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Mitigation of Acetamiprid – Induced Renotoxicity by Natural Antioxidants via the Regulation of ICAM, NF-kB and TLR 4 pathways

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Short Title: Role of Natural Antioxidants in Acetamiprid Renotoxicity

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Male wistar albino rats 150 g were got from the Experimental Animal Center, King Saud University. The animals were allowable to acclimate in the laboratory for one week, at temperature (22°C) and natural light/dark cycle. They were given a standard rat pellet diet and distilled water and libitum. The use of animals was under the order of King Saud University's ethics committee (KSU-SE-18-37).

Abstract

Background: Acetamiprid (ACMP) is a member of the neonicotinoid group of insecticides. It is extensively used worldwide. The misuse of ACMP creates danger hazards to human and animal. Methods: ACMP induced renal damage evidenced by an increase in kidney injury biomarkers. So the goal of this work is to clarify the reno protective effect of Quercetin(Qrctn) and/or Nano-glutathione (N- Gluta) solely or in combination to counterbalance the danger effect of ACMP. All treatments with the previous agents were coadministered orally with ACMP for one month.. Results: ACMP ingestion caused a significant rise in serum creatinin, urea, and uric acid, TNF α along with renal cystatin C, lipid peroxidation and nitric oxide with the concomitant decline in the levels of reduced glutathione and IL-10 levels. Protein expression of ICAM was upregulated as well as mRNA expression of NF-kB while mRNA expression of Nrf2 was down-regulated. Immune histochemistry of TLR 4

revealed strong Immune reaction. The administration of Qrctn or N- Gluta either individually or together modulated all the preceding aforementioned parameters. Conclusion; Fascinatingly Qrctn and N- Gluta combination was the most powerful regimen to frustrate ACMP reno-toxicity and may be deliberate as a hopeful applicant for renal therapy.

Introduction

Pesticides are chemical formulations progressively used in agriculture as well as public health processes to get a ride from insects, fungus and to overwhelmed insect-conducted diseases [1]. The excessive usage of pesticide has established their widespread in the surroundings which may cause pollination throughout the food chain [2].

The misuse of these agrochemicals may pose serious hazards to all human being. ACMP is belonged to the neonicotinoids class of pesticides which have potency and systemic action for crop defense toward piercing-sucking pests [3].

ACMP is being extremely water soluble that facilitate its dispersion in the irrigation water and consequently to the environment. It was confirmed that ACMP exhibits hepato, reno, cardio, ovarian and brain toxic impact at sub-lethal doses. The kidney is still the target organ affected by this agrochemical [4].

ACMP raises oxidative stress biomarkers, as p38 MAPK, and declines antioxidant enzymes activity. It affects male reproductive function through the action of its metabolites in testes [5]. Neutralization of free radicals and reduction or prevention of their dangerous effect takes place via antioxidants treatment.

Mitochondrial hydrogen peroxide scavenging is GSH-dependent route as a result of the absence of the antioxidant enzyme catalase [6]. Maintaining of GSH pool in mitochondria is essential for homeostasis of the redox system in the mitochondria and in evaluating the sensitivity to apoptosis induced by chemicals. Membrane transport processes are critical in controlling renal cellular and subcellular GSH amounts and in deciding susceptibility to cytotoxicity induced by oxidants and electrophiles [6].

Flavonoids, such as Qrctn is antioxidants, can scavenge free radicals, which damage cell membranes, tamper with DNA, and even cause cell death. Qrctn acts like an antihistamine and an anti-inflammatory and may help protect against heart diseases. Qrctn exhibits its anti-inflammatory power by stabilizing histamine-releasing cells. Previous studies proposed that flavonoids like Qrctn may reduce atherosclerosis risk [7] .

Nowadays the use of Nano drug is considered as a novel strategy for the enhancement of bioavailability of the drug, lesser side effects, and improvement of its solubility and hence its excretion and protect it against degradation. This stimulates our interest to make use of the mixture of N- Gluta together with the natural antioxidant Qrctn in the amelioration of oxidative stress and inflammation Little was known about the mechanism underlying ACMP reno

toxicity at the molecular level. This goal was reached by assessing serum TNF, IL-10, cystatin C, mRNA expression of nrf2 and NF-kB in renal tissues along with TLR4protein expression.

