International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 6 Nov-Dec 2021, pp: 233-243 www.ijprajournal.com ISSN: 2249-7781

Piperine Aggravates Ischemia Reperfusion Induced Acute Kidney Injury through Peroxisome Proliferator Activated Receptor Gamma Antagonism in Rats

Gurdeep Kaur, Arashdeep Singh Cheema, Deendyal, Abhishek, Sachleen Kaur Kang,
Rajbir Bhatti, Amrit Pal Singh*

Department of Pharmaceutical Sciences,Guru Nanak Dev University, Amritsar (INDIA)
Assistant Professor,Department of Pharmaceutical Sciences,Guru Nanak Dev University,Amritsar-143005 (INDIA)

Date of Submission: 05-11-2021 Date of Acceptance: 20-11-2021

ABSTRACT
Present study investigated the role of piperine against ischemia reperfusion-induced acute kidney injury (AKI) in rats. AKI was induced in rats by clamping renal pedicles for 40 minutes followed by reperfusion for 24 hours. AKI was assessed by measuring creatinine clearance (CrCl), blood urea nitrogen, plasma uric acid, potassium level, fractional excretion of sodium (Fe_{Na}) and microproteinuria. Oxidative stress in rat kidneys was quantified and hematoxylin-eosin staining was used to assess changes in renal tissues. Piperine (10 mg/kg, p.o.) was administered for 1 and 2 weeks prior to AKI. Administration of piperine per se for 2 weeks had no effect on various parameters employed except significant change in lipid profile and increased oxidative stress in renal tissues. Treatment with piperine for 2 weeks in rats with AKI significantly worsened ischemia reperfusion-induced renal damage. Pretreatment with pioglitazone, peroxisome proliferator activated receptor (PPAR-γ) agonist, markedly reversed piperine mediated renal damage in rats. Hence, it may be suggested that PPAR-γ antagonism could be a major mechanism in piperine mediated aggravation of AKI in rats.
Keywords: piperine, acute kidney injury, oxidative stress, PPAR-γ.

I. INTRODUCTION

Acute kidney injury (AKI) is characterized by abrupt decline in glomerular filtration rate, which results in accumulation of nitrogenous and other biochemical waste in body. It serves as one of the major reasons for mortality and morbidity in hospitalized patients undergoing medical and surgical procedures [1]. AKI is a severe complication in intensive care units with prevalence of 30-50% [2]. Ischemia reperfusion is the leading cause of AKI. Ischemia reperfusion injury (IRI) is witnessed in various clinical conditions such as renal transplantation, partial nephrectomy, sepsis and urological interventions [3]. Pathogenesis of IRI-induced renal damage majorly includes depletion of adenosine triphosphate, activation of phospholipase A_2, neutrophil infiltration and production of reactive oxygen species (ROS) [4].

Piperine is an active phenolic component in black pepper that is noted to have diverse biological properties including anti-oxidant, anti-apoptotic and permeation enhancing activity. Piperine serves as good anti-inflammatory agent through inhibition of prostaglandin E_2 (PGE_2), cyclo-oxygenase-2 (COX-2), nitric oxide synthase-2 (NOS-2), nuclear factor kappa B (NF-κB), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and IL-1 [5, 6 ,7, 8]. Role of piperine has been explored in neurological disorders such as epilepsy and depression [9, 10]. Moreover, piperine is reported to reduce cholesterol and triglyceride level in rats fed on high fat diet [11].

Peroxisome proliferator activated receptor-γ (PPAR-γ) belongs to class of nuclear receptors regulating fat storage and glucose metabolism in body. The role of PPAR-γ agonists have been widely explored in various disorders because of their anti-oxidant and anti-inflammatory properties apart from their involvement in glucose metabolism. Pioglitazone, a PPAR-γ agonist attenuates IRI induced renal damage in diabetic rats through antioxidant activity and amelioration of pro-inflammatory cytokines [12]. Moreover, it exhibited reno-protection by inhibiting NF-κB activation, intercellular adhesion molecule-1

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 6 Nov-Dec 2021, pp: 233-243 www.ijprajournal.com ISSN: 2249-7781

(ICAM-1) expression and macrophage infiltration in diabetic kidney [13]. Pioglitazone reduced renal fibrosis and damage through inhibition of matrix metalloproteinase-2 (MMP-2) and MMP-9 expressions [14]. Piperine is reported to reduce expression of PPAR-γ in vitro. Moreover, piperine inhibits rosiglitazone induced PPAR-γ transcriptional activity [15]. Literature regarding interplay between piperine and PPAR-γ and its relevance in various pathological conditions is inadequate. Moreover, the role of piperine in renal IRI is yet to be explored. Hence, the present study is designed to investigate role of piperine against ischemia reperfusion induced AKI.

II. MATERIALS AND METHODS

Present study was carried out in accordance with the guidelines framed by committee for the purpose of control and supervision of experiments on animals (CPCSEA), Government of India. Institutional Animal Ethics Committee of Guru Nanak Dev University, approved the experimental protocol (IAEC/GNDU/2013/06). Male Wistar albino rats weighing 200-250 g were employed in the present study. They were maintained on standard chow and water ad libitum and were exposed to 12 hours light and dark cycles. Before performing surgery, the rats were allowed to acclimatize in metabolic cages for 24 hours.

