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Original Paper

Therapeutic Effect of Chrysin on Adenine-Induced Chronic Kidney Disease in Rats

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Key Words
Rat • Adenine • Chronic kidney disease • Chrysin • Oxidative damage • Inflammation

Abstract
Background/Aims: To study the therapeutic effect of chrysin, a flavonoid with strong antioxidant and anti-inflammatory activities, on adenine-induced chronic kidney diseases (CKD) in rats. ***Methods:*** Chrysin, in three graded oral doses (10, 50 and 250 mg/kg), was given for 10 consecutive days to rats after the induction of CKD by feeding them adenine (0.25%$^{w/w}$ for 35 days). Several plasma and urine biomarkers and tissues morphology were used the investigate chrysin effect on kidney structure and function. ***Results:*** Adenine lowered creatinine clearance and elevated the concentrations of urea, creatinine, plasma neutrophil gelatinase-associated lipocalin and urinary *N*-Acetyl-beta-D-glucosaminidase activity, and increased the concentrations of the uremic toxin indoxyl sulfate, in addition to some inflammatory cytokines. Renal histopathological markers of inflammation and fibrosis were significantly increased. Renal catalase and superoxide dismutase activities, total antioxidant capacity and reduced glutathione were all adversely affected. Most of these adenine – induced actions were moderately mitigated by chrysin, especially at the highest dose. Compared to control, chrysin did not cause any overt adverse effects on the treated rats. ***Conclusion:*** Different doses of chrysin produce variable therapeutic salutary effects in rats with CKD, and that, pending further studies, its usability as a possible therapeutic agent in human CKD should be considered.

Introduction

Chronic kidney disease (CKD) is a growing global health problem that is increasing in incidence and prevalence [1, 2]. In both humans and animals, the pathophysiological basis

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of the disease and its complications include two main features: inflammation and oxidative stress [3, 4]. This was evident in patients and laboratory animals with CKD in whom there are high plasma concentrations of inflammatory mediators (such as C-reactive protein, tumor necrosis factor and other cytokines) and several markers of oxidative stress [5, 6].

Chrysin (5, 7-dihydroxyflavone, Fig. 1), a flavonoid that is found in abundance in the flower extract of several plants as well as edible items such as mushrooms, honey and bee propolis, have several important and diverse pharmacological activities [7-10]. In rats and mice, chrysin has been shown to abate the nephrotoxicity of cisplatin and doxorubicin [11] and the hepatotoxicity of methotrexate and carbon tetrachloride [12].

Previously we studied the ameliorative effect of chrysin against adenine-induced CKD in rats, in which rats were treated with adenine to induce CKD and concomitantly given chrysin as a preventive agent [13]. Chrysin significantly and dose-dependently mitigated some of adenine biochemical and pathological toxic effects such as raised serum creatinine, increased inflammatory cytokines levels and oxidative stress markers without causing any overt adverse effect on the treated rats. However, it was not able to ameliorate significantly the adenine –induced morphological damage.

In the present work, we studied the possible therapeutic effect of chrysin on adenine-induced CKD, where chrysin was given after the end of adenine treatment (established CKD).

Materials and Methods

The studied animals, biochemical methods, Western blot analysis, histopathology studies and statistical analysis (p values < 0.05 considered significant) is similar to the previously published work (ameliorative effect of chrysin) [13].

The experimental design was also similar except for treatment protocol, which was as following:

Rats (n = 48) were randomly divided into eight equal groups and left for one week to acclimatize to their groups before treating them for 45 days as follows:

The 1st (control) group continued to receive the powdered diet without treatment until the end of the study after 45 days.

The 2nd group was switched to a powdered diet containing adenine (0.25%$^{w/w}$in feed) for 35 days. Thereafter, adenine was stopped and rats were given normal powdered feed plus saline (1 ml/kg) for 10 days more.

The 3rd, 4th and 5th groups were given normal powdered diet as in group one, and then given chrysin orally by gavage at doses of 10, 50 and 250 mg/kg, daily for 10 days, respectively.

The 6th, 7th and 8th groups were treated as in groups 3, 4 and 5 except that normal food was replaced by adenine.

