

Gentiopicroside ameliorates lipopolysaccharide-induced acute kidney injury by inhibiting TLR4/NF- κ B signaling in mice model

Saja Majeed Shareef¹, Sarmed Hashim Kathem²

¹Al-Esraa University College, Department of Pharmacology/Iraq

²College of Pharmacy, University of Baghdad, Baghdad/Iraq

Email: Sajashareef90@gmail.com

Abstract

Background: Inflammation is a major factor in the development of acute renal damage and the Toll-like receptor 4 (TLR4) signaling pathway is a major contributor to inflammation in the kidney. Lipopolysaccharide (LPS), a specific ligand for TLR4, has been shown to induce acute kidney injury in human and animal models. Gentiopicroside (GENT) is a natural secoiridoid glycoside with various biological activities. **Aim of study:** This study aimed to investigate the renoprotective effect of gentiopicroside on LPS-induced AKI in mice model.

Method: Real-Time Quantitative qPCR method was used to measure mRNA level of the promising kidney biomarker KIM-1, TLR-4, transcription factors NF- κ B, AP-1, IRF3 and proinflammatory markers IL-1 β and iNOS. Furthermore, TNF- α was measured by ELISA analysis and biochemical analysis and histological analysis were used to detect renal function.

Results: The results showed that gentiopicroside significantly ($p < 0.05$) inhibited LPS-induced TNF- α , and IL-1 β . Furthermore, medium- and high-dose gentiopicroside significantly suppressed the Toll-like receptor 4 (TLR4) / myeloid differential protein-88 (MyD88) / nuclear factor-kappa (NF- κ B) signaling pathway. Urea and creatinine levels were also reduced with gentiopicroside. Kidney injury molecule -1 KIM-1 expression which was the early indicator for AKI was also inhibited by gentiopicroside compared to LPS group.

Conclusion: Our data suggest that gentiopicroside protects against LPS-induced AKI via suppressing the TLR4-MYD88 dependent and independent signaling pathway as well as improving renal function and structural kidney damage. As a result, gentiopicroside may have therapeutic potential for LPS-induced SA-AKI.

Keywords: Acute kidney injury, Lipopolysaccharides, Gentiopicroside.

INTRODUCTION

Acute kidney injury (AKI) one of the most severe and clinically life-threatening syndromes, increases mortality risk during uncontrolled systemic inflammatory response(1)(2). The kidney maintains homeostasis, regulates the extracellular environment (detoxification), and excretes waste products(3). In AKI a higher levels of serum urea and creatinine were revealed since the glomerular filtration rate dropped suddenly (4).

AKI can be caused by a number of events, such as heart surgery, sepsis or organ transplantation (5)(6). Sepsis seems to be the most important cause of acute renal damage, and lipopolysaccharide (LPS) is still the second most important cause of systemic inflammatory response syndrome (SIRS)(7).

Address for correspondence: Saja Majeed Shareef, Al-Esraa University College, Department of Pharmacology/Iraq, Email: Sajashareef90@gmail.com

Received date: 19 August 2022

Accepted: 11 September, 2022

Published: 07 October, 2022

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: pnrjournal@gmail.com

How to cite this article: Shareef M S, Kathem H S, Gentiopicroside ameliorates lipopolysaccharide-induced acute kidney injury by inhibiting TLR4/NF- κ B signaling in mice model., J Pharm Negative Results 2022;13(4):135-145.

Access this article online

Quick Response Code:



Website:

www.pnrjournal.com

DOI:

10.47750/pnr.2022.13.04.018

Saja Majeed Shareef *et al*: Gentiopicroside ameliorates lipopolysaccharide-induced acute kidney injury by inhibiting TLR4/NF- κ B signaling in mice model

Flurry of evidences proposed that pathogen-associated molecular patterns (PAMPs) plays important roles in the pathogenesis of sepsis (8). PAMPs such as LPS are originate from microorganisms and encourage the inflammation in response to infections (9). In fact LPS is an endotoxin found on the surface of Gram-negative bacteria(10) which released into the bloodstream and traveled to various organs including kidney then binds to Toll-like receptor 4 (TLR4), which is present on the plasma membrane of immune cells and on the surface of kidney tubular epithelial cells(10). TLR4 forms homodimers when it binds to a ligand. This starts intracellular signaling through two major pathways: The first, from the plasma membrane, the MyD88-dependent pathway, which leads to early NF-B activation and the production of IL-1, TNF-a and IL-6. The second, from the endosome, the TRIF-dependent (MyD88-independent) pathway, which raises type I IFNs and late-phase NF-B.(11)(12).

As supportive treatment, patients with sepsis-associated acute kidney injury at present get fluid replacement, intravenous antibiotics, vasoactive drugs, and diuretics (13) However, the current treatment for septic AKI is nonspecific and reactive, necessitating the development of innovative therapeutic agents. Furthermore the mortality and frequency of SA-AKI have remained high (14). Hence, it is essential to explore effective therapeutic agents to reduce the mortality of SA-AKI.

Natural products have long been utilized to treat a variety of diseases in humans, and they are now acknowledged as essential sources of therapeutic compounds (15). Gentiopicroside (GENT) is a natural secoiridoid glycoside that has been proven to be the key chemical ingredients for Gentiana plants' significant pharmacological activities (16). It is isolated from plants of the genus Gentiana eg. (Gentiana macrophylla Pall, Gentiana lutea and Gentiana scabra) (17) (18). A vast number of pharmacological studies have revealed that gentiopicroside has a range of activities, including wound-healing, anti-proliferative, and analgesic actions(19). Importantly, gentiopicroside has anti-inflammatory properties, which could attenuate an IL-1 β -induced inflammation in chondrocytes (20) Inflammatory cytokines such as NF κ B1, TNF α , IL6 were likewise reduced by Gentiopicroside (17).

The objectives This work was to investigate the molecular mechanisms and protective effects of GENT against LPS-induced AKI in a mouse model via inhibiting TLR4 signaling pathways.

MATERIALS AND METHODS

Chemicals and reagents

Gentiopicroside was purchased from Hangzhou HyperChem LTD CO (China) with CAS no. 20831-79-9. The purity of the compound was certified to be 98% by high-performance liquid chromatography (HPLC). Lipopolysaccharide (LPS) from Escherichia coli O55:B5 was purchased from Sigma Chem. Co. (St. Louis, MO,

USA). TNF- α ELISA kits were purchased from Shanghai, China. Serum Urea and creatinine assay kit reagents were supplied by AGAPPE DIGNOSTICS SWITZERL AND GmbH (Germany). KIM-1, NF- κ B, IRF3, IL-1B, AP, and iNOS, Primers were purchased from Macrogen / South Korea. RNA extraction kit was purchased from TransGen biotech/ China. EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix were purchased from TransGen, biotech / China. TransStart® Top Green qPCR Super Mix was purchased from TransGen, biotech. /China.

