

## Análisis de la función y estructura renal en ratas hipertensas tratadas con terapia láser de baja intensidad

### *Analysis of renal function and structure in hypertensive rats treated with low level laser therapy*

Briteño-Vázquez M<sup>a</sup>, Fernández-Tamayo NM<sup>b</sup>, González-Pérez M<sup>c</sup>, Pecos-Martín D<sup>d</sup>, Cisneros E<sup>e</sup>, Gallego-Izquierdo T<sup>d</sup>.

<sup>a</sup> Licenciatura en Fisioterapia, Facultad de Medicina de la Benemérita Universidad Autónoma de Puebla (BUAP). Puebla. México

<sup>b</sup> Laboratorio de Anatomía Patológica. Facultad de Medicina de la Benemérita Universidad Autónoma de Puebla (BUAP). Puebla. México

<sup>c</sup> Posgrado en Ciencia de la Ingeniería Biomédica. Universidad Popular Autónoma del Estado de Puebla. Puebla. México

<sup>d</sup> Departamento de Enfermería y Fisioterapia. Universidad de Alcalá. Alcalá de Henares. España

<sup>e</sup> Faculty of Health Sciences. Centro Universitario Internacional de Madrid (CUNIMAD) and International University of La Rioja (UNIR).

#### Correspondencia:

Tomás Gallego Izquierdo  
tomas.gallego@uah.es

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#### RESUMEN

**Introducción:** el objetivo de este estudio fue investigar el efecto de la terapia con láser de baja intensidad en la progresión de la enfermedad renal crónica (ERC) en ratas endogámicas SHR (*spontaneously hypertensive rats*). **Material y método:** se incluyeron en el estudio ratas de 20 semanas de edad de las cepas SHR y Wistar y se dividieron en 4 grupos, a saber: control normotenso (NTC), control hipertenso (HTC), experimental normotenso (NTE) e hipertenso experimental (HTE). Las ratas de los grupos control no fueron irradiadas con láser mientras que los riñones de las ratas de los grupos experimentales fueron irradiados transcutáneamente en puntos predeterminados con una onda continua de 850 nm (100 mW/cm<sup>2</sup>, 12 J/cm<sup>2</sup>) durante 2 minutos durante 6 semanas. Se midieron los parámetros de orina y sangre, y se realizaron procedimientos histológicos para evaluar la función y estructura renal en los 4 grupos, antes y después del tratamiento. **Resultados:** la concentración de albúmina y proteínas totales en orina y las concentraciones de glucosa y potasio en sangre cambiaron significativamente como consecuencia de la interacción de grupo y tiempo. Sin embargo, las variaciones no pueden atribuirse al efecto de la terapia con láser de baja intensidad, sino que son consecuencia de cambios relacionados con la edad tanto en ratas hipertensas como normotensas. El análisis histológico reveló que la estructura de los glomérulos, túbulo intersticial y los vasos eran regulares en todos los grupos. No se observaron cambios en la función o estructura renal en ratas normotensas irradiadas en comparación con los controles normotensos. **Conclusiones:** bajo condiciones experimentales y modelo de hipertensión, la terapia con láser de baja intensidad no tuvo efecto sobre ninguna de las variables estudiadas, por lo que este procedimiento demostró ser seguro para el riñón. Los experimentos futuros deben centrarse en explorar el efecto de la terapia con láser de baja intensidad en otras etapas de la ERC.

**Palabras clave:** enfermedad crónica del riñón, hipertensión, terapia láser de baja intensidad, ratas endogámicas SHR.

### ABSTRACT

*Introduction: the object of this study was to investigate the effect of low level laser therapy on the progression of chronic kidney disease (CKD) in spontaneously hypertensive rats (SHR). Material and method: twenty weeks-old rats from SHR and Wistar strains were included in the study and were divided into four groups, namely: normotensive control (NTC), hypertensive control (HTC), normotensive experimental (NTE.), and hypertensive experimental (HTE). Rats in control groups were not laser irradiated whereas the kidneys of rats in experimental groups were transcutaneously irradiated in predetermined spots with a continuous wave of an 850 nm (100 mW/cm<sup>2</sup>, 12 J/cm<sup>2</sup>) for 2 minutes for 6 weeks. Urine and blood parameters were measured, and histological procedures were performed to test renal function and structure in the four groups before and after treatment. Results: albumin and total proteins concentration in urine and glucose and potassium concentrations in blood changed significantly a consequence of the interaction of group and time. Nevertheless, variations could not be attributable to LLLT effect, but rather were the consequence of age-related changes both in hypertensive and normotensive rats. Histological analysis revealed that structure glomeruli, tubulointerstitial and vasculature were regular in all the groups. No changes were observed in renal function or structure in normotensive rats irradiated when compared to normotensive controls. Conclusions: under experimental conditions and hypertension model, LLLT had no effect on any variable studied but this procedure demonstrated to be safe for kidney. Futures experiments should be focused on exploring LLLT effect on other stages of CKD.*

