ISSN 0975-2366
DOI:https://doi.org/10.31838/ijpr/2020.12.01.177

**Research Article**

# Nephroprotective Effect of Octreotide and Liraglutide in Renal Ischemia Reperfusion Injury in Mice

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Received: 22.11.19, Revised: 24.12.19, Accepted: 26.01.20

## ABSTRACT

Renal ischemia reperfusion injury is a pathological event that can lead to acute renal failure (ARF), and found that 45% of the cases of ARF in the intensive care unit due to IRI in one study. It is also a common cause of graft failure after kidney transplant and increase both morbidity and early loss of transplanted kidney. Several previous studies had been reported that the underlying path physiological mechanisms of acute kidney IR injury are largely due to the generation of oxidative stress and reactive oxygen species (ROS), intense inflammatory response, and enhancement of cellular apoptosis after prolonged or even transient IR injury. Experimental studies had been demonstrated that prevention of inflammatory reaction and inhibition of the production of pro-inflammatory cytokines and oxidative stress using immunological or pharmacological therapy significantly provide protection against ischemic acute kidney injury. Mice were divided randomly into five groups (6 mice in each group): sham group: mice were underwent anesthetic and surgical procedures the same as that in ischemia group except the induction of ischemia, ischemia reperfusion group (control group): mice were underwent 30 minutes bilateral renal ischemia by clamping of right and left renal arteries and 2 hours reperfusion, vehicle group: IRI + normal saline (for both drugs) by intraperitoneal injection, 30minutes before the induction of ischemia and underwent bilateral renal ischemia for 30 minutes followed by 2 hours reperfusion, octreotide treated group: treated with a dose of octreotide (30µg/kg) by intraperitoneal injection, 30 minutes before ischemia and underwent 730 minutes bilateral renal ischemia followed by 2 hours reperfusion, Liraglutide treated group: treated with a dose of Liraglutide (1mg/kg) by intraperitoneal injection, 30min before ischemia and underwent 30 minutes bilateral renal ischemia followed by 2 hours reperfusion. At the end of the research, we observed that mean serum and kidney tissue levels of MCP-1, IL-1β, P2X7 and renal function indices (blood urea and serum creatinine) were significantly increased in I/R (control) group in compare with sham group ($P<0.05$). All mice in the control group showed a significant ($P<0.05$) renal injury and increase in renal histology score. Both of Liraglutide and Octreotide drugs cause significant lowering of serum and tissue levels of MCP-1, IL-1β, P2X7 in addition to cause significant reduction in renal function indices (blood urea and serum creatinine) ($P<0.05$). Histological found that both of drugs markedly decreased the renal injury in ischemia reperfusion injury ($P<0.05$). We concluded that both of octreotide and Liraglutide have a protective effect in renal I/R injury in male mice via their effects mainly as anti-inflammatory and anti-oxidant effects. Renal ischemia/ reperfusion injury (IRI), is a harmful event that result in acute kidney injury (AKI) [1]. Ischemic injury happens when there is impairment of blood flow to the tissues of the kidney, nevertheless it increase in severity when the blood flow is restored on reperfusion [2]. Also there is either localized or generalized impairment in the delivery of oxygen(O2) and nutrients to tissues of the kidney, accumulation of cell waste product [3]. There is unevenness of local tissue o2 supply and demand and the aggregation of waste products of anabolism and catabolism.

**Key words:** Octreotide, Liraglutide, RIR

## INTRODUCTION

As a result of this mismatch, the renal tubular epithelial cells undergo inflammation and injury and, if its severe, tubular epithelial cells undergo death by means of apoptosis and necrosis (acute tubular necrosis [ATN]), with organ failure that leads to disturbance of water and electrolyte homeostasis and decrease the excretion of waste products of metabolism [4]. Renal IRI occur when the flow of the blood is returned after transient interruption of the renal blood supply ,which may cause more severe tissue injury to the kidney than ischemia itself [5]. Various clinical conditions can cause it such as: shock, sepsis, vascular surgery and organ transplantation [6-7].The main path