Animals and experimental Protocol

Thirty-two healthy adult Albino mice BALB/c weighing between 20–30 gm were brought from and maintained in the house of animals in the College of Pharmacy, University of Baghdad. Mice were maintained at constant room temperature with a 12:12 h light-dark cycle combined with ad libitum feeding with a standard diet for rodents and tap water. All of the experiments in this study were approved by the scientific and ethical committee of the College of Pharmacy, University of Baghdad. After two weeks of acclimatization, animals were weighed and their first weights were recorded. The animals were randomly divided into four groups and each group contained eight mice (n=8): Group I (Negative control group): Mice received 0.1 ml normal saline, Group II (Model of LPS-induced acute kidney injury): mice received normal saline by for five consecutive days. On the fifth day, mice received a single dose of intraperitoneal LPS in dose (10mg/kg) and then euthanized 24 hours after LPS administration. Group III (Treatment group): mice received low dose gentiopicroside (40 mg/kg/day) orally for five consecutive days. On the fifth day, mice received a single dose of intraperitoneal LPS (10mg/kg) and Group IV (Treatment group): mice received high dose gentiopicroside (80 mg/kg/day) orally once daily for five constitutive days. On the fifth day, mice received a single dose of intraperitoneal LPS (10mg/kg).

Euthanization was done by diethyl ether followed by cervical dislocation. Retro-orbital sampling is used to collect blood and subsequently centrifuged for 20 minutes at 3000 rpm and 4°C. The serum was then stored at -20 degrees Celsius for the estimation of serum urea and creatinine.

Kidney tissue preparation

Right kidneys were removed from all mice. Then, kidney tissue was divided into two parts for ELISA and PCR analysis.

Renal Histology Staining and Evaluation

Twenty-four hours after LPS challenge, mice were sacrificed and their left kidneys were removed for histopathological examination. For morphological examination, each tissue was fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

The semi-quantitative score for kidney injury was estimated for each animal observed in blinded manner. The percentage of tubules in renal cortex that showed tubular sloughing, tubular dilation and loss of the brush border, were scored as follows: 0, normal kidney; 1, <25%; 2, 25–50%; 3, 50–75%; 4, >75% (21).

Biochemical measurements

Renal function was assessed in mice of all groups at the end of experiment. Serum urea and creatinine, as important index of renal injury severity, were measured for the assessment of renal function. After 24 hours of treatment, serum was drawn from each animal and the concentrations of urea and creatinine were analyzed by semi-automated biochemical analyzer using commercial kit reagents following the manufacturer’s instructions.

RNA Extraction and Real-Time Quantitative qPCR

Real-Time PCR was used to determine the mRNA expression levels of NF-κB, IRF3, iNOS, IL-1b, AP and KIM-1. The kidney tissue had been isolated and washed to remove any blood then the tissue minced to small pieces. 50-100 mg from tissue was put in each tube containing 1 ml of TRIzol and frozen for later use and total RNA was isolated from the kidney tissue by using TransZol Up Plus RNA Kit (TransGen, biotech. ER501-01) according to the instructions of the manufacturer. Then DNA-free total RNA was reverse-transcribed and the complementary DNA was synthesized by using of EasyScript® one-step gDNA removal and cDNA synthesis supermix (TransGen, biotech. AE311-02). The mRNA expression levels of NF-κB, IRF3, iNOS, IL-1-b, AP and KIM-1 were analyzed using qRT-PCR and the SYBR Green Supermix (TransGen, biotech. AQ131-01). For the purposes of qRT-PCR analysis, GAPDH was used as the housekeeping for RNA quality and differences among samples. The primers were synthesized by, Macrogen / South Korea and the sequences are shown in table 1.

Table 1: Sequences of the primers for quantitative real-time PCR.

<i>Primer</i>	<i>Sequence (5'→3' direction)</i>
F-GAPDH	CGGGTTCCTATAAATACGGACTG
R-GAPDH	CCAATACGGCCAAATCCGTTCC
F-NF-κB	AAGACAAGGAGCAGGACATG
R- NF-κB	AGCAACATCTTCACATCCC
F-TLR4	TCCCTGCATAGAGGTAGTTCC
R-TLR4	TCAAGGGGTTGAAGCTCAGA
F- Kim-1 (Havr1)	GGCTCTCTCCTAACTGGTCA CCACCACCCCCTTTACTTCC
R- Kim-1 (Havr1)	AG GCTGCAGGATGATGCGAT TTCTAGCCAGGACGACTTGC
F-AP-1	CAATTCCTCCCCTGGCTAGA
R-AP-1	GGGATCCTGAACCTCGTTCCG
F-IRF3	GGTGAAGGGACTGAGCTGTT
R-IRF3	ACGTTCTCCGTTCTCTTGCAG
F- iNOS	TGCCACCTTTTGACAGTGATG
R-iNOS	TGATGTGCTGCTGCGAGATT
F- IL-1β	
R- IL-1β	

Enzyme-Linked Immunosorbent Assay analysis of TNF-α

Various groups of kidney tissues were homogenized with PBS (pH7.4) on ice, and then separated by centrifugation for 20 minutes at 2000-3000 RPM. The supernatants were

subsequently collected to determine the concentration of TNF-α accordance with the manufacturer’s instruction.

