Med. J. Cairo Univ., Vol. 93, No. 2, Accepted 23/3/2025
DOI: 10.22608/MJCU. 885-894, June 2025
www.medicaljournalofcairouniversity.net

The Effect of Therapeutic Low-Intensity Pulsed Ultrasound on the Modulation of Macrophage Phenotypes in Different Renal Diseases

SARAH A.A. GOUDA, M.D.[1]; BASMA E. ABOULHODA, M.D.[2]; OMAIMA MOHAMMED ABDELWAHED, M.D.[1]; HEND ABDALLAH, M.D.[2]; LAILA RASHED, M.D.[3]; MANSOUR A. ALGHAMDI, M.D.[4,5]; NIVIN SHARAWY, M.D.[1]; MIRA HANNA, M.D.[1] and TAREK SHOUKRY, M.D.[1]

The Department of Medical Physiology[1], Anatomy Department[2], Medical Biochemistry & Molecular Biology Department[3], Faculty of Medicine, Cairo University, Department of Anatomy[4], College of Medicine, King Khalid University, Abha, Saudi Arabia and Genomic & Personalized Medicine Unit[5], College of Medicine, King Khalid University, Abha, Saudi Arabia

Abstract

Background: Therapeutic low-intensity pulsed ultrasound (LIPUS) has been found to have anti-inflammatory effects in many diseases, attributed to its immune-modulating effects.

Aim of Study: This study aimed to examine thepotential anti-inflammatory impact of LIPUS through modulation of macrophages' plasticityin different acute kidney injury (AKI) models.

Material and Methods: The study was conducted on 90 rats (250-300 gm body weight) which were divided into 3 groups: (a) Control group; (b) Untreated group including subgroups of different AKIs (Rhabdomyolysis, lipopolys accharide, and ischemia-reperfusion-induced AKI); and (c) AKI-treated with LIPUS group which was subdivided according to the previously mentioned models. Renal functions, levels of cytokines (IL-1β, TNF-α, and IL-10), Macrophage -related markers (M1) (iNOS and CD38), and M2-related markers [arginase-1 (Arg-1), CD206, and FIZZ-1] expressions were assessed in renal tissues using Enzyme-Linked Immunosorbent Assay (ELISA) and real-time reverse-transcriptase-polymerase chain reaction (RT-PCR) respectively, in addition to histopathological examinations of renal tissue.

Results: The study reported the ability of LIPUS to switch macrophages towards the M2 anti-inflammatory phenotype in different treated -AKI models with restoration of renal function and structure.

Conclusion: LIPUS has anti-inflammatory properties via modulating macrophage towards M2 phenotype which could be a protective approach in mitigation of different models AKI.

Key Words: Low-intensity pulsed ultrasound – Macrophage plasticity – Acute kidney injury.

Correspondence to: Dr. Nivin Sharawy,
E-Mail: nivinsharawi@gmail.com
nivin.sharawi@cu.edu.eg

Introduction

ACUTE kidney injury (AKI) is a global health issue with a high risk of developing chronic kidney disease (CKD) and renal failure. Despite progress in understanding renal diseases, there are no treatments for renal recovery and repair. Unresolved inflammation leads to chronic inflammation **[1,2].**

Pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) trigger pattern recognition receptors and transcription factor activity, triggering pro-inflammatory or anti-inflammatory pathways. In kidney injury, macrophages engulf DNA, activating Absent in melanoma 2 (AIM2) and enhancing the switch from anti-inflammatory to pro-inflammatory microenvironment with subsequent formation of extracellular matrix and induction of fibrosis **[1-3].** Thus, a potential therapeutic addition in nephrology medicine is to target inflammation, particularly macrophages.

Despite the identification of anti-inflammatory therapies targeting immune cells, definitive evidence linking targeting immune cells to slowing renal injury progression and chronic kidney disease development remains inconclusive. Ultrasound waves have been tested in pre-clinical studies and have shown a positive effect on renal protection through the regulation of pathophysiological processes, including inflammation **[4-7].** Low-intensity ultrasound waves that have little thermal effect have not been defined yet, but the most frequently used parameters are spatial and temporal average intensity between 0.03 and 1 W/cm^2. The effects of ultrasound therapy are

likely to be mediated by mechanosensing proteins [such as Piezo Type Mechanosensitive Ion Channel Component 1 (PIEZO1)] involved in the regulation of transcription factors. Because of its non-invasive nature, targeting ability, mechanical potency, and lack of hepatic and kidney burden, ultrasound waves appear to be the perfect treatment for renal inflammatory disorders **[8]**.

Renal inflammation necessitates the production of sufficient immune cells to combat damage or infection and replenish those that have been disrupted by the inflammatory challenge. In terms of immune modulation therapy, macrophages, neutrophils, and dendritic cells (DCs) are attractive candidates because of their high level of plasticity and diversity. Their biological functions are diverse and they can either enhance tissue repair or induce tissue destruction by either inhibiting or promoting the inflammatory response **[9-12]**.

Among the two major subsets of macrophages, M1 is the pro-inflammatory subset, but M2 is the tissue-repairing subset **[9]**. In the early stages of inflammation, M1 is primarily regulated by toll-like receptor ligation and interferon (IFN)-γ, but M2 differentiation is influenced by interleukin (IL)-4 and IL-13 and is skewed in the later stages of renal injury. M2 macrophages promote tissue healing by releasing GM-CSF, intrinsic IL-10, colony-stimulating factor 1 (CSF1), and IL-1 receptor-associated kinase-M (IRAK-M) **[12]**.

Aim of the Work: The aim of this study was to estimate the potential modulating effects of ultrasound therapy on macrophage polarization, renal function, and structure in different models of acute kidney injury.

Material and Methods

Animals:

Following ethical approval of Institutional Animal Care Committee, Cairo, Egypt (CU III F 14 22) for the use of animals, 90 rats (250-330gm) werebought and housed in 27 × 38 × 17 cm cages with chip beds at Cairo University's Faculty of Medicine's animal house (from May 2022 till May 2024). All of the animals were conditioned for one week at room temperature with a typical day-night cycle prior to the experiment. Additionally, they had free access to water and chow for the duration of the experiment.