physiological processes that occur during IRI include: oxidative stress, increase intracellular calcium concentration ,inflammation and apoptosis [8]. Despite many years of heavy workup, the underlying path physiological mechanisms of RIRI remain vague. Recently, a lot of anti-inflammatory and antioxidant drugs were effective in lessen injury caused by IRI [9-10]. RIRI is a pathological event that can lead to acute renal failure(ARF), and found that 45% of the cases of ARF in the intensive care unit due to IRIin one study [11]. It is also a common cause of graft failure after kidney transplant and increase both morbidity and early loss of transplanted kidney [12-14]. Acute renal failure (ARF) represent acute decline in renal function that lead to elevation of nitrogenous wastes like serum level of blood urea, nitrogen & serum creatinine which is a main clinical issue with elevated incidence rate, dangerous consequences, undesirable therapeutic options, and increase hospital stay and cost [15].Among these subtypes intrinsic ARF the most common and severe one in admitted patient with a MR of( 40-80%) in the ICU and associated with acute tubular necrosis (ATN) [16]. A hallmark of intrinsic ARF is a severe and persistent constriction of renal blood vessels that decrease renal blood flow to 50% of normal [17]. There are many mechanisms for ischemic ARF, the most important one is the renal tubular epithelial injury which plays a key role and there is recent studies show additional mechanisms including renal vascular endothelial damage and dysfunction, have a main role in expanding renal tubular epithelial damage and subsequently participate to the ongoing pathogenesis of ischemic ARF [18]. In the ischemic stage of acute kidney injury, hypoxia contributes to kidney injury, while the inflammation occurs in the reperfusion phase. During reperfusion phase, the innate immune response is activated through the ischemic tissue and exacerbates damage. It is well established that an intense inflammatory response happens following ischemic-reperfusion injury [19]. Inflammation has a significant role in the mechanism of acute kidney injury that has been result from ischemia. The inflammatory reaction that occurs in renal ischemia reperfusion injury cause activation of the endothelium, leukocyte entrapment, and impairment of micro vascular blood flow [20]. Cytokines are low molecular weight (LMW) around 25 kDa plasma proteins inflammatory mediators or glycoprotein's that has been liberated from different cell types, mostly from leukocytes in response to different activating stimuli, and have a role in the development and functions of the immune system. Ischemia up regulate the synthesis of inflammatory chemoattractant cytokines like IL-1, IL-6, and TNF-α in the ischemic AKI [21].IL-1β considered as one of the chemoattractant protein that recruit leukocytes into the zone of the inflammation. There is significant evidence that the polymorph nuclear leukocytes have an important role in the IRI in brain and heart[22], but the importance of PMN in renal ischemic reperfusion injury still in doubt [23].In IRI, hypoxia itself causes interaction between cytokines and the transcriptional reaction directly lead to production of cytokine, so production of IL-1 activates renal tubular epithelial cells to produce proinflammatory mediators for example TNF-α and IL-6 [24]. In general, cytokines have an effect on both local and remote organs in AKI[25].MCP-1 is known as powerful chemoattractant protein mainly for monocyte recruitment. One of the significant features of IRI is the infiltration of monocyte/ macrophage into the kidney tissue. The infiltration of monocyte/macrophages are triggered through the action of chemotactic factors that have been generated by renal cells[26]. It found that the infiltrated macrophages can increase the inflammatory reaction due to the production of vasoconstrictors, cytokines & toxic substances like free radicals. Elevated numbers of monocyte & macrophages had been observed in the transplanted kidneys of cases with" acute tubular necrosis" (ATN) or graft rejection [27] also in the rat kidneys that had been subjected to renal ischemic reperfusion insult[28].P2X receptors (P2XRs) are membrane ligand-gated ion channels that have been gated by the binding of appropriate ligand such as ATP binding. The P2XR family is consist of seven members (P2X1R–P2X7R), these receptors trimerize in chromomeric or heterogenic texture to form ion channels [29]. The binding affinity of ATP to P2X7 receptor (P2X7R) is low which has been required (10-100) times increasing concentrations of ATP for stimulation in compare with other subtypes of P2X receptors [30].In the previous study, it had been found that the level of P2X7R was higher in the kidney after I/R damage and administration of a selective inhibitor of P2X7 receptor early after the onset of reperfusion phase lead to improvement of kidney function", decrease renal tubular epithelial damage, and prevented cell death and also inhibited the production of chemokine in the harmed kidney. So P2X7R activation play a significant function in quickening AKI by inducing renal tubular epithelial cell death and inflammatory reaction and the suppression of it provide protection against ischemic AKI in mice model[31].*Octreotide* is a synthetic somatostatin analogue that consist of 8-amino acid peptide that have greater pharmacological activity than the naturally occurring polypeptide molecule (somatostatin) and have long duration of action (1-2hours) in contrast with somatostatin that has very short half-life (2-3 minutes) which limits its clinical use [32]. It inhibits growth hormone secretion [33]. Somatostatin and Oct also inhibit

gastro pancreatic secretion like glucagon, insulin, gastric, pancreatic polypeptide, gastric inhibitory polypeptide and decrease the secretion of exocrine pancreatic content like amylase, lipase, and trypsin. It also has been affected on the activities of leukocyte infiltration, chemo taxis, leukocyte adhesion and prohibit the release of ROS from leukocytes [34].Depot preparations of octreotide are available which is long-acting allow for one dosage every 4 weeks. It can be used in the treatment of excess growth hormone secretion (acromegaly) and in the flushing and frequent loose stool associated with characinoid syndrome. The intravenous infusion of octreotide can be used for the management of bleeding associated with esophageal varices [35]. Somatostatin in which the octreotide is the synthetic analogue of it found mainly in the gastrointestinal neuroendocrine cells and the sensory nerve endings [36]. Octreotide has the same pharmacological activities of somatostatin in addition, it can regulate the inflammatory and oxidative pathways in numerous biological processes and it has been used as a therapeutic agents in many clinical cases such as :bowel obstruction, acute pancreatitis, neuroendocrine tumors, gastrointestinal diseases and intestinal ischemia reperfusion injury [37-38]. Another study of Octreotide in retinal I/R damage in guinea pigs model has been showed that Octreotide and somatostatin both provide protection against hypoxic ischemic cell injury due to their endocrine and cytoprotective effects and decrease leukocyte oxygen free radicals-induced lipid peroxidation in I/R injury of the retina [39]. There is also a recent study that was done by Wang *et al*. [34] and was observed that octreotide may also give protection against retinal ischemia reperfusion injury. In previous study, it was found that octreotide can be used clinically in treating intestinal ischemia reperfusion injury due to the early activation of Heme oxygenase-1 (HO-1) [40].In the recent times studies showed that octreotide have an organ protective effect via many mechanisms including anti-inflammatory effect by down-regulation of inflammatory mediators, direct cell protection and decrease end toxin levels [33, 41]. For further evaluation of octreotide, study was done on ischemia reperfusion injury in a rabbit liver model to show the effect of octreotide and the mechanism of it and showed that Oct reduced the hemodynamic changes and hepatic cell injury through inhibition of hepatocytes apoptosis and decrease the level of end toxin and serum proinflammatory mediators like TNF-alpha and IL-1beta. Based on the result data that obtained from the study they suggest that octreotide can be used in decreasing hepatic injury after liver surgery and/or liver transplantation and may serve as an effective therapeutic agent for pharmacological pretreatment against I/R injury [33]. A several studies were performed to examine the effect of octreotide in acute inflammation of the pancreases in experimental and clinical study however the data that obtained from the these studies are debatable, in part due to variation in the experimental model that used in the studies or the stages of human acute pancreatitis studied. Ko *et al*. [42]observed that Oct had not been ameliorated the impacts of acute severe bile induced pancreatitis in dogs as estimated by histological examination. Augelli *et al*. [43]found that only when give Oct before induction of pancreatitis had a beneficial effect in pancreatitis in canine model Toriumi *et al*. [44] and Zhu *et al*. [45]demonstrated that Oct have a prophylactic effects in two different studies on pancreatitis model in rats. Another research [46] had been reported that somatostatin can be used as prophylactic therapy to reduce the occurrence and severity of acute pancreatitis after pancreatic duct sphincter hydrostatic balloon dilation. The application of Oct in pancreatic transplantation have good results by decreasing morbidity and excellent graft success [47]. On the light of the previous studies, they were designed to examine the effect of Oct in acute pancreatitis that result from complete IRI of the pancreas in rat model and found that Oct positively affected both the micro vascular perfusion failure and enhanced leukocyte-endothelial interaction[32]. In rats with IRI of the pancreases, study was done to examine the effect of Oct when given before the induction of ischemia and observe that Oct was protective by improvement of microcirculatory impairment and decrease leukocyte adherence to venues this give a hope that Oct could be protective in clinical setting of pancreatic transplantation by prevention of post ischemic pancreatitis[48]. Liraglutide (LG) is a glucagon-like peptide 1 receptor analogue (GLP-1), that has been used recently for the management of non- insulin dependent type 2 diabetes mellitus. Glucagon like peptide 1 (GLP-1) is an incretion hormone that has been released postprandial from the gut into the blood stream and binds to the GLP-1 receptors [49]. Liraglutide binds to GLP-1 receptor, which augment endogenous insulin secretion from beta cell of the pancreases in a glucose-dependent manner, it decreases glucagon secretion in the presence of high glucose concentration and increase β cell mass by causing β cell proliferation and differentiation[50]. GLP-1 also cause delay in gastric emptying rate [51]. Previous studies has been reported that Liraglutide and Exendin-4, two GLP-1analogues, have numerous cellular protective effects, including the preservation of endothelial cells against senescence mainly through antioxidant effect [52-53]and anti-inflammatory pathways[54]. In different