**Keywords:** *chronic kidney disease, hypertension, low level laser therapy, spontaneously hypertensive rats.*

### INTRODUCTION

Chronic kidney disease (CKD) is defined as an abnormality of kidney structure or function that has been present for 3 or more month. Diagnostic of CKD requires a glomerular filtration rate (GFR) of less than 60 ml/min × 1.73 m<sup>2</sup> and/or the presence of kidney damage (i.e., proteinuria, albuminuria, pathologic abnormalities evidenced by biopsy or imaging, genetic disorders or a history of renal transplantation)<sup>(1)</sup>. Usually, CKD is a progressive disease leading to end-stage renal disease (ESRD), point in which kidney function is no longer able to sustain life and patients require kidney replacement therapy<sup>(2)</sup>.

Hypertension is one of the leading causes of CKD in all developed and many developing countries. In fact, untreated hypertension renal damage has a high prevalence and is a major cause of end-stage renal disease (ESRD)<sup>(3, 4)</sup>. It is known that kidney auto-regulatory mechanisms protect renal microvasculature from episodic or sustained arterial pressure elevation. Nevertheless, if

the arterial pressure increase becomes more severe, regulatory mechanisms fail and pressure rise is transmitted to glomerular capillaries causing kidney damage proportional to the degree of arterial pressure exposure. Earliest responses to glomerular hypertension include structural changes, such as wall thickness increase and lumen diameter narrowing. This adaptation is intended to reduce vascular wall stress but also to maintain and amplify hypertension. Moreover, wall hypertrophy increases diffusion distance of oxygen causing ischemic injury of both glomeruli, tubular and interstitial structures. In turn, the resulting capillary stretching triggers adaptive mechanisms (proliferation, activation of renin-angiotensin-aldosterone system or RAAS, cellular remodeling and changes in signaling pathways) become maladaptive in the long term leading to inflammation, glomerulosclerosis, tubular atrophy and interstitial fibrosis<sup>(5)</sup>.

Due to its high prevalence (estimated to be 8-16 % worldwide) and associated complications, CKD is a major health issue<sup>(3)</sup> and, therefore, identification of strategies to slow its progression is a priority.

RAAS inhibitors are considered as first line therapy for hypertensive patients as they reduce proteinuria and CKD progression, besides reducing blood pressure. However, combination of RAAS blockers has been associated with adverse effects including risk of severe increase in serum potassium concentration and acute renal failures and, therefore, should be avoided<sup>(6)</sup>.

On the other hand, the effect of these drugs, as others antihypertensive treatments, are measured as the blood pressure reduction, but the biologically relevant parameter for hypertensive kidney damage is the blood pressure to which the kidney is exposed. Hence, therapeutic approaches focused on control of renal blood pressure might be a good prevention strategy<sup>(7)</sup>.

Low-level laser therapy (LLLT) is a non invasive, painless therapeutic strategy which has been applied to a wide spectrum of disorders because its dose-dependent ability to increase cell proliferation and tissue regeneration, reducing chronic pain and attenuating inflammation and fibrosis<sup>(8)</sup>. Moreover, some experiments in normotensive and hypertensive animals support that light induces vasodilation by oxide nitric release from blood vessels<sup>(9, 10)</sup>.

In this way, the purpose of this study is to evaluate the effect of LLLT applied to the kidney region on the onset and progression of CDK in spontaneously hypertensive rats (SHR). SHR is the most widely used animal model of human essential hypertension, seeing that they develop high blood pressure spontaneously with age, as is observed in humans, and the change in blood pressure results in end-organ damage<sup>(11)</sup>. Because of all of this, this model is ideal to explore therapeutic preventive strategies, since there are very few studies with similar objectives. Even though there is a pharmacological treatment available, effectiveness is not guaranteed. Therefore is necessary to look for alternatives with minimal side effects, such as LLLT.

## MATERIALS AND METHOD

### Animals

Experiments were performed on 18-20 weeks old rats weighing 350-500 g. Rats were normotensive (Wistar strain, mean systolic pressure of 118 mmHg) or sponta-

neously hypertensive (SHR, mean systolic pressure of 154 mmHg).