Statistical analysis

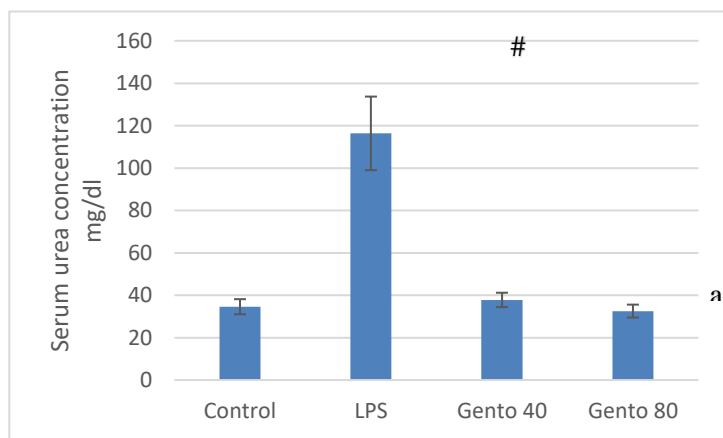
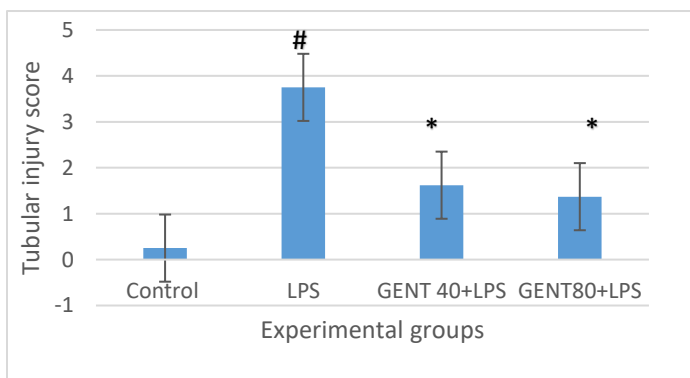
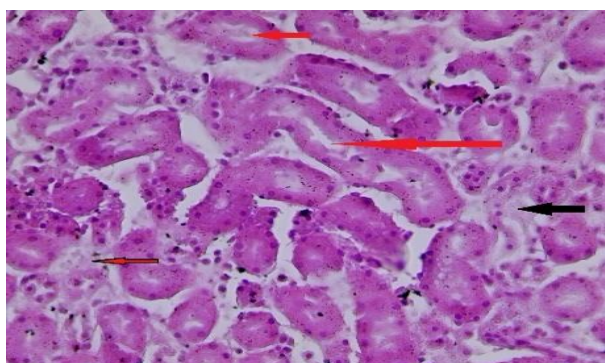
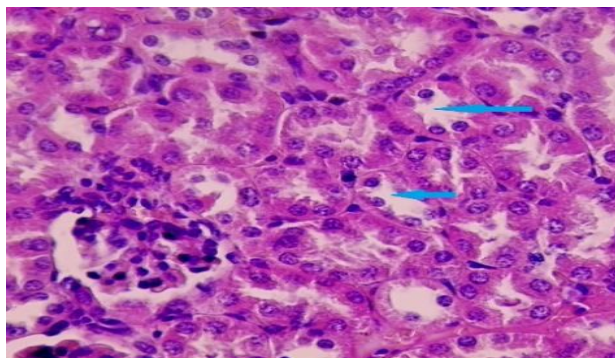
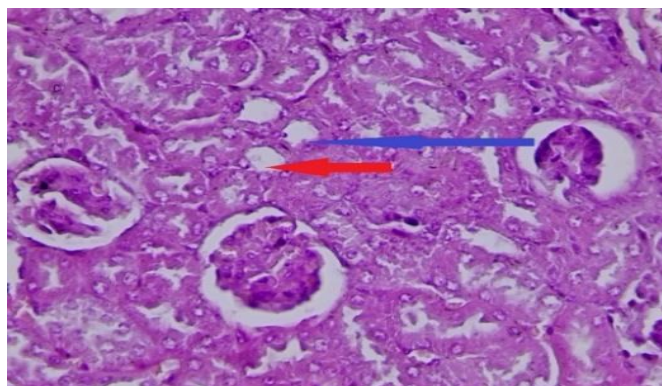
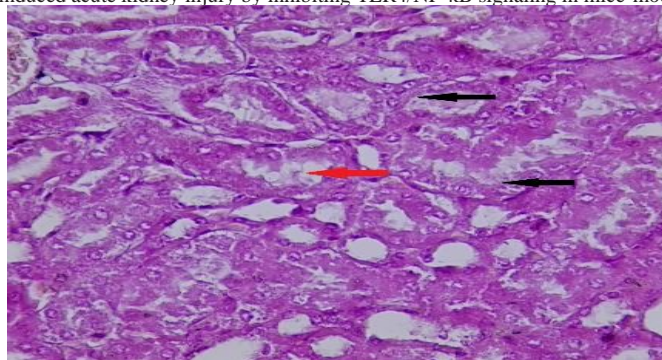
The data were presented as the mean ± standard error of mean (SEM). The analysis was performed using Statistical Package

for the Social Sciences, version 25 (SPSS, version 25). One-way analysis of variance (ANOVA) and the Tukey test were utilized to assess the group differences. Independent t-test was used to compare the means among treatment groups. The differences between the groups were considered statistically significant when the P value was less than 0.05 (P<0.05).

RESULTS

Effects of Gentiopicroside on LPS-induced renal dysfunction in mice

After 24 hours of LPS administration, the serum levels of urea and creatinine significantly increased in the mice. Strikingly, serum urea and creatinine levels were significantly reversed by GENT treatment. Parallel to the worsening of renal function, histological evaluation of the LPS group revealed obvious signs of tubular injury, including tubular cell sloughing, brush border loss, and tubular dilation in the cortex compared to normal group and as a result of improved renal function, these histological lesions were significantly diminished in the (GENT40mg/kg + LPS group) & (GENT 80 mg/kg + LPS).



(F)

#

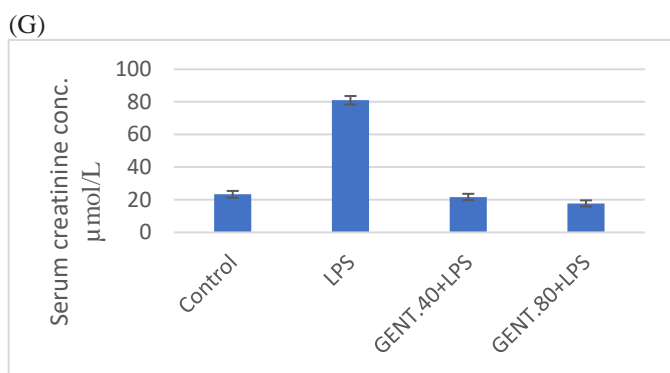


Fig. 1: Effects of GENT. on LPS-induced pathological kidney changes. Representative histopathological images (X40) Blue arrow: normal proximal tubules. Black arrow: tubular cells sloughing. Red arrow: Tubular dilation and loss of brush border. normal (B) LPS group (C) GENT 40 mg+ LPS (D) GENT 80 mg +LPS (E) Tubular injury score (F) Serum urea level (G) Serum creatinine level

*denotes significant difference (P<0.05) vs LPS group.
denotes significant difference (P<0.05) vs control group.

Effect of gentiopicroside on Kidney injury molecule-1 (KIM-1) in LPS induced AKI model

According to the qRT-PCR results, LPS significantly upregulated the mRNA levels of KIM-1 in kidney tissues of model group compared to normal control group (54.95 ± 7.38 vs 3.38 ± 1.00). The mRNA levels upregulation was attenuated by the small and high dose of GENT treatments compared with the LPS model group [$(4.68 \pm 1.273082$ & 2.19 ± 0.78) vs (LPS, 54.95 ± 7.38)] respectively. On the other hand, the results showed that, there was a significant difference (P<0.05) in KIM-1 expression in mice group treated with low dose (40mg /kg) compared to the high dose of GENT 80mg/kg.

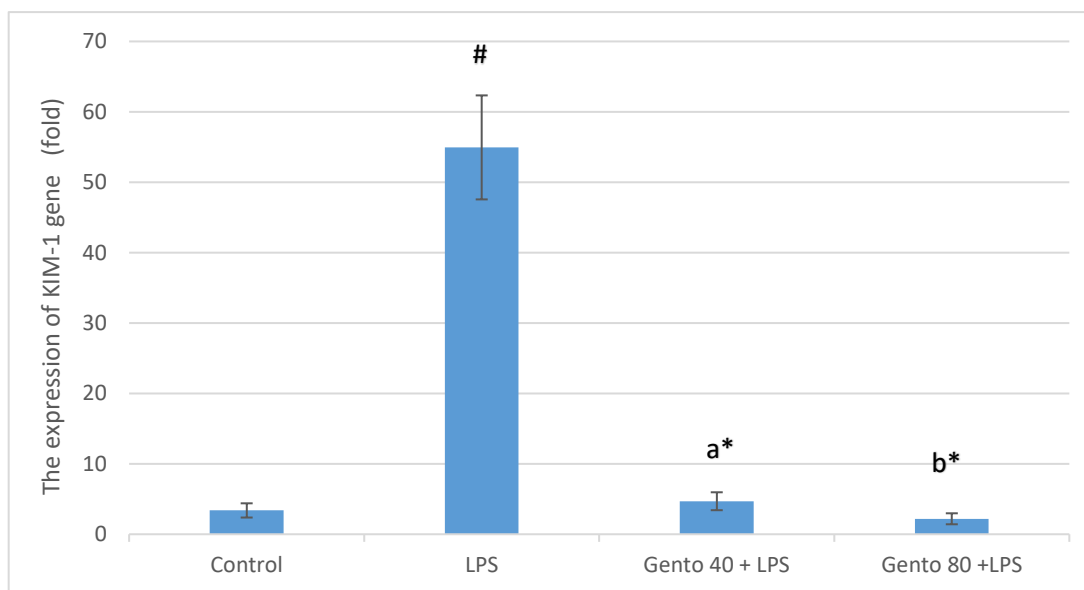


Fig.2: Effects of GENT on KIM-1 gene expression level in kidney tissue in LPS induced AKI.