experimental models, Liraglutide has been found to have Neuroprotective effect. Another study was done to look for the effect of Liraglutide in ischemic stroke by causing middle cerebral artery (MCA) occlusion in rat model and possible protective mechanisms. They found that Liraglutide has been significantly decreased the volume of infracted area, improved neurological deficits, & decreased stress hyperglycemia without causing hypoglycemia. Liraglutide prevented programmed cell death apoptosis via decreasing expression of excessive reactive oxygen species (ROS) and make the mitochondrial function better in neurons under O2 glucose deprivation (OGD) in vitro and MCA occlusion in vivo. These results of the study proposed that Liraglutide has neuroprotective effect against ischaemia-prompted apoptosis. So Liraglutide can be used as a therapy in patients with stroke, particularly in those with type 2 diabetes mellitus or stress-induced hyperglycemia [55].Studies have been observed that Liraglutide has beneficial effect in ischemic stroke and Alzheimer's disease (AD), Liraglutide decreases amyloidal plaque deposition and improves cognitive ability in AD in mouse model, and it is now in the clinical trials to be tested in AD patients [56]. Several studies have been showed the GLP-1 analogs have neuroprotective effects on animal models of neurological diseases, including stroke, Parkinson disease, Alzheimer disease(AD), and amyotrophic lateral sclerosis(ALS)s [57-58].

### *Preparation of Animal*

Pathogen-free thirty adult male Swiss Albino Mice 3-5 months ago with an average weight between 35 and 45 gram were purchased from Animal Resource Center in Baghdad. The study was performed at Department of Pharmacology & Therapeutics in Al-Kufa Medical Collage from November 2017 to March 2018. Mice were placed in the animal =house of Babylon University/ College of Science/ in a controlled conditions with temperature (24 ± 2°C) with changing 12-hours light/12-hours dark cycles. They were get free access to water & standard food till the beginning of experimental procedures. And all experimental researches were done in accordance with the instructions for Care & Utilization of Lab Animals and had been confirmed by Animal Care Committee.

### *Study Design*

Following two weeks of adaptation, mice were divided randomly into five groups as showing below:

1. Sham group (n=6 mice): mice were subjected to the same anesthetic and surgical processes as that in ischemia group except the induction of ischemia.
2. Ischemia reperfusion group (control group): mice were underwent 30 min bilateral renal ischemia by clamping of right and left renal arteries and 2 hours reperfusion.
3. Vehicle group: IRI + normal saline (for both drugs) through i.p injection, 30minutes before ischemia and underwent bilateral renal ischemia for 30 minutes followed by 2 hours reperfusion [59-60].
4. Octreotide treated group: treated with a dose of octreotide (30$\mu$g/kg) [59] by intraperitoneal injection, 30 minutes before ischemia and underwent 30 minutes bilateral renal ischemia followed by 2 hours reperfusion.
5. Liraglutide treated group: treated with a dose of Liraglutide (1mg/kg)[61] by intraperitoneal injection, 30 minutes before ischemia and underwent 30 minutes bilateral renal ischemia followed by 2 hours reperfusion.

### *Experimental Model of Renal Ischemia / Reperfusion Injury*

Animals were anesthetized with intraperitoneal injection of ketamine in a dose of 100mg/kg and Xylazine in a dose of 10mg/kg. After anesthesia of (5-10 minutes) mice were putted on its back, their limbs and tails were fixed with stickers to make sure that they remained stable during all the time of the experimental procedure. Hair in chest was shaved and the skin was sterilized with disinfectant. The reflexes were checked frequently during the period of anesthesia by squeezing the tail and the hind to ensure that mice are adequately anesthetized. After that, little midline abdominal incision was done to show the abdominal structures; also the intestine was retracted in order to expose the kidney & renal pedicles. The renal pedicles were occluded bilaterally with a no traumatic microvascular clamps for 30 minutes, during that time the abdomen was closed with sutures. The time of ischemia was selected to maximize clonally of renal insufficiency while reducing animal mortality in these animals. After clamps were released, the kidneys were inspected for an additional 5 min to look for color changes which indicate reperfusion, after that the incision has been closed in 2 layers with 2-0 surgical sutures. After that, mice were returned back & allowed to recover in their cages to get better with food and water, and left for 2 hours for reperfusion [62]. After reperfusion , mice were killed using high dose of anesthesia, blood samples has been drawn from carotid artery for measurement of inflammatory markers in serum [33], the left kidney (as a standard for all groups) has been removed and divided into two parts, the upper half was prepared for analysis of inflammatory parameters in tissue and the lower half was prepared for histological evaluation [63].

## Samples preparation

### *Blood Sampling*

At end of the experimental procedure, 1-1.5ml of right carotid artery blood samples has been drawn **[33]**. The sample of the blood was put in plane tube at 37c without any anticoagulant and left for 30 min, then centrifuged at 3000rpm for 10 min and the serum obtained is used for measurement of inflammatory markers (MCP-1, P2X7, IL-1β,) and renal function test (urea and creatinine) levels by enzyme linked immunosorbent assay (ELISA).