Results

Physiological data

The general appearance of the rats with adenine – induced CKD was subjectively judged to be improved by chrysin treatment, especially at the highest dose (250 mg/kg). The kidneys from the control and chrysin-treated rats appeared normal. However, the kidneys of adenine-treated rats were pale and with white crystals, similar to that described before [14-16]. The appearance of the kidneys of rats treated with adenine plus the three doses of chrysin were improved compared with the kidneys of rats treated with adenine alone.

Fig. 1. Chemical structure of chrysin (5,7 dihydroxy flavone).

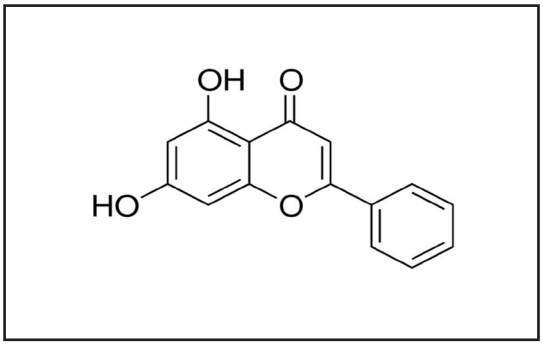

Table 1. Effect of chrysin treatment on some physiological parameters in rats with adenine-induced chronic kidney disease. Values in the tables are mean ± SEM (n = 6). Chronic kidney disease (CKD) was induced by the inclusion of adenine in the feed at a concentration of 0.25 % w/w for 35 days and then chrysin (10, 50, and 250 mg/kg) was given orally by gavage for 10 days after induction of CKD. On the last day of the treatment, the rats were placed in metabolic cages to collect urine. Values with superscripts are statistically different *p* value [a] vs control; [b] groups treated with chrysin alone vs. its corresponding groups treated with adenine+chrysin

Group	Body weight change (%)	Relative kidney weight (%)	Water intake (mL)	Urine output (mL)
Control	27.4±2.7	0.7±0.0	17.1±1.5	8.7±1.4
Adenine	-3.8±1.2[a]	2.4±0.1[a]	65.8±5.5[a]	52±4.1
Chrysin (10mg/Kg)	23.9±6.9	0.7±0.1	19.6±0.7	11.2±1.1
Chrysin (50mg/Kg)	25.9±10.4	0.8±0.0	16.1±2.0	9.5±0.6
Chrysin (250mg/Kg)	9.6±1.0[d]	0.6±0.0	15.3±1.1	7.5±0.4
Chrysin (10mg/Kg)+Adenine	-0.4±1.1[ab]	1.8±0.2[ab]	42.8±1.7[ab]	35.0±1.2[a]
Chrysin (50mg/Kg)+Adenine	1.9±0.9[a]	1.7±0.1[ab]	41.3±3.4[ab]	34.8±1.5[a]
Chryins (250mg/Kg)+Adenine	-1.9±0.4[ab]	1.5±0.1[ab]	37.2±3.7[ab]	28.8±1.2[a]

