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SHORT COMMUNICATION

Renoprotective effect of tectorigenin glycosides isolated from *Iris spuria* L. (Zeal) against hyperoxaluria and hyperglycemia in NRK-49Fcells

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ABSTRACT

Oxidative stress has been identified as an underlying factor in the development of insulin resistance, β-cell dysfunction, impaired glucose tolerance and type 2 diabetes mellitus and it also play major role in kidney stone formation. The present study is aimed to elucidate the *in vitro* nephroprotective activity of two isoflavonoid glycosides, tectorigenin 7-*O*-β-D-glucosyl-(1→6)-β-D-glucoside (**1**) and tectorigenin 7-*O*-β-D-glucosyl-4′-*O*-β-D-glucoside (**2**) isolated from the *n*-BuOH fraction of *Iris spuria* L. (Zeal) rhizome MeOH extract against oxalate and high glucose-induced oxidative stress in NRK-49F cells. The results revealed that compounds **1** and **2** significantly increased the antioxidant enzyme activities and decreased MDA levels in both oxalate and high glucose stress. Treatment with these phytochemicals effectively down-regulated expression of crystal modulator genes and pro-fibrotic genes in oxalate and high glucose-mediated stress respectively. This study indicates cytoprotective, antioxidant, anti-urolithic and anti-diabetic effects of compounds **1** and **2** against oxalate and high glucose stress.

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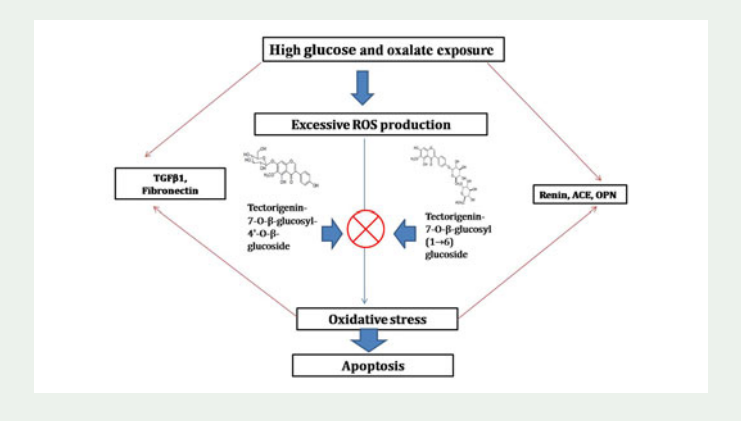

1. Introduction

Oxidative stress is linked to many pathological disorders and associated with elevated intracellular levels of reactive oxygen species (ROS) that cause damage to lipids, proteins and DNA. ROS are produced during the interactions between the oxalate and renal cells which are responsible for the various cellular damage (Khan 2014). The most important events in the pathogenesis of Type 2 diabetes (T2D) induced nephropathy are oxidative stress, inflammation, and fibrosis (Kanwar et al. 2011).

Unfortunately, existing therapeutic options for kidney stone disease have several limitations including recurrence of stone formation, severe pain and also it is expensive. Similarly drugs targeting diabetes induced nephropathy mainly reduce proteinuria and glomerulosclerosis. However novel therapeutic approach targeting oxidative stress and fibrosis for both kidney stone disease and diabetes associated renal damage is necessary. Antioxidants can attenuate the damaging effects of ROS and delay many events that contribute to cellular aging (Poljsak et al. 2013). Phytochemicals facilitate the stimulation of various defense mechanisms against oxidative stress induced tissue damage. Plant-derived secondary metabolites, including bioflavonoids, have beneficial health effects and their intake can reduce the risk of chronic diseases. The genus *Iris spuria* L. has been reported to have diverse pharmacological activities and is well known for its flavonoid content and the flavonoid constituents include mainly isoflavones and their glycosides (Singab 2004, Venditti et al. 2017). Most *Iris* plants are cultivated in the Egyptian gardens as ornamental herbs. Their underground parts are also used in traditional folk medicine in some parts of Egypt and Turkey (Wollenweber et al. 2003). It is also used in traditional Chinese medicine. *Iris Spuria* L. is a rhizomatous plant having purple flowers and slender elongated leaves. *Iris* rhizomes have been used as anti-inflammatory, antioxidant, anti-neurodegenerative, hypolipidemic agents. They also showed hepatoprotective effect against liver toxicity (Akther et al. 2014). To the best of our knowledge, there are a few reports on the antioxidant, antidiabetic and anti-urolithic activity of isoflavonoids isolated from *Iris spuria* L. Therefore, the present study was designed to evaluate the protective effect of compounds tectorigenin 7-*O*-β-D-glucosyl-(1→6)-β-D-glucoside (**1**) and tectorigenin 7-*O*-β-D-glucosyl-4′-