General protocol:

Following seven days of acclimatization, 90 rats were divided into 9 groups (n=10) at random: (a) Control group; (b) Untreated acute kidney injury; and (c) Acute kidney injury treated with LIPUS. Each group was further subdivided into three equal subgroups. The control group of rats (group a), which is neither exposed to disease nor LIPUS treatment, was sub-divided into group a.1 Rhabdomyolysis-control (R-Control): Rats were injected only with a normal saline intramuscular, group a. 2 Lipopolysaccharide-control (LPS-Control): Rats were injected only with a saline intraperitoneal, and group a.3 Ischemic-reperfusion control group (IRI-Control: Rats were subjected to sham surgery in which the kidney was exposed but without clamping of the renal artery. Rats in the untreated acute kidney injury group (group b) were sub-divided into group b.1 Rhabdomyolysis-induced acute kidney injury group b.2 Lipopolysaccharide-induced acute kidney injury and group b.3 ischemic-reperfusion acute kidney injury group c include the treated AKI group in which it was subdivided into group c.1 treated R-AKI, group c.2. treated LPS-AKI and group c.3 IRI-AKI.

Induction of AKI models:

Rhabdomyolysis induced AKI:

It was induced by intramuscular injection of 50% glycerol (8 ml/kg B.W.) diluted in a 0.9% normal saline solution and equally divided in both hind limbs **[13]**, and were sacrificed 4 days after injection.

Lipopolys accharide-induced AKI:

A single dosage of lipopolys accharide (LPS) from E. coli strain 055:B5 (5mg/kg) was administered intraperitoneally (i.p.) to rats **[14,15]** and were sacrificed 4 days later.

Ischemia reperfusion-induced AKI:

Rats were anesthetized with an intraperitoneal mixture of ketamine (80mg/kg) and xylazine (16mg/kg). The animal was placed on a heating pad and the body temperature was monitored throughout the procedure. Following depilation and disinfection, the abdomen was opened with a midline incision and the left kidney was exposed.The renal pedicle was carefully dissected and the blood vessels for the renal pedicle were clamped. Successful ischemia was characterized by a color change of the kidney from red to dark purple. The vascular clip was released at 45 minutes to start reperfusion. The abdomen was closed after verification of kidney color to change back to red. Thereafter, rats were observed and sacrificed on day 4 **[16]**.

Protocol for low-intensity pulsed ultrasound (LIPUS):

Rats were anesthetized intraperitoneally with a ketamine (90mg/kg) and xylazine (9mg/kg) mixture

prior to ultrasonic exposure. A depilatory was used to shave off the fur. For the application of LIPUS, preheated ultrasonic gel was applied to the depilated skin. A rectal thermometer and a heating pad were used to keep the rats' body temperature at 37°C. LIPUS was performed using the Evo Touch ultrasound machine, with a 15L8w transducer (Quantel Medical, Paris, France). The left kidney was located in real time using standard B-mode imaging at a frequency of 14 MHz and an on-screen imaging mechanical index of 0.99. The two main determinants of the ultrasonic wave's energy are intensity and mechanical index. We used an ultrasonic wave with a spatial average and a temporal average (SATA; the average intensities over the cross-sectional area of an ultrasound beam and the pulse repetition period, respectively) to produce low-intensity (energy) pulsed ultrasound. Rats in LIPUS-treated acute kidney injury were divided into c.1 LIPUS-Rhabdomyolysis-AKI (LIPUS-R-AKI), c.2 LIPUS-lipopolysaccharide-AKI (LIPUS-LPS-AKI), and c.3 LIPUS-ischemic reperfusion-AKI (LIPUS-IRI-AKI). Rats in each subgroup received LIPUS24 hours before the induction of acute kidney injury (administered at an intensity of 3W/cm^2; 80% duty cycle; 7 MHz frequency, and bursting mechanical index 1.2 for 2s, repeated every 6s for 2min for a total exposure time of 20s per treated animal). The control animals did not receive LIPUS, although they underwent the identical preparatory methods ***[17]***.

Assessment of kidney functions:

At the end of the experiment, serum, urine, and renal samples were collected. Serum and urine samples were tested by colorimetric for creatinine and blood urea nitrogen (BUN) levels 24 hours post- R-AKI and LPS-AKI, and 4 days post-IRI-AKI. Creatinine clearance was estimated using the following formula. Ccr (ml/min/kg) = [urinary Cr (mg/dl)X urine volume (ml)/serum Cr (mg/dl)] X [1000/body weight(g)] X [1/1440(min)] ***[18]***.

Enzyme-linked immunosorbent assay (ELISA):

In accordance with the manufacturer's instructions, cytokines levels, includeIL-1β, TNF-α, and IL-10 were measured in the kidney using ELISA kits developed specifically for rats (Solarbio, Beijing, China). Kidney samples were stored at –80°C, thawed, homogenized, and centrifuged at 10000g for 90min. The supernatant was used to measure the levels of cytokines (IL-1β, TNF-α, and IL-10).

Real-time polymerase chain reaction (RT PCR):

A quantitative real-time polymerase chain reaction was used to measure the expression of genes. The pro- and anti-inflammatory macrophage phenotype was measured by Applied Biosystem using software version 3.1 (StepOneTM, USA) and SYBR Green I. M1 expression markers (iNOS and CD38) and M2 expression markers (arginase-1 (Arg-1), CD206, and FIZZ-1) were measured in renal tissues, respectively. The real-time PCR workflow for gene expression analysis includes RNA isolation, reverse transcription, real-time PCR assay creation, and data processing. The PCR primer pair sequences for each gene are shown in Table (1).

Table (1): The primer sequences for real-time polymerase chain reaction (real-time PCR).

GENE	PRIMER SEQUENCES (5'-3')
CD 38	Forward 5'-TGTAAAACGACGGCCAGT-3'
	Reverse 5'-CAGGAAACAGCTATGACC-3'
INOS	Forward 5'-ACCCAAGGTCTACGTTCAGG-3'
	Reverse 5'-CGCACATCTCCGCAAATGTA-3'
FIZZ 1	Forward 5'-ATGAACAGATGGGCCTCCTG-3'
	Reverse 5'-CCAAGATCCACAGGCAAAG-3'
CD206	Forward 5'-TCTTTGCCTTTCCCAGTCTCC-3'
	Reverse 5'-TGACACCCAGCGGAATTTC-3'
ARG-1	Forward 5'-ACAAGACAGGGCTCCTTTCA-3'
	Reverse 5'-AGCAAGCCAAGGTTAAAGCC-3'

Histopathological Examinations:

Haematoxylin and eosin were used to stain the kidney slices after they were embedded in paraffin wax, fixed in 10% formalin, and cut into 5-mm sections. The tissues underwent light microscopy evaluation. A pathologist who was not aware of the experimental protocol conducted the histopathological assessment to determine the extent of renal tubular injury.

Statistical analysis:

The results were analyzed using Prism 5 (GraphPad Software, La Jolla, CA, USA), and the Kolmogorov-Smirnov test was employed to ascertain whether the data was normally distributed. If a normal distribution was found, one-way analysis of variance (ANOVA) was applied. If significant differences were found, a post hoc analysis using the Newman-Keuls multiple comparison tests was conducted. The statistical significance was established at a two-tailed *p*-value of 0.05. Means and standard deviations of the mean (SD) are used to express the results.