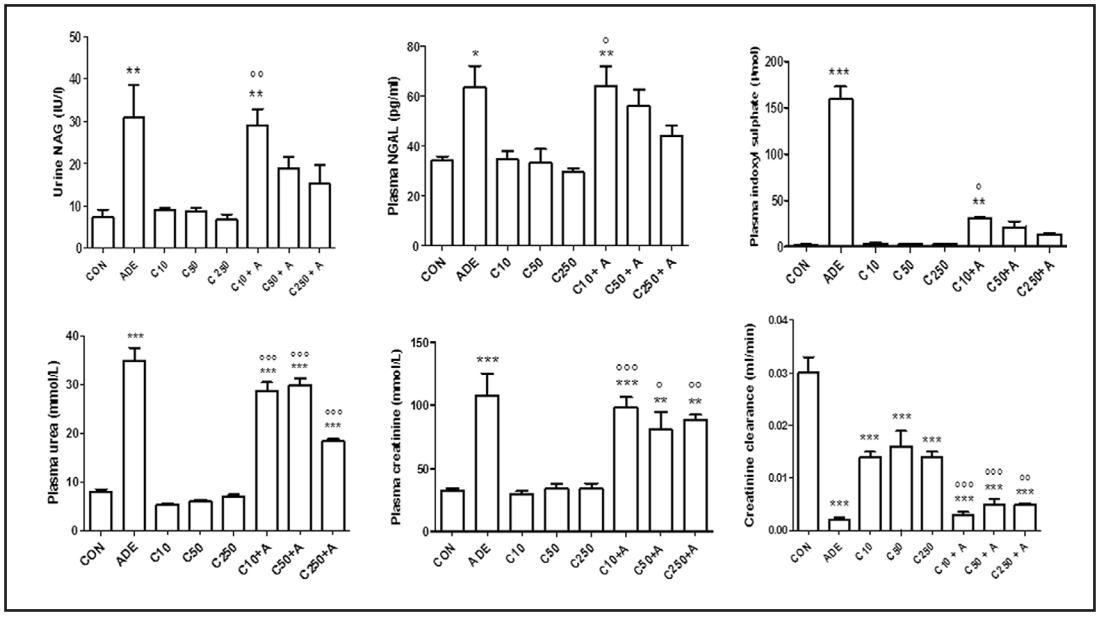

Fig. 2. Effect of chrysin treatment on creatinine clearance, and plasma concentrations of creatinine, urea, indoxyl sulfate, and neutrophil gelatinase-associated lipocalin (NGAL), and urinary N-acetyl-β-D-glucosaminidase (NAG) activity in control rats (CON) or rats treated singly or concomitantly with adenine (ADE, A), chrysin (C) at doses of 10, 50 or 250 mg/kg. Each column and vertical bar is a mean ± SEM (n = 6). Different superscripts indicate significance as follows: * denotes significance of different groups *vs* control group: where *$p \leq 0.05$, **$p < 0.001$, ***$p < 0.0001$. ° denotes significance of groups treated with chrysin alone *vs*. its corresponding groups treated with chrysin + adenine, where °$p \leq 0.05$, °°$p < 0.001$, °°°$p < 0.0001$.

The physiological data of the eight groups of rats in the experiment are shown in Table 1. Adenine treatment significantly reduced the growth of rats, and increased the absolute and relative kidney weight, the water intake and urine output. Treatment with the three doses of chrysin increased the body weights of rats and did not significantly affect the water intake of rats, or their urine output. However, chrysin, given after the induction of adenine CKD mitigated the effects of adenine treatment, which were mostly dose – dependent and most prominent with the highest dose of chrysin.

