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NEPHRO-PROTECTIVE ROLE OF MORIN AGAINST EXPERIMENTALLY INDUCED DIABETIC NEPHROPATHY

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Diabetic nephropathy (DN) is known as one of the most difficult types of diabetic complications to treat. DN is associated with reactive oxygen species (ROS) generation due to hyperglycemia resulting in oxidative damages to renal tissues. The present study was designed to evaluate the possible nephro-protective effects of morin, a potent antioxidant, on streptozotocin (STZ)-induced diabetes in rats. Morin treatment (15 and 30 mg/kg/day) was continued to diabetic rats by gavage for five consecutive weeks. Serum creatinine and uric acid levels were estimated. Furthermore, renal levels of thiobarbituric acid reactive substances (TBARS), total glutathione (T-GSH), non-protein sulphydral (NP-SH), superoxide dismutase (SOD) activity, catalase (CAT) activity, nucleic acids and total protein were estimated. Histopathological changes were also observed in kidney sections. Elevated serum creatinine and uric acid levels were significantly attenuated in diabetic rats by morin. In renal tissue, morin significantly ameliorated the elevated TBARS levels as well as the reduced levels and activities of nucleic acids, T-GSH, NP-SH and CAT in diabetic rats. These results are validated by histopathological investigations. Present results clearly demonstrate the nephro-protective effects of morin to be mediated through attenuation of oxidative stress and suggest it as a potential drug for the prevention of DN.

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Keywords: Morin, streptozotocin, diabetes, nephropathy

1. Introduction

Diabetes mellitus (DM) is a major health issue in all countries and its prevalence has increase sharply worldwide during the past few decades [1]. Suffering from diabetes for long time is known to cause numerous complications such as nephropathy, retinopathy and neuropathy [2-3]. DN is a challenge in clinical practice because of its severity, chronicity and resistance to therapies.

Many studies suggested oxidative stress as one of the major pathophysiological route during DN. Persistent hyperglycemia causes increased production of free radicals especially ROS from glucose auto-oxidation and protein glycosylation [2, 4]. In diabetic rat kidneys, activities of antioxidant enzymes such as CAT, SOD and glutathion peroxidase (GSHPx) were found to be reduced. However, by using antioxidants such activities can be enhanced [5]. In addition, diabetes is usually accompanied with an increase in lipid peroxidation (LPO), alteration of the glutathione redox state, a decrease in the content of individual natural antioxidants and decreased induction of antioxidant enzymes [6]. Despite the evidence are now available about the possible role of oxidative stress in the development of diabetic complications, further studies are still required [7]. Therefore, there is a need to further identify antioxidants that would be effective in attenuating hyperglycemia-induced renal damage in diabetes.

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Morin is one of the naturally occurring bioflavonoids, originally isolated from members of the Moraceae family. It has exhibited several pharmacological properties including antioxidant, anti-inflammatory, chemoprotective, anticancer, and anti-promotion [8]. Furthermore, morin demonstrated a potent antioxidant effects and decreased oxidative damage in several biological tissues [8]. Indeed, several antioxidants such as quercetin, n-acetylcysteine and α-lipoic acid have already been reported for their protective effects against DN [9-11]. Thus, it could be speculated that morin may have a potential renal preventive effects against experimentally induced DN, which is the aim of the current study.