## Tissue Sampling

### *Tissue Sampling for Measurements of TNF-α, P2X7, IL-1β*

In preparation of tissue, the kidney was washed with normal saline to remove any blood or clots and stored in deep freeze (-80°C). Then the homogenization of kidney tissues was done with high intensity ultrasonic liquid processor in 1:10 (w/v) 0.1 M potassium phosphate buffer saline(pH7.4) which contain protease inhibitor cocktail & 0.2% Triton X-100 [64]. The homogenate was placed in the centrifuge and centrifuged at 3000rpm for 10 minutes at 4°C. The supernatant was used for the measurement of (MCP-1, P2X7, and IL-1β) and renal function test urea and creatinine by ELIZA with a commercially available ELIZA kits.

### *Sampling of kidney Tissue for Histopathological Scoring*

The lower half of the kidney sample was fixed in 10% buffered formalin & processed by routine histological procedures. Paraffin-embedded tissue pieces were prepared and the tissue slide sections were cut about 5µm-thick level areas were cut and re-colored with hematoxylin-eosin (H&E) stain, then sent to histopathologies for subsequent histological examination. The scoring system was performed in a blinded fashion. The tissue slides were examined by light microscope and graded for renal tubular epithelial injury by using quantitative measurements. The morphological changes of renal tubular injury has been identified as tubular epithelial swelling, loss of brush border, vascular degeneration, necrotic tubules, cast development, & desquamation [65]. The degree of renal tubular epithelial damage in RIRI was described as the following: 0, represents no damage; 1, represents less than 25% of the damage of the renal tubules; 2, represents 25%-50% of the damage of the renal tubules; 3, represents 51-75% of damage of the renal tubules; 4, represents 76-100% of damage of the renal tubules [66-67].

### *Statistical analysis*

Data in this study were analyzed using (SPSS version 22) and expressed as mean and standard deviation (SD). Analysis of Variance one way (ANOVA) test followed by Post-Hoc test LSD method were used for the multiple comparisons between more than two groups. The obtained results were considered significant if (P- Value < 0.05). The Mann-Whitney U test was used to assess the statistical significance of the difference between 2 groups in total renal histology score.

### *Comparisons of Variables between Groups that included in Our Experimental Study*

Control group has mean level of MCP-1 in serum more than that in sham group (984±64 against 98±48, P value<0.001), while the mean level of serum MCP-1 in Octreotide group is remarkably lower than the control group (532±61 versus 984±64, P value<0.001) also the mean level of serum MCP-1 in Liraglutide – treated group is remarkably lower in compare with that in ischemia/reperfusion group (502±52 vs.984±64, P<0.001). No significant variation was found between vehicle & control group (912±54 ng/ml vs.984±64 ng/ml, P=0.2) as shown in figure (1).

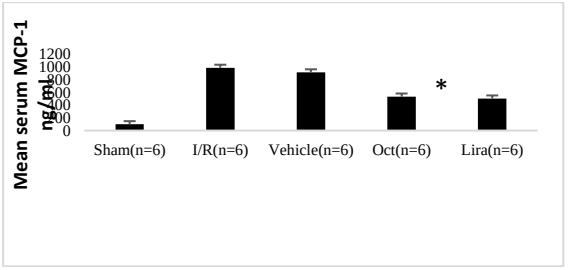



**Fig. 1: Serum MCP-1 in studied groups (value is presented as mean ± SD)**

(* represent significant versus sham; # represent significant versus control; ¥ represent significant versus Vehicle). The level of mean Kidney tissue MCP-1 in I/R group was remarkably higher than that in sham group (623±54 vs. 45±16, P<0.001), while octreotide-treated group has mean level of MCP-1 in kidney tissue less than that in I/R group (346±68 vs. 623±54, P<0.001) also the level of mean Kidney tissue MCP-1 in Liraglutide group was significantly lower than the control group (332±65 against 623±54, P<0.001). Insignificant variation was found between vehicle and control group (603±67 vs.623±54, P=0.4) as shown in figure (2).

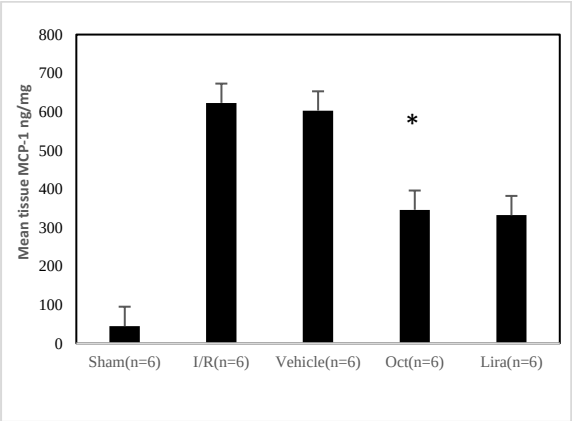



Fig. 2: Kidney tissue MCP-1 in studied groups (value is presented as mean ± SD)

(*represent significant versus sham; # represent significant versus control; ¥ represent significant versus vehicle)

The mean level of serum IL-1β in I/R group was remarkably higher than the sham group (412±47 vs. 51±10, P value<0.001), while the mean level of serum IL-1β in Octreotide-treated group is remarkably low in compare with that in I/R group(199±31 against 412±47, P value<0.001) also the mean level of serum IL-1β in Liraglutide group was remarkably lower than the I/R group(184±26 vs. 412±47, P value<0.001). Insignificant variation was found between vehicle and control group (432±51ng/ml against 412±47ng/ml, P=0.2) as shown in figure (3).