Results

Low-intensity pulsed ultrasound restored the renal function and structure after AKI:

In comparison to the control group, it was found that renal function was significantly impaired after

different types of AKIs. This effect was significantly ameliorated in rats treated with LIPUS 24h before induction of AKI (Fig. 1). Histological assessment of kidney tissue from all AKI rats revealed an increase in indices of acute tubular necrosis (ATN), such as necrotic cells in the tubular lumen, loss of the tubular cells brush border, and dilated tubular lumen, compared to controls. All previous indices were reduced when AKI rats were treated with LIPUS 24hrs before AKI (Fig. 2).

Low-intensity pulsed ultrasound mitigated M1 macrophages expression and its proinflammatory cytokines after AKI:

The expressions of the pro-inflammatory macrophage (M1) markers (CD 38 and iNOS) and the renal levels of its related pro-inflammatory (IL-1β, and TNF-α) cytokineswere dramatically increased in all injured kidney models. Administration of LIPUS 24 hours before kidney injury in different AKI groups impeded both pro-inflammatory macrophage (M1)-related markers expression and IL-1β, and TNF-α levels in renal tissues in the different models of AKI (Fig. 3).

Low-intensity pulsed ultrasound switched macrophages towards M2 polarization in AKI treated groups:

The treatment with LIPUS 24 hours before induction of AKI facilitated M2 macrophage polarization, as was observed by the significant increase in the anti-inflammatory (M2) markers (Arg-1, CD206, and FIZZ-1) as well as IL-10 levels in renal tissues of the treated groups (Fig. 4).

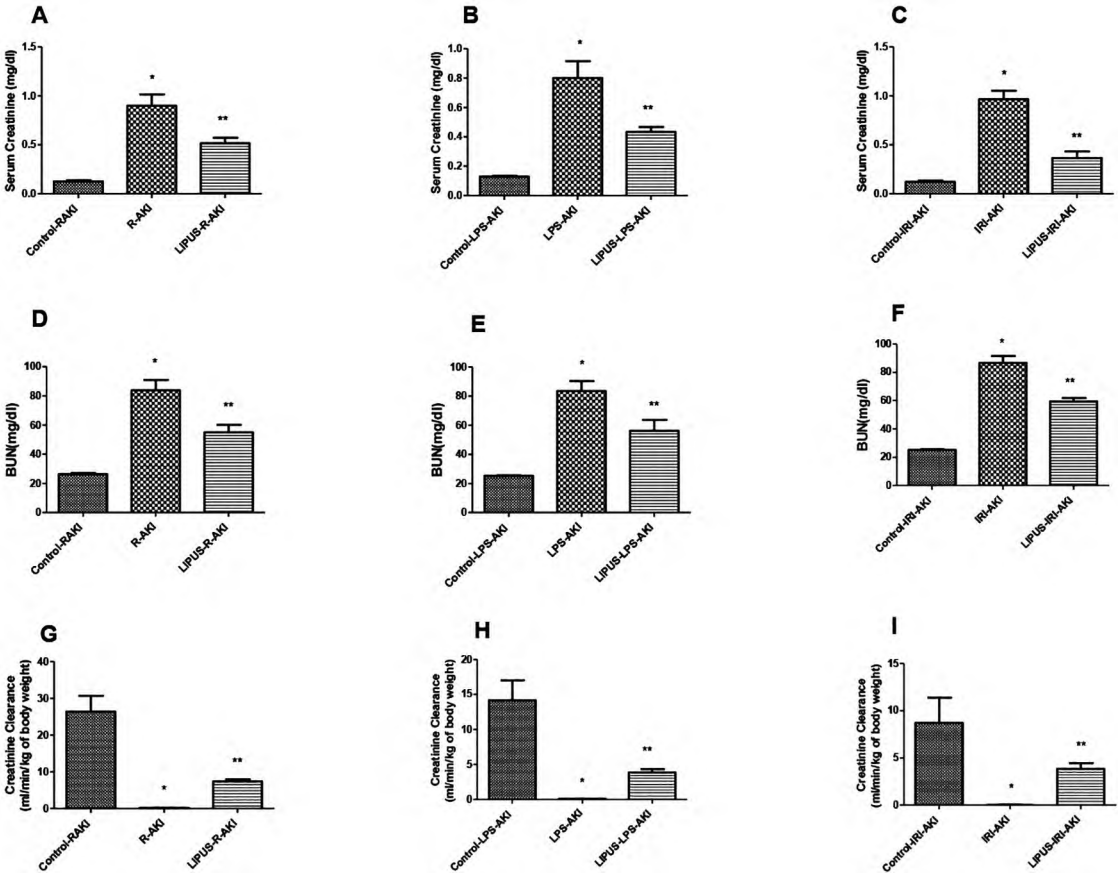

Fig. (1): Low-intensity pulsed ultrasound restored the renal functions after AKI.

(A-I) Representative colorimetric assessment of Creatinine (A-C), blood urea nitrogen (BUN) (D-F), and estimation of creatinine clearance (Ccr) (G-I), following low-intensity pulsed ultrasound therapy (LIPUS) in rhabdomyolysis (R-AKI), lipopolysaccharides (LPS-AKI), and ischemic reperfusion (IRI-AKI) renal injury models. Data are presented as the mean ± SD (n=10 rats/group). *p<0.05 vs corresponding controls, **p<0.05 vs corresponding AKI groups.