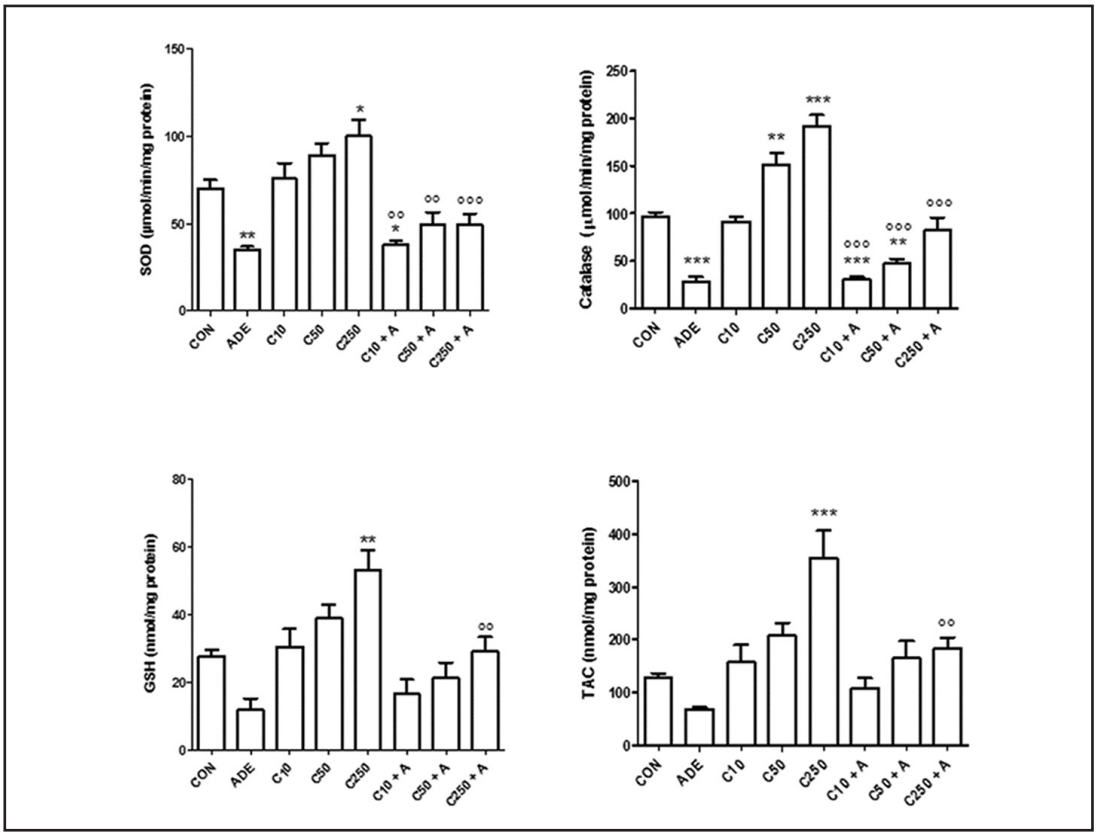

Fig. 3. Effect of chrysin treatment on renal concentration or activity of reduced glutathione (GSH), superoxide dismutase (SOD), total antioxidant capacity (TAC) and catalase in control rats (CON) or rats treated singly or concomitantly with adenine (ADE, A), chrysin (C) at doses of 10, 50 or 250 mg/kg. Each column and vertical bar is a mean ± SEM (n = 6). Different superscripts indicate significance as follows: * denotes significance of different groups *vs* control group: where *$p \leq 0.05$, **$p < 0.001$, ***$p < 0.0001$. ° denotes significance of groups treated with chrysin alone *vs*. its corresponding groups treated with chrysin + adenine, where °$p \leq 0.05$, °°$p < 0.001$, °°°$p < 0.0001$.

Biochemical measurements

As shown in Fig. 2, adenine treatment increased creatinine and urea concentrations in plasma, and decreased creatinine clearance. The albumin urinary concentration in the adenine –treated rats (29.1 ± 3.1) was higher than in the control rats and rats treated with the three doses of chrysin (ranging from 4.2 ± 0.3 to 6.30± 0.5). Adenine also elevated the plasma neutrophil gelatinase-associated lipocalin (NGAL) and urinary N-acetyl-beta-D-glucosaminidase (NAG) activities, and the plasma concentration of the uremic toxin indoxyl sulfate (IS). Treatment with the three doses of chrysin alone did not significantly affect any of the above indices, and were not different from that of the controls. Administration of chrysin at the three graded doses (10, 50 and 250 mg/kg) following the end of the adenine treatment caused a dose-dependent amelioration of all above measured indices.

The effect of chrysin treatment on antioxidant indices in kidney homogenates from control rats and treated rats is shown in Fig. 3. Adenine treatment depressed superoxide dismutase (SOD) and catalase activities, glutathione (GSH) concentrations, and total antioxidant capacity. Treatment with chrysin alone at the three graded doses enhanced these indices in a dose-dependent manner. Concomitant treatment of rats with adenine and the three doses of chrysin dose-dependently abated the adenine-induced oxidative stress, especially at the highest dose of chrysin, which almost returned the anti-oxidants to what observed in control group.