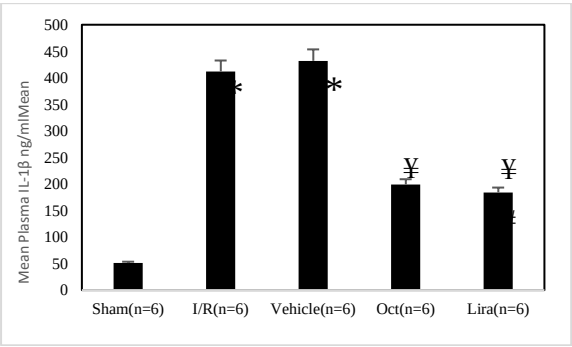



**Fig. 3: Plasma IL-1β in studied groups (value is presented as mean ± SD)**

(*represent significant versus sham; # represent significant versus control; ¥ represent significant versus vehicle). The mean level of Kidney tissue IL-1β in I/R group was remarkably higher than the sham group (321±97 vs. 45±13, P value<0.001). The mean level of Kidney tissue IL-1β in Octreotide group was remarkably lower than I/R group (133±86 ng/ml vs. 321±97ng/ml, P<0.001) also the mean level of Kidney tissue IL-1β in Liraglutide group was remarkably lower than I/R group (141±89 vs. 321±97, P<0.001). Insignificant variation was found between vehicle and control group (301±91ng/ml versus321±97ng/ml, P=0.3) as shown in figure (4).

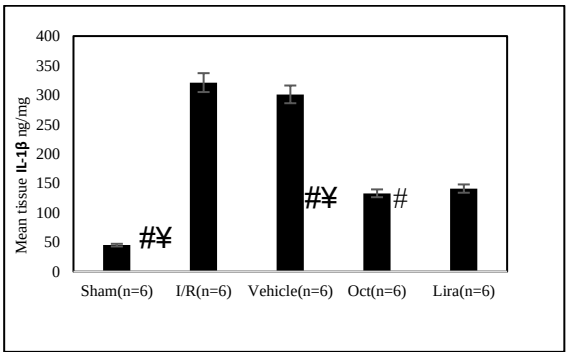
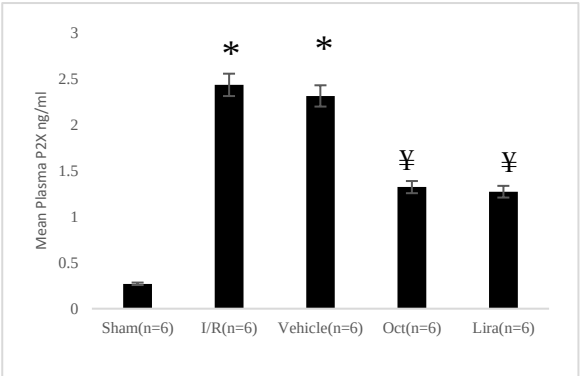



**Fig. 4: Kidney tissue IL-1β in studied groups (value is presented as mean ± SD)**

(*represent significant versus sham; # represent significant versus control; ¥ represent significant versus vehicle) The mean level of serum P2X7 in I/R group was significantly higher than sham group (2.43±0.09 versus 0.27±0.01, P value<0.001) .The mean level of serum P2X7 in Octreotide group was remarkably lower than I/R group (1.32±0.02 ng/ml vs. 2.43±0.09ng/ml, P<0.001) also the mean level of serum P2X in Liraglutide group was remarkably lower than the I/R group (1.27±0.02ng/ml vs. 2.43±0.09ng/ml, P<0.001). Insignificant variation was found between vehicle and control group (2.31±0.08 ng/ml vs.2.43±0.09ng/ml, P=0.1) as shown in figure (5).

**Fig. 5: Plasma P2X in studied groups (value is**




**expressed as mean ± SD)**

(*represent significant versus sham; # represent significant versus control; ¥ represent significant versus vehicle). The mean level of BUN in control group is remarkably higher than sham group, (22±1.5 against 9.5±0.9, P value<0.001), while the mean level of blood urea in Octreotide group was remarkably lower than that of I/R group (14.3±1 vs. 22±1.5, P value<0.05) and also the mean level of blood urea in Liraglutide group was remarkably lower than that of I/R group (13.7±0.9 vs. 22±1.5, P value<0.05). Insignificant variation was found between vehicle and control group (20.5±1.2 vs. 22±1.5, P value=0.2). as shown in figure (6). The mean level of serum creatinine in control group was significantly high in compare with sham (2.9±0.2 versus 0.85±0.1, P value<0.001) while the mean level of serum creatinine in OCT group was

remarkably low in compare with that of I/R group(1.2±0.09 versus 2.9±0.2, P value<0.05) also the mean level of serum creatinine in LIRA group was remarkably low in compare with that of I/R group(1.32±0.09 versus 2.9±0.2, P value<0.05). Insignificant variation was found between vehicle & control group (2.78±0.1 mg/dl 2.9±0.2 mg/dl versus, P=0.3) as shown in figure (6).

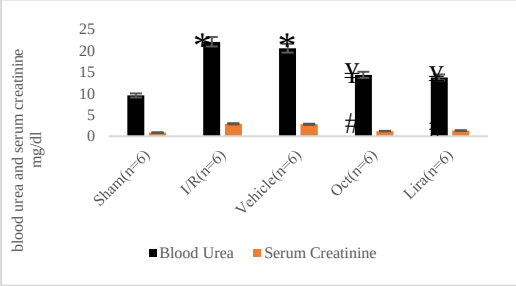



**Fig. 6: Blood urea and serum creatinine in studied groups (value is presented as mean ± SD)**

(*represent significant versus sham; # represent significant versus control; ¥ represent significant versus vehicle)

### *Comparison of Histological Scores among Experimental groups*

Mean histological score was remarkably high in control group in compare with sham (3±0.0 vs. 0±0, P=0.001); meanwhile mean score of Liraglutide group was (1.66±0.53) which is significantly lower than control group (P value=0.002) also mean score of Octreotide-treated group was(1.16±0.53) which is remarkably low in compare with that of control group (P=0.002). Mean histological score of vehicle group was (3±0.0), with no remarkable variation from that in control group (P=0.5) however it was remarkably high in compare with that of Liraglutide group (P=0.002) and also it is remarkably higher in compare with Octreotide-treated group (P value=0.002) as shown in figure (3-7).

| Score | Sha | Cont | Vehi | Lira | Octr |
|---|---|---|---|---|---|
| Score 0 | 6(10 | 0 | 0 | 0 | 0 |
| Score 1 | 0 | 0 | 0 | 2(33 | 5(83. |
| Score 2 | 0 | 0 | 0 | 4(66 | 1(16. |
| Score 3 | 0 | 6(10 | 6(10 | 0 | 0 |
| Total | 6(10 | 6(10 | 6(10 | 6(10 | 6(10 |
| Mean ±SD | 0±0 | 3±0 | 3±0 | 1.66 ±0. 53 | 1.16 ±0.5 3 |