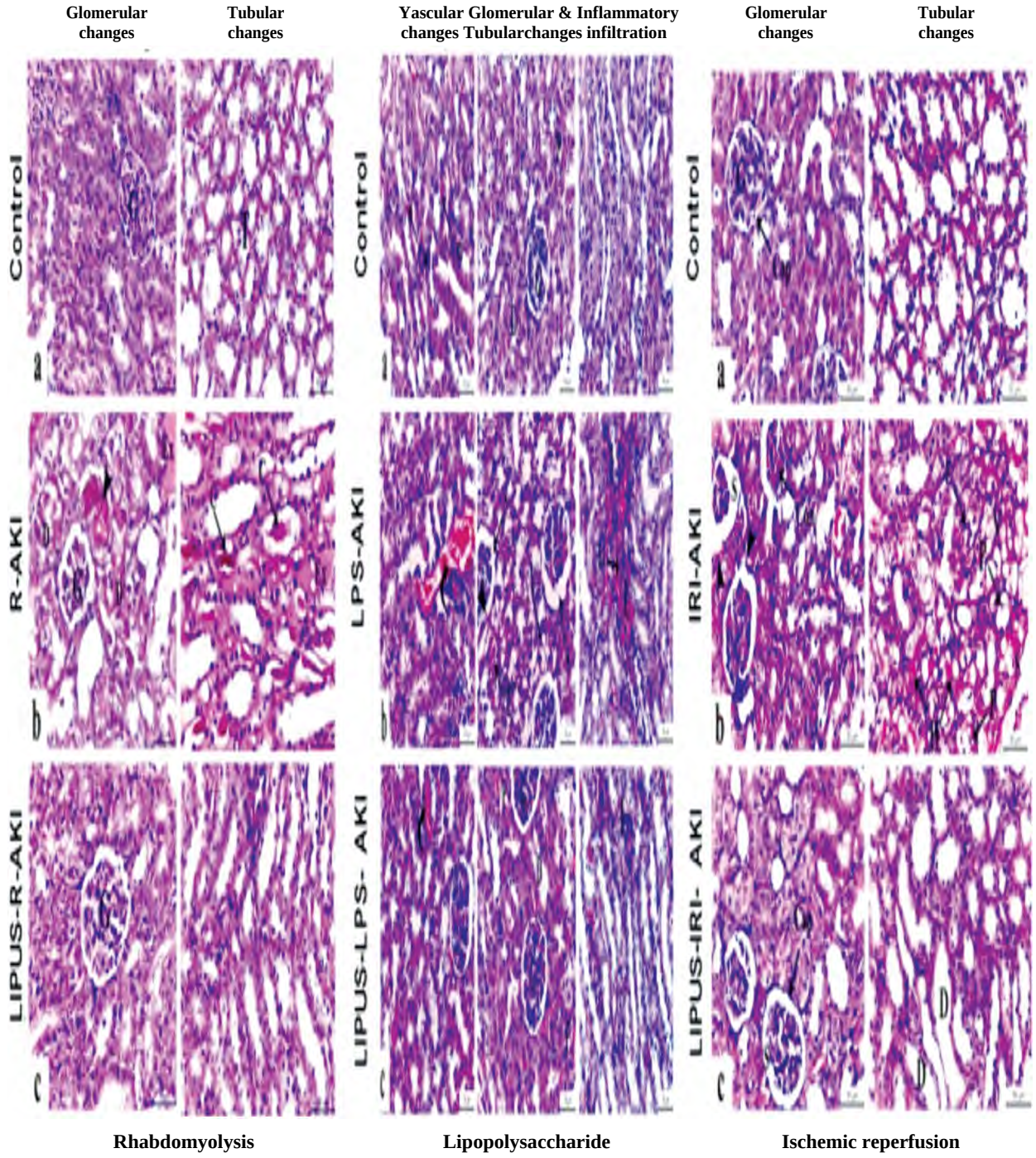

Fig. (2): Low-intensity pulsed ultrasound restored the renal structure after AKI.

Representative of hematoxylin and eosin-stained sections of the kidney. (a) Control groups displayed normal histological structure of glomeruli (G) and tubules (T), (b) Acute kidney injury (AKI) groups displayed degeneration of some tubules (D) and others appear distorted with intra-luminar casts (C) in rhabdomyolysis model (R-AKI). In the Lipopolysaccharide (LPS-AKI) group, a marked dilatation and congestion of the renal cortical blood vessels (curved arrow) was observed. The renal glomeruli show shrunken glomerular capillaries with retraction of the capillary tuft and widening of the capsular space (S). The Bowman's capsule appears disrupted and irregular (arrow head). The tubules are distorted with cytoplasmic vacuoles (V) in their epithelial lining and cellular casts (C) in their lumina. Inflammatory cellular infiltration (I) in the vicinity of interstitial exudate (Ex) and blood extravasation can also be observed. In ischemia-reperfusion (IRI-AKI) group displaying shrinkage of the renal glomeruli with occasional glomerulosclerosis, widening of the Bowman's space (S) and discontinuity of the Bowman's capsule (arrow heads). Some glomeruli show marked congestion of their capillaries (Con). The tubules show marked hyaline degeneration (H) with their epithelium displaying highly-vacuolated cytoplasm (V) and numerous pyknotic nuclei (P). The tubular lumina are filled with hyaline casts and epithelial casts (E) from the exfoliated tubular cells. (c) The Low intensity pulsed ultrasound (LIPUS) -treated groups shows restoration of the normal glomerular and tubular architecture with minimal vascular dilatation and congestion (curved arrow). The glomeruli show intact capillary tuft with normal urinary space between the parietal and visceral layers of the Bowman's capsule. In between the renal corpuscles, the proximal convoluted tubules (P) are seen lined by pyramidal cells, showing narrow lumen, acidophilic granular cytoplasm, apical brush border, and rounded vesicular nuclei. The distal convoluted tubules (D) display a wide lumen and are seen lined by cuboidal cells with rounded nuclei and acidophilic cytoplasm. Very few inflammatory cells (I) were observed.

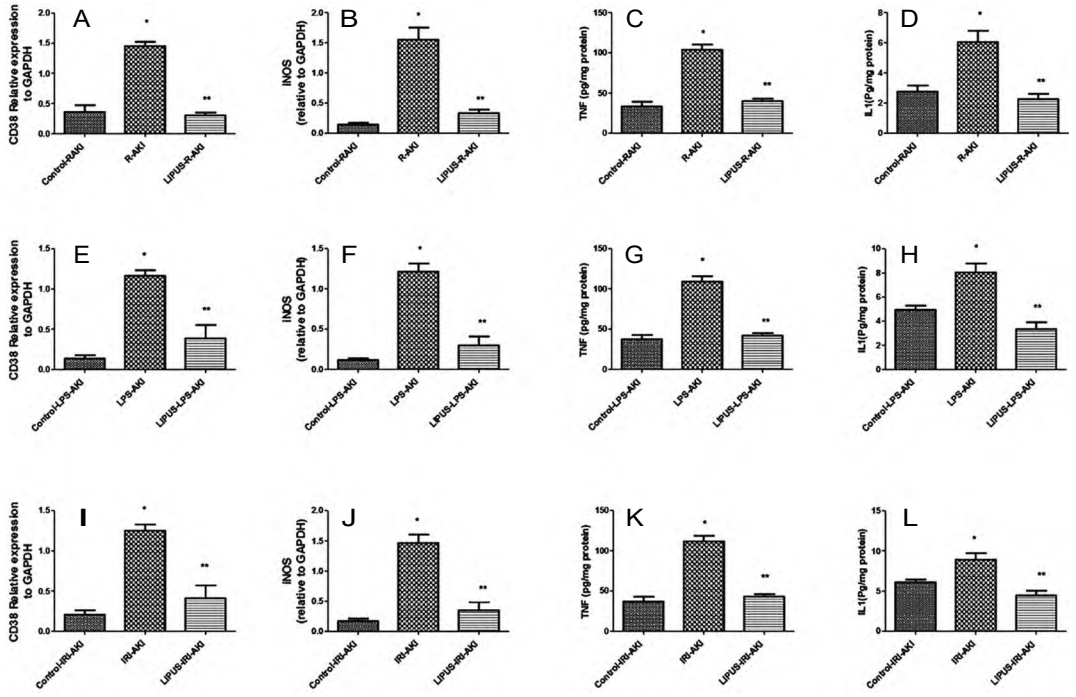

Fig. (3 A-L): Low-intensity pulsed ultrasound mitigated M1 macrophages expression and its proinflammatory cytokines after AKI.