AKI was induced by using bilateral renal ischemia reperfusion model in rats. Rats were anaesthetized with intraperitoneal injection of ketamine (50 mg/kg) and xylazine (10 mg/kg). Rat kidneys were exposed and occluded for 40 minutes followed by reperfusion for 24 hours. In sham group, the animals were exposed to similar surgical procedure but renal pedicles were not occluded. Post-surgery, rats were returned to metabolic cages for urine collection.

After 24 hours, rats were anaesthetized using ketamine (50 mg/kg, i.p.). Blood was collected using retro-orbital puncture and rats were sacrificed by cervical dislocation. Plasma was used for estimating creatinine, urea nitrogen (BUN), uric acid and electrolytes (sodium/ potassium). The levels of glucose, triglycerides, cholesterol and high-density lipoprotein (HDL) level were quantified in plasma. In addition, the creatinine, sodium and protein content in urine were determined. Kidneys were removed and washed with saline. A part of kidney tissue was preserved in formalin for histopathological studies, a part was used for estimating superoxide anion generation (SAG) and remaining tissue was minced and homogenized (10% w/v) in 1.17% potassium chloride solution using Teflon homogenizer. Contents were centrifuged at 800 × g for 20 minutes and the resultant pellet was used for estimating myeloperoxidase (MPO) activity, whereas clear supernatant was used to quantify lipid peroxides and reduced glutathione (GSH) levels.

2.1. Estimation of renal parameters

The quantification of creatinine in plasma and urine samples was done by using kit (Angstrom Biotech Ltd., Vadodara, India) and creatinine clearance was calculated using standard formula. CrCl was presented as mL/min/kg. The BUN and plasma uric acid level was estimated using kit (Transasia Biomedicals Ltd., Solan, India). Potassium level was estimated in the plasma sample using kit (Crest Biosystems, Goa, India) and presented as mM. Sodium level was estimated in the plasma and urine samples and fractional excretion of sodium (Fe_{Na}) was calculated using standard formula. Result was presented as percentage changes in values. Urine microprotein level was determined using pyrogallol method and expressed as milligrams per day.

2.2. Estimation of plasma glucose and lipid levels

The glucose, triglycerides, cholesterol and HDL levels were estimated in plasma samples using kits (Medsource Ozone Biomedicals Ltd., Faridabad, India). Results were presented as mg/dL of plasma.

2.3. Estimation of oxidative stress

MPO activity was measured using method described in our previous studies [3]. Quantitative measurement of thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation in kidney was performed as previously described [3]. Results were expressed as nM/mg protein. nanomoles per milligram of protein. SAG in renal tissue was assayed in terms of measuring reduced nitrobluetetrazolium (NBT) [16]. Results were expressed as reduced NBT in pM/min/mg of tissue. GSH content in renal tissue was estimated using established method [3]. The results were expressed as reduced GSH in µM/mg protein

2.4. Hematoxylin and eosin staining

Kidney tissues preserved in 10% formalin were embedded in paraffin, cut (4 µm thick) and stained with hematoxylin-eosin staining. Slides were observed for gross morphological alterations including increase in glomerular and tubular changes and cellular necrosis.

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 6 Nov-Dec 2021, pp: 233-243 www.ijprajournal.com ISSN: 2249-7781

2.5. Experimental Protocol

Eight groups were employed in the present study, each comprising 6 rats. Piperine and pioglitazone were suspended in 0.5% carboxy methyl cellulose (CMC). Group 1 (Control): No surgery was performed on rats. Group 2 (Sham operated): The surgery was performed to expose both kidneys but ischemia was not given. Group 3 (Piperine 10 mg/kg per se): Piperine (10 mg/kg, p.o.) was administered once daily for 2 weeks and no surgery was performed. Group 4 (Ischemia reperfusion injury, IRI): Both kidneys were occluded for 40 minutes followed by reperfusion for 24 hours. Group 5 (Piperine, 1 week treated): Piperine (10 mg/kg, p.o.) was administered once daily for 1 week and surgery was performed on 7th day after 1 hour of piperine administration. Group 6 (Piperine, 2 weeks treated): Piperine (10 mg/kg, p.o.) was administered once daily for 2 weeks and surgery was performed on 14th day after 1 hour of piperine administration. Group 7 (Pioglitazone 10 mg/kg treated): Pioglitazone (10 mg/kg, p.o.) was administered once daily for 2 weeks and surgery was performed on 14th day after 1 hour of pioglitazone administration. Group 8 (Piperine + Pioglitazone treated): Pioglitazone (10 mg/kg, p.o.) was given 2 hours after administration of piperine (10 mg/kg, p.o.) for 2 weeks and surgery was performed on 14th day after 1 hour of pioglitazone administration.

2.6. Drugs and chemicals

Piperine was purchased from Acros Organics, Belgium. Pioglitazone was procured from Panacea Biotec, Lalru, India. Ketamine and xylazine were obtained from Neon Pharmaceuticals, Mumbai, India and Indian Immunologicals Ltd, Hyderabad, India. GSH and NBT were obtained from Loba Chemie, Mumbai, India. All other reagents used in the study were of analytical grade.

2.7. Statistical analysis

Results were expressed as mean ± S.E.M. The data obtained from various groups were statistically analyzed using one way analysis of variance followed by Tukey-Kramer post hoc test using Graphpad Instat software. The $p< 0.05$ was considered to be statistically significant.