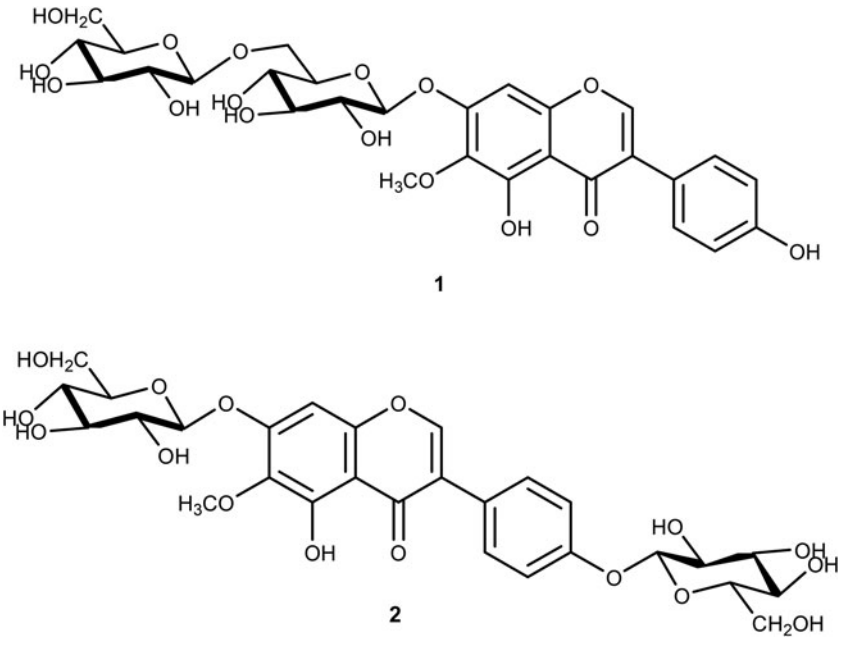

Figure 1. Chemical Structures of compounds Tectorigenin 7-*O*-β-D-glucosyl-(1→6)-β-D-glucoside (**1**) and Tectorigenin 7-*O*-β-D-glucosyl-4′-*O*-β-D-glucoside (**2**).

O-β-D-glucoside (**2**) obtained from the *n*-BuOH fraction of *I. spuria* rhizome MeOH extract against oxalate and high glucose-induced oxidative stress in NRK-49F cells.

2. Results and discussion

Chromatographic separation of the *n*-BuOH fraction obtained from the 70% MeOH extract of *I. spuria* rhizomes resulted in the isolation of 2 isoflavonoid glycosides. The compounds were identified as described by Singab (2004). The structures of the isoflavonoids are represented in Figure 1. The results showed that compounds tectorigenin 7-*O*-β-D-glucosyl-(1→6)-β-D-glucoside (**1**) and tectorigenin 7-*O*-β-D-glucosyl-4′-*O*-β-D-glucoside (**2**) protected the renal fibroblasts against both oxalate and high glucose induced oxidative stress by increasing cell viability. MTT assay (Figure 2, 3) showed that NRK-49F cells treated with compound **2** (25 μM) enhanced cell viability on oxalate exposure and compound **1** (50 μM) documented enhanced protective effect during high glucose stress.

In this study, supplementation of compounds **1** and **2** significantly ameliorated the levels of intracellular ROS generation (Figure S1). Treatment with compounds **1** and **2**, significantly improved the antioxidant enzyme activities of catalase and superoxide dismutase. Similarly, lipid peroxidation levels were restored close to normal levels after treating the cells with compounds **1** and **2**. N-acetylcysteine (NAC), an antioxidant that attenuates oxidative stress, was used as a positive control in this assay (Table S1, Table S2).

Under hyperoxaluric condition, oxalate ions and crystal deposition leads to angiotensin II (Ang II) activation, which subsequently leads to ROS production (Sharma et al.

2016). The results revealed that supplementation of compounds **1** and **2** with high glucose and oxalate significantly decreased ROS generation in NRK49F cells. Studies suggested that the use of exogenous antioxidants conferred a protective role in oxalate stress (Zhai et al. 2013, Ganesan et al. 2018).

The decrease in enzyme activity results in increased ROS generation and consequently activates the Renin – Angiotensin System (RAS) which leads to the renal disease progression. Also, renal injury and inflammation caused by RAS subsequently causes calcium oxalate stone formation. In this study, cell shrinkage and disorganised