2. Materials and methods:

2.1 Animals and experimental design:

Twenty four adult male Wistar albino rats, weighing 250–270 g, were used in this study. All animals were maintained under controlled conditions of temperature (22±1°C), humidity (50-55%), and light (12 h light/12 h dark cycle). Animals had free access to Purina rat chow and drinking water. Experimental diabetes was induced by a single intraperitoneal STZ (Sigma) injection to overnight fasted rats in 65 mg/kg dose. Using a glucometer (ACCU-CHEK ACTIVE, Roche, Germany), fasting blood glucose was measure 72 hr later and glucose levels ≥ 300 mg/dL considered to be diabetic. In control group, normal healthy rats were used as vehicle (10 % CMC). Diabetic rats were randomly divided into three groups (six rats in each group); untreated diabetic vehicle group and diabetic rats treated with two individual doses of morin (15 and 30 mg/kg/day) by gavage for five consecutive weeks. At end of the treatment, fasting blood samples were obtained under light anesthesia via cardiac puncture, then serum samples were separated and preserved in -20°C till analysis. Animals were then scarified and kidneys were dissected, weighed, dipped in liquid nitrogen for one minute and kept in -80ºC till analysis. A cross-section of kidney from each group was preserved in 10% formalin for histopathology. All experimental procedures are in accordance with the ethical guidelines of the experimental animal care centre, College of Pharmacy, King Saud University.

2.2 Serum analysis:

In serum, creatinine, uric acid levels were estimated by using commercially avaiblabe diagnostic kits (Randox Lab Limited, U.K. and Human GmbH, Germany).

2.3 Determination of nucleic acids and total protein levels in renal tissues:

The method described by Bregman, (1983) [12] was used to estimate DNA and RNA levels in kidney homogenate. In addition, the modified Lowry method by Schacterle and Pollack (1973) [13] was used to estimate levels of total protein in kidney using bovine plasma albumin as a standard.

2.4 Determination of renal redox status:

A TBARS assay kit (ZeptoMetrix) was used to measure the LPO products, malondialdehyde (MDA) equivalents. Furthermore, concentrations of T-GSH and NP-SH were measured using the method described by Sedlak and Lindsay (1968) [14]. The activity of the antioxidant enzyme SOD in kidney was estimated using the method described by Kono, (1978) [15] with the aid of nitroblue tetrazolium as the indicator. Moreover, CAT activity was determined in renal tissues using the method described by Aebi, (1978) [16].

2.5 Histopathological examination of kidney tissues:

Cross sections of kidney were fixed in 10 % neutral buffered formalin, embedded in paraffin wax and sectioned at 3 µm. Sections were then stained with Hematoxylin and Eosin (H & E) stain and placed in slides for light microscopic examination. Slides were evaluated by a histopathologist who was blinded to the treatment groups to avoid any kind of bias.

2.6 Statistical analysis

All data were expressed as mean±Standard Deviation (SD). Data were statistically analyzed using one-way ANOVA followed by Student-Newman-Keuls multiple comparisons test. Six rats were used in each group. The differences were considered statistically significant at $P<0.05$.

3. Results

Mean kidney weights were significantly ($P<0.01$) increased in diabetic group as compared to control animals. Morin treatments with both the doses (15 and 30 mg/kg/day) to diabetic rats could not correct kidney weights increase as compared to untreated diabetic rats (Tab. 1). Serum creatinine and uric acid levels were significantly ($P<0.05$ and $P<0.01$, respectively) increased in diabetic rats as compared to control animals. Morin supplementation in both doses significantly ($P<0.05$) inhibited the STZ-induced increase in creatinine and uric acid levels (Tab. 1).

Table 1: Effects of morin on kidneys weights, serum creatinine and uric acid levels following experimentally induced diabetes by STZ in rats

	Kidney weights (g/100g body weight)	**Creatinine** (µM/L)	**Uric acid** (µM/L)
Control	0.62±0.09	64.71±22.11	66.31±25.86
STZ	0.96±0.07 ^{**a}	85.97±6.72 ^{*a}	183.30±51.22 ^{**a}
Morin (15)+STZ	1.06±0.22	54.58±28.88 ^{*b}	129.85±11.15 ^{*b}
Morin (30)+STZ	0.97±0.12	61.71±14.99 ^{*b}	131.67±27.03 ^{*b}

$^{'a'}$ STZ group was compared with control. $^{'b'}$Morin treated groups were compared with STZ group. Statistical significance was considered as $^{*}P<0.05$ and $^{**}P<0.01$.