(A-L) Representative qRT-PCR analysis of pro-inflammatory M1-related markers (CD38 (A, E, I), inducible nitric oxide synthase (iNOS) (B, F, J), and Enzyme-linked immunosorbent quantifications oftumor necrotic factor alpha (TNF-α,) (C, G, K), and Interleukin-1β, (IL-1β,) (D, H, L)) in renal tissues following low-intensity pulsed ultrasound therapy (LIPUS) in rhabdomyolysis (R-AKI), lipopolysaccharides (LPS-AKI), and ischemic reperfusion (IRI-AKI) renal injury models. Data are presented as the mean ± SD (n=10 rats/group).p*<0.05 vs corresponding controls, **p<0.05 vs corresponding AKI groups.

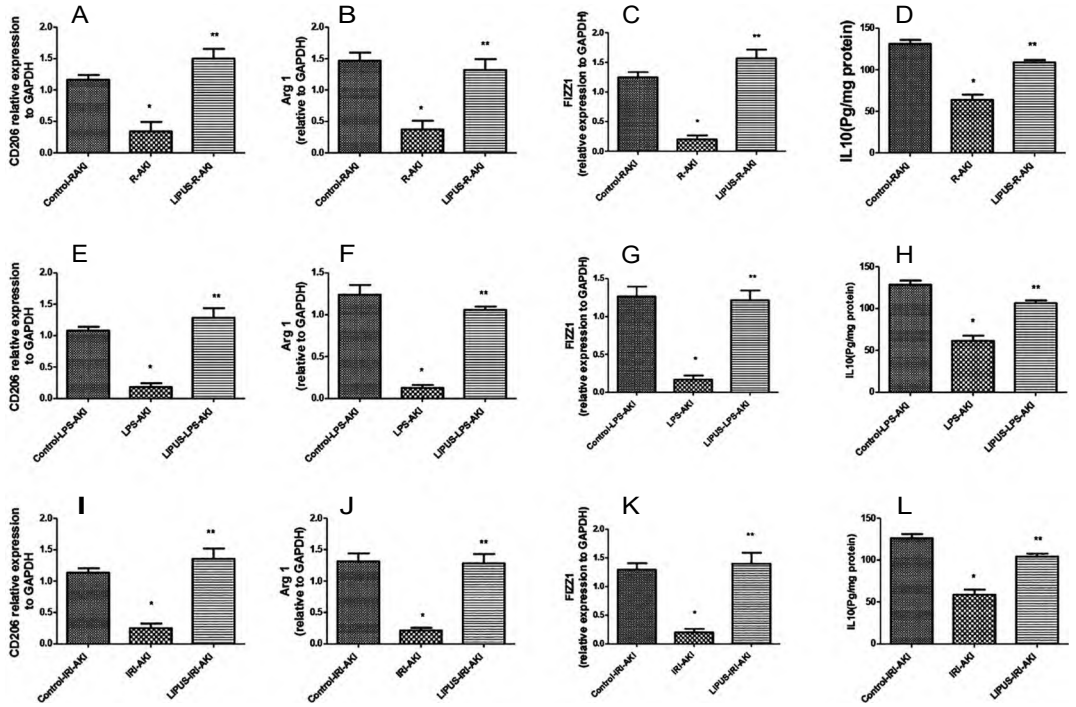

Fig. (4 A-L): Low-intensity pulsed ultrasound switched macrophages towards M2 polarization in AKI-treated groups.

(A-L) representative qRT-PCR evaluation of M2 anti-inflammatory macrophages-related markers (CD206 (A, E, I), arginase-1 (Arg-1) (B, F, J), FIZZ-1 (C, G, K), and Enzyme-linked immunosorbent quantifications of interleukin-10 (IL-10) (D, H, L)) in renal tissues following low-intensity pulsed ultrasound therapy (LIPUS) in rhabdomyolysis (R-AKI), lipopolysaccharides (LPS-AKI), and ischemic reperfusion (IRI-AKI) renal injury models. Data are presented as the mean ± SD (n=10 rats/group). *p<0.05 vs corresponding controls, **p<0.05 vs corresponding AKI group.

Discussion

Our results revealed that LIPUS administration before induction of AKI has a positive impact onrenal structure and function by reducing inflammationand modulation of macrophage polarization toward theanti-inflammatory phenotype (M2). The role of inflammation in acute and chronic renal disease is well-established [19]. Acute kidney injury dramatically affects the proximal tubule due to an initial insult and immune cellinfiltration, which primarily results in loss of cell cytoskeletal integrity and mislocalization of adhesion molecules [20]. Since it is necessary for healing and can also cause damage through differently released mediators (cytokines, eicosanoids, and reactive oxygen species), inflammation is a double-edged sword. In agreement with previous data, we observed that induction of AKI with different methods (LPS, ischemia-reperfusion injury, and rhabdomyolysis) is associated with the pro-inflammatory (M1) infiltration concomitant with renal function and structure deterioration [21-23].Regarding our study, the macrophages played a pivotal role in different models of AKI pathophysiology and deterioration of kidney functions and structure. Neutrophils and macrophages proliferate in the early hours following injury, interacting with interstitial cells and damaged kidney epithelium. Subsequently, tubular necrosis is exacerbated by the activation of lymphoid lineage immune cells, including T cells, B cells, and NK cells. T cells' predominant function plays a major part in the persistence of pro-inflammatory macrophage activation [24].

It is noteworthy that, in renal diseases, the activation of M1 macrophages is triggered by either TNF-α or interferon gamma (IFN-γ). This leads to the release of various inflammatory mediators, which, in turn. Trigger the initiation of tubular necrosis and the development of chronic renal disease. Renal protection linked to a decrease in M1 macrophages through genetic depletion or inhibition of monocyte chemoattractant protein (MCP-1)has confirmed the harmful role of M1 macrophages in renal disease. While M2 macrophage scavenging cellular debris aids in renal recovery, prolonged M1 activation may tilt the M1/M2 balance in favour of exacerbating inflammation [25].