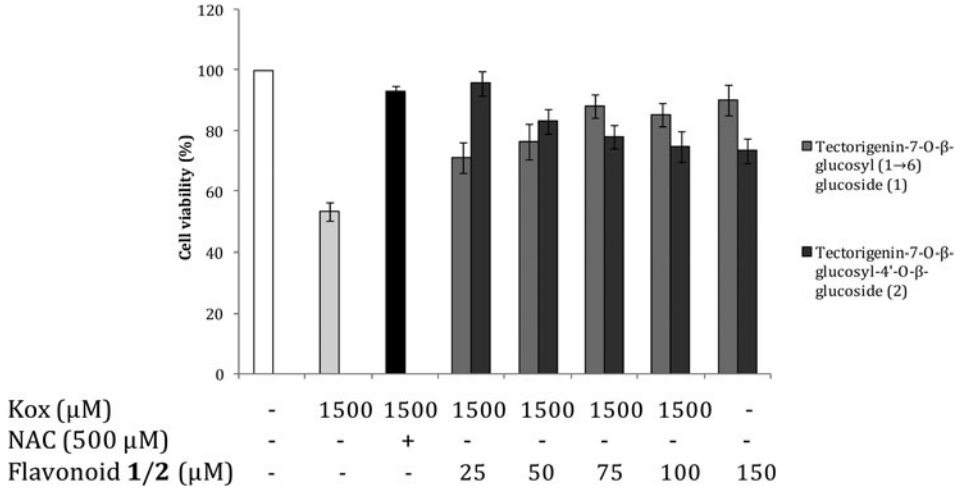

Figure 2. Cytotoxic effect of KOx on cell viability and cytoprotective effect of compounds 1 and 2 against KOx. NRK-49F cells co-treated with oxalate (1.5 mM) and varying concentrations of isoflavonoids **1** and **2**. The data expressed mean value of three independent experiments.

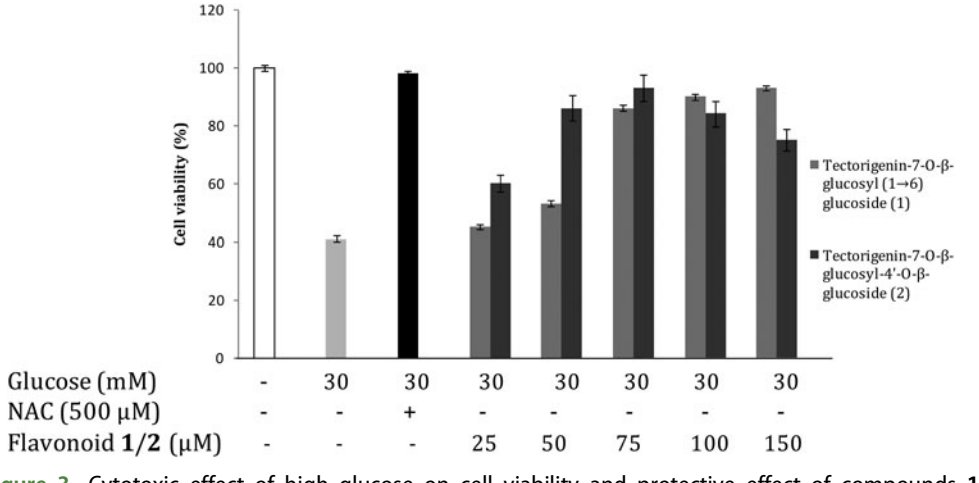

Figure 3. Cytotoxic effect of high glucose on cell viability and protective effect of compounds **1** and **2** against hyperglycemia. NRK-49F cells co-treated with high glucose (30 mM) and varying concentrations of isoflavonoids **1** and **2**. The data expressed as mean value of three independent experiments.

structures were observed in NRK-49F cells when exposed to oxalate. Whereas, the cell morphology changes were restored when treated with compound **1** and **2**, and CaOx crystal aggregation was reduced in cells treated with compounds indicating its anti-lithic property (Figure S2).

Semi-quantitative PCR results showed a significant up-regulation of Osteopontin (OPN), renin and ACE mRNA levels in NRK-49F cells exposed to oxalate stress. Whereas, mRNA expression levels of these genes were significantly down-regulated in NRK-49F cells treated with compounds **1** and **2**. Interestingly, compound **2** exhibited relatively higher effect in down-regulating the over-expressed genes than compound **1** (Figure S4). OPN expression level was significantly up-regulated in NRK-49F cells exposed to oxalate and enhances crystal retention. Treatment with flavonoids prevented the over-expression of OPN, a downstream process of RAS pathway and thus may have enabled crystal clearance and cell viability.

High glucose stress resulted in a phenotypic conversion of normal fibroblasts to spindle-shaped enlarged NRK-49F cells when compared to control cells (devoid of glucose stress), while co-treatment with compounds **1** and **2** restored the morphological architecture of NRK-49F cells (Figure S3). Semi quantitative results showed that the mRNA expression level of TGF-β1 and fibronectin was up-regulated in high-glucose condition. On the other hand, supplementation of compounds **1** and **2** down-regulated both TGF-β1 and fibronectin expression when compared to hyperglycemic cells (Figure S5). The results of the present study revealed the up-regulation of TGF-β1 in renal fibroblast cells which were subjected to high glucose stress is comparable to previous reports (Zhu et al. 2007). Since ROS play a critical role in pro-fibrotic pathways and TGF-β1 stimulation, treatment with flavonoids with antioxidant and anti-fibrotic property, enabled the protection of cell morphology and cell viability.

3. Conclusion

In conclusion, it was evident from the results of the present study that the isoflavonoids tectorigenin 7-*O*-β-D-glucosyl-(1→6)-β-D-glucoside (**1**) and tectorigenin 7-*O*-β-D-glucosyl-4′-*O*-β-D-glucoside (**2**) have therapeutic potential in the management of CaOx stone disease and Type 2 Diabetes mellitus. Despite these promising results, further detailed studies with experimental based *in vivo* models are required to elucidate the protective role compounds **1** and **2** in oxalate and glucose mediated renal damage.

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Disclosure statement

No potential conflict of interest was reported by authors.

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