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The Influence of N-Acetylcysteine Alone and in Combination with Angiotensin Converting Enzyme Inhibitor and Angiotensin Receptor Antagonist on Systemic and Tissue Levels in Rats with Experimentally-Induced Chronic Renal Failure

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AOS, SS, AVO, NS, EED, BY, FE and GS conceived the study interpreted the data and wrote the manuscript. AOS, SS, AVO and FE performed laboratory tests. AOS performed statistical analyses. AOS, SS, AVO, NS, EED, BY, FE and GS.

Key words
Chronic renal failure, Oxidative stress, N-acetylcysteine, Captopril, Valsartan

ABSTRACT

The protective effects of ACE inhibitor, Captopril, and angiotensin receptor blocker, Valsartan, were evaluated in the treatment of chronic renal failure (CRF) with and without the presence of N-acetylcysteine (NAC). The renal mass of Wistar albino rats was reduced at a rate of 5/6. Captopril, Valsartan and NAC were applied intra-peritoneal alone or in combination. Blood pressure and heart rate were monitored at weekly intervals over a period of six weeks. Serum creatinine, blood urea nitrogen (BUN), lactate dehydrogenase (LDH) activity, cytokines (TNF-α, IL-1β, IL-6) concentrations, urinary volume, creatinine, and both serum and urinary electrolyte levels were measured. In addition, the apoptosis rate of white blood cells was analysed from plasma samples. Tissue samples from the brain, heart, aorta and kidneys were used for analysis of the collagen content besides tissue luminol, lucigenin, malondialdehyde (MDA) and glutathione (GSH) levels. A significant difference was determined between the CRF group and the control group with regard to heart rate, blood pressure, serum creatinine, BUN, LDH, cytokines and urinary electrolyte levels. Furthermore, monocyte and neutrophil apoptosis, tissue luminol, lucigenin, malondialdehyde and collagen levels were found to increase. Tissue glutathione levels were found to decrease indicating oxidative damage. These results indicate that oxidative mechanisms induce tissue damage in CRF, and the angiotensin receptor blocker, Valsartan, improved oxidative tissue damage when used in combination with the ACE inhibitor, Captopril or NAC, yielded better results and could be a novel approach for the treatment of CRF when used in combination with anti-oxidants.

INTRODUCTION

Chronic renal failure is a common and worldwide health problem affecting both human beings and companion animals (Ravarotto *et al*., 2018; Shipov *et al*., 2018; Wang *et al*., 2019). Oxidative stress is reported to be an important factor in the pathogenesis of chronic renal failure and related multi-organ damage such as the heart,

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aorta and brain (Osikov *et al*., 2015; Popolo *et al*., 2013). The balance between oxidant-antioxidants changing in favour of oxidants leads to renal failure-related local and systemic tissue damage. Oxidative stress is discernible and measurable at stages of chronic renal failure (Liu *et al*., 2011; Ravarotto *et al*., 2018).

Prevention of oxidative stress can be achieved by reducing oxidant load and enhancing the antioxidant systems (Deniz *et al*., 2011). Captopril was shown to have a potent anti-oxidant activity due to containing both sulfhydryl or thiol groups and being an ACE inhibitor (Bagchi *et al*., 1989; Guzman-Hernandez *et al*., 2015). The angiotensin AT1-receptor blocker, Valsartan, has comprehensive anti-inflammatory effects in plasma and at the cellular level. These effects are known to result from the inhibition of transcription of pro-inflammatory cytokines together with reactive oxygen species (Cardoso *et al*., 2018; Touyz *et al*., 2007).

N-acetylcysteine (NAC), acts as a potent free radical-scavenger compound. NAC may easily be hydrolysed to cysteine and may also widen the natural anti-oxidant defences through increasing the glutathione levels (GSH) (Fujita *et al*., 2019). NAC has been used both as a mucolytic agent for more than 30 years and also for reducing GSH or oxidative stress conditions such as ischemic damage and toxicity (Aldini *et al*., 2018). NAC has also been used for the treatment of ischemic damage and toxicity in the lungs, liver and experimental cutaneous flaps (Sehirli *et al*., 2003).

"Renal mass reduction model" is the most preferred model in experimental studies of chronic renal failure. The left kidney is cut through superior and inferior pole ligation following a right nephrectomy, with failure developing due to the reduced renal mass and nephron count. Nephrosclerosis and local and systemic damage develops as a result of "renal mass reduction model" (Fukui *et al*., 2011; Oyan *et al*., 1999; Wu-Wong *et al*., 2015).

Under the light of the above mentioned data, the present study aimed to investigate, compare and assess the effects of an angiotensin-converting enzyme (Captopril), an angiotensin AT1-receptor blocker (Valsartan) and an anti-oxidant agent (N-acetylcysteine), alone or in combination, against oxidants and the associated damage to heart, kidney, aorta and cerebral tissue and the hemodynamic outcomes in chronic renal failure.

MATERIALS AND METHODS

Animals

Wistar albino rats weighing 200-250 g from both genders were kept in a room at +22 ±2°C constant temperature, under 12 h dark/light cycle conditions. The subjects were fed with water and standard pellet ad libitum. All test protocols were approved by the Marmara University Medical School Animal Care and Use Committee (Approval No: 23.2004.mar).

Surgery and experimental procedures

The animals were divided into two major groups as the chronic renal failure group and the control (C) group (n = 8). Then, each animal in the CRF group underwent 5/6 nephrectomy (Vaziri *et al*., 2002) and divided into 6 sub-groups; Saline, Captopril (1 mg/kg; CAP) (Jahovic *et al*., 2005), Valsartan (1 mg/kg; VAL) (Sironi *et al*., 2004), N-acetylcysteine (150 mg/kg; NAC) (Sener *et al*., 2003), CAP (1 mg/kg) + NAC (150 mg/kg) and VAL (1 mg/kg) + NAC (150 mg/kg) was administered intraperitoneal to the animals in the CRF group. Each group consisted of 8 animals. Procedures were applied using haemostasis and aseptic techniques under ketamine anaesthesia (100 mg/kg ketamine and 0.75 mg/kg chlorpromazine, intraperitoneal). The sham-operated rats were administered only saline solution during 6 weeks of follow-up. Blood pressure and heart rate were measured weekly during this period. At the end of 6 weeks, 24-h urine samples were collected, the animals were decapitated and trunk blood, heart, brain, kidney and aorta tissue samples were collected. Blood and tissue samples were stored at -70 °C until the time of analysis. The urine samples were analysed on day of sampling. Additional tissue samples were placed within 10% formaldehyde for detection of tissue collagen content and histological examination.

