4-week Apocynin treated group compared to 8-week Apocynin treated group:** insignificant change ( $P > 0.05$ ).
- **Both Apocynin treated groups versus Sham operated group:** significant increase ( $p < 0.001$ ) was reported in GPx level.

Table (5): Renal catalase activity (U/g) in all studied groups

Groups Number	Sham operated	Ischemia reperfusion	Apocynin treated for 4 weeks	Apocynin treated for 8 weeks	
Mean ± S.D	79.01 ± 19.94	63.75 ± 9.77	83.97 ± 8.84	79.49 ± 11.28	
f test	4.894				
p value	0.05*				
<b>Tukey's test</b>					
G I & G II	G I & G III	G I & G IV	G II & G III	G II & G IV	G III & G IV
0.044*	0.829	0.942	0.005*	0.039*	0.867

\*Denotes statistical significance at p value ( $p < 0.05$ )

**6. Kidney malondialdehyde level (MDA) (nmol/g) in all studied groups:**

Renal MDA level is an index of lipid peroxidation, in table 6 we can conclude that:

- **Sham group versus I/R group:** a significant increase ( $p < 0.001$ ) in Renal MDA level showed was observed.
- **4-week Apocynin treated group compared to I/R group:** a significant decrease ( $p < 0.001$ ) in GPx level resulted.

- **8-week Apocynin treated group compared to I/R group:** showed a significant decrease ( $p < 0.001$ ) in GPx level.
- **4-week Apocynin treated group compared to 8-week Apocynin treated group:** insignificant difference ( $P > 0.05$ ).
- **Sham operated group versus both treated groups:** insignificant change in mDA level ( $p > 0.05$ ) was reported.

**Table (6): Renal GPx activity (U/mg ptn) in all studied groups:**

Groups Number	Sham operated	Ischemia reperfusion	Apocynin treated for 4 weeks	Apocynin treated for 8 weeks	
Mean ± S.D	2.55 ± 0.42	1.3 ± 0.28	3.25 ± 0.53	3.31 ± 0.53	
f test	43.526				
p value	0.001*				
<b>Tukey's test</b>					
G I & G II	G I & G III	G I & G IV	G II & G III	G II & G IV	G III & G IV
0.001*	0.05*	0.001*	0.001*	0.001*	0.727

\*Denotes statistical significance at p value ( $p < 0.001$ )

**DISCUSSION**

The major oxidant system in leukocytes is constituted by NADPH oxidase and MPO, which are the key enzymes in a cascade of reaction leading to ROS as  $H_2O_2$ , hypochlorous acid (HOCL), hypobromous acid (HoBr), and hypothiocyanous acid<sup>[10]</sup>.

Apocynin as a NOX inhibitor is a widely investigated anti oxidative and anti-inflammatory agent, which alleviate oxidative stress after I/R injury<sup>[11]</sup>.

Ischemia reperfusion injury is a complex phenomenon, which is often encountered in vascular surgery and organ transplantation and can lead to structural and functional cell damage<sup>[12]</sup>.

The result of the present work showed that ischemia reperfusion led to significant increase in BUN, creatine, MPO, MDA level and significant decrease in creatinine clearance, catalase and GPx level. This indicate that ischemia reperfusion lead to renal damage. The mechanism of this renal damage may be explained by increase the inflammatory cells infiltration that may produce systemic inflammatory response which may be due to release of cytokines and generation of free radicals. These free radicals

may induce lipid peroxidation and release of ROS which may produce cell death (apoptosis).

**Tsutahara *et al.***<sup>[13]</sup>reported that, bone marrow-derived cells, as T lymphocytes, may be involved in I/R and have been shown to participate early in its pathogenesis.

Moreover, **Matsuyama *et al.***<sup>[14]</sup>suggested that, I/R trigger a series of reactions, it could elicit a systemic inflammatory response by the release of cytokines & inflammatory mediators, it cause the generation of free radicals.

Furthermore, **Junior *et al.***<sup>[15]</sup>demonstrated that, I/R is commonly associated with lipid peroxidation, it induce autocatalytic mechanisms leading to oxidative destruction which could lead to production of toxic reactive metabolites & cell death. Moreover, **Junior *et al.***<sup>[15]</sup>added that, Lipid peroxidase as a free radical generating system has been suggested to be closely related to I/R induced tissue damage.

In addition, **Iravani and Zolfaghari**<sup>[16]</sup>reported that, the reactive oxygen species such as superoxide anion ( $O_2^-$ ) hydroxyl radical (OH• & hydrogen peroxide ( $H_2O_2^-$ ) have a causal relationship with oxidative stress.

Reperfusion of the ischemic renal tissues may be typically more damaging than ischemia itself and it occurs when the blood supply return to kidney tissues after a period of ischemia because the absence of oxygen and nutrient supply from the blood may create a condition in which restoration of the circulation results in release of free radical.