So the goal of this project is directed towards improving the health of individuals who subjected to subtoxic doses of ACMP throughout stopping the disorders from getting worse and reducing its complications in kidneys.

Materials and methods

Drugs

ACMP was purchased from Sigma Chemical Co., USA, Qrctn was bought from iherb and N- Gluta was obtained from Lipolife, Drakes Lane Industrial Estate, Drakes Lane, (UK).

Experimental Design

Male wistar albino rats 150 g were allowable to acclimate in the laboratory for one week, at temperature (22°C) and natural light/dark cycle. They were given a standard rat pellet diet and distilled water and libitum.

Thirty rats were separated into five collections six rats each. Group I, negative control rats received 1% Carboxy Methylcellulose (CMC). Group II, rats were administered 100 mg/kg/day of ACMP [1]. Group III, ACMP intoxicated rats treated with Qrctn 100mg/kg/day [8]. Group IV, ACMP intoxicated rats were administered 300mg/kg/day of N- Gluta [9]. Group V, rats were administered a mixture of N- Gluta and Qrctn as the same previous doses. All treatments with the previous agents were co administered orally with ACMP for one month. There after animals were subjected to CO_2 in gradual concentration, sacrificed by decapitation; blood samples were collected for serum separation. Right kidneys were collected, weighted, and then were homogenized in phosphate buffered to yield 20% homogenates. Parts of right kidneys will be kept under liquid nitrogen for Western blot analysis another right kidneys were kept in 10% formalin for immunohistological examination

Biochemical serum and tissue analysis

Serum urea, creatinine and uric acid, renal nitric oxide (NO), GSH and lipid peroxide (MDA)

Serum urea creatinine and uric acid were assessed using the kits(Randox Laboratories). Renal MDA and GSH levels were measured according to Uchiyama and Mihara (1978)[10] and Ellman (1959)[11] methods, respectively. Total nitrite will be valued following the method for Moshag et al. (1995) [12].

Evaluation of TNF-α and IL-10 **levels**

TNF-α level and IL-10 and cystatin C were assessed using ELISA kits obtained from R&D Co.

Quantitative Real-Time Polymerase Chain Reaction for Analysis of mRNA expression of renal Nrf2 and NF-kB

Total RNA were isolated from renal tissue homogenates using RNeasy Purification Reagent (Qiagen, Valencia, CA) according to manufacturer's instruction. The purity (A260/A280 ratio) and the concentration of RNA were evaluated using spectrophotometry (GeneQuant 1300, Uppsala, Sweden). RNA quality will be confirmed by gel electrophoresis on a 1% agarose gel stained with ethidium bromide [13].

Evaluation of Protein Level

Western Blot

Renal ICAM-1protein expression was done according to Mahmood, et al, (2012) [14].

Immuno Histological investigation

Kidneys sections wad cut and examined for immuno histopathological **investigation** using anti TLR4 [15].

Statistical analysis

Data were expressed using GraphPad Prism (GraphPad Software, San Diego, CA, USA), and all statistical comparisons were made by means of the one-way analysis of variance test followed by Tukey's test post hoc analysis. Results were expressed as mean ± standard error of the mean and a P-value ≤ 0.05 were be considered significant.

Results

Table (1) revealed that the serum creatinine, urea, and uric acid were elevated upon ACMP administration matched with to the normal control group. Whereas treatment with the antioxidants in question solely or together declined almost of the previous distorted parameters compared to ACMP administered group. Serum levels of TNFα, and cystatin C were increased while IL-10 was decreased in ACMP administration group compared matched with the control group. Whereas treatment of the antioxidants in question alone or in combination declined almost of the preceding distorted parameters compared to ACMP administered group (Fig.1). Ingestion of ACMP increased the levels of renal NO and MDA levels, whilst the level of GSH level was declined compared to the control group. Treatment with N-Gluta and /or N-Gluta & Qrctn consecutively controlled the changed parameters versus ACMP administered group (Fig.2).

Protein expression of ICAM-1 and mRNA expression of NF-kB were up regulated whereas mRNA expression of Nrf2 was down regulated in renal tissue upon ACMP treatment.

Inversely treatment with the antioxidants individually or in combination modulated these expressions compared to ACMP administered group (Fig. 3 & 4).