There was a significant decrease in both renal DNA and RNA concentrations ($P<0.05$ and $P<0.001$, respectively) in STZ group as compared to controls animals (Tab. 2). The reduced levels of kidney DNA and RNA were significantly ($P<0.05$) increased by both doses of morin (Tab. 2). Kidneys total protein levels remained unchanged by STZ and morin groups (Tab. 2).

Table 2: Effects of morin on renal levels of nucleic acids and total protein following experimentally induced diabetes by STZ in rats:

	DNA (µg/100mg tissue)	**RNA** (µg/100mg tissue)	**Total protein** (µg/100mg tissue)
Control	240.70±16.07	233.47±26.46	12.23±3.53
STZ	183.95±31.90 ^{*a}	142.56±32.40 ^{**a}	11.07±1.11
Morin (15)+STZ	212.91±21.25 ^{*b}	161.49±17.60 ^{*b}	10.66±1.80
Morin (30)+STZ	226.35±28.61 ^{*b}	227.26±40.35 ^{*b}	13.93±4.62

$^{'a'}$ STZ group was compared with control. $^{'b'}$Morin treated groups were compared with STZ group. Statistical significance was considered as $^{*}P<0.05$ and $^{**}P<0.01$.

In kidneys of diabetic animals, TBARS levels were significantly (P<0.01) increased, while T-GSH and NP-SH levels were significantly (P<0.05 and P<0.01; respectively) decreased as compared to control rats. Also, activity of SOD and CAT was significantly (P<0.05 and P<0.01; respectively) reduced in kidneys of STZ treated animals as compared to control group (Fig. 1). Morin treatment in both doses significantly (P<0.05) reduced the elevated TBARS levels as compared to untreated diabetic rats (Fig. 1). Moreover, only the high doses of morin (30mg/kg/day) significantly (P<0.05) attenuated the elevated levels of renal T-GSH as compared to STZ group. Both doses of morin significantly (P<0.01) inhibited STZ induced elevation of renal NP-SH (Fig. 1). The decreased renal SOD activity was partially, but not significantly, restored by morin treatment. In addition, renal CAT activity showed a significant (P<0.05) enhancement only in morin 30 mg/kg/day treated group as compared to diabetic untreated animals (Fig. 1).