Measurement of blood pressure (BO) and heart rate (HR)

Blood pressure (BP), systolic blood pressure (mmHg) and heart rate (HR) (bpm) were measured with the tail-cuff method (Rhma-Labor Technique, 2 channel blood pressure monitor 8002). The rats were accustomed to these procedure 7 days before the surgical procedure. In the beginning, the rats were placed into a receptacle, which was warmed to 35 °C for 10 min. Blood pressure and heart rate were measured automatically and the mean value of 3 records was recorded (Sen *et al*., 1979).

Biochemical analyses

Tumour necrosis factor-alpha (TNF- α), interleukin-1β (IL-1 β) and interleukin-6 (IL-6) levels were analysed using rat-specific ELISA test kits, and sodium (Na) (respectively KRC3014, KRC0011, KRC0061, Biosource International, Nivelles, Belgium), potassium (K), inorganic phosphorus (PO_4^{-3}), creatinine (Crea) and blood urea nitrogen (BUN) concentrations and the serum lactate dehydrogenase (LDH) activity were measured using commercial kits in conjunction with a automatized biochemistry analyser.

Crea, Na, K and PO_4^{-3} concentrations and urinary excretion rates were evaluated from the urine samples.

Evaluation of apoptosis

Heparinized blood samples were used for the investigation of white blood cell apoptosis. Apoptosis induction was performed using phorbol 12-myristate 13-acetate (PMA) (Sigma-Aldrich, Taufkirchen, Germany) (Takei *et al*., 1996).

Tissue MDA and GSH concentrations

Malondialdehyde (MDA), which is an end product of lipid peroxidation, glutathione (GSH), which is an important anti-oxidant, were measured in the heart, brain, kidney and aorta samples. Homogenized tissue samples were used for the determination of malondialdehyde (MDA) and glutathione (GSH) levels. Homogenization was performed with ice-cold 150 mM KCl. MDA levels were measured using the thiobarbituric acid (TBA) reactivity method as previously described. MDA forms a colour complex, which gives maximum absorbance at 532 nm in a hot, alkaline environment through a reaction with TBA (Buege and Aust, 1978). The results were expressed as nmol MDA/g tissue. GSH levels were assessed using the modified Ellman procedure (Beutler, 1975) with results expressed as µmol GSH/g tissue.

Evaluation of tissue collagen content

Tissue collagen content was measured as a free radical-related fibrosis marker. Tissue samples were cut using a razor blade, immediately fixed with 10% formalin and embedded in paraffin and sectioned at a thickness of 15 µm. Collagen content was evaluated as previously described (Lopez-De Leon and Rojkind, 1985). 540 and 605 nm absorbance were used for the determination of collagen and protein levels.

Chemiluminescence experiment

The chemiluminescence of luminol and lucigenin were measured to assess the role of reactive oxygen species in tissue damage. Measurements were made at room temperature using a Junior LB 9509 luminometer (EG and G Berthold, Germany). The samples were placed into vials containing PBS-HEPES buffer (20 mM HEPES, pH 7.2, 0.5 M PBS). Reactive oxygen species were measured after adding enhancers lucigenin or luminol at 0.2 mM final concentration. Luminol detects reactive species; namely, it is selective for -$OH^{\cdot}$, H_2O_2, HOCl radicals, and lucigenin is selective for O−2 (Davies *et al*., 1992; Ohara *et al*., 1993). Measurements were made at one-minute intervals and results were given as the area under the curve for measurement duration of 5 min. The results were corrected for wet tissue weight (relative light units (rlu)/mg tissue) (Haklar *et al*., 1998).

Statistical analyses

Statistical analyses were performed using the GraphPad Prism 7.0 software (GraphPad Software Inc., San Diego; CA; ABD). Each group was composed of 8 animals. All data were expressed as mean ± standard deviation. The groups were compared with the analysis of variance (ANOVA) and then post hoc Tukey's multi-comparison tests. A p level of <0.05 was accepted as statistically significant.

RESULTS

No significant difference in blood pressure was determined between all the test groups during the first and second weeks post renal surgery ($p>0.05$). The mean systolic blood pressure significantly increased in all groups except in the combination therapy groups (CAP +NAC and VAL+NAC) from the 3rd week ($p<0.001$; Table I). However, the mean systolic blood pressures of experimental groups were found to be significantly higher than the sham group from the 4th week. Besides, Captopril, Valsartan or NAC therapies and their combinations were found to relieve CRF-related hypertension ($p<0.05$-0.001).

Induced CRF led to a significant elevation in serum urea and creatinine levels after the 6th week ($p<0.001$, Table II). Consistent with these results, serum sodium and phosphate levels were found to increase in this group and potassium levels decreased in the CRF group treated with saline solution compared to the control group ($p<0.001$). A significant decrease was determined in serum urea, creatinine, sodium and phosphate levels in Captopril, Valsartan, NAC, Captopril+NAC and Valsartan+NAC groups ($p<0.05$-0.01). However, a reduction in serum potassium levels was prevented with Captopril, Valsartan, NAC, CAP+NAC and VAL+NAC treatments ($p<0.01$-0.001). Alternatively, urinary sodium, phosphate and creatinine excretion was found to decrease CRF groups and an increased potassium loss accompanied this in the CRF group treated with saline ($p<0.001$, Table III). Urinary sodium, phosphate and creatinine excretion was found to significantly increase in the groups treated with Captopril, Valsartan, NAC, CAP+NAC and VAL+NAC compared to the CRF group treated with saline ($p<0.05$-0.001), and potassium regressed to control group values in all treatment groups ($p<0.001$).