III. RESULTS

The control, sham and piperine per se group did not observe any significant difference among themselves except in oxidative stress parameters, triglycerides, total cholesterol and HDL level. Therefore, all the comparisons were made with respective to control group except aforementioned parameters.

3.1. Effect of piperine and pioglitazone on renal parameters

A significant decrease in CrCl was observed in IRI, IRI + piperine (1 and 2 weeks) and IRI + piperine + pioglitazone groups as compared to control group. Moreover, no significant difference in CrCl was noted between IRI and IRI + piperine (1 week) groups. However, the IRI + piperine (2 weeks) group observed a significant fall in CrCl as compared to IRI + piperine (1 week) group. IRI + pioglitazone group witnessed significant elevation in CrCl as compared to IRI, IRI + piperine (1 and 2 weeks) groups. A significant reduction in CrCl was observed in IRI + piperine + pioglitazone group as compared to IRI + pioglitazone group (Figure 1).

The IRI, IRI + piperine (1 and 2 weeks) and IRI + piperine + pioglitazone groups observed a significant increase in BUN level as compared to control group. The difference in BUN level between IRI and IRI + piperine (1 week) was not significant. The elevation of BUN level was significant in IRI + piperine (2 weeks) as compared to IRI and IRI + piperine (1 week) groups. A significant reduction in BUN level was observed in IRI + pioglitazone group as compared to IRI, IRI + piperine (1 and 2 weeks) groups. Pretreatment with piperine abolished protective effect of pioglitazone (Figure 1).

Plasma uric acid was significantly increased in IRI, IRI + piperine (1 and 2 weeks), and IRI + piperine + pioglitazone groups as compared to control group. The IRI + piperine (2 weeks) group witnessed significant elevation in plasma uric acid as compared to IRI + piperine (1 week) group. Moreover, IRI + pioglitazone group showed significant reduction in plasma uric acid as compared to IRI, IRI + piperine (1 and 2 weeks) groups. The plasma uric acid in IRI + piperine + pioglitazone group was significantly less than IRI + piperine (2 weeks) however; it remained significantly higher than IRI + pioglitazone group (Figure 1).

The potassium level in plasma was significantly elevated in IRI, IRI + piperine (1 and 2 weeks) and IRI + piperine + pioglitazone groups as compared to control group. No significant difference observed in plasma potassium level among IRI and IRI + piperine (1 week) groups. The IRI + piperine (2 weeks) group witnessed significant increase in plasma potassium level as compared to IRI and IRI + piperine (1 week) groups. The IRI + pioglitazone group showed

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 6 Nov-Dec 2021, pp: 233-243 www.ijprajournal.com ISSN: 2249-7781

renoprotection as plasma potassium level was significantly reduced as compared to IRI, IRI + piperine (1 and 2 weeks) groups. Reduction in plasma potassium level was significant in IRI + piperine + pioglitazone group as compared to IRI + piperine (2 weeks) group. Pretreatment with piperine significantly deteriorated renoprotective effect of pioglitazone group (Figure 1).

A significant increase in Fe_{Na} was observed in IRI, IRI + piperine (1 and 2 weeks) and IRI + piperine + pioglitazone groups as compared to control group. The piperine (1 week) group did not show any significant difference in Fe_{Na} as compared to IRI group. The IRI + piperine (2 weeks) group observed significant increase in Fe_{Na} as compared to IRI and IRI + piperine (1 week) groups. However, the Fe_{Na} was significantly reduced in IRI + pioglitazone group as compared to IRI group witnessed renoprotection. The reduction in Fe_{Na} was significant in IRI + pioglitazone and IRI + piperine + pioglitazone groups as compared to IRI + piperine (2 weeks) group. However, the increase in Fe_{Na} was significant in IRI + piperine + pioglitazone group as compared to IRI + pioglitazone group indicating that pretreatment with piperine abolishes reno-protective effect of pioglitazone (Figure 1).

The microproteinuria was significantly increased in IRI, IRI + piperine (1 and 2 weeks), as well as IRI + piperine + pioglitazone groups as compared to control group. A significant increase in microproteinuria observed in IRI + piperine (2 weeks) group as compared to IRI and IRI + piperine (1 week) groups. The IRI + pioglitazone group witnessed significant reduction in microproteinuria as compared to IRI, IRI + piperine (1 and 2 weeks) groups. The IRI + piperine + pioglitazone group observed significant decrease in microproteinuria as compared to IRI + piperine (2 weeks) group although it remained significantly higher than IRI + pioglitazone group (Figure 1).

3.2. Effect of piperine and pioglitazone on glucose and lipid levels

There was no significant decrease in blood glucose and HDL level between various groups employed in the present study (Table 1). A significant reduction in triglyceride level (TG) observed in piperine per se, IRI + piperine (2 weeks) and IRI + piperine + pioglitazone group as compared to control group. Moreover, a significant decrease in TG level observed in IRI + piperine (2 weeks) and IRI + piperine + pioglitazone groups as compared to IRI as well as IRI + piperine (1 week) group. The TG level was significantly raised in IRI + pioglitazone group as compared to IRI + piperine (2 weeks) group. The IRI + piperine + pioglitazone group witnessed significant reduction in TG level as compared to IRI + pioglitazone group (Table 1).