Animals were maintained in Claude Bernard house facility (Benemérita Autonomous University of Puebla) at 24,2 °C on a 12 hour light / dark cycle with ad libitum access to food and water. All procedures followed Official Mexican Standard NOM-062-Z00 (Technical specifications for the production, care and use of laboratory animals) and were approved by Benemérita Autonomous University of Puebla.

### Study design

An experimental study was conducted in order to evaluate the renal protective effect of laser irradiation therapy on hypertensive nephropathy.

Animals were divided into four groups, namely: normotensive control (NTC, n = 2), hypertensive control (HTC, n = 2), normotensive experimental (NTE, n = 3), and hypertensive experimental (HTE, n = 3). Control groups included non-laser irradiated rats whereas experimental groups included laser irradiated rats.

### Physiological and biochemical measures

Systolic blood pressure was assessed using a sphygmomanometer.

To evaluate renal function, blood and urine were analyzed. To collect urine, rats were housed individually in metabolic cages for 24 hours. Creatine, albumin, creatinine, total proteins, urea, glucose and electrolytes were quantified.

Blood was obtained from the retro-orbital plexus and creatine, albumin, creatinine, total proteins, urea, glucose and electrolytes were measured. Creatine clearance (C) was calculated using the equation  $C = U \times V / P$ , where U is urinary concentration, V urinary flow rate and P plasma concentration. On the other hand, fractional excretion (FE) of electrolytes was calculated as:

$$FE_{\text{electrolyte}} = U_{\text{electrolyte}} \times S_{\text{creatinine}} / U_{\text{creatinine}} \times S_{\text{electrolyte}}$$

where S is serum concentration.

Urine and blood analyses were performed before and after the laser irradiation therapy in the experimental groups or at the same time points in the control groups.

Thus, biochemical measures were performed twice in the four groups.

### Laser irradiation procedure

Animals were anesthetized by intra-peritoneal injection of ketamine/xylazine. Kidneys positions were determined according to a rat atlas<sup>(12)</sup>, and then were irradiated through shaved skin using an Gallium Arsenide (GaAs) laser (KLD Biosistemas, Sao Paulo, Brasil). Laser was applied in a punctual manner as a continuous wave of an 850 nm (100 mW/cm<sup>2</sup>, 12 J/cm<sup>2</sup>) for two minutes<sup>(13)</sup>. Irradiation of both kidneys was carried out once a day for 40 days.

### Tissue collection and histochemistry

All rats were euthanized with an overdose of isoflurane. Kidneys were dissected, fixed in 10 % phosphate-buffered formalin and embedded in paraffin. Sections were cut at thicknesses of 10 µm and processed for hematoxylin-eosin or Gomori staining.

Samples were observed in a biological binocular biological binocular microscope CX-31, OLYMPUS®. The images were acquired with 4x magnification.

### Statistical analysis

Results are presented as mean ± standard error of the mean (SEM). Statistical analyses were performed with IBM SPSS statistics 25. Differences between normotensive and hypertensive rats were analyzed with Student t-test with or without the Welch's correction as appropriate. Four groups repeated measures were compared using a two-factor mixed analysis of variance (ANOVA) followed by a post hoc Bonferroni. Significant difference was accepted for  $p < 0.05$ .

## RESULTS

### Characterization of renal function in hypertensive rats

First of all, we characterized 18-20 weeks-old SHR. As expected, mean systolic pressure in hypertensive rats (153.8 ± 9.9 mmHg,  $n = 5$ ) was significantly higher than that observed in the age-matched normotensive rats (118 ± 6.4 mmHg,  $n = 5$ ; t-test,  $p < 0.05$ ).

In order to evaluate renal function in hypertensive rats, we quantified several biomarkers in urine (table 1) and blood (table 2) and calculated creatine clea-

TABLA 1. Urine analysis in normotensive and hypertensive rats.

Parameter	Normotensive	Hypertensive	p value
Creatinine (mg/dl)	100.0 ± 41.5	137.5 ± 41.0	ns
Albumin (mg/l)	1.5 ± 0.1	7.8 ± 0.8	< 0.01
Total proteins (mg/l)	1647.8 ± 198.4	1985 ± 257.5	ns
Urea (mg/dl)	3428.8 ± 1470.9	5150 ± 1473.0	ns
Glucose (mg/dl)	5.5 ± 2.0	12 ± 3.4	ns
Sodium (mEq/l)	23.2 ± 9.3	32.4 ± 9.8	ns
Potassium (mEq/l)	30.6 ± 1.0	27.8 ± 3.5	ns
Chloride (mEq/l)	128.4 ± 77.5	164.1 ± 61.0	ns

Mean levels ± SEM of parameters measured in blood, p values were obtained from comparison of normotensive ( $n = 5$ ) and hypertensive rats ( $n = 5$ ) using impaired Student t-test with or without the Welch's correction as appropriate. ns: not significant.

rance as well as fractional excretion of electrolytes (table 3).

