Nano Glutathione and Quercetin Abates the Toxicopathic Effect of a Neonicotinoid in Rats Kidneys: Role of DNA Damage and VCAM-1/KIM-1 Protein Expressions

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INTRODUCTION

Pesticides are systemic toxicants that arouse extensive ecological dramatic health problems. The widespread increase of their appliance in crop protection and pests control induce a hazardous effect to all living organisms. The current study is focused on the prophylactic impact of Quercetin (Qrct) and / or Nano glutathione (N- Glta) alone as well as their combination to counteract the harmful effect of Mospilan (Mosp) - induced reno toxicity.

Mospilan is a neonicotinoid pesticides that act in a similar way to nicotine (Kimura Kuroda et al., 2012). Mosp exerts its effect on the nervous system of insects by excessive activation of acetylcholine receptors. The misuse of Mosp by the farmers causes this pesticide to enter into food chain that may cause toxicity to human (Mondal et al., 2009) . Kidney is considered a target of Mosp.

Quercetin is a natural flavonoid, It has been shown to exert antioxidant, anti-inflammatory, anti-carcinogenic and antiviral activities. (Wang et al., 2016).

The kidneys depend on a specific amount of glutathione (GSH) to maintain normal function.

(Lash L, 2005). Exogenous GSH may protect the kidney against oxidative stress while brush-border plasma membrane functions physiologically in turnover of cellular GSH. However, GSH has poor water solubility. This initiates our interest to use N-Glta and /or Qrct to ameliorate Mosp hazard effect on the kidney, few information were known about Mosp induced renal damage as well as concerning its toxicological action at cellular and molecular levels.

According to the toxicological information, there is no specific antidote for Mosp that is why the current study was designed to assess the role of Qrct and N-Glta alone or in combination, this is a novel strategy to reduce the risk of this pesticide on rat's kidney. Also to discover new molecular pathways involved in Mosp toxicity and treatment.

RESEARCH OBJECTIVES

1-To induce Mosp toxicity in rats.

2-To measure the biochemical and molecular mechanisms underlying Mosp toxicity in serum and kidney of rats.

3-To treat rats with Qrct and N-Glta and to elucidate their nephro-protective effect.

4- To study the efficacy of the two antioxidants in question in reducing the drastic biochemical effects in Kidney through measuring the biomarkers of kidney damage and inflammatory mediator including TNF-α and interleukin -6 also lipid peroxides, glutathione (GSH), superoxide dismutase (SOD), Malondialdehyde (MDA) and nitric oxide.

5- Protein expression of vascular adhesion molecule-1 (VCAM-1), kidney injury molecule-1 (KIM-1) was assessed using Western Blot technique. as well as mRNA expression of nuclear factor kappa B (NF-κB) and DNA fragmentation were performed.

6- Histopathological examination was also performed.

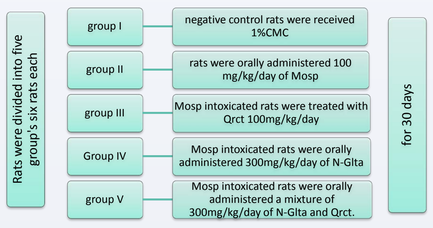
MATERIALS AND METHODS

Drugs

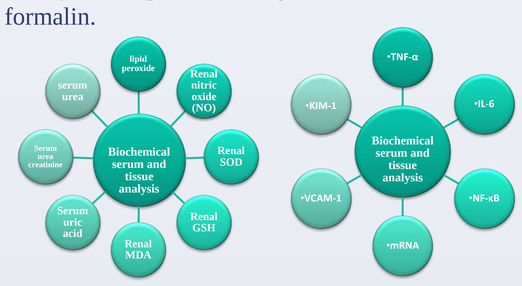
Mospilan 100mg\kg (Mondal et al., 2014) was obtained from Sigma Chemical Co., USA, Quercetin 100mg\kg (El-Nicety et al., 2014) was obtained from MRM USA manufacture and Nano Glutathione 200mg\kg (Tredici et al., 1994) was obtained from Lipolife, Drakes Lane Industrial Estate, Drakes Lane, (UK).