Fig.5 revealed that the immunohistochemistry of Toll-like receptor 4 protein expression was absent in normal glomeruli in the control group, and very faintly expressed in tubules. While TLR4 protein expression in ACMP treated group was detectable in the glomeruli mainly localized to podocytes, moderately expressed along the proximal tubule and strongly expressed along the distal tubule. In ACMP treated with Qrctn group. TLR4 protein expression was detectable weakly in the glomeruli mainly localized to podocytes, mild- moderately expressed along the proximal tubule, weakly expressed along the distal tubule. In ACMP treated with N-Gluta group. Such protein was absent in the glomeruli, mildly expressed along the proximal tubule, weakly expressed along the distal tubule. TLR4 protein expression was absent in the glomeruli, very faintly expressed along the proximal tubule weakly expressed along the distal tubule in Qrctn and N-Gluta treated group.

Discussion

ACMP; 1E-N-[(6-Chlor-3-pyridinyl) methyl]-N'-cyan-N-methylethanimidamid; is a new nicotine agonist it has the ability to run off outward water, thus it may be reached to non-target organisms via the pesticide remains in air and water [16].

ACMP is very operative for aphids, and pests of leafy vegetables [17]. The electronegativity of nitroguanidine, nitromethylene, and nitroamine enhances the selectivity of ACMP to bind with the subsite in nicotine -acetylcholine receptors, It acts on the central nervous system of insects and causes excitation of the nerves and final paralysis, and consequently death [18].

The misuse of these agrochemicals may pose serious hazards to human and animal health [3].

The kidney is well-thought-out as a target of various toxic constituents, as environmental chemicals. The sensitivity of the kidney to various environmental substances may be due to it's anatomical, biochemical or physiological features. Factors that lead to the sensitivity of the kidney include increased blood flow, the presence of different types of xenobiotic carriers and metabolizing enzymes in the kidney [19]. For this reason, there is a critical need for the usage of natural products for the treatment of numerous diseases since they are highly available and show fewer side effects.

The Administration of Qrct could largely ameliorate kidney interstitial fibrosis and macrophage accumulation in the kidneys with obstructive nephropathy.Qrct can also reduce the levels of oxidative stress and apoptosis in kidney cells in rats [20].

The suitable GSH supply to the kidneys is essential to keep its regular function. This may be due to the great levels of aerobic metabolism, mainly in the proximal tubules. Additionally, the

function of some carriers in the kidney still not known, it is expected that several carriers are accountable for GSH plasma membrane transport [21].

The antioxidant effectiveness of exogenously administered GSH is hindered by its instability when crossing cell membranes and it's rapidly hydrolysis in the circulation [22]. In circulation, GSH is degraded rapidly by gamma-glutamyltranspeptidase, an enzyme found in the extracellular surfaces of cells, yielding glutamate (Glu), cysteine (Cys), and glycine . Although Cys is a critical amino acid for the synthesis of GSH, it is sufficiently reactive in circulation for large amounts of Cys to be oxidized immediately to cystine (Cys2) [23]. To increase the bioavailability of GSH, its formulation in lipid nanoparticles significantly increases its penetration into the cell.

ACMP increase markers of oxidative stress and down-regulated the activity of antioxidant enzymes [5]. It was observed that ACMP has the toxic potential on liver, kidney, heart, ovary, and brain at sub-lethal doses. The kidney is the most damaged organ via the exposure to this insecticide [4].

Serum cystatin C (CYS) has the potential to be a more precise marker for glomerular filtration rate (GFR) and can be used as a reliable and precise marker for renal function in mouse models.CYS is more sensitive than creatinine, and it shows renal damage earlier than serum creatinine and blood urea nitrogen [24].

Herein, ACMP-induced oxidative renal-injury was recognized by the rise in serum creatinine, urea, uric acid and cystatin C which represented cellular outflow and alteration of the cell membranes efficacy of the kidneys. Administration of Qrctn and/or N- Gluta fruitfully decreased their levels.

The present study demonstrated that ACMP -ingestion elevated renal MDA and NO levels with the concomitant decreased GSH level. Park et al. (2007) [25] indicated that oxidative stress prompted by a disparity between the antioxidants and the generation of reactive oxygen species (ROS) and hence ROS is the main cause of ACMP induced cytotoxicity [5]. Renal MDA, NO, and GSH were ameliorated by the co-administration of Qrctn and N- Gluta, they were reported to exert renoprotective effects against lipid peroxidation induced by toxic chemicals [26, 27].