Urine analysis revealed that albumin levels were significantly higher in hypertensive rats ( $7.82 \pm 0.81$  mg/l) when compared to normotensive rats ( $1.52 \pm 0.11$  mg/l; t-test,  $p < 0.01$ ). No significant changes were found in any other urine parameter (table 1).

Blood glucose levels decreased significantly from  $371.75 \pm 18.71$  mg/dl in normotensive rats to  $206.20 \pm 6.57$  mg/dl in hypertensive rats (t-test,  $p < 0.001$ ). On the other hand, sodium content diminished in samples from hypertensive rats ( $5.21 \pm 0.09$  mEq/l) as compared to normotensive rats ( $6.41 \pm 0.19$  mEq/l; t-test,  $p < 0.001$ ).

No significant changes were observed in any other blood parameter (table 2).

#### Low level laser effect on renal function

In order to test the effect of LLLT on kidney function, we analyzed physiological parameters in SHR (HTE group) and Wistar rats (NTE group) before and after irradiation of kidney. Because hypertension and CDK progress with age, we also evaluated renal function at the same time points in untreated SHR (HTC group) and Wistar rats (NTC).

TABLA 2. Blood analysis in normotensive and hypertensive rats.

Parameter	Normotensive	Hypertensive	p value
Creatinine (mg/dl)	$0.6 \pm 0.0$	$0.6 \pm 0.0$	ns
Albumin (mg/l)	$3.3 \pm 0.1$	$3.2 \pm 0.1$	ns
Total proteins (mg/L)	$6.3 \pm 0.2$	$6.6 \pm 0.1$	ns
Urea (mg/dl)	$52.6 \pm 2.1$	$44.0 \pm 3.2$	ns
Glucose (mg/dl)	$371.8 \pm 18.7$	$206.2 \pm 6.6$	$<0.001$
Sodium (mEq/l)	$140.2 \pm 1.1$	$140.4 \pm 0.8$	ns
Potassium (mEq/l)	$6.4 \pm 0.2$	$5.2 \pm 0.1$	$<0.001$
Chloride (mEq/l)	$105.9 \pm 0.7$	$104.9 \pm 0.4$	ns

Mean levels  $\pm$  SEM of parameters measured in blood, p values were obtained from comparison of normotensive (n = 5) and hypertensive rats (n = 5) using impaired Student t-test with or without the Welch's correction as appropriate. ns: not significant.

TABLA 3. Creatine clearance and fractional excretion of electrolytes in normotensive and hypertensive rats.

Parameter	Normotensive	Hypertensive	p
Creatinine clearance (ml/min)	$14.5 \pm 1.9$	$17.4 \pm 5.5$	ns
FENa	$0.1 \pm 0.0$	$0.1 \pm 0.0$	ns
FEK	$4.8 \pm 1.0$	$3.6 \pm 0.8$	ns

Mean values  $\pm$  SEM of calculated parameters, p values were obtained from comparison of normotensive (n=5) and hypertensive rats (n=5) using impaired Student t-test with or without the Welch's correction as appropriate. ns: not significant.

Results of quantification of urine and blood markers as well as calculated creatine clearance and fractional excretion in all the groups at the two time points are shown in tables 4, 5 and 6.

In urine samples, albumin and total protein levels were significantly affected by the interaction of group and time (table 4; two-factor mixed ANOVA). Before treatment, HTE rats displayed albumin levels significantly higher than that observed in normotensive groups, i.e., NTE and NTC (pairs comparisons with Bonferroni post hoc,  $p < 0.05$ ) whereas no significant differences were detected after laser application. We got the same results when compared HTC with NTC rats (figure 1A). In HTE and HTC groups, total protein levels raised significantly after treatment or at the equivalent time point, respectively (pair comparison with Bonferroni post hoc,  $p < 0.05$ ). On the contrary protein levels did not change in NTE and NTC groups (figure 1B).