Serum LDH activity increased three-fold in the CRF group treated with saline. This result indicates general tissue damage ($p<0.01$). LDH activity was suppressed through the blocking of angiotensin AT1 receptors,

Table I. Systolic blood pressure (mmHg) values of experimental groups (n=8).

Systolic blood pressure (mmHg)		Baseline	3rd week	4th week	5th week	6th week
Control		116 ± 3.4	118 ± 3.8	118 ± 3.9	119 ± 3.8	119 ± 2.6
Chronic Renal Failure (CRF)	Saline	117 ± 5.9	140 ± 6.9 ***	144 ± 7.1 ***	151 ± 7.3 ***	157 ± 7.6 ***
	CAP	112 ± 5.7	129 ± 5.8 ***, ++	132 ± 5.8 ***, ++	135 ± 5.5 ***, +++	139 ± 4.8 ***, +++
	VAL	113 ± 7.2	130 ± 3.9 ***, +	134 ± 4.7 ***, ++	136 ± 4.3 ***, +++	137 ± 4.6 ***, +++
	NAC	115 ± 6.6	133 ± 4.5 ***	137 ± 4.6 ***	139 ± 4.5 ***, +++	142 ± 3.8 ***, +++
	CAP+NAC	114 ± 6.3	126 ± 5.8 +++	129 ± 5.3 **, +++	130 ± 5.6 **, +++	131 ± 5.9 **, +++
	VAL+NAC	116 ± 6.1	125 ± 6.1 +++	128 ± 5.2 **, +++	129 ± 5.8 **, +++	130 ± 6.1 **, +++

** p<0.01 and *** p <0.001 compared to control, + p<0.05, ++ p<0.01, +++ p<0.001 compared to saline-treated CRF group. CAP, Captopril; VAL, Valsartan; NAC, N-acetylcysteine.

Table II. Blood urea nitrogen (BUN), serum creatinine, Na+, K+, PO43- levels of experimental groups (n=8).

		BUN (mg/dl)	Creatinine (mg/dl)	Na^+ (mmol/l)	K^+ (mmol/l)	PO_4^{3-} (mmol/l)
Control		38.75 ± 2.45	0.63 ± 0.04	134 ± 4.7	5.3 ± 0.3	5.3 ± 0.4
Chronic Renal Failure (CRF)	Saline	95 ± 2.20 ***	1.97 ± 0.11 ***	188 ± 5.2 ***	2.2 ± 0.2 ***	11.6 ± 0.5 ***
	CAP	73.63 ± 2.25 ***, +++	1.23 ± 0.05 **, +++	163 ± 4.1 **, ++	4.1 ± 0.3 ++	8.8 ± 0.2 ***, +++
	VAL	74.88 ± 2.22 ***, +++	1.25 ± 0.04 **, +++	165 ± 4.3 ***, +	4.2 ± 0.4 ++	8.9 ± 0.3 ***, +++
	NAC	75.75 ± 2.21 ***, +++	1.27 ± 0.05 **, +++	166 ± 4.5 ***, +	3.9 ± 0.3 ++	9.1 ± 0.3 ***, +++
	CAP+NAC	62.75 ± 2.62 ***, +++, φ	1.03 ± 0.04 **, +++	150 ± 4.8 +++	4.7 ± 0.3 +++	7.6 ± 0.3 ***, +++
	VAL+NAC	63.38 ± 3.22 ***, +++, γ	1.04 ± 0.04 **, +++	151 ± 5.1 +++	4.5 ± 0.3 +++	7.8 ± 0.3 ***, +++

** p<0.01, *** p<0.001, compared to control group; + p<0.05, ++ p<0.01, +++ p<0.001, compared to saline--treated CRF group; φ p<0.05, compared to CRF-CAP group; γ p<0.05, compared to CRF-VAL group. For abbreviations, see Table I.

Table III. Urine excretion and creatinine (Ucr), sodium (UNa), potassium (UK), phosphate (UPO4) levels in the urine samples of experimental groups (n=8).

		Urine excretion (ml/h)	U_{cr} (mol/h)	U_{Na} (mol/h)	U_K (mol/h)	U_{PO4} (mol/h)
Control		0.53 ± 0.10	2.78 ± 0.52	43.6 ± 3.4	177.8 ± 7.8	2.36 ± 0.13
Chronic Renal Failure (CRF)	Saline	0.23 ± 0.06 ***	1.08 ± 0.25 ***	17.5 ± 1.8 ***	256.3 ± 7.3 ***	1.11 ± 0.12 ***
	CAP	0.37 ± 0.02 **, +	1.94 ± 0.46 **, ++	33.1 ± 2.0 *, +++	205 ± 7.9 +++	1.73 ± 0.10 **, ++
	VAL	0.41 ± 0.07 ++	2.05 ± 0.43 *, ++	34.3 ± 2.1 +++	207 ± 7.7 +++	1.88 ± 0.12 +++
	NAC	0.36 ± 0.03 **, +	1.80 ± 0.44 **, +	31.1 ± 2.0 **, ++	201.5 ± 5.9 +++	1.61 ± 0.10 ***, +
	CAP+NAC	0.49 ± 0.04 +++, φ	2.34 ± 0.47 +++	37.3 ± 2.5 +++	184.6 ± 7.9 +++	2.14 ± 0.12 +++
	VAL+NAC	0.51 ± 0.09 +++	2.51 ± 0.46 +++	39.3 ± 2.5 +++	192.8 ± 7.1 +++	2.19 ± 0.13 +++

For statistical details and abbreviations, see Table I.

inhibition of angiotensin converting enzyme (ACE) or treating with NAC (p<0.001, Table IV). Chronic renal failure led to significant increases in serum levels of pro-inflammatory cytokine TNF-α, IL-1β and IL-6 in parallel with the increase in serum LDH activity (p<0.001). All measured pro-inflammatory mediators were seen to be significantly suppressed/regressed after the 6th week of Captopril, Valsartan, NAC, Captopril+NAC and Valsartan+NAC treatment (p<0.01-0.001).