NF-E2-related factor 2 (Nrf2), is a transcription factor, Keap1 shows a vital rule in Nrf2-response regulation, which then trans activates antioxidant response element (ARE)-driven antioxidant genes. Keap1 is the redox-sensitive protein that on stimulus allows the activation of Nrf2. It was documented Nrf2 is down-regulated by fluoride treatment [28].

Nrf2-ARE is the main pathway that regulates detoxifying and the activation of antioxidant enzymes in circumstances of electrophilic or oxidative stress. Toll-like receptors

(TLR) are located in the endoplasmic reticulum, lysosomes, and endosomes, they play an essential role in initiating the immune responses [29]. TLRs are expressed in heart cells, kidney, and liver [30].

TLRs are integral glycoproteins that exhibit vital role in signaling within and outside the cell. Extra cellular, it binds the ligand to TLR that activates intracellular Toll/interleukin-1 receptor domains (TIR) that triggers different signaling [31]. TLR4 activation causes cardiac apoptosis, pro-inflammatory response, and oxidative stress. TLR4 activation triggers nuclear factor-kappa B (NF-κB) transcriptional activity in the cytoplasm and the release of NF-κB from its inhibitor IkBα and then it is translocated into the nucleus that in turn stimulates pro-inflammatory cytokines IL-6 and TNF-α production [28, 32], which are proteins regulator for tissues inflammation process [33].

TNF-α upregulates IL-6 via the signal transduction factor NF-kB, and IL-6 activates STAT3. STAT3 is signal transduction factor that activates a large number of genes important in hepatocyte regeneration [34, 35]. Interleukin 10 (IL-10) is a cytokine with potent anti-inflammatory properties that plays a central role in limiting host immune response to pathogens, thereby preventing damage to the host and maintaining normal tissue homeostasis. Dysregulation of IL-10 is associated with enhanced immunopathology in response to infection as well as increased risk for development of many autoimmune diseases [36].

Activation of adhesion of the leukocyte to the endothelium via TNF-α is facilitated by the elevation of adhesion molecules at the endothelium of the cell surface. TNF-α activates the vascular adhesion molecule-1 (VCAM-1), and the intracellular adhesion molecule-1 (ICAM-1), expression in several organs; such as lung, liver, and kidney [36]. Together, the data propose that the TNF-α receptor facilitates the initiation of ICAM-1 and VCAM-1 expression and is vital plays an important role in controlling the infiltration of leukocyte [37]. The present study confirmed marked increases in the levels of TNF α as well as protein expression of ICAM-1 and mRNA expression of NF-kB while, IL-10 level and mRNA expression of Nrf2 was down-regulated, also strong Immune reaction of TLR 4 was observed upon ACMP ingestion. The administration of Qrctn or N- Gluta alone or in combination modulated all the preceding aforementioned parameters.

It was concluded that the administration of Qrctn or N- Gluta either individually or in a mixture modulated all the preceding aforementioned parameters. Fascinatingly the mixture of Qrctn and N- Gluta was the most efficient protocol to frustrate ACMP renotoxicity and could be considered as a hopeful applicant for renal therapy.