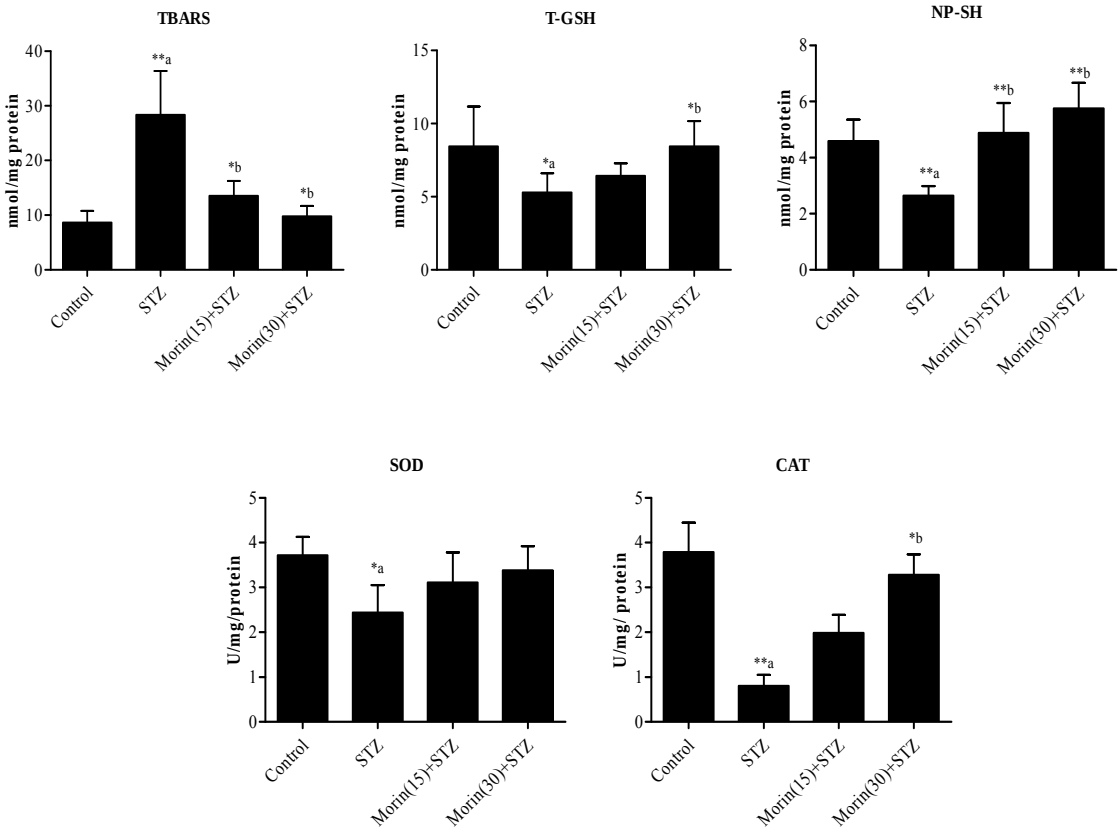

Fig. 1: Effects of morin on renal levels and activities of oxidative stress biomarkers (TBARS, T-GSH, NP-SH, SOD and CAT) following experimentally induced diabetes by STZ in rats $^{'a'}$ STZ group was compared with control. $^{'b'}$Morin treated groups were compared with STZ group. Statistical significance was considered as $^{*}P<0.05$ and $^{**}P<0.01$.

Histopathological evaluation revealed the following: normal and benign looking kidney tissues with prominent cortex and medulla with perfectly presence of glomeruli and tubules in control group (Fig. 2-A); renal tubules with swollen epithelial cells with occasionally occluded lumen associated with scattered intraluminal eosinophilic sections in STZ group (Fig. 2-B); preserved cortex and medulla with scattered few hypercellular glomeruli, renal tubules with swollen cells partially occluding the lumen associated with some eosinophilic intratubular secretions in morin (15 mg/kg/day) treated group (Fig. 2-C); benign looking glomeruli with preservation of cortex and medulla as well as few cloudy degeneration of renal tubules and intra-tubular eosinophilic secretions in morin (30 mg/kg/day) treated group (Fig. 2-D).

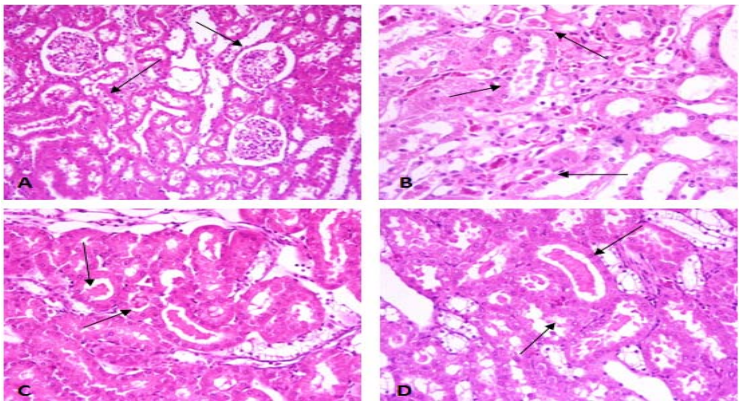

Fig. 2. Histopathological kidney sections showing: [A] normal and benign looking kidney for control group; [B] swollen and inflamed renal tubules for STZ group; [C] preserved cortex and medulla with partially inflammation for morin (15)+STZ group; [D] benign looking and preserved of cortex and medulla with few cloudy degeneration for morin (30)+STZ group.