Values are indicated as mean ± SEM. (n=8) for each group
* denotes significant difference (P<0.05) vs LPS group.
denotes significant difference (P<0.05) vs control group (GPI).
(a & b) non -identical small letters denotes significant difference (P<0.05) among treatment groups

Effect of gentiopicroside on Toll-like receptor 4 TLR-4 expression level in LPS induced -AKI model

After 24h of LPS injection TLR-4 gene expression

significantly up-regulated compared to control group (15.74 ± 2.92 vs 1.98 ± 0.72). The results revealed that, mice received GENT 40mg/kg showed TLR4 mRNA downregulation significantly (P<0.05) compared to LPS group (4.75 ± 0.65 vs. 15.74 ± 2.92). Furthermore, the increment in GENT dose to 80mg/kg/day showed a significant drop in TLR4 level (1.77 ± 0.48) compared to LPS group (15.74 ± 2.92). Interestingly GENT seems to have dose-dependent effect on TLR4 renal expression with further attenuation in TLR-4 was achieved with high dose.

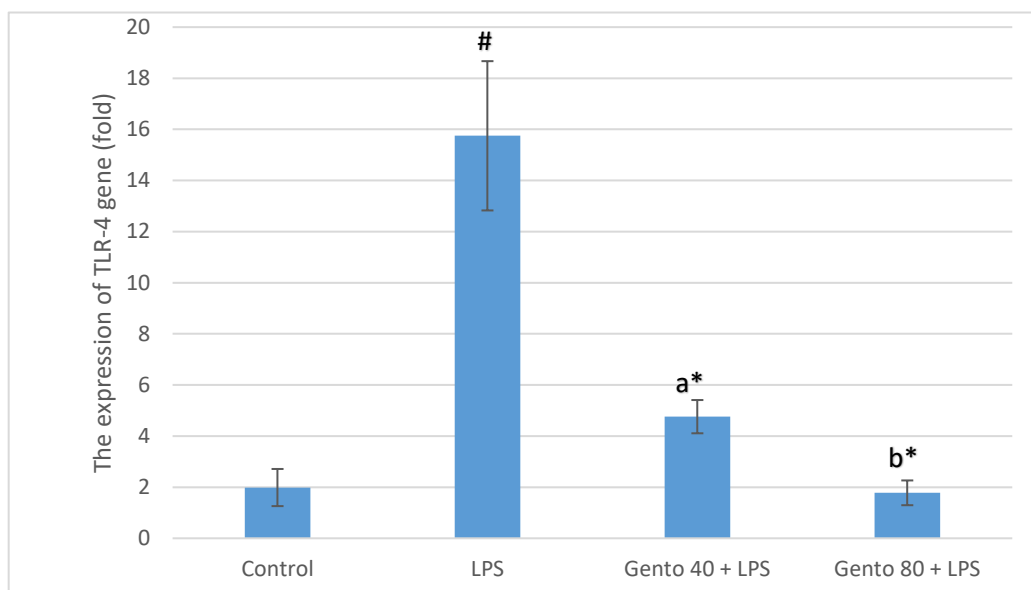


Fig.3: Effects of GENT on TLR4 gene expression in renal tissue in LPS induced AKI. Values are indicated as mean ± SEM. (n=8) for each group

* denotes significant difference (P<0.05) vs LPS group.

denotes significant difference (P<0.05) vs control group (GPI).

(a & b) non-identical small letters denotes significant difference (P<0.05) between treatment groups

Effect of gentiopicroside on gene expression of NF-κB in LPS-induced AKI model

The level of NF-κB was intensely increased after 24 hours of LPS injection in model group compared to control group

(p<0.05) (21.70± 2.50 Vs 1.37 ±0.39) . Moreover, the results of our study showed that NF-κB levels in tissue homogenate for mice in all treated groups (40 mg/kg &80 mg/kg GENT) were significantly reduced (P<0.05) (0.99±0.18 & 0.50±0.28) compared to NF-κB levels in tissue homogenate of mice in LPS group (21.70± 2.50) as showed figure (). Furthermore, doubling the dose of GENT to 80 mg/kg showed no significant reduction (P>0.05) in NF-κB renal level compared to low dose 40mg/kg of GENT, and both doses showed extremely reduction in NF-κB level.

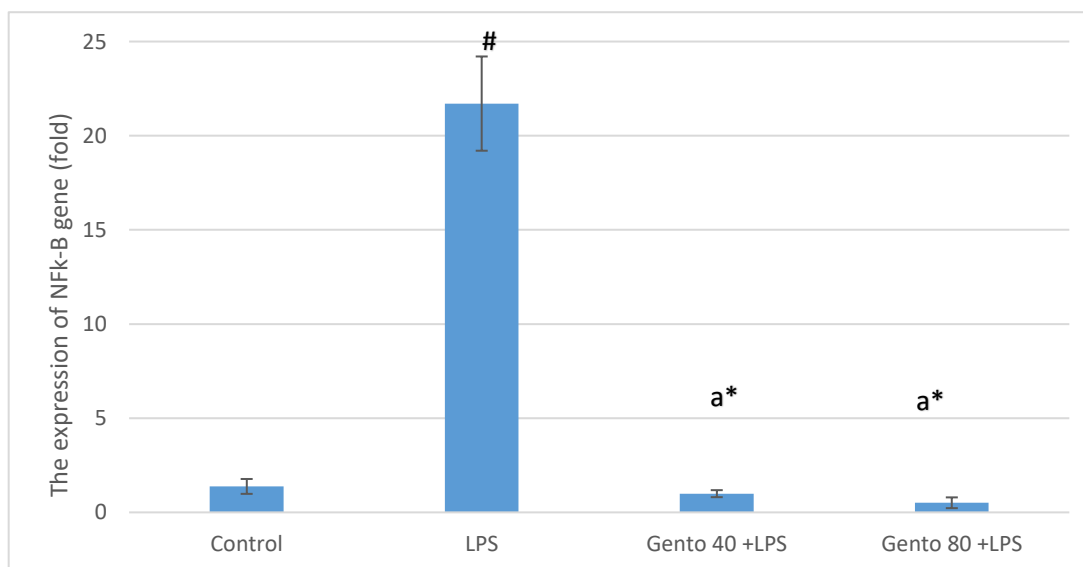


Fig.4: Effects of GENT on NF-κB gene expression level in renal tissue in LPS induced AKI .