Table 2. Effect of chrysin on the activities of some enzymes in plasma rats with adenine-induced chronic kidney disease. Values in the tables are mean ± SEM (n = 6). Chronic kidney disease (CKD) was induced by the inclusion of adenine in the feed at a concentration of 0.25 % w/w for 35 days and then chrysin (10, 50, and 250 mg/kg) was given orally by gavage for 10 days after induction of CKD. On the last day of the treatment, rats were killed for blood collection. AST: aspartate aminotransferase, ALT: alanine aminotransferase, CK: creatinine kinase, GGT: gamma glutamyl transferase, LDH: lactate dehydrogenase. Values with superscripts are statistically different *p* value [a] vs control; [b] groups treated with chrysin alone vs. its corresponding groups treated with adenine+chrysin

Enzyme	ALT (IU/L)	AST (IU/L)	CK (IU/L)	GGT (IU/L)	LDH (IU/L
Control	37.8±3.0	75.7±9.1	273.7±35.7	1.1±0.2	205.7±28.8
Adenine	82.2±4.1[a]	110.8±4.9[a]	396.7±37.9[a]	4.6±0.5[a]	313.2±16.2[a]
Chrysin (10mg/Kg)	52.0±3.0	91.3±5.1	242.8±32.8	1.7±0.5	192.0±28.4
Chrysin (50mg/Kg)	56.6.0±4.4	87.6±13.7[a]	240.6±31.4	1.7±0.4	197.8±38.8
Chrysin (250mg/Kg)	40.2±6.9	74.0±9.9	203.5±31.5	2.2±0.4	158.0±18.0
Chrysin (10mg/Kg)+Adenine	73.5±9.9	112.2±9.4[a]	276.0±48.7	2.3±0.6[b]	313.2±19.1[a]
Chrysin (50mg/Kg)+Adenine	70.5±9.9	92.6±9.3[a]	231.0±18.6	3.0±0.5	305.7±23.2
Chryins (250mg/Kg)+Adenine	56.3±7.5	80.8±9.3	227.2±39.3	3.0±0.4	154.6±9.2