4. Discussion

The effects of morin on STZ-induced DN in male Wistar albino rats were investigated in the present study. We found STZ administration-induced renal injury and nephrotoxicity. Morin supplementation (15 and 30 mg/kg/day) to diabetic rats for five consecutive weeks protected against the STZ-induced DN, which was also supported by the histopathological investigations.

Hyperglycemia is known to be implicated in the development of diverse diabetic complications, such as retinopathy, nephropathy, neuropathy [17]. In the present study, mean kidney weights were significantly increased in diabetic rats and these results are in agreement with earlier reports [18-19]. This may be because of loss or degradation of structural proteins and unavailability of carbohydrate as an energy source within cells [20]. In addition, increased kidney weight in STZ treated rats was not alleviated by morin treatment for five weeks in both doses. Measurement of creatinine can be considered as a marker of renal dysfunction [21-22]. In addition, uric acid is a well-known mediator of diabetic kidney injury [23-25]. Higher levels of serum creatinine and uric acid observed in STZ group indicate the presence of DN with renal hyperfiltration. These results were also consistent with the previous reports [21-22]. Impairments in serum creatinine and uric acid was attenuated by morin administration for five weeks possibly through reducing glomerular hyperfiltration in STZ-induced diabetes.

Oxidative stress is suggested to be a potential donor to the progression diabetic complications [26]. It also coupled to a decrease in the antioxidant status, which can increase the deleterious effects of free radicals [27]. In the current study, kidney levels of TBARS, a specific LPO marker, were elevated while T-GSH and NP-SH levels were decreased in diabetic rats, which is in correspondence with earlier reports [28]. Moreover, the renal activities of the endogenous antioxidant enzymes CAT and SOD were found to be reduced in diabetic rats. It has been reported that hyperglycemia leads to generation of ROS in tissues from glucose auto-oxidation and protein glycosylation [2, 4, 29] thereby alters normal cellular defence mechanisms and eventually leads to increased oxidative stress. Furthermore, the increased levels of ROS in diabetes, due to their increased production and/or decreased destruction, has been linked to altered activity of nonenzymatic and enzymatic antioxidants. This includes T-GSH, NP-SH, CAT and SOD [30]. The present study also demonstrated that STZ caused significant reduction in renal DNA and RNA contents. It could be due to formation of DNA adducts and strand breaks [31-32]. Indeed, Imaeda et al. (2002) [33] reported that nucleic acids damage in the kidney can be significantly induced following STZ injection. Therefore, we suggest that STZ induced-DN reported in our study was due to increased rate of renal oxidative stress and LPO, which is kwon to provoke ROS generation and injury as well as cytotoxicity.

Bioflavonoids such as morin are now widely accepted as physiologic antioxidants, which are known to have effective free radical scavenging activity [34-35]. In the present study, STZ induced renal injury was significantly attenuated by morin administration. The antioxidant and anti-diabetic properties of morin might explain these nephroprotective effects. Our study showed that morin can significantly decrease the LPO product, TBARS, as compared to its altered levels in STZ group. Furthermore, morin attenuated the decreased renal levels of endogenous antioxidants e.g. T-GSH and NP-SH as well as CAT activity by STZ. However, only SOD activity was gradually, but not significantly, restored by morin. These speculations were supported by the histopathological evaluation, where morin prevented STZ-induced nephrotoxicity. Morin is a potent antioxidant, which was found to exert strong inhibitory effect on ROS generation [36]. It is also an effective free radical scavenger [37]. Morin was reported to restore the expression, activities of antioxidant enzymes and levels of GSH [36]. Previous data reported that morin decreases the oxidative damage of in several biological systems such as cardiovascular, lung fibroblasts, hepatocytes, and neurons [8]. Moreover, measurements of kidney levels of nucleic acids revealed that morin can prevent renal cytotoxic damage induced by STZ supplementation to the rats. This may be due to the inhibitory effect of morin on the adduct formation by STZ. Therefore, it can be concluded from this study that the ameliorative effects of morin against experimentally induced DN are attributed to its antioxidant, cytoprotective and free radical scavenging capability.

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