Conflict of Interest declaration

Authors declare that there is no Conflict of Interest

Acknowledgment

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Figure1

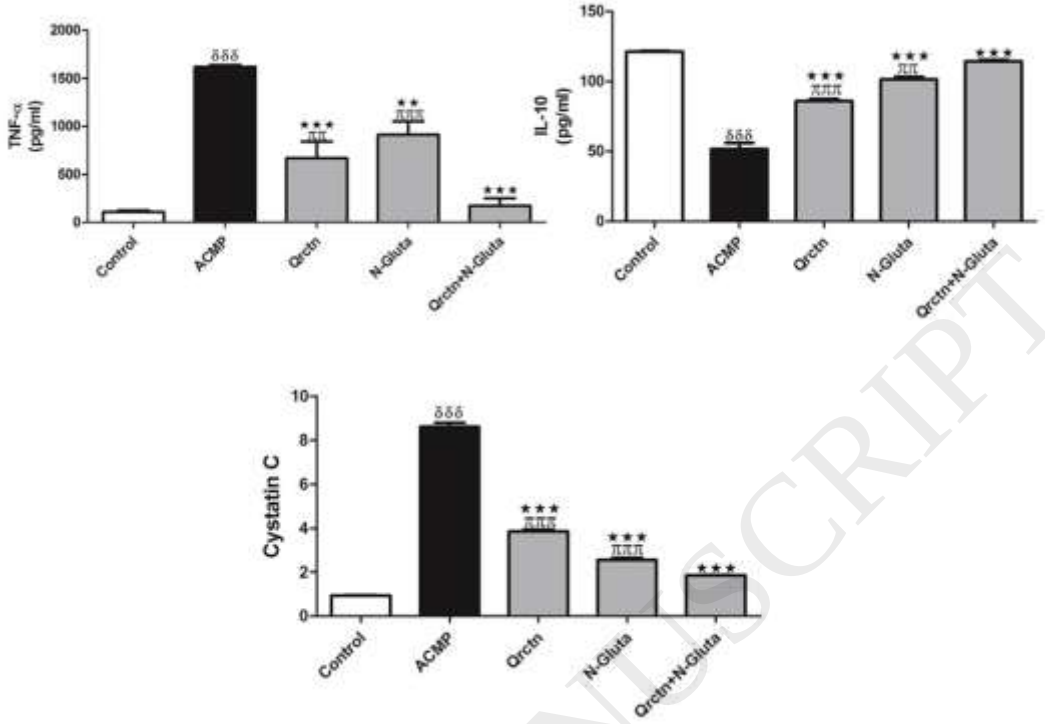

Figure 2

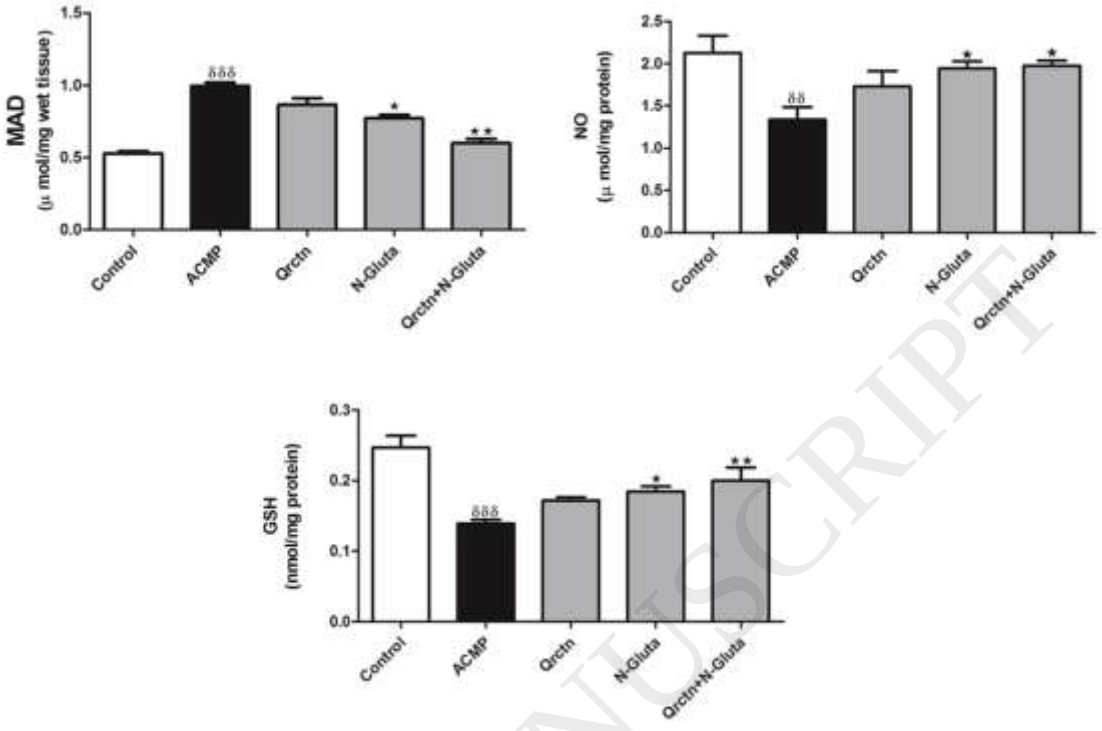

Figure3

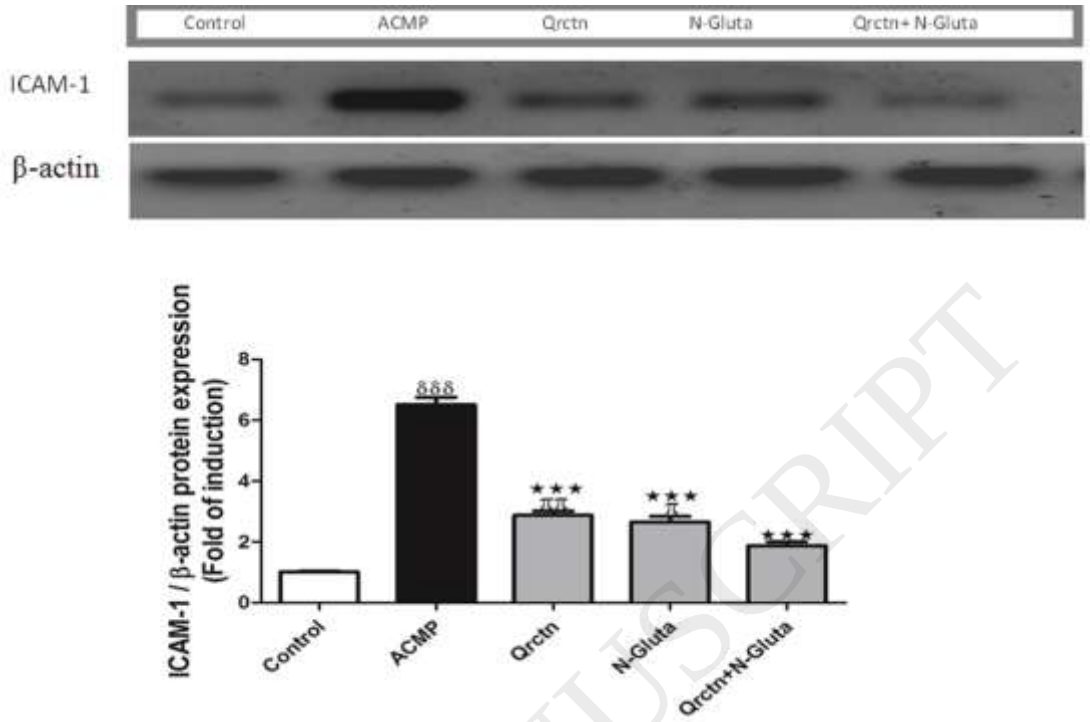

Figure4