In blood samples, glucose and potassium levels were significantly affected by the interaction of group and time (table 5; two-factor mixed ANOVA). Before treatment, glucose levels in hypertensive groups, HTE and HTC, were

significantly lower than that observed normotensive groups, NTE and NTC (pairs comparisons with Bonferroni post hoc,  $p < 0.05$ ). After treatment, no differences were observed between any of the four groups (figure 1C). Before treatment, potassium concentration was significantly diminished in HTE and HTC groups when compared with NTE group (pairs comparisons with Bonferroni post hoc,  $p < 0.05$ ). After treatment, potassium levels fall in normotensive groups, in such a way that concentration in HTC was significantly elevated when compared with NTE (pair comparison with Bonferroni post hoc,  $p < 0.05$ ). No other significant differences were detected at this time point (figure 1D).

Finally, creatine clearance or fractional excretion of electrolytes were not significantly affected by the interaction of group and time (table 6; two-factor mixed ANOVA).

#### Low level laser effect on renal structure

After treatment, or at the equivalent time points, kidneys from HTE, HTC, NTE and NTC groups were processed for hematoxylin-eosin or Gomori staining.

TABLA 4. Urine analysis in HTE, HTC, NTE and NTC groups.

		Creatinine (mg/dl)	Albumin (mg/l)	Total proteins (mg/l)	Urea (mg/dl)	Glucose (mg/dl)	Sodium (mEq/l)	Potassium (mEq/l)	Chloride (mEq/l)
HTE n=3	Pre	91.5 ± 37.2	8.1 ± 0.6	1788 ± 378.7	4033.3 ± 2176.8	8.3 ± 3.5	20.4 ± 5.6	24.85 ± 4.8	121.5 ± 93.7
	Post	111.7 ± 44.2	1.5 ± 0.2	2688.7 ± 421.5	3083.3 ± 474.6	9.27 ± 2.1	31.7 ± 11.0	39.39 ± 1.6	99.1 ± 32.6
HTC n=2	Pre	206.5 ± 69.0	7.3 ± 2.3	2280.5 ± 295.5	6825.0 ± 1675.0	17.5 ± 5.5	50.5 ± 18.0	32.2 ± 4.4	228.0 ± 63.9
	Post	110.6 ± 0.6	1.7 ± 0.2	3111.4 ± 253.6	3562.5 ± 287.5	10.5 ± 0.7	36.6 ± 3.0	42.5 ± 1.9	113.7 ± 8.8
NTE n=3	Pre	46.5 ± 4.2	1.40 ± 0.1	1395.0 ± 59.0	1530.0 ± 35.3	3.00 ± 0.6	11.27 ± 1.2	29.5 ± 1.2	28.4 ± 5.1
	Post	57.7 ± 13.8	1.58 ± 0.2	1484.7 ± 194.4	1683.3 ± 486.8	3.7 ± 1.2	26.0 ± 9.3	42.2 ± 2.4	66.7 ± 24.1
NTC n=2	Pre	180.3 ± 80.3	1.7 ± 0.2	2026.9 ± 379.1	6276.9 ± 2848.1	9.3 ± 3.8	41.2 ± 18.0	32.2 ± 1.7	278.2 ± 149.9
	Post	44.1 ± 8.1	1.3 ± 0.2	1307.4 ± 106.4	1125.0 ± 335.0	2.0 ± 1.0	20.0 ± 3.6	36.5 ± 3.4	44.6 ± 13.2
time* group		F=1.8; ns	F=18.5; p<0.05	F= 6.2; p<0.05	F=1.8; ns	F=1.2; ns	F=1.7; ns	F=1.4; ns	F=2.1; ns
time		F=3.0; ns	F=52.7; p< 0.001	F=5.4; ns	F=6.7; p<0.05	F=2.5; ns	F=0.1; ns	F=32.9; p<0.05	F=7.2; ns
group		F=2.9; ns	F=13.5; p<0.05	F=4.2; ns	F=2.1; ns	F=6.9; p<0.05	F=2.1; ns	F=0.8; ns	F=1.4; ns

Mean levels ± SEM of parameters measured in 24 hours urine. For comparison between groups and pre and post treatment time points we used two-factor mixed ANOVA. ns: not significant.

TABLA 5. Blood analysis in HTE, HTC, NTE and NTC groups.