Values are indicated as mean ± SEM. (n=8) for each group
 * denotes significant difference (P<0.05) vs LPS group.
 # denotes significant difference (P<0.05) vs control group (GPI).
 (a& b) non -identical small letters denotes significant difference (P<0.05) between treatment groups

Effect of gentiopicroside on activator protein-1 (AP-1) in LPS induced AKI model

LPS injection also increased the expression of transcription factor AP-1 mRNA level in renal tissue compared to negative control group (18.16 ±1.98 vs 1.41±0.47). The administration of GENT (40 mg/kg (Group III) & 80mg/kg (Group IV) revealed a significant attenuation in AP-1 mRNA expression when compared to LPS group [(2.01±0.40 & 0.84±0.25) vs (LPS, 18.16 ±1.98)]. Additionally, significant difference (p<0.05) was demonstrated between (GENT 40mg/kg) and (GENT 80mg/kg) in mRNA level of AP-1 (2.01±0.40 vs 0.84±0.25) respectively.

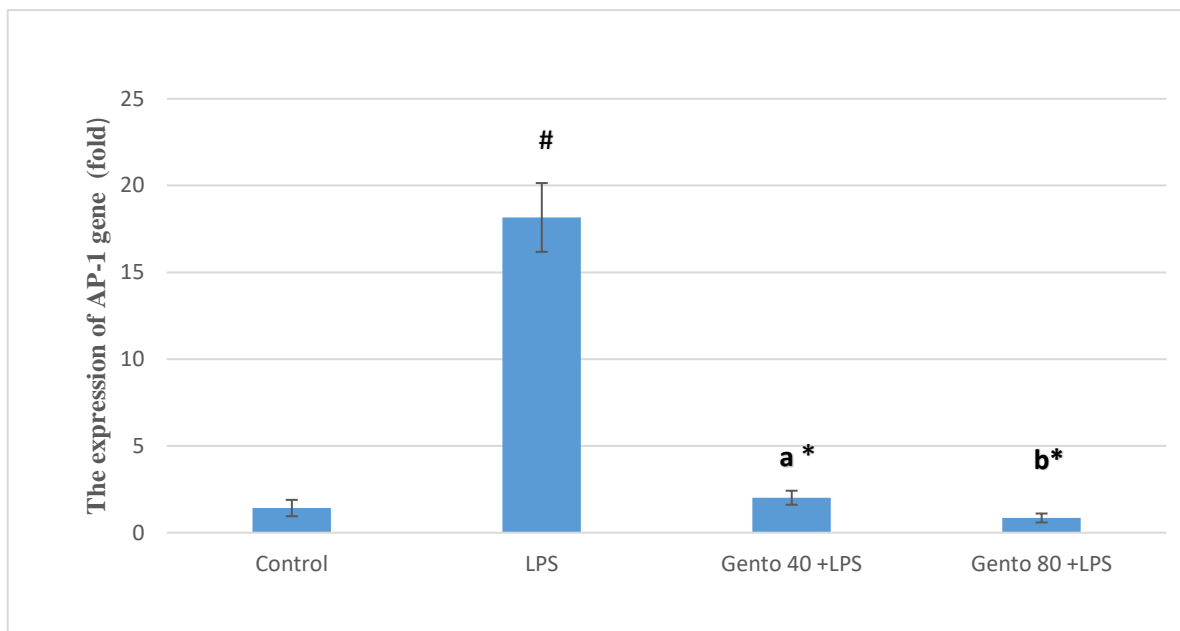


Fig.5: Effect of GENT on gene expression levels of AP-1 in kidney tissue in LPS induced AKI. Values are indicated as means ± SEM. (n=8) for each group.

* denotes significant difference (P<0.05) vs LPS group.
 # denotes significant difference (P<0.05) vs control group (GPI).
 (a, &b) non -identical small letters denotes significant difference (P<0.05) between treatment groups.

Effects of Gentiopicroside on TNF-α and interleukin-1β(IL-1β) levels of kidney tissue in LPS -induced AKI model

LPS injections also significantly increased TNF-α concentration and IL-1β gene expression compared to a

normal control group who received normal saline (p<0.05) (655.97±19.13 Vs 191.29 ±13.08) for TNF-α and (49.08±5.23 Vs 3.58±1.98) for IL-1β. GENT administration significantly reduced these increases (TNF-α: LPS, 655.97±19.13 ng/L vs. GENT 40mg, 229.96 ±19.73 ng/L and GENT 80 176.43±10.06 ng/L) (IL-1β: LPS, 49.08±5.23 vs. GENT 40mg 21.66 ± 2.17 and GENT 80mg 7.13±1.50 Figure A, B). Additionally, doubling the GENT dose to 80 mg/kg/day, revealed significant attenuation in (TNF-α) and (IL-1β) levels compared to low dose (40 mg/kg).

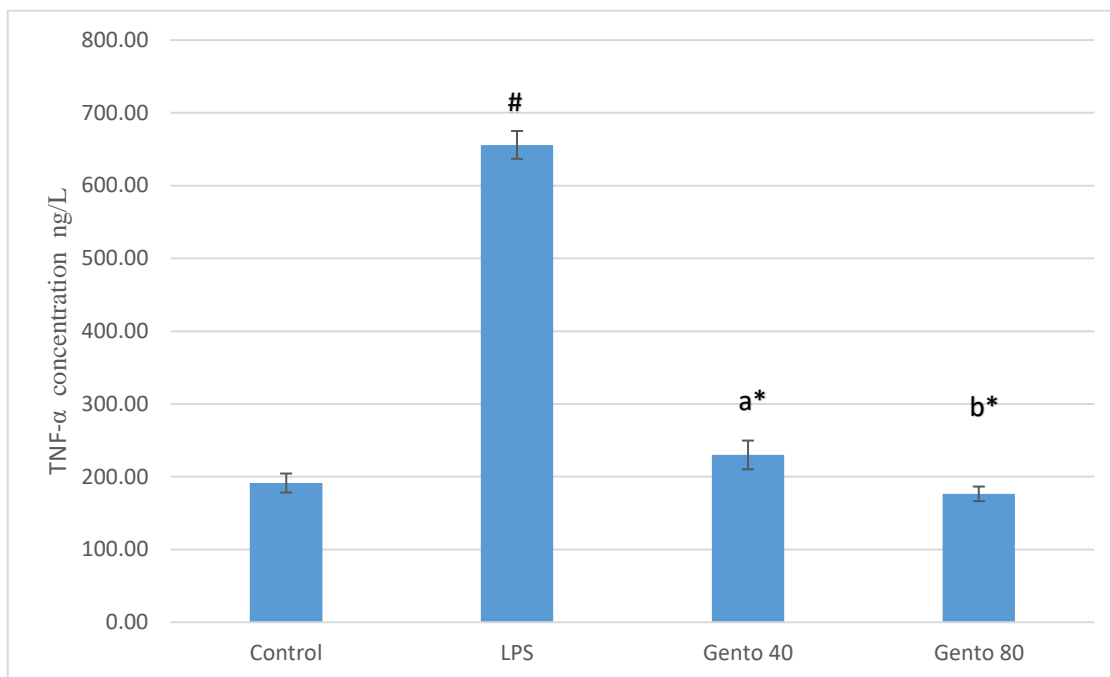


Fig.6A: Effects of GENT on TNF-α level in kidney tissue in AKI mice model.