The CL levels in tissue samples were investigated by both luminol and lucigenin probes in order to show that reactive oxygen species are involved in CRF-related tissue

Table IV. Serum lactate dehydrogenase (LDH) activity and plasma TNF-α, IL-1β and IL-6 levels (n=8).

		LDH (U/l)	TNF-α (pg/ml)	IL-1β (pg/ml)	IL-6 (pg/ml)
Control		2226 ± 143	4.6 ± 0.5	9.6 ± 0.1	3.7 ± 0.4
Chronic Renal Failure (CRF)	Saline	6923 ± 159 ***	25.1 ± 2.4 ***	70.5 ± 1.9 ***	11.5 ± 0.7 ***
	CAP	5526 ± 156 ***, +++	13.6 ± 1.1 ***, +++	47.5 ± 1.5 ***, +++	7.4 ± 0.5 ***, +++
	VAL	5499 ± 174 ***, +++	13.2 ± 1.1 ***, +++	48.2 ± 1.5 ***, +++	7.7 ± 0.3 ***, +++
	NAC	5361 ± 182 ***, +++	13.90 ± 1.1 ***, +++	48.8 ± 1.6 ***, +++	7.9 ± 0.3 ***, +++
	CAP+NAC	3855 ± 200 ***, +++,φφφ	9.6 ± 0.8 +++	25.9 ± 2.1 ***, +++, φφφ	6.2 ± 0.4 *, +++
	VAL+NAC	4031 ± 235 ***, +++, γγγ	8.9 ± 0.6 +++	28.2 ± 1.6 ***, +++, γγγ	6.3 ± 0.6 **, +++

For statistical details and abbreviations, see Table I.

Table V. Luminol and lucigenin CL levels in the brain, heart, aort and kidney tissues (n=8).

Chemiluminescence levels (rlu/mg)			Brain	Heart	Aorta	Kidney
	Control	luminol	7.81 ± 0.99	10.95 ± 0.82	34.04 ± 4.18	12.62 ± 1.38
		lucigenin	9.72 ± 1.07	11.60 ± 0.76	27.70 ± 4.18	10.69 ± 1.35
Chronic Renal Failure (CRF)	Saline	luminol	26.71 ± 2.92 ***	46.42 ± 7.34 ***	147.40 ± 11.15 ***	125.10 ± 12.82 ***
		lucigenin	26.89 ± 1.92 ***	52.99 ± 4.96 ***	141.40 ± 11.73 ***	109.40 ± 8.94 ***
	CAP	luminol	14.10 ± 1.94 +++	16.65 ± 2.08 +++	77.50 ± 9.40 +++	17.89 ± 2.41 +++
		lucigenin	12.21 ± 2.24 +++	18.32 ± 2.22 +++	76.12 ± 8.14 *, +++	28.01 ± 5.62 +++
	VAL	luminol	13.30 ± 2.18 +++	19.91 ± 2.25 +++	55.46 ± 10.43 +++	26.03 ± 4.74 +++
		lucigenin	13.65 ± 2.14 +++	20.44 ± 2.79 +++	70.74 ± 8.77 *, +++	30.73 ± 4.27 +++
	NAC	luminol	14.75 ± 2.13 ++	22.83 ± 3.15 +++	70.89 ± 7.65 +++	34.19 ± 4.95 +++
		lucigenin	14.25 ± 1.82 +++	22.72 ± 2.96 +++	73.33 ± 10.62 *, +++	35.24 ± 5.63 *, +++
	CAP + NAC	luminol	10.76 ± 1.69 +++	13.34 ± 1.41 +++	53.48 ± 12.80 +++	15.29 ± 3.05 +++
		lucigenin	10.84 ± 1.41 +++	14.73 ± 2.26 +++	57.68 ± 9.37 +++	18.16 ± 4.38 +++
	VAL + NAC	luminol	11.46 ± 1.66 +++	12.94 ± 1.21 +++	57.92 ± 12.83 +++	23.16 ± 4.42 +++
		lucigenin	11.70 ± 1.58 +++	13.62 ± 1.61 +++	67.24 ± 11.93 +++	21.73 ± 4.62 +++

For statistical details and abbreviations, see Table I.

Table VI. Collagen contents in the brain, heart aorta and kidney tissues (n=8).

Collagen contents (µg/mg)		Brain	Heart	Aorta	Kidney
Control		6.84 ± 1.54	7.60 ± 0.76	8.96 ± 1.03	9.93 ± 0.93
Chronic Renal Failure (CRF)	Saline	22.65 ± 1.47 ***	22.23 ± 1.51 ***	22.76 ± 1.60 ***	23.14 ± 1.98 ***
	CAP	14.86 ± 1.16***, +++	13.60 ± 1.37 *, +++	15.49 ± 1.20 **, ++	15.01 ± 1.13 ***, +++
	VAL	15.15 ± 1.17 ***,+++	13.95 ± 1.20 *, +++	15.68 ± 1.24 **, ++	15.35 ± 1.14 ***, +++
	NAC	14.96 ± 1.21 ***, +++	13.81 ± 1.37 *, +++	15.54 ± 1.21 **, ++	15.21 ± 1.11 ***, +++
	CAP+NAC	9.53 ± 1.13 +++, φ	10.93 ± 1.21 +++	9.93 ± 1.11 +++, φ	11.03 ± 0.92 +++
	VAL+NAC	9.69 ± 1.11 +++, γ	11.21 ± 0.99 +++	10.11 ± 1.10 +++, γ	11.18 ± 0.91 +++

For statistical details and abbreviations, see Table I.

damage and a significant elevation was determined in the CRF group compared to the control group ($p<0.001$; Table V). Strikingly, angiotensin receptor blocker or angiotensin converting enzyme (ACE) inhibitor alone or together with an antioxidant treatment prevented radical formation in the tissues damaged as a result of the systemic effects of renal failure ($p<0.01$-0.001).

As a measure of developed fibrotic activity, the

collagen content of all tissues increased in the CRF group treated with saline (p<0.001). Alternatively, treatment with Captopril, Valsartan or NAC suppressed the fibrotic activity and the collagen content of the tissues in rats treated with combination therapy did not differ compared to the animals in the control group (Table VI).