Moreover, **de Groot and Rauen**<sup>[17]</sup> explained that the damaged enzymes which have role in electron transferring in the mitochondria cause electron transfer to oxygen in turn resulting in generation of superoxide and ROS. The over product of ROS cause membrane lipid peroxidation.

Furthermore, **Yildirimi et al.**<sup>[18]</sup> reported that, ROS are generated early during reperfusion where the initial cell death triggers an inflammatory response with activation of tissue macrophages and recruitment of neutrophils, both of which cause cell damage by further ROS generation.

Moreover, **Hadi et al.**<sup>[19]</sup> suggested that, the apoptotic program is initiated shortly after the onset of ischemia but there is evidence that the process is amplified during reperfusion. The results of the present work showed that I/R lead to significant increase of BUN and Cr and significant decrease in creatinine clearance that is improved significantly by Apocynin treatment. The significant increase in BUN and Cr and significant decrease in creatinine clearance may be explained by that I/R produced renal damage or may lead to acute renal failure and impairment of glomerular function that disturb BUN and Cr and resulted in their elevation. Also increased urea and creatinine may be due to renal ischemia that increase oxidative stress and free radicals which may progress to tissue damage and apoptosis.

Moreover Apocynin treatment improved BUN and Cr levels may be due to reduction of leukocyte infiltration and reduction of oxidative stress and reduced lipid peroxidation and may prevent apoptosis. So Apocynin may protect the podocytes density which may improve proteinuria.

**Sahna et al.**<sup>[20]</sup> stated that, Apocynin improved the elevation of serum levels of BUN and Cr is caused by impairment of glomerular function.

These finding are also supported by those found by **Yayi et al.**<sup>[21]</sup> who stated that, Apocynin has beneficial effect on renal function and improved the kidney damage which occurred after I/R injury. Furthermore, **Munshi et al.**<sup>[22]</sup> declared that, Apocynin improved the increased urea and creatinine in I/R may be attributed to depletion of energy rich phosphates (ATP) caused by shortage of

oxygen during ischemia, which leads to apoptosis and necrosis of tubular cells.

In addition, **Mohamed and Mubarak**<sup>[23]</sup> detected that, Apocynin prevented ATP depletion that causes tubular epithelial cells to undergo apoptosis or necrosis, and both apoptotic and necrotic tubular epithelial cells may be seen in ischemic acute renal failure. Moreover, Apocynin protect the podocytes densities that lead to progressive proteinuria.

Moreover, **Kinsey et al.**<sup>[24]</sup> detected that, Apocynin treatment lead to improvement of renal I/R that initiated changes in vascular endothelial cells, tubular epithelial cells and leukocytes that resulted in the loss of immune system homeostasis in the kidney. The present work revealed that I/R injury produced significant increase in MPO level which decreased significantly by Apocynin treatment. The mechanism by which oral Apocynin causes inhibition of MPO level could be explained that Apocynin reduced the neutrophils infiltration as MPO is present in neutrophil and this may decrease ROS.

**Kaçmazet et al.**<sup>[25]</sup> suggested that, Apocynin decreased I/R injury which elicit an acute inflammatory response that characterized by activation of neutrophils as evidenced by increase in periportal neutrophil infiltration.

**Altunoluk et al.**<sup>[26]</sup> also added that, Apocynin reduced neutrophils which are the inflammatory cells, which produces abundantly ROS during I/R injury. MPO is found in neutrophils and catalyzes the formation of hypochlorous acid (HOCl), a toxic agent to cellular components and initiates oxidative injury. Furthermore, **Sener et al.**<sup>[27]</sup> reported that, Apocynin reduced MPO activity that used as an indirect evidence of neutrophil infiltration in oxidant-induced tissue injury.

This is supported by **Altintas et al.**<sup>[28]</sup> who stated that Apocynin inhibits MPO which is a particular oxidase in PMNL and the MPO activity in tissue is used to estimate the PMNL chemotaxis and infiltration.

PMNL infiltration during the reperfusion period may cause generation and releasing of additional large amount of oxidants that exacerbates this harmful cascade. Apocynin, which is also, reduces the generation of inflammatory mediators by inhibiting NOX.

The results of the present work showed a significant reduction of catalase level in I/R which improved after Apocynin treatment.

These results could be explained by that catalase is an antioxidant enzyme that reduces oxidative stress and may reduce the formation of ROS activity produced by I/R. so it may act as a defense mechanism against tissue damage which may be due to NOX inhibition.

**Altintas *et al.*** <sup>[28]</sup> suggested that, Apocynin treatment improved the level of catalase enzyme. As CAT is antioxidant enzyme that is a component of the defense mechanism against ROS activities. The levels of the enzymes within host increase to protect the tissues during I/R injury. Moreover, **Candelario-Jalil *et al.*** <sup>[29]</sup> suggested that, Apocynin decrease the over production of reactive oxygen species that can be detoxified by endogenous antioxidants.