There is growing interest in the role of immune cells and mediators in renal impairment, and different immune pathways (such as NF-κB) contribute to tubular necrosis and chronic renal failure [26]. An essential component of the inflammatory response is the transcription factor NF-κB. As a multitude of genes involved in inflammation, such as adhesion molecules, chemokines, and cytokines, are induced to be expressed when NF-κB is activated. This process facilitates the infiltration of immune cells into the renal tissue [27].

Research on ultrasound therapy for kidney disease has recently gained popularity resulting from the discovery of the immunomodulating mechanism of ultrasound [28]. In the current experimental study, LIUPS mitigated the inflammatory markers IL-1β, TNF-α, and IL-10 in different AKI disease models. According to previous studies, therapeutic ultrasound, either alone or in combination with other treatments, could change the biomechanical characteristics in different tissues to speed up the healing process as reported in nerve injury and bone fractures models [4-7].

In agreement with the current work, it was reported that ultrasound could dramatically affect the signaling pathways associated with inflammation (most notably NF-κB) which results in a reduction of NF-κB activation and inflammatory cytokines release (primarily of IL-1β, TNF-α, and IL-6) [29-32]. The authors added that ultrasound was reported to modulate the immune cells (infiltration and polarization) in different models Previous studies found that ultrasound decreased macrophage infiltration which was reported by decreased expression of F480 and CD 68 [31,33,34], while other studies found that it decreased leukocyte, T cells, and dendritic cells infiltrations [17,29]. Moreover, following US therapy, neutrophils and lymphocytes were found to be reduced in the renal tissues of septic rats [30]. Furthermore, favourable results from the ultrasound were obtained when it was used as a therapeutic as well as a preventive tool [31]. Although there is increasing evidence that ultrasound has therapeutic benefits, more significant attempts are being undertaken to elucidate the signaling-modulating mechanisms of ultrasound. However, because cell activities and regulation systems differ, it was difficult to make definitive conclusions about the pathways that therapeutic ultrasound affected.

It is worth noting that, High-intensity (>3W/cm^2) therapeutic ultrasound treatments primarily take advantage of their thermal effect, whereas the efficacy of low-intensity (<3W/cm2) treatments is predominately driven by non-thermal effects, such as acoustic cavitation, microbubbles, and biological signaling [35]. The biological signaling effects of LIPUS were previously demonstrated in two distinct AKI and CKD models [30,34]. Meanwhile, HIPUS-induced microbubbling and cavitation have been observed in the cisplatin AKI model. In their studies, Ullah and Burks [36,37] focused on using

HIPUS to modulate the kidney's inflammatory environment and facilitate the delivery of pluripotent mesenchymal stem cells. A limited number of studies have demonstrated that the anti-inflammatory properties of ultrasound may stem from its impact on cholinergic anti-inflammatory pathways located in the spleen. Acute ultrasound activation of adrenergic receptors on CD4+ splenocytes results in the release of acetylcholine and the suppression of inflammation **[38]**.

Conclusion:

To reduce the risk of death and prevent the development of CKD, an anti-inflammatory therapy that reduces tubular necrosis would be ideal. Based on our data, ultrasound therapy could successfully reprogram macrophages from M1 proinflammatory to M2 anti-inflammatory phenotypes in all studied models of acute renal injury. Ultrasound has long been known to have anti-inflammatory properties; however, there is limited evidence to support its role in the modulation of immune cells. Further studies are needed to understand the mechanisms that enhance or restrict immune cell modulation, as well as to identify the types of immune cells involved in chronic renal diseases.

Acknowledgment:

This paper is based upon work supported by Science Technology & Innovation Funding Authority (STDF) under a grant (N°26276). We thank Jamal Mahmoud and Naglaa Hamdy for their contribution to this research. The authors are also grateful to Dr. Hazem Mahmoud Ali for his skilful technical assistance.

Conflict of interest:

All the authors declared no Conflict of interest.

Data Availability:

The data used to support the findings of this study are available from the corresponding author upon request.

References

1- FURMAN D., CAMPISI J., VERDIN E., CARRERA-BASTOS P., TARG S., FRANCESCHI C., FERRUCCI L., GILROY D.W., FASANO A., MILLER G.W., MILLER A.H., MANTOVANI A., WEYAND C.M., BARZILAI N., GORONZY J.J., RANDO T.A., EFFROS R.B., LUCIA A., KLEINSTREUER N. and SLAVICH G.M.: Chronic inflammation in the etiology of disease across the life span. Nat. Med., 25: 1822–32, 2019. https://doi.org/10.1038/S41591-019-0675-0.

2- SPEER T., DIMMELER S., SCHUNK S.J., FLISER D. and RIDKER P.M.: Targeting innate immunity-driven inflammation in CKD and cardiovascular disease. Nat. Rev. Nephrol., 18: 762–78, 2022. https://doi.org/10.1038/S41581-022-00621-9.

3- CHAI D., ZHANG Z., SHI S YUCHEN, QIU D., ZHANG C., WANG G., FANG L., LI H., TIAN H., LI H. and ZHENG J.: Absent in melanoma 2-mediating M1 macrophages facilitate tumor rejection in renal carcinoma. Transl Oncol., 14 (4), 101018, 2021. https://doi.org/10.1016/J.TRANON.2021.101018.

4- KUSUYAMA J., NAKAMURA T., OHNISHI T., EIRAKU N., NOGUCHI K. and MATSUGUCHI T.: Low-Intensity Pulsed Ultrasound (LIPUS) Promotes BMP9-Induced Osteogenesis and Suppresses Inflammatory Responses in Human Periodontal Ligament-Derived Stem Cells. J. Orthop. Trauma, 31 (7), 2017. https://doi.org/10.1097/01.BOT.0000520897.92470.70.

5- HARRISON A. and ALT V.: Low-intensity pulsed ultrasound (LIPUS) for stimulation of bone healing - A narrative review. Injury, 52 (Suppl 2): S91–6, 2021. https://doi.org/10.1016/J.INJURY.2021.05.002.

6- TANG L., LI N., JIAN W., KANG Y., YIN B., SUN S., GUO J., SUN L. and TA D.: Low-intensity pulsed ultrasound prevents muscle atrophy induced by type 1 diabetes in rats. Skelet Muscle, 7: 1-10, 2017. https://doi.org/10.1186/S13395-017-0145-7.

7- LI Z., YE K., YIN Y., ZHOU J., LI D., GAN Y., PENG D., ZHAO L., XIAO M., ZHOU Y., DAI Y. and TANG Y.: Low-intensity pulsed ultrasound ameliorates erectile dysfunction induced by bilateral cavernous nerve injury through enhancing Schwann cell-mediated cavernous nerve regeneration. Andrology, 11 (6): 1188-1202, 2023. https://doi.org/10.1111/ANDR.13406.