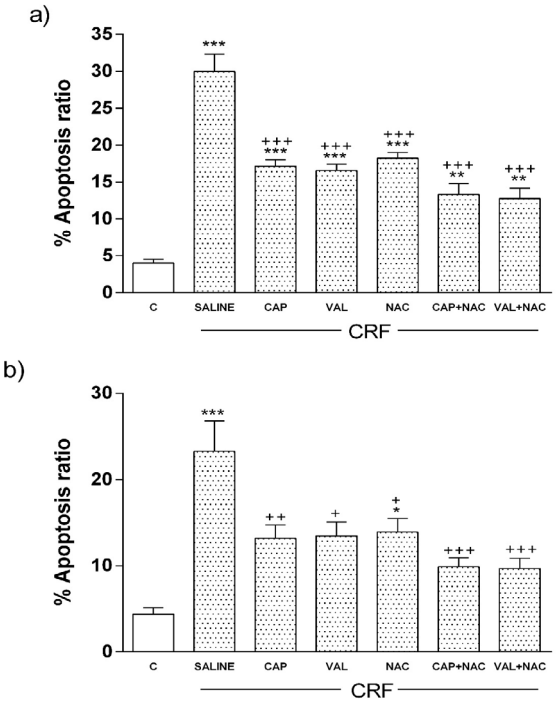

Fig. 1. Apoptosis ratio in a) neutrophils, and b) lymphocytes in chronic renal failure (CRF). Control (C), Saline (SF) treated, Captopril (CAP) treated, Valsartan (VAL) treated, N-acetyl-L-cysteine (NAC) treated, CAP+NAC treated, VAL+NAC treated CRF groups. Apoptosis ratios were calculated by dividing the values of after-stimulation to the values obtained prior to phorbol myristate acetate stimulation. * p<0.05, ** p<0.01 and *** p <0.001 compared with control, + p<0.05, ++ p<0.01, +++ p<0.001 compared with SF treated CRF group.

The apoptosis rate of both neutrophils and lymphocytes significantly increased in the rats with CRF compared to the control group (p<0.05; Fig. 1). However, the rate of apoptotic white blood cells significantly decreased in the Captopril, Valsartan, NAC, Captopril+NAC and the Valsartan+NAC groups (p<0.05; Fig. 1).

The MDA levels, which are measured as an important degradation product of lipid peroxidation in renal, cardiac, cerebral and aortic tissues, were determined to be significantly higher in the saline-treated CRF group compared to the control group (p<0.001). The MDA levels were found to significantly decrease in the Captopril, Valsartan, NAC,

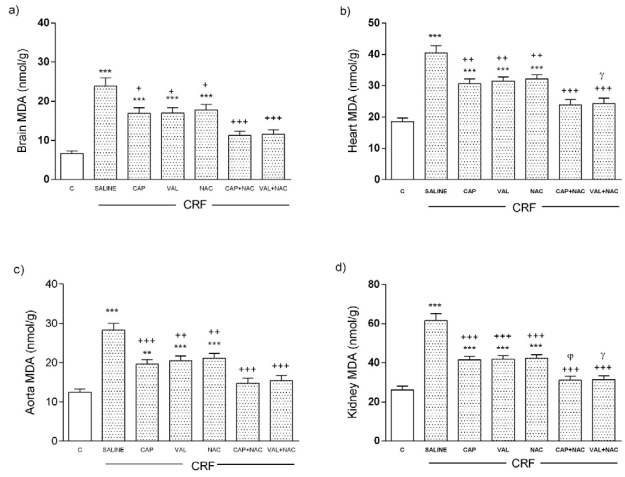

Fig. 2. Malondialdehyde (MDA) levels in the a) brain, b) heart, c) aorta and d) kidney tissues in chronic renal failure (CRF). Control (C), Saline (SF) treated, Captopril (CAP) treated, Valsartan (VAL) treated, N-acetyl-L-cysteine (NAC) treated, CAP+NAC treated, VAL+NAC treated CRF groups. ** p<0.01 and *** p <0.001 compared with control, + p<0.05, ++ p<0.01, +++ p<0.001 compared with SF treated CRF group, φ p<0.05 compared with CAP treated CRF group, γ p<0.05 compared with VAL treated CRF group.

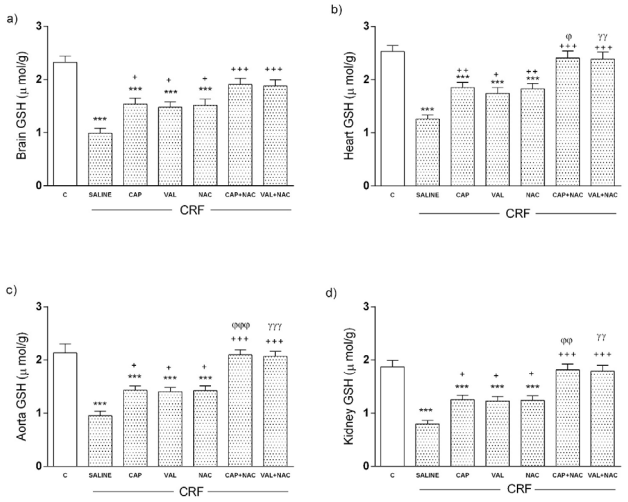

Fig. 3. Glutathione (GSH) levels in the a) brain, b) heart, c) aort and d) kidney tissues in chronic renal failure (CRF). Control (C), Saline (SF) treated, Captopril (CAP) treated, Valsartan (VAL) treated, N-acetyl-L-cysteine (NAC) treated, CAP+NAC treated, VAL+NAC treated CRF groups. *** p <0.001 compared with control, + p<0.05, ++ p<0.01, +++ p<0.001 compared with SF treated CRF group, φ p<0.05, φφ p<0.01, φφφ p<0.001 compared with CAP treated CRF group, γγ p<0.01, γγγ p<0.001 compared with VAL treated CRF group.