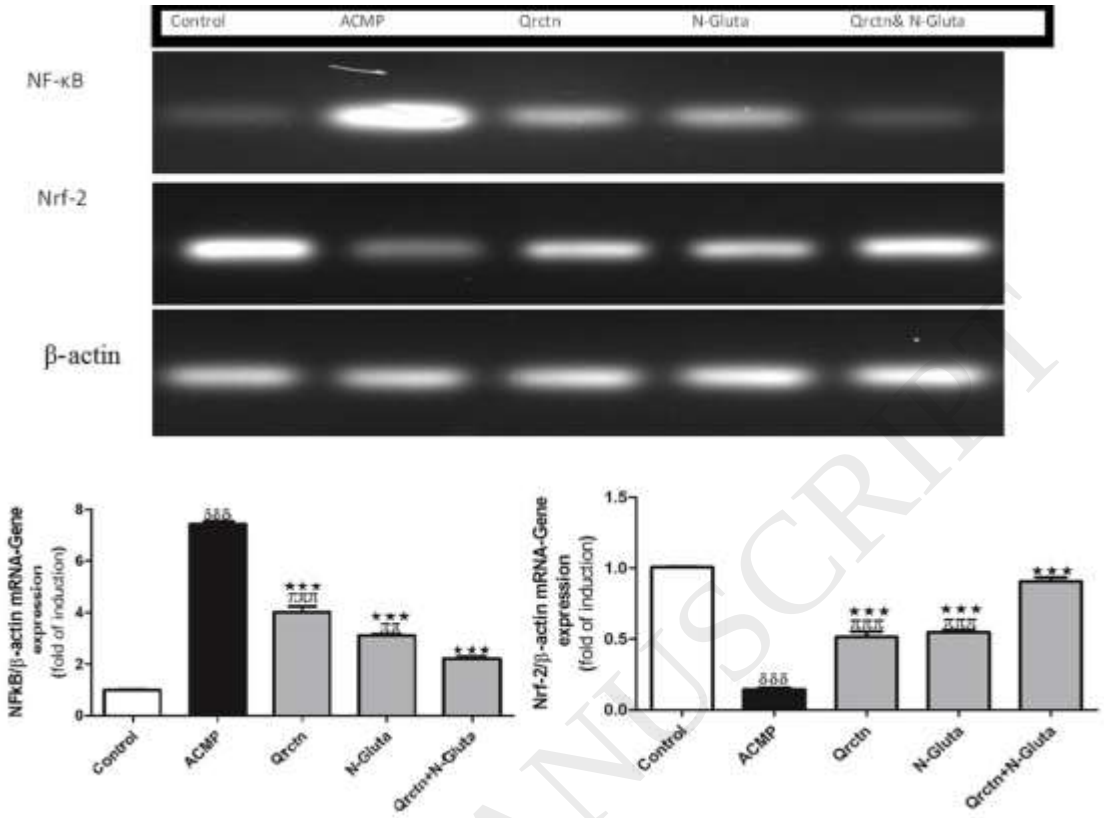

Figure4

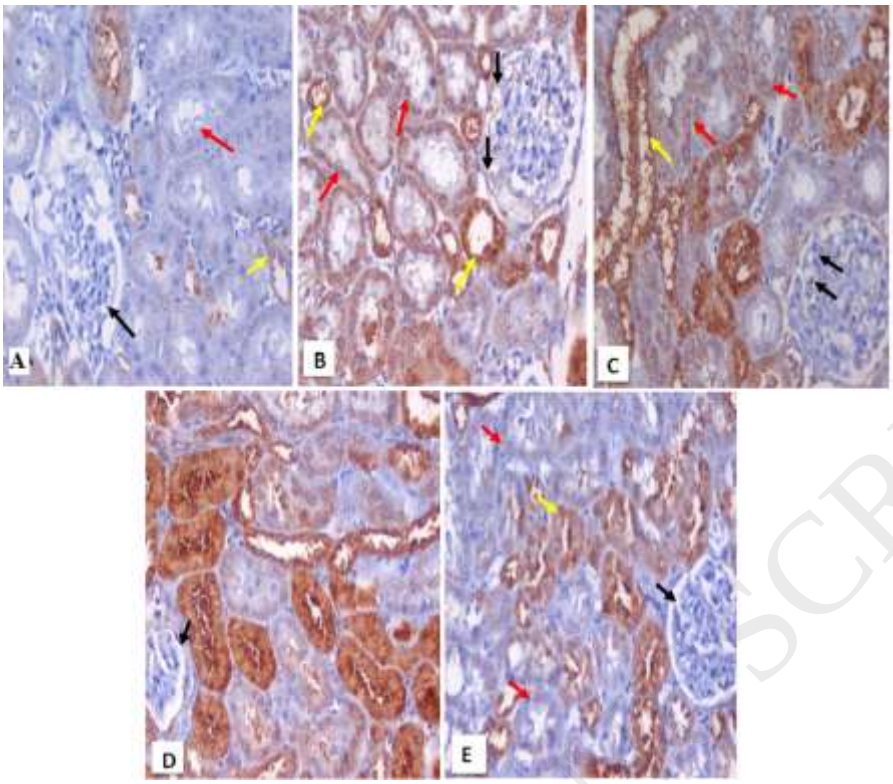

Table (1): Serum levels of urea, creatinine and uric acid in control and in all groups.

	Control	ACMP	Qrctn	N-Gluta	Qrctn & N-Gluta
Urea (mg/dl)	18±0.95	32±1.5 $^{\delta\delta\delta}$	24±0.82**	21±0.81***	21±1.2***
Creatinine (mg/dl)	1.63±.08	16.6±1.9 $^{\delta\delta\delta}$	5.5±1.05***	3.5±0.4***	2.7±0.5***
Uric acid (mg/dl)	4±0.27	13.8±1.3 $^{\delta\delta\delta}$	5.98±0.6***	6.87±0.74***	5.6±0.4***

Notes: Data are shown as mean ± SEM (N=6). $^{\delta\delta\delta}P\leq 0.001$ compared to control group, and $^{***}P\leq 0.001$ compared to ACMP group.