The cholesterol level was significantly lower in piperine per se, IRI + piperine (1 and 2 weeks), as well as in IRI + piperine + pioglitazone group as compared to control and IRI group. No significant difference in cholesterol level observed among piperine treated groups. The cholesterol level observed significant increase in pioglitazone group as compared to IRI + piperine (2 weeks) group. A significant decrease in cholesterol level observed in IRI + piperine + pioglitazone group as compared to IRI + pioglitazone group (Table 1).

3.3. Effect of piperine and pioglitazone on renal oxidative stress

A significant increase in MPO activity in renal tissue was observed in all groups except IRI + pioglitazone group as compared to control group. The MPO activity was significantly increased in IRI + piperine (2 weeks) groups as compared to piperine per se, IRI and IRI + piperine (1 week) groups. The IRI + pioglitazone group witnessed reduction in MPO activity as compared to piperine per se, IRI, IRI + piperine (1 and 2 weeks) groups. The IRI + piperine + pioglitazone group observed a significant increase in MPO activity as compared to IRI + pioglitazone group (Table 2).

The lipid peroxidation measured in terms of thiobarbituric acid reactive substances (TBARS) was significantly increased in all groups except pioglitazone treated group as compared to control group. The piperine per se, IRI and IRI + piperine (1 week) groups did not show significant difference in GSH level. The IRI + piperine (2 weeks) group witnessed the elevation in TBARS as compared to piperine per se, IRI and IRI + piperine (1 week) groups. A significant reduction in TBARS was observed in IRI + pioglitazone group as compared to piperine per se, IRI, IRI + piperine (1 and 2 weeks) groups. However, the IRI + piperine + pioglitazone group showed a significant increase in TBARS as compared to IRI + pioglitazone group (Table 2).

The SAG measured in terms of reduced NBT was significantly elevated in all groups except pioglitazone treated group. Moreover, in comparison to piperine per se group, the SAG was significantly increased in IRI, IRI + piperine (1 and 2 weeks) and IRI + piperine + pioglitazone groups. The IRI, IRI + piperine (1 and 2 weeks) groups did not observe any significant change in SAG. A significant decrease in amount of reduced NBT was observed in IRI + pioglitazone group as compared

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 6 Nov-Dec 2021, pp: 233-243 www.ijprajournal.com ISSN: 2249-7781

to piperine per se, IRI, IRI + piperine (1 and 2 weeks) groups. The reduction in SAG was significant in IRI + piperine + pioglitazone group as compared to IRI + piperine (2 weeks) group. The increase in reduced NBT was significantly higher in IRI + piperine + pioglitazone group as compared to IRI + pioglitazone group (Table 2).

The piperine per se, IRI, IRI + piperine (1 and 2 weeks) and IRI + piperine + pioglitazone groups witnessed significant decrease in GSH level as compared to control group. No significant difference in GSH level was observed in piperine per se, IRI and IRI + piperine (1 week) groups. Moreover, the GSH level was significantly declined in IRI + piperine (2 weeks) group as compared to IRI + piperine (1 week) group. A significant increase in GSH level was observed in IRI + pioglitazone group as compared to piperine per se, IRI, IRI + piperine (1 and 2 weeks) groups thereby indicating protective role of pioglitazone in renal IRI. However, the pretreatment with piperine abolished renoprotective effect of pioglitazone (Table 2).

3.4. Effect of Piperine and pioglitazone on renal tissues

Hematoxylin and eosin staining demonstrated significant morphological changes in renal tissue including tubular dilatation, moderate necrosis, tubular atrophy, detachment of basement membrane from glomerulus and hyaline cast formation in IRI group as compared to control group. The per se treatment of piperine had no effect on renal tissues whereas its treatment in rats with renal IRI did not observe any protection. The pioglitazone observed marked protection against renal IRI that was abolished with piperine treatment (Figure 2).

IV. DISCUSSION

Ischemia reperfusion is the leading cause of AKI in humans worldwide. The IRI initiates a cascade of events that involve tubular epithelial injury, inflammation, and altered microvascular functions that finally lead to death of renal cells [17]. There are several factors that are involved in pathogenesis of renal damage such as depletion of ATP, generation of ROS, activation of phospholipase A_2 and neutrophil infiltration. In the present study, significant changes in various parameters including CrCl, BUN, uric acid, electrolytes, microproteinuria, renal oxidative stress and histological changes indicated AKI in rats subjected to IRI as compared to control group. Piperine is an alkaloid present in the fruits of black pepper and other species of Piperaceae family [18]. Piperine is noted to possess diverse biological properties apart from its permeation enhancer activity. It is used in disorders of central nervous system such as depression through its monoamine oxidase inhibitory activity [9], epilepsy through gamma amino butyric acid agonism, suppression of presynaptic glutamate release and calcium overloading [10, 19]. Piperine is reported as an anti-oxidant through inhibition of lipid peroxidation [20], anti-apoptotic [8], anti-genotoxic and anti-mutagenic agent thus preventing damage of DNA [21]. Piperine plays an important role in regulating genes associated with lipid metabolism [15]. The administration of piperine for two weeks per se as well as in rats with IRI resulted in significant decrease in total cholesterol and triglycerides that is in accordance with other studies [11].