8- LI X., ZHONG Y., ZHANG L. and XIE M.: Recent advances in the molecular mechanisms of low-intensity pulsed ultrasound against inflammation. J. Mol. Med. (Berl)., 101 (4): 361-374, 2023. https://doi.org/10.1007/S00109-023-02302-X.

9- PATEL A.A., GINHOUX F. and YONA S.: Monocytes, macrophages, dendritic cells and neutrophils: An update on lifespan kinetics in health and disease. Immunology, 163 (3): 250-261, 2021. https://doi.org/10.1111/IMM.13320.

10- RAO X., ZHONG J. and SUN Q.: The heterogenic properties of monocytes/macrophages and neutrophils in inflammatory response in diabetes. Life Sci., 116 (2): 59-66, 2014. https://doi.org/10.1016/J.LFS.2014.09.015.

11- ROSALES C.: Neutrophil: A Cell with Many Roles in Inflammation or Several Cell Types? Front Physiol., 9: 324475, 2018. https://doi.org/10.3389/FPHYS.2018.00113.

12- LEE S.A., NOEL S., SADASIVAM M., HAMAD ARA. and RABB H.: Role of Immune Cells in Acute Kidney Injury and Repair. Nephron, 137 (4): 282-286, 2017 https://doi.org/10.1159/000477181.

13- KAUR T., SINGH D., PATHAK D., SINGH A.P. and SINGH B.: Umbelliferone attenuates glycerol-induced myoglobinuric acute kidney injury through peroxisome proliferator-activated receptor-γ agonism in rats. J. Biochem. Mol. Toxicol., 35.11: e22892, 2021 https://doi.org/10.1002/JBT.22892.

14- BOSSÙ P., CUTULI D., PALLADINO I., CAPORALI P., ANGELUCCI F., LARICCHIUTA D., GELFO F., DE BARTOLO P., CALTAGIRONE C and PETROSINI L.: A single intraperitoneal injection of endotoxin in rats induces long-lasting modifications in behavior and brain protein levels of TNF-α and IL-18. J. Neuroinflammation, 9: 1-12, 2012. https://doi.org/10.1186/1742-2094-9-101/FIGURES/5.

15- FENG L.X., ZHAO F., LIU Q., PENG J.C., DUAN X.J., YAN P., WU X., WANG H.S., DENG Y.H., and DUAN S. BIN.: Role of Nrf2 in Lipopolysaccharide-Induced Acute Kidney Injury: Protection by Human Umbilical Cord Blood Mononuclear Cells. Oxid Med. Cell Longev., 1: 6123459, 2020. https://doi.org/10.1155/2020/6123459.

16- LE CLEF N., VERHULST A., D'HAESE P.C. and VERVAET B.A.: Unilateral Renal Ischemia-Reperfusion as a Robust Model for Acute to Chronic Kidney Injury in Mice. PLoS One, 11 (3): e0152153, 2016. https://doi.org/10.1371/JOURNAL.PONE.0152153.

17- GIGLIOTTI J.C., HUANG L., BAJWA A., YE H., MACE E.H., HOSSACK J.A., KALANTARI K., INOUE T., ROSIN D.L. and OKUSA M.D.: Ultrasound modulates the splenic neuroimmune axis in attenuating AKI. Journal of the American Society of Nephrology, 26 (10): 2470-2481, 2015. https://doi.org/10.1681/ASN.2014080769.

18- HAMZAWY M., GOUDA S.A.A., RASHID L., ATTIA MORCOS M., SHOUKRY H. and SHARAWY N.: The cellular selection between apoptosis and autophagy: roles of vitamin D, glucose and immune response in diabetic nephropathy. Endocrine, 58: 66-80, 2017. https://doi.org/10.1007/S12020-017-1402-6.

19- ORTEGA L.M. and FORNONI A.: Role of cytokines in the pathogenesis of acute and chronic kidney disease, glomerulonephritis, and end-stage kidney disease. Int. J. Interferon Cytokine Mediat Res., 49-62, 2010. https://doi.org/10.2147/IJICMR.S10111.

20- BONVENTRE J.V. and YANG L.: Cellular pathophysiology of ischemic acute kidney injury. J. Clin. Invest.,121 (11): 4210-4221, 2011. https://doi.org/10.1172/JCI45161.

21- WANG Q., SU Y.Y., LI Y.Q., ZHANG Y. F., YANG S., WANG J.L. and LI H.Y.: Atorvastatin alleviates renal ischemia-reperfusion injury in rats by promoting M1-M2 transition. Molecular Medicine Reports, 15 (2): 798-804, 2017. https://doi.org/10.3892/mmr.2016.6074.

22- LIU CM., LI B.H., TANG K.H., DONG X.N., XUE L.G., SU G. and JIN Y.: Aquaporin 1 alleviates acute kidney injury via PI3K-mediated macrophage M2 polarization. Inflammation Research, 69: 509–21, 2020. https://doi.org/10.1007/S00011-020-01334-0/FIGURES/8.

23- WANG S., ZHANG C., LI .J., NIYAZI S., ZHENG L., XU M., RONG R., YANG C. and ZHU T.: Erythropoietin protects against rhabdomyolysis-induced acute kidney injury by modulating macrophage polarization. Cell Death & Disease, 8 (4): e2725-e2725, 2017. https://doi.org/10.1038/cddis.2017.104.

24- BLACK L.M., LEVER J.M. and AGARWAL A.: Renal Inflammation and Fibrosis: A Double-edged Sword. Journal of Histochemistry and Cytochemistry, 67: 663–81, 2019. https://doi.org/10.1369/0022155419852932.

25- IMIG J.D. and RYAN M.J.: Immune and Inflammatory Role in Renal Disease. Compr Physiol., 3 (2): 957, 2013. http://doi: 10.1002/cphy.c120028.

26- MEZZANO S.A., BARRÍA M., DROGUETT M. A., BURGOS M.E., ARDILES L.G., FLORES C. and EGIDO, J.: Tubular NF-κB and AP-1 activation in human proteinuric renal disease. Kidney international, 60 (4): 1366-1377,2001. https://doi.org/10.1046/j.1523-1755.2001.00941.x.

27- SANCHEZ-LOPEZ E., RAYEGO S., RODRIGUES-DIEZ R., RODRIGUEZ J.S., RODRIGUES-DIEZ R., RODRIGUEZ-VITA J. and RUIZ-ORTEGA M.: CTGF promotes inflammatory cell infiltration of the renal interstitium by activating NF-κB. Journal of the American Society of Nephrology, 20 (7): 1513-1526, 2009. DOI: 10.1681/ASN.2008090999.