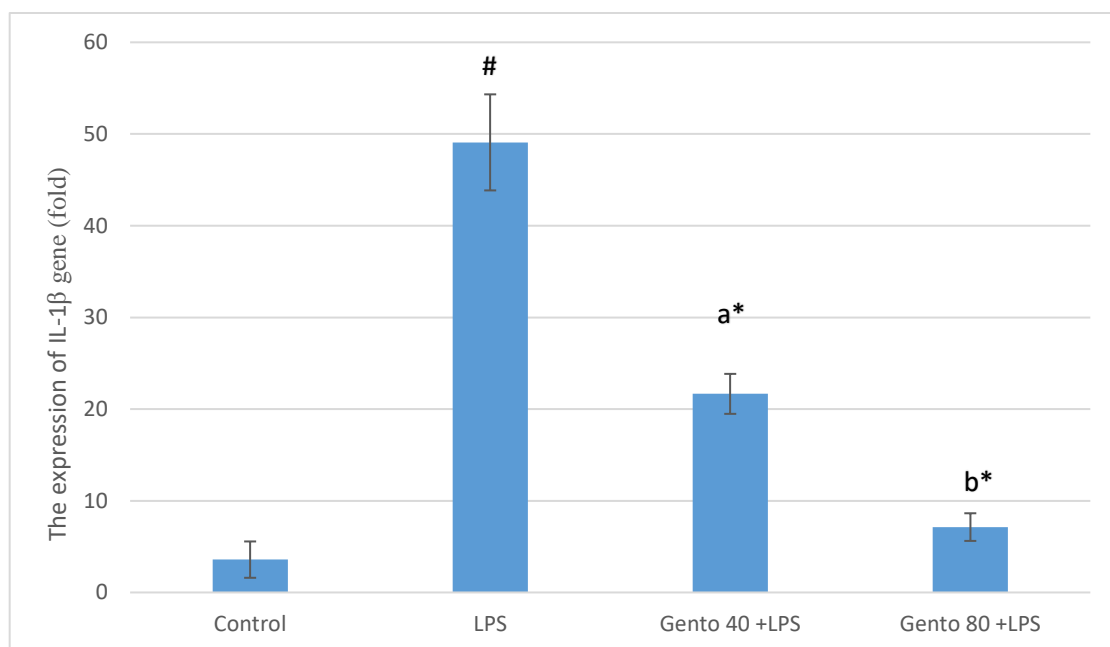


Fig.6B: Effects of GENT on IL-1β level in kidney tissue in AKI mice model.

Values are indicated as mean ± SEM. (n=8) for each group
 * denotes significant difference (P<0.05) vs LPS group.
 # denotes significant difference (P<0.05) vs control group (GPI).
 (a, &b) non -identical small letters denotes significant difference (P<0.05) between treatment groups

Effect of Gentiopicroside on Interferon Regulatory Factor 3 (IRF3) in kidney tissue of LPS induced AKI model

Our results revealed that administration of LPS in group II resulted in a significant increase in renal IRF3 levels as compared to the negative control group (P<0.05) (37.14 ±2.99 vs 2.10 ± 0.84) respectively. Besides, the pretreatment with 40 mg/kg and 80 mg/kg GENT exhibited significant attenuation in IRF3 level comparing with LPS group ((6.40±0.57& 3.69±0.3) vs (37.14 ±2.99) respectively. At the same time, no significant differences (P>0.05) was exhibited in the levels of IRF3 between the treatment groups. Hence the effect of GENT on IRF3 renal expression was dose-

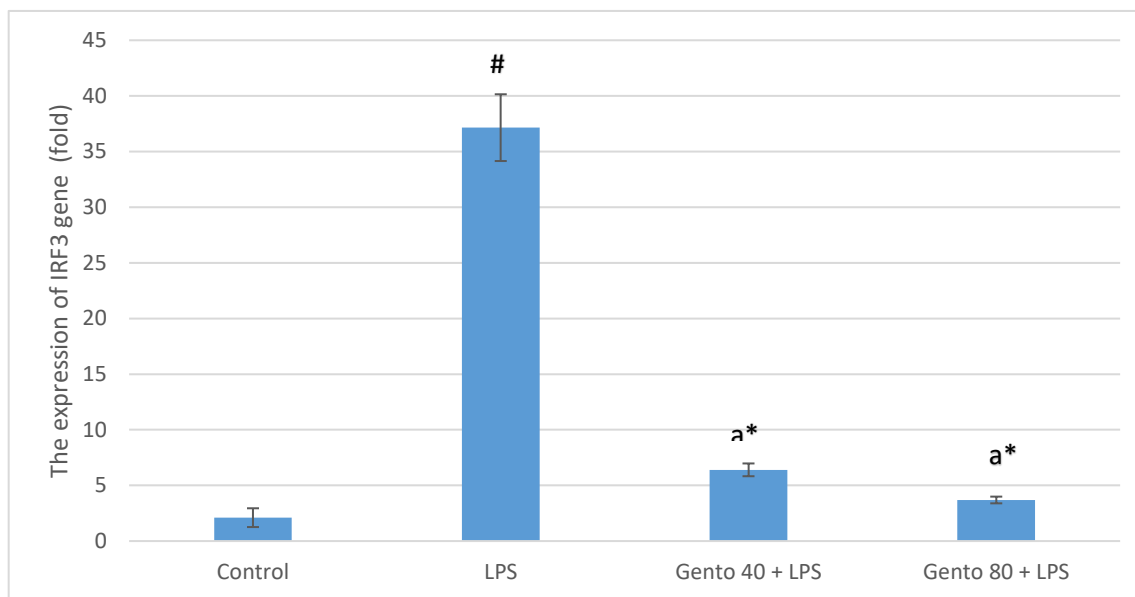


Fig.7: Effects of GENT on IRF3 gene expression in renal tissue in AKI mice model.

Values are indicated as mean \pm SEM. (n=8) for each group
 * denotes significant difference (P<0.05) vs LPS group.
 # denotes significant difference (P<0.05) vs control group (GPI).
 (a, &b) non -identical small letters denotes significant difference (P<0.05) between treatment groups.

Effect of gentiopicroside on inducible nitric oxide synthase (iNOS) in LPS induced AKI model

The Data presented in figure (11) showed a significant elevation (P<0.05) in iNOS gene expression in renal tissue after 24 hours from LPS administration as compared with

the control group (40.87 \pm 5.59 vs 2.23 \pm 1.00). Interestingly the pretreatment with GENT. at a dose of (40 mg/kg) produced a significant reduction (P<0.05) in iNOS gene expression compared with the LPS model group (9.73 \pm 1.85 vs 40.87 \pm 5.59). Furthermore, multiplication the dose of GENT to 80 mg/kg mg/kg exhibited significant reduction (P<0.05) in iNOS levels as compared to the LPS model group (3.98 \pm 1.44 vs 40.87 \pm 5.59) respectively. Moreover, the results exhibited that, there was significant variation (P<0.05) in iNOS mRNA level between the low and high dose of GENT administered to mice and the effect is dose - dependent.