Furthermore, **Asaga *et al.*** <sup>[30]</sup> reported that Apocynin improves catalase content which significantly reduced due to ischemic insult. This could be explained by the consumption of catalase to scavenge of the rapidly generating ROS due to ischemia.

The results of the present work showed significant reduction in GPx level which improved by Apocynin treatment. These results could be explained by that the reduction of GPx by I/R is may be due to increase MPO levels that may increase free radicals. Also, the mechanism by which Apocynin increase GPx may be due to reduction of lipid peroxidation and oxidative stress which may reduce active free radicals.

Moreover, **Altintas *et al.*** <sup>[28]</sup> showed that, significant increase in GPX activity in I/R group treated by Apocynin.

Furthermore, **Van Haaften *et al.*** <sup>[29]</sup> explained that, Apocynin increased GPX which is important component of the protective mechanism of the cell against lipid peroxidation and oxidative stress, which occur during I/R injury and GPX convert hydrogen peroxide to water thus, they prevent the formation of more reactive-free radicals. Moreover, **Gezginci-Oktayoglu *et al.*** <sup>[30]</sup> explained that, glutathione peroxidase plays primary role in minimizing the oxidative stress that increased by Apocynin treatment.

The results of the present work showed a significant increase in MDA levels in the renal tissue of rats after induction of renal I/R injury which is major index of lipid peroxidation and oxidative stress. These results can be explained by that, ischemia may be associated by change of nerve supply which may produce necrosis of renal tissue and lipid oxidation products which may be MDA.

The increase of MDA could be explained also by excessive production of ROS due to oxidative stress, which may be due to activation of xanthine oxidase or leukocytes infiltration. So the generation of free radicals may cause cell damage during reperfusion.

**Al-Omar *et al.*** <sup>[31]</sup> stated that, ischemia affect the nervous supply of tissues causing more necrosis and production of lipid peroxides that arise from damaged cells.

Moreover, **Erdogan *et al.*** <sup>[32]</sup> found that, Renal I/R causes tissue injury by oxygen radicals and oxidative stress caused by an imbalance between production of ROS and the antioxidant capacity.

Additionally, **Hamidian *et al.*** <sup>[33]</sup> explained that, reperfusion of an ischemic tissue leads to ROS generation which arise from xanthine oxidase activation or from leukocytes penetration in the interstitial tissue.

In addition, **Raju *et al.*** <sup>[34]</sup> showed that during reperfusion of the tissue, oxygen needed for the conversion of hypoxanthine to a uric acid becomes available resulting in generation of enormous amount of free radicals which react with lipid in the cell and mitochondrial membranes leading to disruption of integrity of the cell.

Oral Apocynin treatment showed a significant decrease in MDA level. These results can be explained by that oral Apocynin may act as antioxidant prevent cell damage, lipid peroxidation and may reduce the production of ROS.

The results was agreed by **Altintas *et al.*** <sup>[28]</sup> who showed that Apocynin inhibits the production of MDA which is a major indicator of oxidative stress, which increases due to lipid peroxidation, which is one of the harmful consequences of I/R injury. Furthermore, **Yayi *et al.*** <sup>[35]</sup> also stated that Apocynin reduced MDA level that show significant increase in renal I/R injury suggesting that oxidative balance was disrupted after renal I/R and improved by Apocynin administration.

In addition to that, **Sahna *et al.*** <sup>[36]</sup> showed that, Apocynin administration inhibit lipid peroxidation which is the main pathway of oxidative stress, regardless of the source of free radicals, by blocking of this pathway may be an effective strategy to prevent ROS-mediated kidney damage.

**Moreover,** A shrinkage of nearly all the glomeruli with enlargement of the Bowman's space in I/R group was observed. Such damage was however alleviated in both treated group which was manifested as a slight cortical damage, moderate

form of tubular desquamation, tubular necrosis, interstitial congestion, a few damaged glomeruli, mild form of tubular desquamation, necrosis and interstitial congestion in Apocynin treated for 4 weeks group. Nevertheless, the glomeruli were normal in Apocynin treated for 8 weeks group.

### CONCLUSION

In both treated groups, apocynin showed improvement of the kidney damage, which occurred after I/R injury, serum creatinine, blood urea nitrogen and creatinine clearance return to levels approximate to the normal levels. MDA and MPO also showed significant decrease in their level when compared by I/R group. GPx and catalase levels showed significant increase which prevent the formation of more reactive-free radicals. The histopathological examination confirmed our results which showed slight cortical damage, moderate form of tubular desquamation, tubular necrosis, and interstitial congestion. There were some damaged and normal glomeruli. There was no significant difference in results between the group treated for 4 weeks and the group treated for 8 weeks.

According to all the findings and outcome, we conclude that Apocynin could be used to prevent the kidney damage induced by I/R injury occurring in many ways.

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