Experimental Design

In this study, the use of animals was under strict compliance to the local ethics committee in King Saud University (KSU-SE-18-37). Thirty adult male albino rats 150 g were obtained from the Experimental Animal Center, at King Saud University. The animals were allowed to acclimate in the laboratory for one week, under standard environmental conditions. They were given a standard rat pellet diet and distilled water and libitum and divided into five group's six rats each.



Rats were subjected to CO2 gas; blood samples were collected for serum separation by centrifugation at 3000xg. Right kidneys were collected, and then were homogenized in phosphate buffered saline to yield 20% homogenates. Parts of right kidneys were kept under nitrogen for Western blot analysis another right kidney was kept for histological examination in 10% formalin.



Biochemical serum and tissue analysis

Serum urea, creatinine and uric acid and renal SOD were evaluated using the kits purchased from Randox Laboratories. Renal MDA and GSH levels were assessed following Uchiyama and Mihara (1978) and Ellman (1959) methods, respectively. Total nitrite was determined according to the method described by Moshag et al. (1995).

TNF-α and IL-6 levels were estimated using ELISA kits obtained from R&D Co.

Detection of Gene Expression of renal NF-κB Using Real-Time PCR mRNA expression

Total RNA Extraction

mRNA expression was performed according to the method of (Mahmood, et al, 2012

Determination of Protein Level

Western Blot

Western blot of renal VCAM-1and KIM-1 protein expression was performed according to the method of (Mahmood, et al, 2012).

DNA percentage was carried using DNA fragmentation quantitated by measuring oligonucleosome bound DNA using an ELISA kit (Boehringer, Mannheim, Germany) (Leist et al., 1994).

Histological examination

Sections of kidneys were cut and used for histopathological examination using hematoxylin and eosin (H&E) stain.

Statistical analysis

It was performed using GraphPad Prism (GraphPad Software, San Diego, CA, USA), using one-way analysis of variance test followed by Tukey's test post hoc analysis.

RESULTS

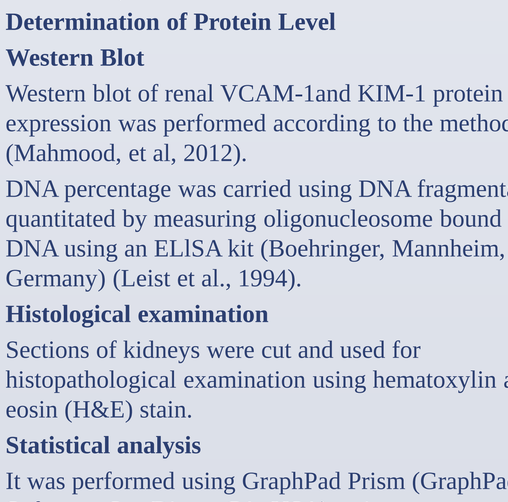


Figure (1): Revealed that the administration of Mosp induced elevation in serum urea ,creatinine and uric acid while the treatment with the antioxidants in question alone or in combination decline their levels .

Figure (2): The ingestion of the afore mentioned antioxidants down regulated TNFα , and IL-1β levels in Mosp treated rats

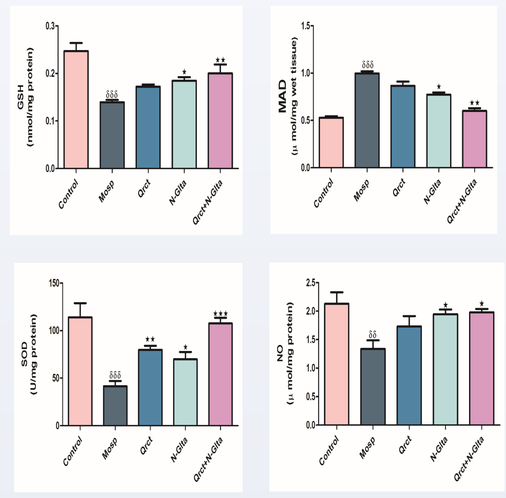


Figure (3):) Renal NO and MDA levels were elevated while level of GSH and the activity of SOD were decreased. Treatment with the antioxidants in question successively modulated the latter altered parameters versus Mosp administered group .