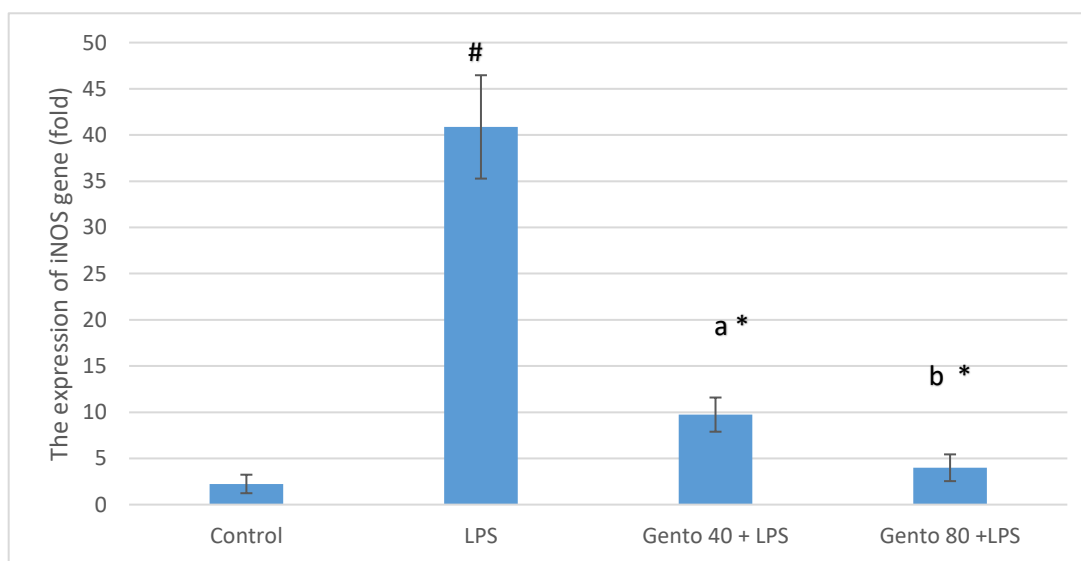


Fig.8: Effects of GENT on iNOS mRNA level in renal tissue in LPS induced AKI mice model.

Values are indicated as mean \pm SEM. (n=8) for each group
 * denotes significant difference (P<0.05) vs LPS group.
 # denotes significant difference (P<0.05) vs control group (GPI).
 (a, &b) non -identical small letters denotes significant difference (P<0.05) between treatment groups.

DISCUSSION

Patients with sepsis-associated AKI have a high hospital mortality rate, and LPS is one of the most prevalent causes of this condition (22). Therefore, there is an urgent need for therapeutic medicines that can prevent the progression of LPS-induced SA-AK.

In present study, the injection of 10 mg /kg LPS induced renal dysfunction by increasing serum urea and creatinine and induced renal pathological changes, observed as a renal tubular cells sloughing, loss of the brush border, and tubular dilation in the cortex which was consistent with previous studies(23). We found that treatment of GENT 40 and 80 mg/kg/day ameliorated LPS-induced kidney injury, as revealed by a considerable reduction in blood urea and creatinine levels and an improvement in the tubular injury score. Kidney injury molecule-1 (KIM-1) is a promising kidney injury biomarker. At an early stage of AKI, its expression in proximal renal tubular epithelial cells is greatly raised(24). The pretreatment with 40 and 80mg/kg GENT revealed significant attenuation in KIM-1 gene expression compared to LPS group. Altogether, these results indicate that GENT has a protective effect against LPS-induced functional and structural injury. To investigate the anti-inflammatory mechanism of GENT, its effects on TLR4 signaling pathway was studied.

TLR4 receptor on renal tubular epithelial cells, can recognize circulating pathogens and related chemicals, such as lipopolysaccharide (LPS), at the onset of sepsis. Activation of MyD88-dependent signaling induces the phosphorylation of MAPKs and IKK, resulting in the activation of NF-B and AP-1 transcription factors. Upon activation of the MyD88-independent pathway, the complex is activated, resulting nuclear localization of IRF3, thereby initiating the type I IFN response and driving late-phase NF-B activation (25)

Consistent with findings from a previous study, the current study demonstrated that LPS significantly induced the gene expression of TLR4 and the subsequent activation of transcription factors NF-B, AP-1 and IRF3 in the renal tissue of mice models(26). Interestingly the administration of low and high dose of GENT revealed a significant reduction in TLR4, NF- κ B and AP-1 gene expression in renal tissue compared to model group. In present study the MyD88-independent pathway was also studied and the gene expression of IRF3 was measured in renal tissue homogenate in treated group. The results showed that mice received 40mg/kg and 80 mg/kg GENT were significantly attenuated the IRF3 mRNA level compared to LPS model. Transcription of NF-

κ B, AP-1 and IRF3 initiated the production of pro-inflammatory cytokines and effector cytokines such as TNF- α , IFN- γ , IL-1 β , which contributed to the pathogenesis of AKI(12)(27). Our results regarding proinflammatory cytokines revealed that GENT40 mg/kg and 80 mg/kg suppress TNF- and IL-1 significantly when compared to the LPS-injected group. Qiong et al. found that GENT prevented LPS/IFN- γ -induced inflammatory cytokines production by macrophages through the NF- κ B signaling pathway in vitro and diminished the mRNA level of IL-1 β , IL-6 and TNF α in lung tissue(28). Furthermore nitric oxide NO plays a pivotal role in tubular endothelial cells function (29). LPS stimulates the production of endogenous NO by activating an inducible NO synthase (iNOS)(31). Furthermore, a local induction of iNOS in the kidney, particularly in the medulla and proximal tubules, may be the cause of peroxynitrite-related tubular injury as a consequence of local formation of reactive oxygen and nitrogen species. (ROS and RNS) during systemic inflammation(32). However our current study revealed that GENT 40mg/kg and 80 mg/kg significantly decreased the renal tissue level of iNOS which providing evidence in restoring the tubular function and attenuation in ROS and RNS.

Besides, the results of our research showed that doubling the dose of GENT to 80mg/kg lead to additional measurable effect than 40 mg/kg which was statistically significant (P<0.05). Thus the effect of GENT on LPS induced AKI is dose dependent.

CONCLUSION

This is the first study to demonstrate that Gentiopicroside had a promising pharmacological action in the prevention of LPS-induced kidney injury in a mouse model through its potent reno-protective and anti-inflammatory effects. In this study, pretreatment with low and high doses of gentiopicroside improved renal pathological changes and inflammatory responses. These ameliorative effects may have been achieved by downregulating TLR4 (MyD88-dependent and -independent pathways), inhibiting early and late NF- κ B pathways, and thereby suppressing the production of inflammatory cytokines. These findings provide novel insights into a promising candidate for treating kidney damage caused by sepsis.

REFERENCES

- Vaara ST, Pettilä V, Kaukonen KM, Bendel S, Korhonen AM, Bellomo R, et al. The attributable mortality of acute kidney injury: A sequentially matched analysis. *Crit Care Med*. 2014;42(4):878–85.
- Dellepiane S, Marengo M, Cantaluppi V. Detrimental cross-talk between sepsis and acute kidney injury: New pathogenic mechanisms, early biomarkers and targeted therapies. *Crit Care [Internet]*. 2016;20(1). Available from: <http://dx.doi.org/10.1186/s13054-016-1219-3>
- Al-Rakhat IG, Al-Shawi NN. Effects of hydrochlorothiazide on tenofovir disoproxil fumarate-induced nephrotoxicity in rats. *Iraqi J Pharm Sci*. 2019;28(2):58–64.