		Creatinine (mg/dl)	Albumin (mg/l)	Total proteins (mg/l)	Urea (mg/dl)	Glucose (mg/dl)	Sodium (mEq/l)	Potassium (mEq/l)	Chloride (mEq/l)
HTE n=3	Pre	0.6 ± 0.3	3.2 ± 0.9	6.6 ± 0.2	42.9 ± 2.3	208.0 ± 3.6	140.3 ± 0.2	5.3 ± 0.1	105.4 ± 0.3
	Post	0.7 ± 0.0	3.3 ± 0.1	6.0 ± 0.4	58.0 ± 3.2	191.0 ± 14.4	147.0 ± 3.3	5.1 ± 0.1	115.5 ± 3.4
HTC n=2	Pre	0.6 ± 0.1	3.2 ± 0.2	6.6 ± 0.1	45.7 ± 9.2	203.5 ± 19.5	140.7 ± 2.4	5.1 ± 0.1	104.0 ± 0.4
	Post	0.7 ± 0.1	3.2 ± 0.2	6.2 ± 0.0	56.5 ± 4.5	194.5 ± 33.5	146.6 ± 1.0	5.4 ± 0.0	115.9 ± 0.2
NTE n=3	Pre	0.6 ± 0.0	3.3 ± 0.1	6.3 ± 0.3	52.5 ± 3.9	359.0 ± 29.0	140.6 ± 1.9	6.5 ± 0.3	106.3 ± 1.2
	Post	0.7 ± 0.0	3.2 ± 0.1	6.4 ± 0.1	56.3 ± 7.2	173.3 ± 7.1	145.5 ± 2.6	5.0 ± 0.0	115.5 ± 1.7
NTC n=2	Pre	0.6 ± 0.0	3.2 ± 0.0	6.2 ± 0.0	52.8 ± 0.2	390.9 ± 19.2	139.6 ± 0.6	6.3 ± 1.1	105.2 ± 0.6
	Post	0.8 ± 0.1	3.3 ± 0.1	6.3 ± 0.1	55.5 ± 0.5	203.4 ± 72.7	143.3 ± 1.4	5.1 ± 0.0	114.4 ± 0.7
time*group		F=3.1; ns	F=0.3; ns	F=0.9; ns	F=1.8; ns	F=8.0; p<0.05	F=0.2; ns	F=11.6; p<0.01	F=0.2; ns
time		F=55.7; p<0.001	F=0.0; ns	F=0.9; ns	F=12.4; p<0.05	F=30.8; p<0.01	F=12.6; p<0.05	F=25.7; p<0.01	F=76.2; p<0.001
group		F=0.4; ns	F=0.1; ns	F=0.1; ns	F=0.2; ns	F=5.8; p<0.05	F=0.3; ns	F=7.0; p<0.05	F=0.1; ns

Mean levels ± SEM of parameters measured in blood. For comparison between groups and pre and post treatment time points we used two-factor mixed ANOVA. ns: not significant.

TABLA 6. Creatinine clearance and fractional excretion of electrolytes in HTE, HTC, NTE and NTC groups.

		Creatinine clearance (ml/min)	FENa	FEK
HTE n=3	Pre	10.70 ± 3.3	0.1 ± 0.1	4.2 ± 1.1
	Post	15.5 ± 3.6	0.2 ± 0.1	6.3 ± 1.8
HTC n=2	Pre	27.4 ± 10.0	0.1 ± 0.0	2.7 ± 0.9
	Post	16.9 ± 0.9	0.2 ± 0.0	5.79 ± 0.3
NTE n=3	Pre	12.23 ± 1.4	0.1 ± 0.0	6.0 ± 0.9
	Post	7.9 ± 1.0	0.2 ± 0.2	10.8 ± 1.7
NTC n=2	Pre	17.9 ± 3.4	0.1 ± 0.0	3.0 ± 1.8
	Post	8.1 ± 0.1	0.2 ± 0.0	13.3 ± 1.5
time*group		F=3.8; ns	F=0.3; ns	F=2.6; ns
time		F=7.2; p<0.05	F=2.9; ns	F=22.5; p<0.05
group		F=2.6; ns	F=0.03; ns	F=4.4; ns

Mean values ± SEM of calculated parameters. For comparison between groups and pre and post treatment time points we used two-factor mixed ANOVA. ns: not significant.