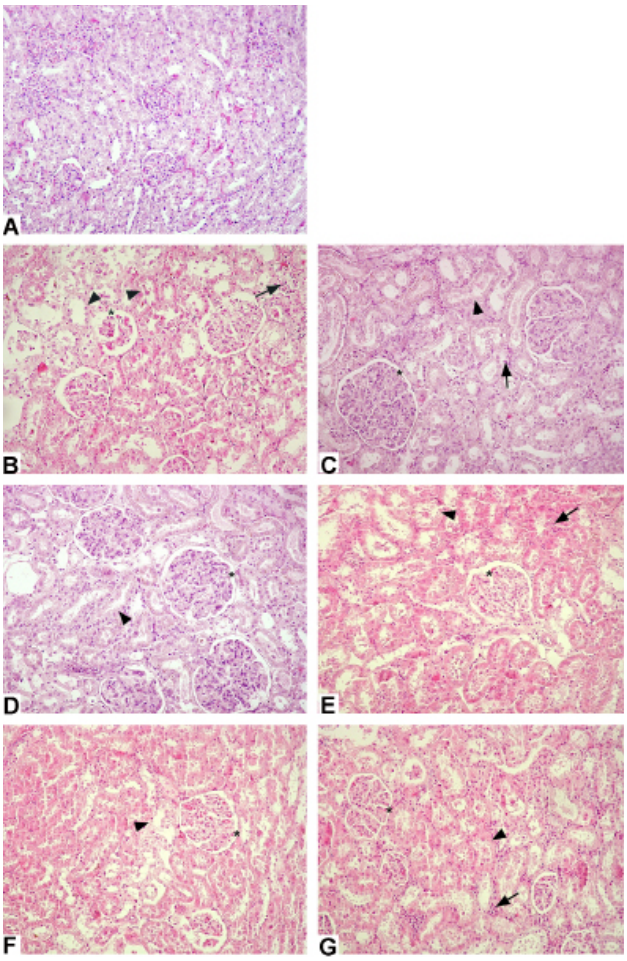

Fig. 4. Representative photomicrographs of kidney in experimental groups. Normal morphology of kidney (A) in control group (A); degenerated glomerulus (*) and tubular structures (arrowhead) and inflammatory cells (arrow) in CRF group (B); quite regular glomerulus (*), a few degenerated tubular structures (arrowhead) and decreased number of inflammatory cells (arrow) in CRF+CAP (C), CRF+VAL (D), CRF+NAC (E), CRF+CAP+NAC (F) and CRF+VAL+NAC (G) groups are seen. H andE staining, Original magnifications: 200x.

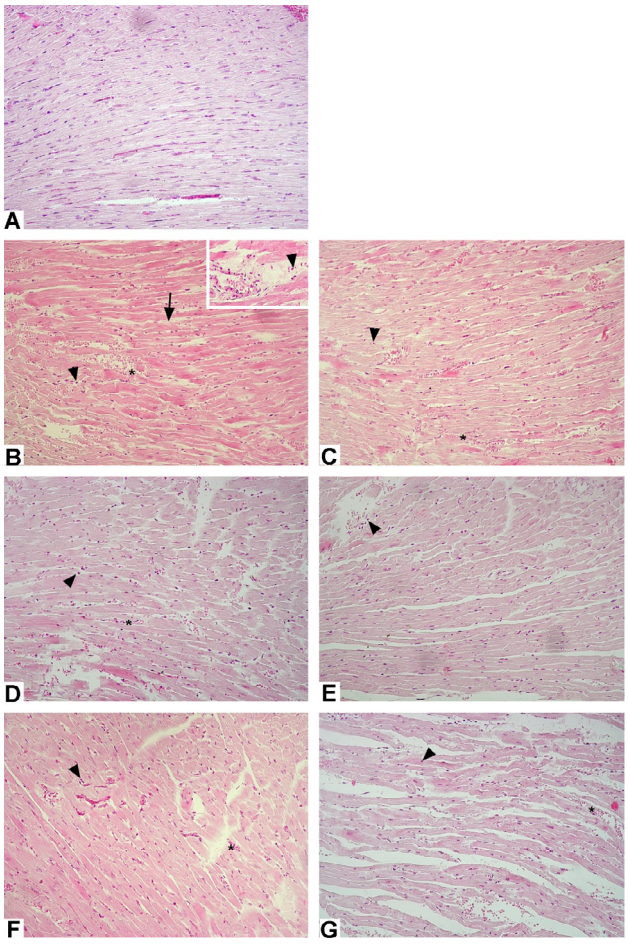

Fig. 5. Representative photomicrographs of heart in experimental groups: Normal morphology of heart in control group (A); degenerated cardiac muscle fibers (arrow), haemorrhage (*) and inflammatory cells (arrowhead) in CRF group (B); quite regular cardiac muscle fibers, decrease of vascular congestion (*) and inflammatory cells (arrowhead) in CRF+CAP (C), CRF+VAL (D), CRF+NAC (E), CRF+CAP+NAC (F) and CRF+VAL+NAC (G) groups are seen. H andE staining, original magnifications: 400x.

Captopril+NAC and Valsartan+NAC groups ($p<0.05$-0.001, Fig. 2). In the saline-treated CRF group, the major cellular anti-oxidant GSH levels were determined to significantly decrease ($p<0.01$-0.001); however, the reduction in GSH levels was found to be lower in CAP, VAL or NAC-treated CRF groups. However, this did not occur in GSH stores when NAC was given in combination with Captopril or Valsartan ($p<0.05$-0.01; Fig. 3).

Normal morphology of kidney (Fig. 4A), heart (Fig. 5A), aorta (Fig. 6A) and cerebral cortex (Fig. 7A) were observed in the control group. Degenerated Bowman capsule, glomerulus and tubular structures and inflammatory cell infiltration in interstitium of kidney (Fig. 4B); degenerated cardiac muscle fibres, severe vascular congestion and inflammatory cell infiltration in cardiac tissue (Fig. 5B); irregularity in endothelial layer and inflammatory cell infiltration in adventitia layer of aortic tissue (Fig. 6B); vascular congestion and degenerated neurons in cerebral cortex (Fig. 7B) were observed in

CRF group. All of these histopathological alterations were moderately decreased in CRF+CAP (Figs. 4C-7C), CRF+VAL (Figs. 4D-7D), CRF+NAC (Figs. 4E-7E), CRF+CAP+ NAC (Figs. 4F-7F), and CRF+VAL+NAC (Fig. 4G-7G) groups.