- Mehta RL, Bouchard J, Soroko SB, Ikizler TA, Paganini EP, Chertow GM, et al. Sepsis as a cause and consequence of acute kidney injury: Program to Improve Care in Acute Renal Disease. *Intensive Care Med.* 2011;37(2):241–8.
- Thiele RH, Isbell JM, Rosner MH. AKI associated with cardiac surgery. *Clin J Am Soc Nephrol.* 2015;10(3):500–14.
- Li PKT, Burdmann EA, Mehta RL. Acute kidney injury: Global health alert. *Transplantation.* 2013;95(5):653–7.
- Stasi A, Intini A, Divella C, Franzin R, Montemurro E, Grandaliano G, et al. Emerging role of Lipopolysaccharide binding protein in sepsis-induced acute kidney injury. *Nephrol Dial Transplant.* 2017;32(1):24–31.
- Peerapornratana S, Manrique-Caballero CL, Gómez H, Kellum JA. Acute kidney injury from sepsis: current concepts, epidemiology, pathophysiology, prevention and treatment. *Kidney Int [Internet].* 2019;96(5):1083–99. Available from: <https://doi.org/10.1016/j.kint.2019.05.026>
- Moriyama K, Nishida O. Targeting cytokines, pathogen-associated molecular patterns, and damage-associated molecular patterns in sepsis via blood purification. *Int J Mol Sci.* 2021;22(16).
- Gabarin RS, Li M, Zimmel PA, Marshall JC, Li Y, Zhang H. Intracellular and Extracellular Lipopolysaccharide Signaling in Sepsis: Avenues for Novel Therapeutic Strategies. *J Innate Immun.* 2021;13(6):323–32.
- Sadiq AT, Zalzal MH. The Possible Protective Effect of Safranal on the Lipopolysaccharide-induced Acute Lung Injury in Mice. *inflammation (ie, drug overdose and Acute pancreatitis).* 2021;2:4..
- Vázquez-Carballo C, Guerrero-Hue M, García-Caballero C, Rayego-Mateos S, Opazo-Ríos L, Morgado-Pascual JL, et al. Toll-like receptors in acute kidney injury. *Int J Mol Sci.* 2021;22(2):1–27.
- Peerapornratana S, Manrique-Caballero CL, Gómez H, Kellum JA. Acute kidney injury from sepsis: current concepts, epidemiology, pathophysiology, prevention and treatment. *Kidney Int.* 2019;96(5):1083–99.
- D.F. G, J.M. E, M.J. K, B.G. C. Benchmarking the incidence and mortality of severe sepsis in the united states. *Crit Care Med.* 2013;41(5):1167–74.
- Newman DJ, Cragg GM. Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. *J Nat Prod.* 2020;83(3):770–803.
- Xiong K, Gao T, Zhang T, Wang Z, Han H. Simultaneous determination of gentiopicroside and its two active metabolites in rat plasma by LC–MS/MS and its application in pharmacokinetic studies. *J Chromatogr B Anal Technol Biomed Life Sci [Internet].* 2017;1065–1066:1–7. Available from: <http://dx.doi.org/10.1016/j.jchromb.2017.09.017>
- Choi RY, Nam SJ, Lee HI, Lee J, Leutou AS, Ri Ham J, et al. Gentiopicroside isolated from *Gentiana scabra* Bge. inhibits adipogenesis in 3T3-L1 cells and reduces body weight in diet-induced obese mice. *Bioorganic Med Chem Lett [Internet].* 2019;29(14):1699–704. Available from: <https://doi.org/10.1016/j.bmcl.2019.05.038>
- Wang Z, Tang S, Jin Y, Zhang Y, Hattori M, Zhang H, et al. Two main metabolites of gentiopicroside detected in rat plasma by LC-TOF-MS following 2,4-dinitrophenylhydrazine derivatization. *J Pharm Biomed Anal [Internet].* 2015;107:1–6. Available from: <http://dx.doi.org/10.1016/j.jpba.2014.12.003>
- Wu S, Yang L, Sun W, Si L, Xiao S, Wang Q, et al. Design, synthesis and biological evaluation of gentiopicroside derivatives as potential antiviral inhibitors. *Eur J Med Chem [Internet].* 2017;130:308–19. Available from: <http://dx.doi.org/10.1016/j.ejmech.2017.02.028>
- Zhao L, Ye J, Wu GT, Peng XJ, Xia PF, Ren Y. Gentiopicroside prevents interleukin-1 beta induced inflammation response in rat articular chondrocyte. *J Ethnopharmacol [Internet].* 2015;172:100–7. Available from: <http://dx.doi.org/10.1016/j.jep.2015.06.031>
- Li J, Zhang Z, Wang L, Jiang L, Qin Z, Zhao Y, et al. Maresin 1 Attenuates Lipopolysaccharide-Induced Acute Kidney Injury via Inhibiting NOX4/ROS/NF- κ B Pathway. *Front Pharmacol.* 2021;12(December):1–15.
- Morrell ED, Kellum JA, Pastor-Soler NM, Hallows KR. Septic acute kidney injury: Molecular mechanisms and the importance of stratification and targeting therapy. *Crit Care.* 2014;18(5):1–10.
- Hu X, Zhou W, Wu S, Wang R, Luan Z, Geng X, et al. Tacrolimus alleviates LPS-induced AKI by inhibiting TLR4/MyD88/NF- κ B signalling in mice. *J Cell Mol Med.* 2022;26(2):507–14.
- Huang Y, Don-Wauchope AC. The clinical utility of kidney injury molecule 1 in the prediction, diagnosis and prognosis of acute kidney injury: A systematic review. *Inflamm Allergy - Drug Targets.* 2011;10(4):260–71.
- Marongiu L, Gornati L, Artuso I, Zanoni I, Granucci F. Below the surface: The inner lives of TLR4 and TLR9. *J Leukoc Biol.* 2019;106(1):147–60.
- Tögel F, Westenfelder C. Recent advances in the understanding of acute kidney injury. *F1000Prime Rep.* 2014;6(September):1–6.
- Zeng M, Li M, Zhang B, Li B, Kan Y, Zheng X, et al. Camellia oil inhibits oxidative stress and inflammatory response to ameliorate LPS-induced acute kidney injury via downregulation of TLR4-mediated activation of the NF- κ B/AP-1/IRF3 and NLRP3 pathways. *J Funct Foods [Internet].* 2020;68(November 2019):103908. Available from: <https://doi.org/10.1016/j.jff.2020.103908>
- Wang Q, Zhou X, Yang L, Luo M, Han L, Lu Y, et al. Gentiopicroside (GENT) protects against sepsis induced by lipopolysaccharide (LPS) through the NF- κ B signaling pathway. *Ann Transl Med.* 2019;7(23):731–731.
- Kathem SH, Mohieldin AM, Abdul-Majeed S, Ismail SH, Altaei QH, Alshimmari IK, et al. Ciliotherapy: A novel intervention in polycystic kidney disease. *J Geriatr Cardiol.* 2014;11(1):63–73.
- Winkler MS, Kluge S, Holzmann M, Moritz E, Robbe L, Bauer A, et al. Markers of nitric oxide are associated with sepsis severity: An observational study. *Crit Care.* 2017;21(1):1–9.
- Feihl F, Waeber B, Liaudet L. Is nitric oxide overproduction the target of choice for the management of septic shock? *Pharmacol Ther.* 2001;91(3):179–213.
- Ratliff BB, Abdulmahdi W, Pawar R, Wolin MS. Oxidant mechanisms in renal injury and disease. *Antioxidants Redox Signal.* 2016;25(3):119–46.