The PPAR-γ belongs to family of nuclear receptors and is involved in regulation of glucose metabolism in cells. Thiazolidinediones such as pioglitazone, rosiglitazone and troglitazone are selective activators of PPAR-γ that are widely used for the treatment of non insulin dependent diabetic mellitus. PPAR-γ is expressed in various tissues including kidneys [22, 23]. It has been documented in several studies that PPAR-γ agonists provide benefit against IRI of various organs including intestine, lung, heart, kidney, sciatic nerve and brain [12, 24, 25, 26, 27, 28]. The pretreatment of pioglitazone reduces oxidative stress, COX-2 expression and inhibits production of proinflammatory cytokines such as TNF-α [26, 29]. Moreover, the role of PPAR-γ agonists in various models of renal dysfunction including IRI, diabetes, hypertension and chemical induced nephropathy is well recognized [30, 31, 32, 33].

Literature regarding crosstalk between piperine and PPAR-γ is limited except an in-vitro study suggesting piperine to downregulate PPAR-γ expression. Piperine is reported to reduce mRNA expression of adipogenic transcription factors such as PPAR-γ, sterol regulatory element binding protein (SREBP) and CCAAT-enhancer binding protein-β (C/EBPβ). Moreover, piperine disrupts rosiglitazone dependent interaction between PPAR-γ and coactivator CBP [15]. Treatment with piperine for two weeks aggravated IRI induced AKI measured in terms of plasma, urine and tissue parameters, whereas one week treatment had no significant effect as compared to IRI group. Furthermore, simultaneous treatment with PPAR-γ agonist, pioglitazone reversed piperine mediated renal damage suggesting PPAR-γ antagonism as a key mechanism in its renal damaging effect. Hence,

the present study highlights novel finding that PPAR-γ antagonistic activity is responsible for renal damaging effect of piperine against IRI induced AKI.

The IRI induces significant oxidative stress as various ROS including superoxide radicals ($O_2^{\bullet}$) and hydrogen peroxide generated during IRI lead to accumulation of neutrophils and release of lytic enzymes. The generated lipid peroxides alter membrane permeability; impair membrane bound ion pumps and cellular functions [17, 34]. In the present study, IRI group witnessed oxidative stress measured in terms of increased levels of TBARS and SAG along with reduced level of GSH that was aggravated to significant extent with piperine treatment. MPO is a peroxidase enzyme expressed in neutrophil granulocytes that produces hypochlorous acid from hydrogen peroxide and chloride anion adding to oxidative stress. MPO activity was significantly increased in IRI and IRI + piperine treated groups whereas pioglitazone treatment abolished piperine mediated oxidative stress. Interestingly, renal tissues of rats with piperine per se treatment for two weeks also observed significant oxidative stress. In the present study, the renoprotection observed with pioglitazone against IRI and IRI + piperine along with restoration of anti-oxidants is supported by other reports [12, 14].

Hence, it is concluded that administration of piperine in rats aggravates IRI induced renal damage. Moreover, the PPAR-γ antagonism might be one of the mechanisms in piperine mediated aggravation of ischemia reperfusion-induced AKI in rats.

REFERENCES

[1]. Uchino S, Kellum JA, Bellomo R, Doig GS, Morimatsu H, Morgera S, Schetz M, Tan I, Bouman C, Macedo E, Gibney N, Tolwani A, Ronco C. Acute renal failure in critically ill patients: a multinational, multicenter study. JAMA. 2005;294(7):813-818.

[2]. Lameire N, Van Biesen W, Vanholder R. Acute renal failure. Lancet. 2005;365(9457):417-430.

[3]. Pundir M, Arora S, Kaur T, Singh R, Singh AP. Effect of modulating the allosteric sites of N-methyl-D-aspartate receptors in ischemia-reperfusion induced acute kidney injury. Journal of Surgical Research. 2013;183(2):668-677.

[4]. Edelstein CL, Ling H, Schrier RW. The nature of renal cell injury. Kidney International. 1997;51(5):1341-1351.

[5]. Sudjarwo SA. The potency of piperine as antiinflammatory and analgesic in rats and mice. Folia Medica Indonesiana. 2005;41(3):190-194.

[6]. Bang JS, Oh DH, Choi HM, Sur BJ, Lim SJ, Kim JY, Yang HI, Yoo MC, Hahm DH, Kim KS. Anti-inflammatory and antiarthritic effects of piperine in human interleukin 1ß-stimulated fibroblast-like synoviocytes and in rat arthritis models. Arthritis Research & Therapy 2009;11(2):1-9.

[7]. Vaibhav K, Shrivastava P, Javed H, Khan A, Ahmed ME, Tabassum R, Khan MM, Khuwaja G, Islam F, Siddiqui MS, Safhi MM, Islam F. Piperine suppresses cerebral ischemia-reperfusion-induced inflammation through the repression of COX-2, NOS2, and NF-κB in middle cerebral artery occlusion rat model. Molecular and Cellular Biochemistry. 2012;367(1):73-84.

[8]. Shrivastava P, Vaibhav K, Tabassum R, Khan A, Ishrat T, Khan MM, Ahmad A, Islam F, Safhi MM, Islam F. Anti-apoptotic and anti-inflammatory effect of piperine on 6-OHDA induced Parkinson's rat model. The Journal of Nutritional Biochemistry. 2013;24(4):680-687.

[9]. Lee SA, Hong SS, Han XH, Hwang JS, Oh GJ, Lee KS, Lee MK, Hwang BY, Ro JS. Piperine from the fruits of Piper longum with inhibitory effect on monoamine oxidase and antidepressant-like activity. Chemical and Pharmaceutical Bulletin. 2005;53(7):832-835.