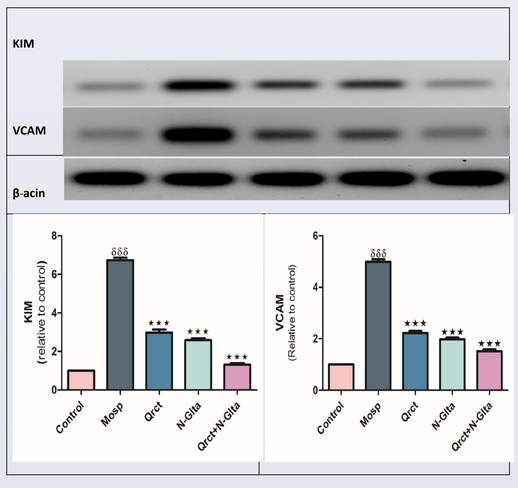


Figure (4): Showed that Mosp exhibited up regulation of the protein expression of VCAM-1 and KIM-1 while the intake of the afore mentioned antioxidants down regulated their expressions.

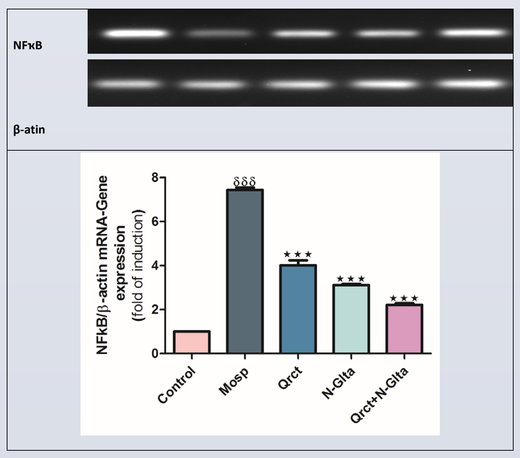


Figure (5): mRNA expression of NF-κB were up regulated whereas mRNA expression of nrf-2 was down regulated in renal tissue. Contrariwise treatment with the antioxidants alone or in combination regulated these expressions compared to Mosp administered group.

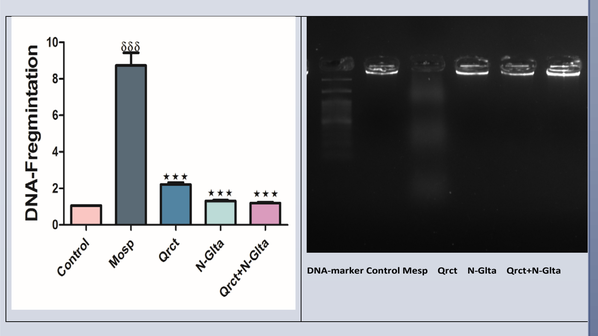


Figure (6): Showed that DNA fragmentation were elevated in Mosp group compared to treatment groups.

Notes: Data are shown as mean ± SEM (N=6). $^{\delta\delta\delta}P \leq 0.001$ compare to control group, $^{*}P \leq 0.05$,$^{**}P \leq 0.01$and$^{***}P \leq 0.001$compare to Mosp group

Interestingly the combination of Qrct and or N- Glta was the most effective regimen to counter act Mosp reno toxicity.