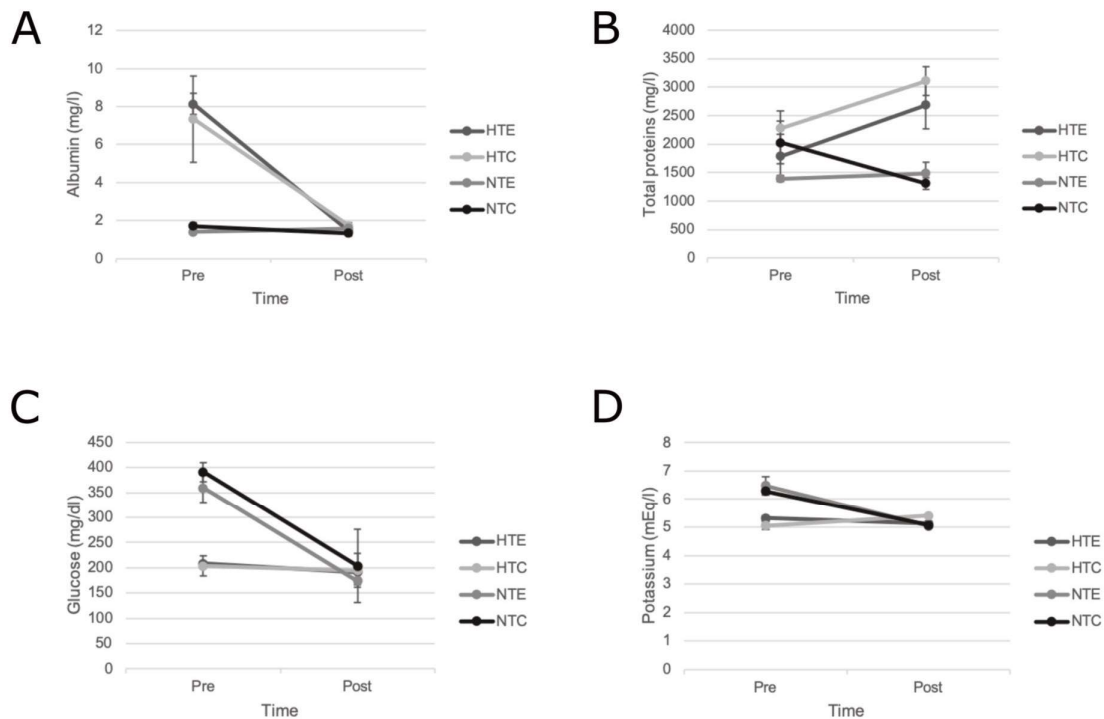


FIGURE 1. Levels of urine parameters in HTE, HTC, NTE and NTC groups pre- and post-treatment. Graphics represent concentration of albumin (A) and total proteins (B) in urine and concentration of glucose (C) and potassium (D) in blood.

Representative images from each group are shown in figure 2. Not relevant finds were observed in the kidneys from HTE, HTC or NTE groups when compared with NTC.

Hematoxylin-eosin staining revealed that the morphological appearance of glomeruli and tubulo interstitium remained normal in HTE, HTC and NTE groups compared with NTC group. We did not observe sclerotic glomeruli neither in hypertensive groups or NTE (figure 2, left column).

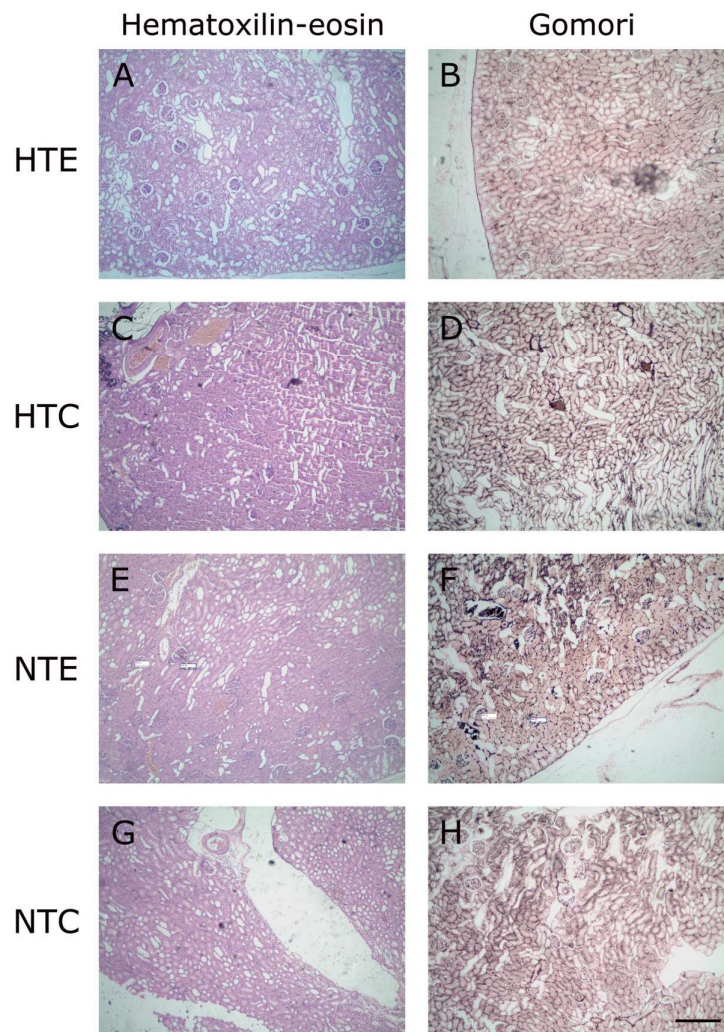
Comparison of Gomori staining between HTE, HTC and NTE groups against NTC group evidenced that vessels were unaltered following hypertension and/or irradiation. Reticulin expression was similar in all groups. No changes in wall thickness or atheroma lesions were found in hypertensive groups or NTE (figure 2, right column).