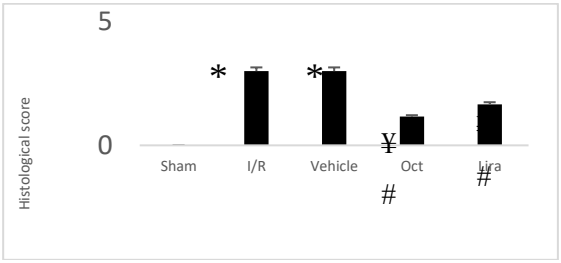



**Fig. 3-7: Mean histological score in studied groups**

(*represent significant versus sham; # represent significant versus control; ¥ represent significant versus vehicle)

### Effects of Octreotide and Liraglutide on Renal Histology after IRI of mice kidney

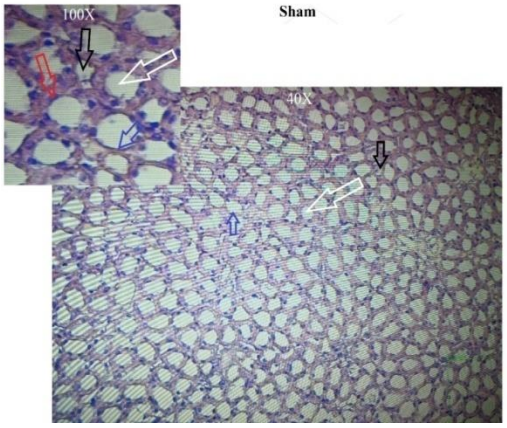



**Fig. 3-8: Representative H and E staining at 40X and 100X magnification of adult male mice kidney specimen**

Mice in sham group without induction of ischemia, showing normal renal tubules (white raw and black raw), intact glomeruli (red raw) and normal Baumann capsule (blue raws)

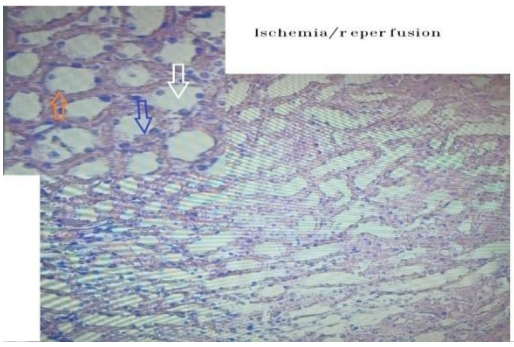



**Fig. 3-9: Representative H and E staining at 40X and 100X magnification of adult male mice kidney**

Mice induced of kidney injury after I/R as control group, showing scattered apoptotic cells with strongly eosinophilic cytoplasm & pyknotic nuclei (red raw, blue raws). Whole field exhibits mostly necrotic renal tubules (white raw and black raw).

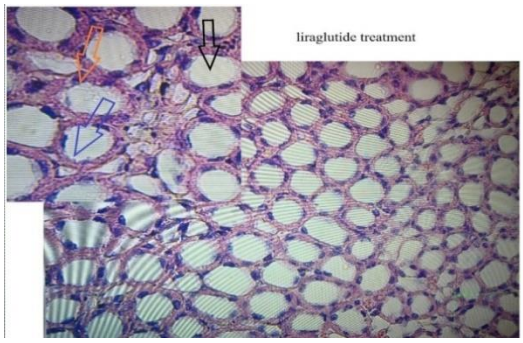



**Fig. 3-10: Representative H and E staining at 40X and 100X magnification of adult male mice kidney**

Mice in vehicle group with induction of kidney injury and pretreated with normal saline, showing scattered individual apoptotic cells with highly eosinophilic cytoplasm &pyknotic nuclei (red raw, blue raws).

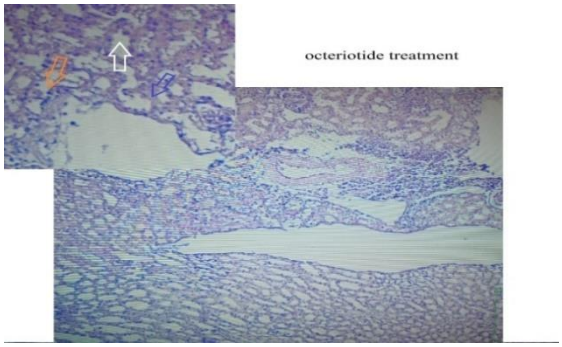
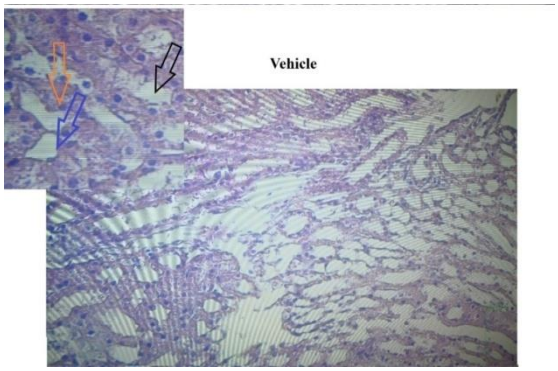



**Fig. 3-11: Representative H and E staining at 40X and 100X magnification of adult male mice kidney**

Mice in Liraglutide- treated group with induction of kidney injury and pretreated with Liraglutide, showing less scattered individual apoptotic/necrotic cells with weak eosinophilic cytoplasm &no pyknotic nuclei (red raw, blue raws). Whole field exhibits mostly non-significant necrotic kidney tubules (white raw and black raw).

**Fig. 3-12: Representative H and E staining at 40X and 100X magnification of adult male mice**




**kidney**

Mice in octreotide-treated group with induction of kidney injury and pretreated with octreotide, showing less scattered individual apoptotic/necrotic cells with weak eosinophilic cytoplasm & without pyknotic nuclei (red raw, blue raws), whole field exhibits moderate necrotic kidney tubules (white raw and black raw).