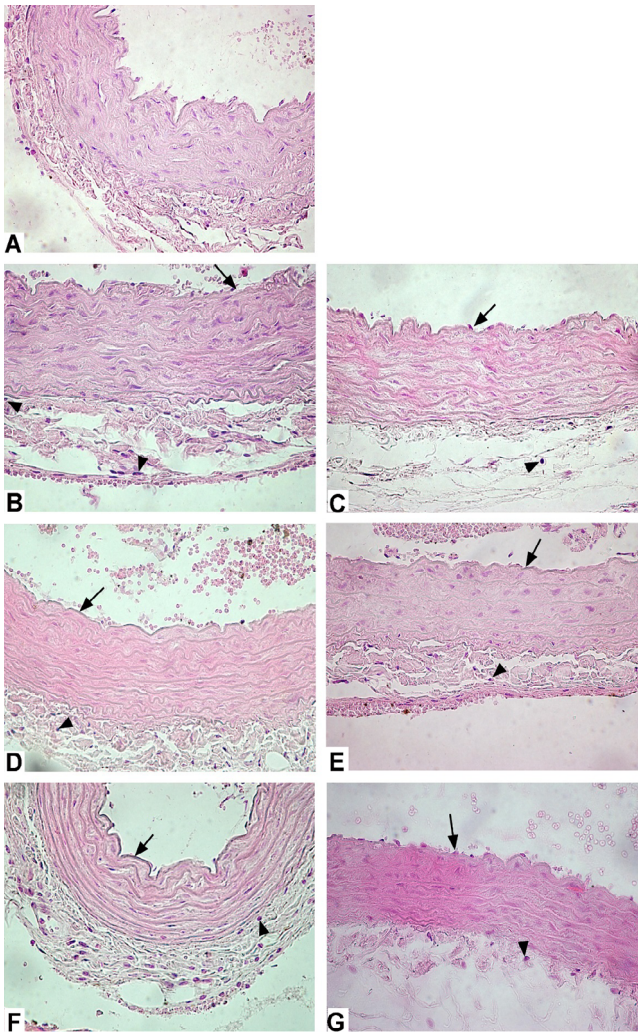

Fig. 6. Representative photomicrographs of aorta in experimental groups: Normal morphology of aorta in control group (A); degenerated endothelium (arrow) and inflammatory cells (arrowhead) in adventitia layer of CRF group (B); quite regular endothelium (arrow) and a decreased number of inflammatory cells (arrowhead) in adventitia layer of CRF+CAP (C), CRF+VAL (D), CRF+NAC (E), CRF+CAP+NAC (F) and CRF+VAL+NAC (G) groups are seen. H andE staining, original magnifications: 200x

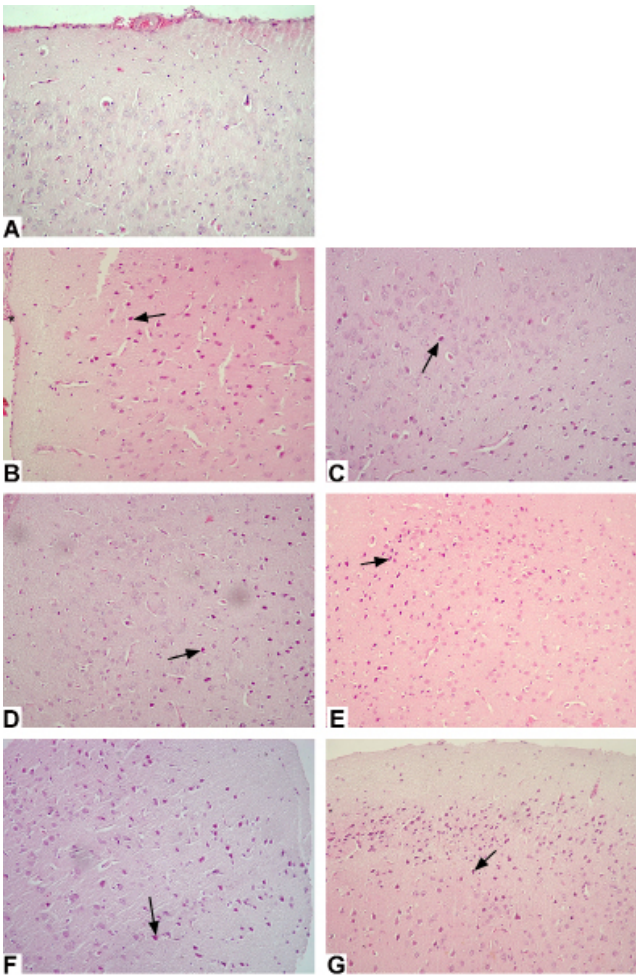

Fig. 7. Representative photomicrographs of cerebral cortex in experimental groups: Normal morphology of cerebral cortex in control group (A); vascular congestion (*) and degenerated neurons (arrow) in CRF group (D); decreased number of degenerated neurons (arrow) in CRF+CAP (C), CRF+VAL (D), CRF+NAC (E), CRF+CAP+NAC (F) and CRF+VAL+NAC (G) groups are seen. H andE staining, original magnifications: 200x.

DISCUSSION

In the current study, CRF led to a significant elevation in blood pressure, apoptosis level, lipid peroxidation, collagen content, luminol and lucigenin levels. The reduction in GSH levels mainly in renal tissue followed by the brain, heart and aorta tissues accompanied this condition is indicative of renal failure-related systematic oxidative damage. In addition, the impairment in renal functions, which was assessed through changes in serum and urine electrolyte levels, increased the serum levels of pro-inflammatory mediators (TNF-α, IL-1β and IL-6).

Administration of ACE inhibitor, Captopril or angiotensin AT1 receptor blocker, Valsartan and/or antioxidant agent, NAC, prevented systemic oxidative damage, healed tissue damage and also preserved renal functions.