28- BEST T.M., WILK K.E., MOORMAN C.T. and DRAPER D.O.: Low Intensity Ultrasound for Promoting Soft Tissue Healing: A Systematic Review of the Literature and Medical Technology. Intern. Med. Rev., 2.11: 271, 2016, doi: 10.18103/imr.v2i11.271.

29- NG Y.Y., HOU C.C., WANG W., HUANG X.R. and LAN H.Y.: Blockade of NFκB activation and renal inflammation by ultrasound-mediated gene transfer of Smad7 in rat remnant kidney. Kidney international, 67: S83-S91, 2005 https://doi.org/10.1111/j.1523-1755.2005.09421.x.

30- TANG W., XIA Y., DENG J., XU H., TANG Y. XIAO X. and WANG Y.: Anti-inflammatory effect of low-Intensity ultrasound in septic rats. Ultrasound in Medicine & Biology, 49 (7): 1602-1610, 2023. https://doi.org/10.1016/j.ultrasmedbio.2023.03.008.

31- CHIANG C.K., LOH J.Z., YANG T.H., HUANG K.T., WU C.T., GUAN S.S. and HUNG K.Y.: Prevention of acute kidney injury by low-intensity pulsed ultrasound via anti-inflammation and anti-apoptosis. Scientific reports, 10 (1): 14317, 2020. https://doi.org/10.1038/s41598-020-71330-1.

32- BURKS S.R., NGUYEN B.A., TEBEBI P.A., KIM S.J., BRESLER M.N., ZIADLOO A. and FRANK J.A.: Pulsed focused ultrasound pretreatment improves mesenchymal stromal cell efficacy in preventing and rescuing established acute kidney injury in mice. Stem Cells, 33 (4): 1241-1253, 2015. https://doi.org/10.1002/stem.1965.

33- LIN C.Y., WANG C.C., LOH J.Z., CHIANG T.C., WENG T.I., CHAN D.C. and LIU S.H.: Therapeutic Ultrasound Halts Progression of Chronic Kidney Disease In Vivo via the Regulation of Markers Associated with Renal Epithelial–Mesenchymal Transition and Senescence. International journal of molecular sciences, 23 (21): 13387, 2022. https://doi.org/10.3390/ijms232113387.

34- AIBARA Y., NAKASHIMA A., KAWANO K. I., YUSOFF F. M., MIZUKI F., KISHIMOTO S. and HIGASHI Y.: Daily low-intensity pulsed ultrasound ameliorates renal fibrosis and inflammation in experimental hypertensive and diabetic nephropathy. Hypertension, 76 (6): 1906-1914, 2020. https://doi.org/10.1161/HYPERTENSIONAHA.120.15237.

35- JIANG X., SAVCHENKO O., LI Y., QI S., YANG T., ZHANG W. and CHEN J.: A review of low-intensity pulsed ultrasound for therapeutic applications. IEEE Transactions on Biomedical Engineering, 66 (10): 2704-2718, 2018. DOI: 10.1109/TBME.2018.2889669.

36- ULLAH M., LIU D.D., RAI S., DADHANIA A., JONNAKUTI S., CONCEPCION W. and THAKOR A.S.: Reversing acute kidney injury using pulsed focused ultrasound and MSC therapy: A role for HSP-mediated PI3K/AKT signaling. Molecular Therapy Methods & Clinical Development, 17: 683-694, 2020. DOI: 10.1016/j.omtm.2020.03.023.

37- BURKS S.R., NGUYEN B.A., TEBEBI P.A., KIM S.J., BRESLER M.N., ZIADLOO A. and FRANK J.A.: Pulsed focused ultrasound pretreatment improves mesenchymal stromal cell efficacy in preventing and rescuing established acute kidney injury in mice. Stem Cells, 33 (4): 1241-1253, 2015. https://doi.org/10.1002/stem.1965.

38- INOUE T., ABE C., KOHRO T., TANAKA S., HUANG L., YAO J. and OKUSA M.D.: Non-canonical cholinergic anti-inflammatory pathway-mediated activation of peritoneal macrophages induces Hes1 and blocks ischemia/reperfusion injury in the kidney. Kidney international, 95 (3): 563-576, 2019. https://doi.org/10.1016/j.kint.2018.09.020.

تأثير استخدام الموجات فوق الصوتية النبضية العلاجية منخفضة الكثافة على تحوير أنواع الخلايا البلعمية فى أمراض الكلى المختلفة

الخلفية والهدف: هدفت هذه الدراسة التجريبية إلى دراسة التأثير المُحتمل للموجات فوق الصوتية النبضية منخفضة الكثافة العلاجية (LIPUS) كمضاد للالتهابات من خلال تعديل مرونة الخلايا البلعمية فى نماذج مُختلفة لإصابات الكلى الحادة (AKI).

الطريقة: أُجريت الدراسة على ٩٠ جرذًا، قُسِّمت إلى تسع مجموعات: أ) المجموعة الضابطة؛ ب) المجموعة غير المُعالَجة، بما في ذلك مجموعات فرعية من إصابات الكلى الحادة المُختلفة (انحلال الربيدات، عديد السكاريد الدهنى، وإصابات الكلى الحادة المُستحثة بالإقفار وإعادة التروية)؛ ج) المجموعة المُعالَجة بـ LIPUS لإصابات الكلى الحادة، والتي قُسِّمت وفقًا للنماذج المذكورة سابقًا. تم تقييم وظائف الكلى، ومستويات السيتوكينات (IL–α–TNF، 1β، وIL–10)، والعلامات المرتبطة بـ M1 (iNOS وCD38)، والعلامات المرتبطة بـ M2 (1-arginase) 1-Arg)، CD206، و1-FIZZ) فى أنسجة الكلى باستخدام اختبار الممتز المناعى المرتبط بالإنزيم (ELISA) وتفاعل البوليميراز المتسلسل العكسى فى الوقت الحقيقى (RT-PCR) على التوالى، بالإضافة إلى الفحوصات النسيجية المرضية لأنسجة الكلى.

النتائج: أفادت الدراسة بقدرة LIPUS على تحويل الخلايا البلعمية نحو النمط الظاهرى المضاد للالتهابات M2 فى نماذج مختلفة من الفشل الكلوى الحاد المُعالج مع استعادة وظائف الكلى وبنيتها.

الخلاصة: يتميز LIPUS بخصائص مضادة للالتهابات من خلال تعديل الخلايا البلعمية نحو النمط الظاهرى M2، مما قد يكون نهجًا علاجيًا فى التخفيف من نماذج الفشل الكلوى الحاد المختلفة. هناك حاجة إلى مزيد من الدراسات لفهم عوامل تعديل الخلايا المناعية وتحديد أنواع الخلايا المناعية فى أمراض الكلى المزمنة.