## DISCUSSION

RIRI is a common cause of organ dysfunction that is often leading to acute kidney failure, resulting in significant mortality between patients who admitted to the intensive care unit and require dialysis[68]. The degree of severity of the injury be dependent on the duration of ischemia and causes additional damage. Oxidative stress is also implicated in kidney damage prompted by I/R injury[69]. Ischemic acute renal failure (iARF) is frequently noticed clinically always during kidney surgery & renal transplantation and could cause ARF and is correlated with high morbidity, mortality, and prolonged hospitalization [70]. Acute kidney injury(AKI) is a complex disorder that consists of multiple causative factors and happens in a variety of settings with varied clinical features that range from a minimal but continuous elevation in serum creatinine to anuric renal failure [71]. So there is a need for urgent medical intervention to protect against kidney IRI. In this study, we found that the IL-1β serum and tissue levels were remarkably elevated in active control group more than that in sham group. These finding is in agreement with the results of the study by [72] have revealed that one of the mechanisms that has been involved in I/R is an intense inflammatory response and increased the expression of proinflammatory cytokines (IL-1β) and other biomarkers in renal parenchyma in the control group (I/R group) as compare with sham group, early at 24hrs and also late at 72hrs after reperfusion. The initiation and continuation of the inflammation play a major role in the tissue/organ damage that occur after ischemia /reperfusion injury [73]. In ischemic reperfusion injury of the liver, it was found that ROS and the proinflammatory cytokines for example TNF-α & IL-1β stimulate hepatic sinusoidal endothelial cells, increase the production of the intercellular adhesion molecules which promote the adhesion and recruitment of Neutrophils and endothelial cells to the site of inflammation, causing subsequent hepatic cell damage [74-75].In myocardial ischemia-reperfusion injury, it was also observed that the inflammatory cytokines like (IL-1β, TNF-α & IL-6) were remarkably increased in control and vehicle group as compare to the sham group and the administration of methionine cause significant reduction of it [76]. The up regulation of inflammatory cytokines that occur in myocardial ischemia/ reperfusion event related to the depletion of glutathione (GSH) [77]. Since glutathione have cellular protective effect, and antioxidant effect by preventing peroxidation of lipid and decreasing cell death[76].The MCP-1

serum and tissue levels were remarkably increased in control group more than that in sham group in the present study. This result are in consistent with the results of other research on renal ischemia/ reperfusion insult in which the MCP-1 is elevated in I/R group and the administration of erythropoietin which has anti-inflammatory effect cause significant reduction in the level of MCP-1 [78]. It was found that MCP-1level is increased in control group in comparison with that in sham group in cerebral ischemia-reperfusion injury [79] and the up regulation of chemokine following ischemic insult have adverse effect by driving leukocyte infiltration into the site of injury [80]. We also found that P2X7 levels of in serum are significantly elevated in I/R group more than that in sham group. Previous literature results have been shown that the level of P2X7R was elevated after I/R injury and the administration of a selective P2X7 receptor antagonist early after the onset of reperfusion phase lead to improvement of kidney function, decrease renal tubular epithelial damage, and prevented cell death and also inhibited the production of chemokine such as MCP-1, RANTES in the damaged kidney. So P2X7R activation play a significant role in accelerating AKI by inducing renal tubular epithelial cell death and inflammatory reaction and the prevention of its activation provide protection against ischemic AKI in mice model [31]. Study was done on cerebral I/R injury and had been demonstrated that inhibiting of P2X7Rs by using P2X7R antagonists protects against injury through modulation of inflammation that occur in the rat hippocampus by causing lowering of microglial and astroglial activation and the production of inflammatory mediators which result in reduction in the mortality rate and neuronal cell death [81]. In the present study, blood urea nitrogen and serum creatinine levels were remarkably elevated in control group than that was found in sham group. Xu *et al.* [59] had shown that blood urea and serum creatinine was remarkably increase on control group than in sham group. Evidence proved that the production of ROS and induction of lipid peroxidation lead to increase in renal function parameters[82]. This study showed that administration of single dose of octreotide 30$\mu$g/kg, 30min before the induction of ischemia cause significant lowering of serum and tissue IL-1β in compare with I/R group. These results are consistent with another study [33] which had been demonstrated that octreotide has an organ protective effect through anti-inflammatory effect and suppression of end toxin or direct protection of the cell, cellular evidence was identified and show that the use of Oct in hepatic ischemia reperfusion injury led to prevention of apoptosis of hepatocytes and maintain their normal morphology as well as reduced the levels of end toxin (ETX) and proinflammatory cytokines mediators like TNF-alpha and IL-1β. Since inflammation and the production of proinflammatory mediators are involved in I/R injury[83]. Previous literature reports had been demonstrated that Somatostatin and its synthetic analogue octreotide have immunomodulatory effect by preventing the production of spontaneous TNF-α and nitric oxide by isolated rat KC and TNF-α and IL-1β by human monocyte [84, 85]. Also the administration of a single dose of octreotide 30$\mu$g/kg, 30min before the induction of ischemia cause significant lowering of plasma and kidney tissue MCP-1 in compare with I/R group. MCP-1 one of the chemokine family that on activation result in recruitment of the monocyte/macrophage into the site of tissue injury, subgroups of T-cell and other cells [86], one of the previous study reported that somatostatin and its analogues have immunomodulatory effect regarding cell infiltration [87], so they found that octreotide has a suppressive effect on MCP-1 in LPS-induced chemokine production in Kuepfer cells of rat since MCP-1 has been involved in hepatic inflammation and fibrosis [88].Another research also revealed that octreotide provide protection against kidney and liver damage after hepatic ischemia reperfusion injury (HIRI) and decrease the levels of MCP-1, IL-6, TNF-alpha in kidney after HIR through modulation of anti-inflammatory and ant apoptotic effects and induction of Autophagy [89]. Other study [31]has been showed that the level of P2X7 receptor was increased in the kidney after RIRI and the administration of a selective P2X7R antagonist, early after the beginning of reperfusion lead to improvement of renal function, decrease renal tubular epithelial damage, and inhibit renal cell death. The early administration of P2X7R antagonist also suppressed the production of chemokine in the damaged kidney. These results suggest that the activation of P2X7R plays a vital role in accelerating AKI by causing renal tubular epithelial cell death and inflammatory reaction and the inhibition of P2X7R provides protection against ischemic acute kidney injury in mice model.ATP is one of the appropriate ligand for binding to P2X7R [90]) and the adenosine triphosphate (ATP) levels that produced endogenously as a result of inflammation that occur in tissue injury are sufficient to bind to P2X7R[91]. Regarding the effect of octreotide on P2X7 level, previous study [92] has been suggested that octreotide drug has inhibitory effect on purinergic P2X and P2Y receptors activation because somatostatin and its analogue has modulator effect on the immune system. In this study, we found that serum level of BUN and serum creatinine significantly reduced in octreotide group than in I/R group. This result are