## DISCUSSION

In this work we have studied the effect of LLLT on the progression of CKD. For this purpose, we have used SHR ranging 20 weeks and displaying a mean systolic blood pressure of 154 mmHg. This value was significantly higher than that observed in age-matched controls and was consistent with data reported for SHR rats of similar ages<sup>(14)</sup>.

Blood pressures of the SHR reach hypertensive levels (above 150 mmHg) at week 9 and continue increasing to 200 mmHg<sup>(15)</sup>. It is well accepted that vascular lesions associated with hypertension are the result of the increased pressure<sup>(16)</sup>. Consequently, in the SHR almost in parallel with the progression of hypertension, progressive renal failure develops. The present results show that





**FIGURE 2. Morphology of kidneys from HTE, HTC, NTE and NTC post-treatment. Representative images of sections obtained from HTE (A, B), HTC (C, D), NTE (E, F) and NTC (G, H) kidneys. Sections stained with hematoxilin-eosin (left column) or Gomori (right column) evidenced no changes between groups.**

urinary albumin is significantly increased in 20 weeks-old SHR, indicating an increased endothelial permeability and, therefore, risk for CKD<sup>(17, 18)</sup>. On the other hand, we have detected a significant decrease in blood glucose and potassium levels. Hypoglycemia might be associated with hypertension<sup>(19)</sup> but the cause of low potassium levels in 20 weeks-old SHR is unclear. Even so, it has been reported that chronically low potassium levels can lead to pathologic abnormalities in the renal tubular cells and fibrosis of the renal interstitium<sup>(20)</sup>. No other changes,

as creatine clearance falls or structural pathological findings, were detected in our hypertension model, so these animals would be considered as early stages of kidney disease.

Literature about LLLT impact on kidney is scarce, but a hypotensive effect associated with nitric oxide nitric vasodilation has been reported in both hypertensive and normotensive rats<sup>(10)</sup>. In our experimental conditions and hypertension model, LLLT had no effect on any variable studied.

After irradiation, urine albumin levels decreased in hypertensive rats (HTE) in such way that were no significant different from values detected in normotensive untreated rats (NTC). However, this recovery could not be attributable to LLLT, because results were obtained for hypertensive untreated rats (HTC). Rather, this might be due to other circumstances occurring in hypertensive rats along the 5 weeks of treatment. Noticeably, others authors have described an rapid increase in urine albumin levels from around 20 week<sup>(15)</sup>, but we did not observed this effect when compared 20 and 26 weeks-old hypertensive rats. On the other hand, urine total proteins augmented significantly in both hypertensive groups before treatment or at the same time point, reflecting the progression of renal functional alterations. This is in accordance with previous reports for SHR as well as for untreated humans<sup>(11, 15, 21)</sup>. Changes in blood glucose and potassium parameters also seemed might reflect metabolic and electrolytic disbalance related with hypertension<sup>(22, 23)</sup>. In accordance with subtle biochemical variations, we did not find histopathological alterations in any group. This is in line with previous descriptions of glomerular alterations since week 30<sup>(15)</sup>.

Note worthy, no changes were observed in renal function or structure in normotensive rats irradiated (NTE) when compared to normotensive controls (NTC), demonstrating the security of laser conditions tested here in this study. It is important to note that this research uses an approximation method. Thus not seeing any significant changes is a result of the small size of the samples and the minimal evolutionary conditions in which was found kidney disease by hypertension. Future research could be aimed to explore other irradiation conditions as well as other stages of CKD.

## ETHICAL RESPONSIBILITIES

**Compliance with ethical standards.** Claude Bernard house facility in Benemérita Autonomous University of Puebla approved the present investigation. All procedures performed in this study were in accordance with the ethical standards laid down in the Declaration of Helsinki. The manuscript does not contain clinical studies or patient data. The authors adhere to the ARRIVE guidelines

**Conflict of interest.** The authors declare that they have no conflict of interest and any funding organization to mention.

**Authorship.** All authors have contributed intellectually to the development of the paper, participating sufficiently so as to take full public responsibility for its contents (and all material included within the same: illustrations and tables).

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