[10]. Fu M, Sun ZH, Zuo HC. Neuroprotective effect of piperine on primarily cultured hippocampal neurons. Biological and Pharmaceutical Bulletin. 2010;33(4):598-603.

[11]. Maneesai P, Scholfield NC, Chootip K. Piperine is anti-hyperlipidemic and improves endothelium-dependent vasorelaxation in rats on a high cholesterol diet. Journal of Physiological and Biomedical Sciences. 2012;25(1):27-30.

[12]. Tawfik MK. Renoprotective activity of telmisartan versus pioglitazone on ischemia/reperfusion induced renal damage in diabetic rats. European Review for Medical and Pharmacological Sciences. 2012;16(5):600-609.

[13]. Ohga S, Shikata K, Yozai K, Okada S, Ogawa D, Usui H, Wada J, Shikata Y, Makino H. Thiazolidinedione ameliorates renal injury in experimental diabetic rats through anti-inflammatory effects mediated by inhibition of NF-kappa B activation. American Journal of Physiology-Renal Physiology 2007;292(4):F1141-F1150.
[14]. Reel B, Guzeloglu M, Bagriyanik A, Atmaca S, Aykut K, Albayrak G, Hazan E. The effects of PPAR-γ agonist pioglitazone on renal ischemia/reperfusion injury in rats. Journal of Surgical Research. 2013;182(1):176-184.
[15]. Park UH, Jeong HS, Jo EY, Park T, Yoon SK, Kim EJ, Jeong JC, Um SJ. Piperine, a component of black pepper, inhibits adipogenesis by antagonizing PPARγ activity in 3T3-L1 cells. Journal of Agricultural and Food Chemistry. 2012;60(15):3853-3860.
[16]. Singh R, Singh AP, Singh M, Krishan P. Impact of obesity on hypertension-induced cardiac remodeling: role of oxidative stress and its modulation by gemfibrozil treatment in rats. Free Radical Biology and Medicine. 2011;50(2):363-370.
[17]. Bonventre JV. Mechanism of ischemic acute renal failure. Kidney International. 1993;43(5):1160-1178.
[18]. Wattanathorn J, Chonpathompikunlert P, Muchimapura S, Priprem A, Tankamnerdthai O. Piperine, the potential functional food for mood and cognitive disorders. Food and Chemical Toxicology. 2008;46(9):3106-3110.
[19]. da Cruz GMP, Felipe CFB, Scorza FA, da Costa MAC, Tavares AF, Menezes MLF, de Andrade GM, Leal LKA, Brito GAC, da Graça Naffah-Mazzacoratti M, Cavalheiro EA, de Barros Viana GS. Piperine decreases pilocarpine-induced convulsions by GABAergic mechanisms. Pharmacology Biochemistry and Behavior. 2013;104:144-153.
[20]. Wakade AS, Shah AS, Kulkarni MP, Juvekar AR. Protective effect of Piper longum L. on oxidative stress induced injury and cellular abnormality in Adriamycin induced cardiotoxicity in rats. Indian Journal of Experimental Biology. 2008;46(7):528-533.
[21]. Abo-Zeid MA, Farghaly AA. The anti-mutagenic activity of piperine against mitomycine C induced chromosomal aberrations and sister chromatid exchanges in mice. Nature and Science. 2009;7:72-78.
[22]. Fajas L, Debril MB, Auwerx J. Peroxisome proliferator-activated receptor-gamma: from adipogenesis to carcinogenesis. Journal of Molecular Endocrinology. 2001; 27(1):1-9.
[23]. Yoshihara D, Kurahashi H, Morita M, Kugita M, Hiki Y, Aukema HM, Yamaguchi T, Calvet JP, Wallace DP, Nagao S. PPAR-gamma agonist ameliorates kidney and liver disease in an orthologous rat model of human autosomal recessive polycystic kidney disease. American Journal of Physiology-Renal Physiology. 2011;300(2): F465-F474.
[24]. Naito Y, Takagi T, Uchiyama K, Handa O, Tomatsuri N, Imamoto E, Kokura S, Ichikawa H, Yoshida N, Yoshikawa T. Suppression of intestinal ischemia reperfusion injury by a specific peroxisome proliferator activated receptor gamma ligand, pioglitazone, in rats. Redox Report. 2002;7(2):294-299.
[25]. Wayman NS, Hattori Y, McDonald MC, Mota-Filipe H, Cuzzocrea S, Pisano B, Chatterjee PK. Thiemermann C. Ligands of the peroxisome proliferator-activated receptors (PPAR-α and PPAR-β) reduce myocardial infarct size. FASEB Journal. 2002;16(9):1027-1040.
[26]. Ito K, Shimada J, Kato D, Toda S, Takagi T, Naito Y, Yoshikawa T, Kitamura N. Protective effects of preischemic treatment with pioglitazone, a peroxisome proliferator activated receptor-gamma ligand, on lung ischemia reperfusion injury in rats. European Journal of Cardiothoracic Surgery. 2004;25(4):530-536.
[27]. Rahimian R, Fakhfouri G, Rasouli MR, Nouri M, Nezami BG, Paydar MJ, Asadi Amoli F, Dehpour AR. Effect of pioglitazone on sciatic nerve ischemia/ reperfusion injury in rats. Pediatric Neurosurgery. 2009;45(2):126-131.
[28]. Zhang HL, Xu M, Wei C, Qin AP, Liu CF, Hong LZ, Zhao XY, Liu J, Qin ZH. Neuroprotective effects of pioglitazone in a rat model of permanent focal cerebral ischemia are associated with peroxisome proliferator-activated receptor gamma-mediated suppression of nuclear factor-κB signaling pathway. Neuroscience. 2011;176:381-395.
[29]. Collino M, Aragno M, Mastrocola R, Gallicchio M, Rosa AC, Dianzani C, Danni

O, Thiemermann C, Fantozzi R. Modulation of the oxidative stress and inflammatory response by PPAR-gamma agonists in the hippocampus of rats exposed to cerebral ischemia/reperfusion. European Journal of Pharmacology. 2006;530(1-2):70-80.