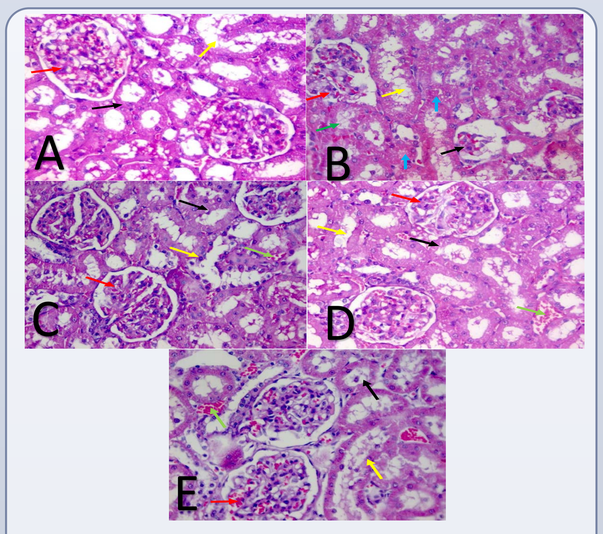


Figure (7): (A) Control group (grp) showed normal architecture. (B) Mosp grp showed few glomeruli corpuscles that are obliterated, diminished and destructed (black arrows) and cellular (hyperplasia of epithelial cells lining the partial layer of Bowman's capsule) (red arrow).Proximal convoluted tubules show destructed epithelial lining with moderate dilatation (yellow arrow), destructed epithelial lining of distal convoluted tubules (green arrow), and proteinaceous debris in the tubules (blue arrow) congested blood vessels (green arrow). (C) Qrct grp showed relative healthy renal cortex showing renal corpuscle with almost normal glomerulus (red arrow), mildly dilated proximal convoluted (black arrow) and distal convoluted tubules (yellow arrow), congested blood vessels (green arrow). (D) N-Glta grp showed relative healthy renal cortex showing renal corpuscle with almost normal glomerulus (red arrow), mildly dilated proximal convoluted (black arrow) and dilated distal convoluted (yellow arrow) tubules. (E) Qrct +N-Glta grp showed almost normal renal cortex showing renal corpuscle with almost normal glomerulus (red arrow), and proximal convoluted (black arrow) and distal convoluted (yellow arrow) tubules.

CONCLUSION

Mosp caused an elevation of kidney injury biomarkers (urea, creatinine and uric acid), inflammatory markers (TNF α , IL-1β)as well as oxidative stress markers (nitric oxide and lipid peroxides ,whereas antioxidants reduced glutathione and super oxide dismutase were declined . More over protein expression of vascular adhesion molecule-1 , kidney injury molecule-1 as well as mRNA expression of nuclear factor kappa B also DNA Damage in renal tissue were upregulated. Histopathological examination of renal tissues revealed a massive destruction. Treatments with Qrct or N- Glta either alone or in combination ameliorated all the previous measured parameters or improved kidney's architecture. Interestingly the combination of Qrct and or N- Glta was the most effective regimen to counter act Mosp reno toxicity and can be considered as a promising candidate for renal therapy. Also molecular pathways of VCAM-1, KIM-1 and DNA damage may be considered as novel mechanism for Mosp toxicity and monitoring its treatment.

REFERENCES

- El-Nicety A, Abdel-Azeem S, Hassan A, Hassan N, Aly S, Abdel-Wahab M. Quercetin inhibits the cytotoxicity and oxidative stress in liver of rats fed aflatoxin-contaminated diet. Toxicology Reports. 2014;1:319-329.
- Lash L. Role of glutathione transport processes in kidney function. Toxicology and Applied Pharmacology. 2005;204(3):329-342.
- Mondal, S., Ghosh, R., Karman, S. and Purohit, K. (2014). Toxic pathological changes on Wistar rat after multiple exposures to Acetamiprid. Veterinary World, 7(12), pp.1058-1065.
- Tredici G, Cavaletti G, Petruccioli M, Fabbrica D, Tedeschi M, Venturino P. Low-dose glutathione administration in the prevention of cisplatin-induced peripheral neuropathy in rats. US National Library of Medicine National Institutes of Health (PubMed).
- Wang W, Sun C, Mao L, Ma P, Liu F, Yang J et al. The biological activities, chemical stability, metabolism and delivery systems of quercetin: A review. Trends in Food Science & Technology. 2016;56:21-38.
- Moshage H. Kok B. Huizenga J. R. Jansen P. L. Nitrite and nitrate determinations in plasma: a critical evaluation.1995, 41(1): 892-896.
- Ellman, G. L. 1959.Tissue sulfhydral group. Arch. Biochem. Biophys. 82, 70-77.
- Kimura-Kuroda, J., Komuta, Y., Kuroda, Y., Hayashi, M. and Kawano, H. (2012).Nicotine-Like Effects of the Neonicotinoid Insecticides Mospilan and Imidacloprid on Cerebellar Neurons from Neonatal Rats. PLoS ONE, 7(2), p.e32432.

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