consistent with other study [59] that has been reported that the administration of octreotide drug after RIRI provide protection against kidney injury and improve renal functions through anti-inflammatory and anti-apoptotic effects. So we concluded that OCT has organ protective effect through anti-inflammatory and anti-apoptotic effect. In this study, we found that the mean of whole scores of the histology sections of the kidney is remarkably decrease in octreotide-treated group in compare with I/R group which indicate significant degree of renal protection from IRI. These result are consistent with a study [59] that established the role of octreotide in protection against kidney injury via anti-inflammatory and ant apoptotic effects. In this study, we found that preconditioning with a single dose of Liraglutide (1mg/kg), 30min before the induction of ischemia cause significant lowering of serum and tissue IL-1β in compare with I/R group. Liraglutide has anti-inflammatory effect on the endothelial cells of blood vessels through increase nitric oxide( NO) production and suppress stimulation of transcriptional-factor NF-κB, to some extent via AMP-associated protein kinase( AMPK) activation [54] and suppression of NF-κB activation leading to decrease in the expression of pro-inflammatory cytokines like: TNF-alpha,IL-1β [93]. Besides, other study show the neuroprotective role of Liraglutide in Parkinson disease by decreasing the level of nigral inflammatory mediators such as: Il-1 β, Il-6, transforming growth factor (TGF-b1) as well as proapoptotic protein BAX were decreased. Also there is significant lowering of serum and tissue MCP-1 level in Liraglutide-treated group as compare to I/R group. It was found that Liraglutide suppresses the production of inflammatory mediators and inhibits the adhesion of monocyte and decrease the atherosclerosis in vivo [94, 95]. Lee *et al.* [96]found that GLP-1 remarkably minimized the generation of inflammatory cytokines like: IL-6 & TNF-alpha and the chemokine MCP-1 in diabetic obese mouse, in addition to lipogenic genes and M1macrophage-specific genes, and directly suppressed the inflammatory pathways NF-kB and JNK. The plasma level of P2X7 was significantly decreased in Liraglutide-treated group as compare to I/R group. No prior study found to examine the influence of Liraglutide on P2X7 level. However, previous study [81] had been reported that the inhibition of P2X7R activation provides protection in transient cerebral ischemia in I/R injury of the hippocampus in rat model by modulation of the inflammatory response via reduction in the formation of inflammatory cytokines & reduction in microglial & astroglial activation. Another study revealed that one of the immunological mechanism that has been involved in hepatic ischemia reperfusion injury (HIRI) through the purinergic system [97] that have immunomodulatory effect on the immune system by ATP & its catabolites [98]. The purinergic signal act via one of the five purinergic receptors on the N Kcell surface to increase interferon gamma production and enhance the inflammatory response [97]. In study that was done on mice with acute lung injury, it had been found that Liraglutide attenuate tissue lung injury due to anti-inflammatory and immunomodulating properties [99]. In this study, we found that level of Blood urea and serum creatinine significantly reduced in liraglutide-treated group than in I/R group. Previous study has been established the role of Liraglutide as anti-inflammatory and anti-apoptotic effect that have organ protective effect and reverse basal ganglia degeneration in Parkinson disease [100]. So Liraglutide has renal protective effect in RIRI and lead to improvement of renal function. Also the mean of whole scores of the histology sections of the kidney is significantly decrease in Liraglutide-treated group as compared to I/R group which indicate that there is a considerable degree of renal protection from IRI. Several previous studies had been demonstrated that Liraglutide has anti-inflammatory effect and immunomodulating effect [101, 102]. Research was done to examine the effect of Liraglutide in lip polysaccharide induced lung injury in mice model and was found that Liraglutide has protective effect by decreasing lung injury which is demonstrated by histological examination via its anti-inflammatory effect by preventing the inflame some pathway that is called nucleotide-binding oligomerization domain-like receptor protein3 (NLRP3) thereby reducing expression of inflammatory mediators( IL-1β and IL-18) and inhibiting the inflammatory reaction [99].Zhu *et al.* [55]had been found that Liraglutide has neuroprotective effect in ischemic stroke because it inhibits cellular apoptosis Furthermore, Liraglutide has antioxidant effect by inhibiting intracellular ROS and improve mitochondrial function therefore, Liraglutide decreased the infarction size and improved the function of motor and somatosensory neurons. So we concluded from the above mentioned previous studies that support our result that Liraglutide have an organ protective effect.

## CONCLUSIONS

1-This study is furthermore supporting the role of pro- inflammatory cytokine (IL-1β), the chemokine (MCP-1) and P2X7 receptor in the involvement of path physiological mechanisms in the renal I/R injury.

2-Both octreotide and Liraglutide cause significant lowering of plasma and tissue levels of IL-1β, MCP-1, P2X7in compare with I/R group.

3- Both octreotide and Liraglutide have anti-inflammatory effect which is proved in the present study through their effects on the inflammatory markers (IL-1β, MCP-1, P2X7).
4-Both octreotide and Liraglutide have protective effect in renal ischemia reperfusion injury by causing significant reduction in renal indices (BUN &serum creatinine) and also cause significant lowering of kidney histological scores.

## RECOMMENDATIONS

1-We need to examine their effect at the time of reperfusion.
2- Give combination of octreotide and Liraglutide to see the effect of combination rather than each one alone.
3-Since both of octreotide and Liraglutide have anti-inflammatory and anti-oxidant properties, we advise to measure the oxidative parameters.
4-increase the duration of ischemia and examine the effect of these drugs in more degree of tissue damage.
5-act more specifically to know the signaling pathway by which these drugs act.

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