Severe renal failure leads to sodium retention, which increases the effective circulating volume (Sinha and Agarwal, 2018). In our study, blood pressure increased at the end of the 3rd week following the reduction in renal mass, the elevated blood pressure levels decreased with the use of anti-hypertensive drugs, Captopril and Valsartan. At the end of the test period, the mean systolic blood pressures were higher in the CRF groups treated with anti-hypertensive and anti-oxidant drugs compared to the control group; however, these values were statistically significantly lower compared to the CRF group treated with saline solution. Angiotensin-converting enzyme inhibitors are known to decrease the blood pressure and one of the first stage drugs in hypertension treatment (Del Vecchio *et al*., 2017; Omboni and Borghi, 2018).

Similar to ACE inhibitors, angiotensin receptor blockers, which are used in small clinical trials, were shown to have beneficial effects on renal functions. In addition, angiotensin receptor blockers induce the natriuretic response, which may contribute to anti-hypertensive effectiveness. Also the up-regulation of AT1r was joined with a significant elevation in angiotensin II positive cell count in 5/6 nephrectomised rats and ACE inhibitors and AT1 receptor antagonists led to renal preservation (Remuzzi *et al*., 2016; Wesson *et al*., 2012). Similarly, angiotensin AT1 receptor blockade was shown to reduce blood pressure, glomerulosclerosis and interstitial fibrosis, attenuated up-regulation of the inflammatory system in remnant renal tissue and thereby slowed down the progression of renal disease (Kario, 2018; Remuzzi *et al*., 2016). This condition indicates that the effects of Captopril and Valsartan on blood pressure are directly related to their anti-hypertensive effects. Conversely, the blood pressure decreased also in the NAC-treated group. A study showed that NAC treatment partially attenuated the development of L-NAME-related hypertension through mildly enhancing vasodilation because high blood pressure results from the imbalance between the vasoconstrictor and the vasodilator systems (Neves *et al*., 2018).

NAC treatment was been shown to decrease oxidative stress in spontaneously hypertensive rat (Pechanova *et al*., 2006) and significantly decreased the oxidative stress and apoptosis through its anti-oxidant effect in lymphocytes of children with CRF (Zachwieja *et al*., 2005). Consistent with the literature, while our study has shown that uremic renal disease and accompanying electrolyte imbalance-related radicals play a role in blood pressure elevation, NAC treatment was shown to relieve the hypertensive response through its anti-oxidant effects. The current data verified that NAC administration decreased the elevated blood pressure, which develops due to renal failure when it is combined with anti-hypertensive substances that act by blocking the renin-angiotensin system. This study revealed that MDA levels in the brain, heart, aorta and renal tissues significantly increased in rats with CRF, however, decreased tissue GSH levels revealed that the antioxidant pool was displaced. Besides, Captopril and Valsartan treatments reversed the MDA and GSH responses and these results indicate the effect of antioxidant treatment. When the animals were treated with Captopril or Valsartan, tissue fibrosis decreased the oxidant-related fibrosis in rats with CRF and it was verified that the anti-oxidant effects of these drugs increased with the use of the anti-oxidant, NAC. Impaired renal functions were also improved through the effects of Captopril, Valsartan and NAC.

Due to the natural limitations of the clinical studies, it was difficult to distinguish the role of renal failure from underlying diseases, co-morbid conditions and therapeutic interventions in humans (Sturgill and Bear, 2019). In addition, it is not possible to deeply investigate the molecular origins of oxidative stress in CRF patients, because the internal organs require an invasive approach (Askari *et al*., 2018). Therefore, the present study has investigated the effect of CRF-related systemic oxidative stress on multiple organs using two chemiluminescence probes luminol (detects H_2O_2, OH-, hypochlorous acid, peroxynitrite, and lipid peroxyl radicals) and lucigenin (sensitive to superoxide radical) (Davies *et al*., 1992; Ohara *et al*., 1993). While the elevated luminol and lucigenin chemiluminescence levels supported the opinion that CRF-induced multi-organ failure contained the formation of toxic oxygen metabolites, their levels significantly decreased through the administration of Captopril, Valsartan, NAC and their combinations. Captopril was reported to clear superoxide, hydroxyl radicals and hypochlorite acid (HOCl) and thereby exhibited an anti-oxidant effect (Bagchi *et al*., 1989; Ghazi-Khansari and Mohammadi-Bardbori, 2007; Guzman-Hernandez *et al*., 2015; Miller *et al*., 2007). Angiotensin receptor antagonists, which have been widely used anti-hypertensive agents, were also shown to have antioxidant activity. Angiotensin II is known to induce reactive oxygen species formation and pro-inflammatory cytokine production. The blockade of these effects is suggested to reduce reactive oxygen species-related tissue damage (Han *et al*., 2017; Sifi *et al*., 2017). Similarly, Valsartan treatment significantly reduced MDA, which affects the oxidative stress and pro-inflammatory status of the kidney in CRF patients (Gamboa *et al*., 2012).

Circulating pro-inflammatory cytokine levels, which have been suggested to play an important role in some uremic complications, significantly increase in end-stage

renal disease (Velasquez *et al*., 2016). Impairment in cytokine production like TNF-α, IL-1β and IL-6 is common in CRF (Wu *et al*., 2018). In our study, elevated circulating inflammatory mediators were shown to result from oxidative damage of distant organs. These pro-inflammatory cytokines are also suppressed with Captopril and Valsartan besides anti-oxidant therapy with NAC. These results indicate the anti-oxidant and anti-inflammatory effects of angiotensin receptor blockers and ACE inhibitors in CRF.

CONCLUSION

In conclusion, both angiotensin receptor blocker and angiotensin-converting enzyme inhibitor were shown to lead to an antioxidant effect in the affected tissues in CRF together with their anti-hypertensive effect. These protective effects were enhanced to a higher extent with the addition of an anti-oxidant, which indicate that while the agents used to block the renin-angiotensin system improved CRF-related renal and distant organ dysfunction, addition of an anti-oxidant enhanced these effects. Hence, we consider that the present study would shed light on developing novel treatment regimens and further research.

Statement of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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