[30]. Ma LJ, Marcantoni C, Linton MF, Fazio S, Fogo AB. Peroxisome proliferator activated receptor-gamma agonist troglitazone protects against nondiabetic glomerulosclerosis in rats. Kidney International. 2001;59(5):1899-1910.

[31]. Sivarajah A, Chatterjee PK, Patel NS, Todorovic Z, Hattori Y, Brown PA, Stewart KN, Mota-Filipe H, Cuzzocrea S, Thiemermann C. Agonists of peroxisome proliferator activated receptor-gamma reduce renal ischemia/reperfusion injury. American Journal of Nephrology. 2003;23(4):267-276.

[32]. Chung BH, Lim SW, Ahn KO, Sugawara A, Ito S, Choi BS, Kim YS, Bang BK, Yang CW. Protective effect of peroxisome proliferator activated receptor gamma agonists on diabetic and non-diabetic renal diseases. Nephrology 2005;10:S40-S43.

[33]. Pereira MG, Câmara NOS, Campaholle G, Cenedeze MA, Teixeira VDPA, dos Reis MA, Pacheco-Silva A. Pioglitazone limits cyclosporine nephrotoxicity in rats. International Immunopharmacology. 2006;6(13-14);1943-1951.

[34]. Paller MS, Hoidal JR, Ferris TF. Oxygen free radicals in ischemic acute renal failure in the rat. The Journal of Clinical Investigation. 1984;74(4):1156-1164.

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 6 Nov-Dec 2021, pp: 233-243 www.ijprajournal.com ISSN: 2249-7781

Table 1: Effect of piperine and pioglitazone on glucose and lipid level. Values are expressed as mean ± S.E.M. a = p<0.05 vs Control; b = p<0.05 vs Piperine per se; c = p<0.05 vs IRI; d = p<0.05 vs IRI + Piperine (1 week); e = p<0.05 vs IRI + Piperine (2 weeks); f = p<0.05 vs IRI + Piogliazone.

Parameter → Groups ↓	**Glucose (mg/dL)**	**Triglycerides (mg/dL)**	**Total cholesterol (mg/dL)**	**HDL (mg/dL)**
Control	116.77 ± 5.34	69.89 ± 3.25	95.52 ± 4.55	45.80 ± 1.32
Sham	111.93 ± 3.95	67.32 ± 1.68	87.02 ± 3.36	44.84 ± 3.67
Piperine per se	105.36 ± 5.73	54.67 ± 2.28[a]	70.12 ± 1.75[a]	47.61 ± 1.89
IRI	107.16 ± 3.64	74.14 ± 2.75[b]	96.91 ± 3.06[b]	52.29 ± 2.04
IRI + Piperine (1 week)	104.61 ± 4.13	68.21 ± 3.01[b]	76.08 ± 2.73[a,c]	45.69 ± 2.51
IRI + Piperine (2 weeks)	98.58 ± 2.62	52.24 ± 2.08[a,c,d]	66.37 ± 2.43[a,c,e]	43.49 ± 1.46
IRI + Pioglitazone (2 weeks)	110.38 ± 3.91	68.01 ± 4.98[b,e]	82.81 ± 2.74[e]	48.25 ± 2.45
IRI + Piperine + Pioglitazone	100.82 ± 2.82	51.08 ± 2.98[a,c,d,f]	66.29 ± 5.26[a,c,f]	45.31 ± 2.37

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 6 Nov-Dec 2021, pp: 233-243 www.ijprajournal.com ISSN: 2249-7781

Parameter → Groups ↓	MPO (U/g of tissue)	TBARS (nM/mg of protein)	SAG (pM/min/mg of tissue)	GSH (µM/mg of protein)
Control	0.38 ± 0.08	0.33 ± 0.05	6.61 ± 1.02	8.36 ± 0.44
Sham	0.45 ± 0.03	0.34 ± 0.04	6.94 ± 1.21	7.83 ± 0.32
Piperine per se	0.92 ± 0.04[a]	0.76 ± .0.06[a]	17.11 ± 1.51[a]	3.09 ± 0.31[a]
IRI	1.26 ± 0.14[a]	0.92 ± 0.03[a]	29.01 ± 2.54[a,b]	3.01 ± 0.17[a]
IRI + Piperine (1 week)	1.10 ± 0.13[a]	0.81 ± 0.07[a]	28.09 ± 1.61[a,b]	3.67 ± 0.25[a]
IRI + Piperine (2 weeks)	1.88 ± 0.11[a,b,c,d]	1.04 ± 0.07[a,b,c,d]	29.92 ± 2.01[a,b]	1.68 ± 0.14[a,d]
IRI + Pioglitazone (2 weeks)	0.41 ± 0.04[b,c,d,e]	0.48 ± 0.03[b,c,d,e]	10.12 ± 0.81[b,c,d,e]	7.52 ± 0.55[b,c,d,e]
IRI + Piperine + Pioglitazone	1.04 ± 0.07[a,e,f]	0.90 ± 0.04[a,f]	24.37 ± 1.24[a,b,e,f]	2.89 ± 0.46[a,f]

Table 2: Effect of piperine and pioglitazone on oxidative stress parameters. Values are expressed as mean ± S.E.M. a = p<0.05 vs Control; b = p<0.05 vs Piperine per se; c = p<0.05 vs IRI; d = p<0.05 vs IRI + Piperine (1 week); e = p<0.05 vs IRI + Piperine (2 weeks); f = p<0.05 vs IRI + Piogliazone.

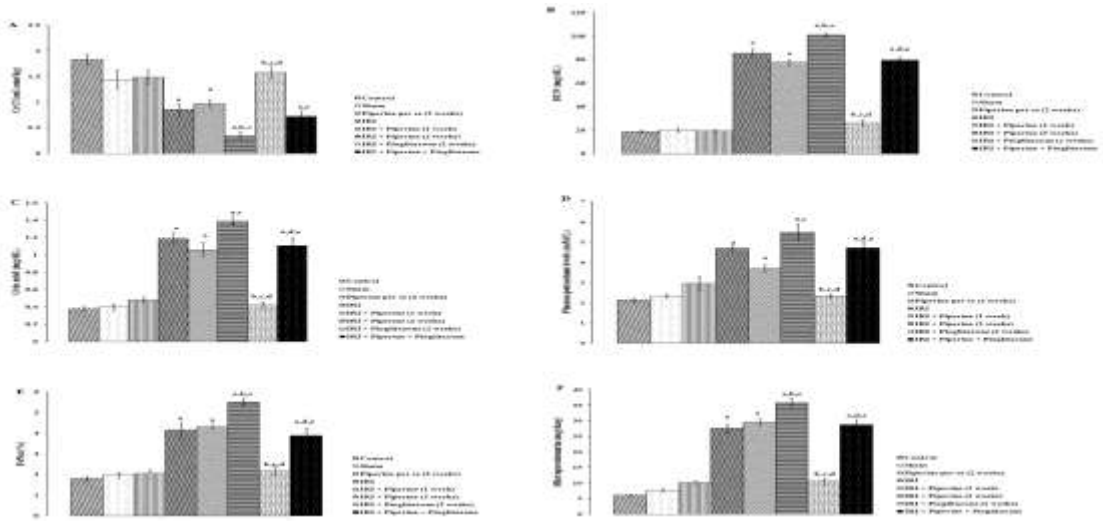

Figure 1: **Figure 1:** Effect of piperine and pioglitazone on renal parameters in rats. Values are expressed as mean ± S.E.M. a = p<0.05 vs Control; b = p<0.05 vs IRI; c = p<0.05 vs IRI + Piperine (1 week); d = p<0.05 vs IRI + Piperine (2 weeks); e = p<0.05 vs IRI + Pioglitazone.

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 6 Nov-Dec 2021, pp: 233-243 www.ijprajournal.com ISSN: 2249-7781

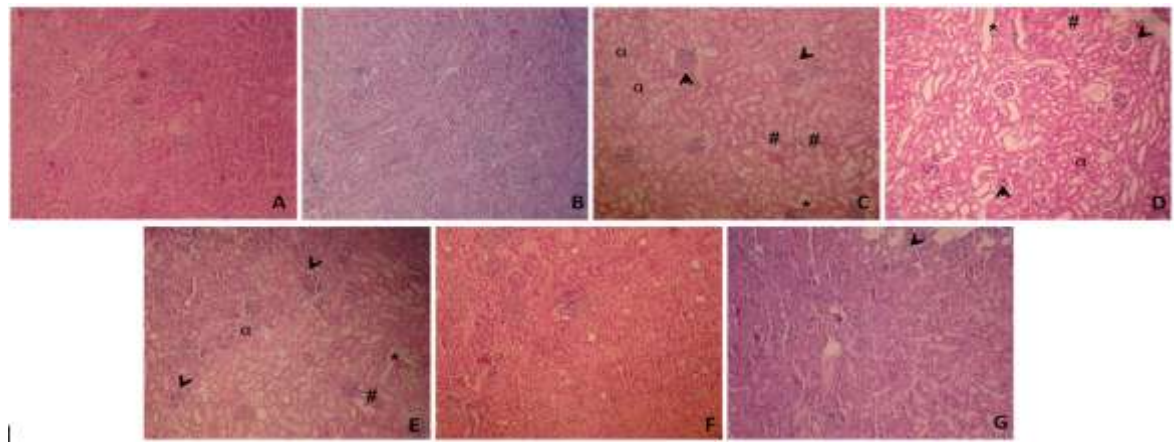

Figure 2: Effect of piperine and pioglitazone on histological changes observed with hematoxylin-eosin staining at 100X magnification. (A- control; B- piperine per se, C- ischemia reperfusion injury (IRI); D- IRI + piperine (1 week); E- IRI + piperine (2 weeks); F- IRI + pioglitazone; G- IRI + piperine + pioglitazone). [Arrows indicate glomerular damage characterised by glomerular hylanisation and detachment from basement membrane, "*" indicate tubular damage characterised by tubular dilatation, "#" represent hylanisation and "α" indicate tubular atrophy].