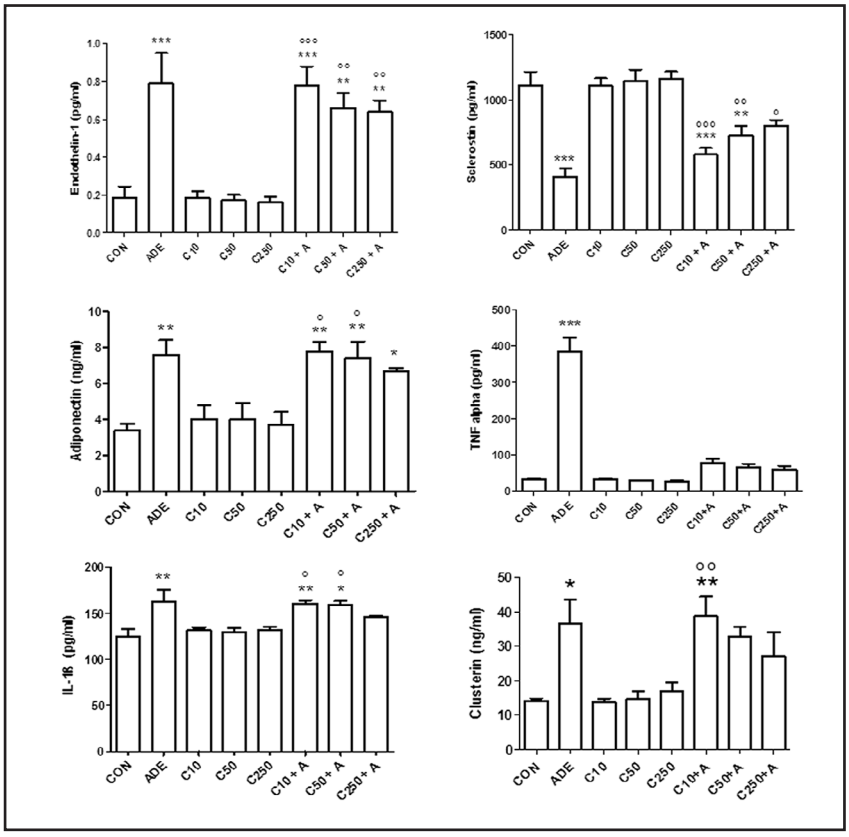

Fig. 4. Effect of chrysin treatment on plasma concentrations of the cytokines tumor necrosis factor alpha (TNF α), sclerostin, adiponectin, interleukin–one beta (IL-1β) and endothelin in control rats (CON) or rats treated singly or concomitantly with adenine (ADE, A), chrysin (C) at doses of 10, 50 or 250 mg/kg. Each column and vertical bar is a mean ± SEM (n = 6). Different superscripts indicate significance as follows: * denotes significance of different groups *vs* control group: where *$p \leq 0.05$, **$p < 0.001$, ***$p < 0.0001$. ° denotes significance of groups treated with chrysin alone *vs*. its corresponding groups treated with chrysin + adenine, where °$p \leq 0.05$, °°$p < 0.001$, °°°$p < 0.0001$.

Table 2 shows the effect of the three doses of chrysin (with and without adenine co-treatment) on the activities of some enzymes in plasma. Adenine treatment caused marked increases (about 2-4 -fold) in the enzymes aspartate transaminase (AST), alanine transaminase (ALT), creatine kinase (CK), L-γ-glutamyltransferase (GGT) and lactate

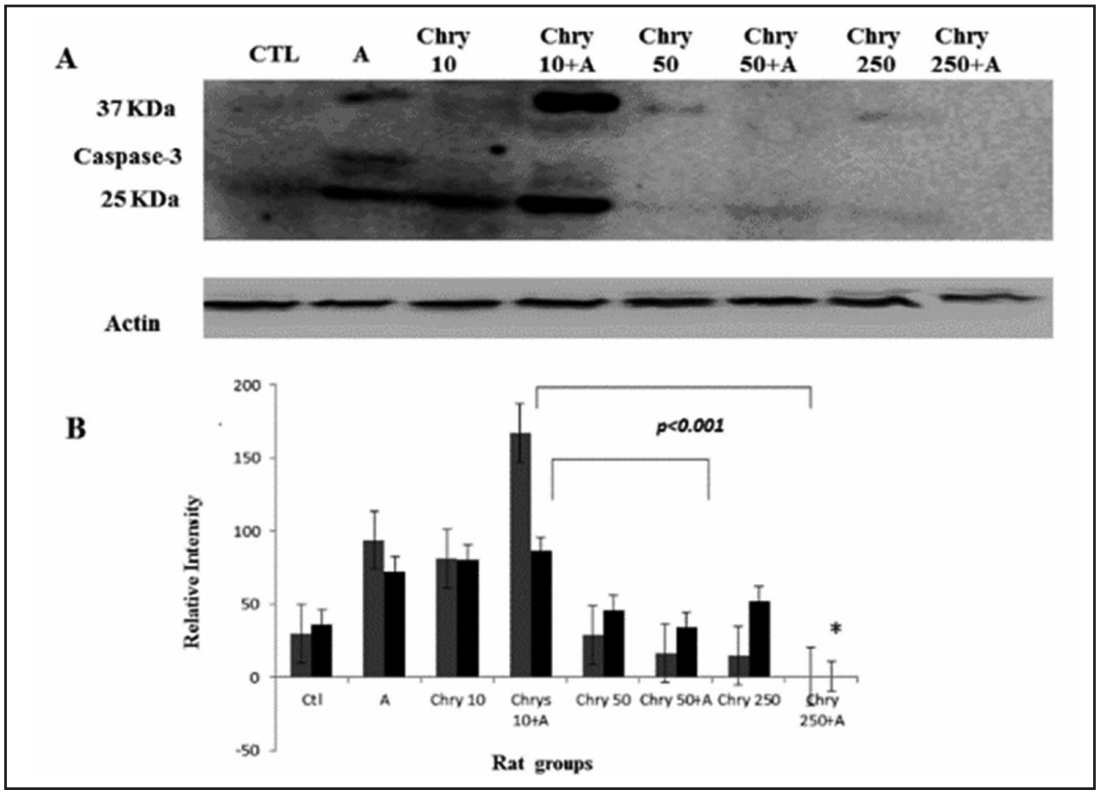

Fig. 5. (A) Western blot showing the apoptotic activity through the expression of Caspase-3 protein and the quantification of the Caspase-3 bands (37 KDa and 25 KDa) after their normalization to the house keeping protein. Actin was calculated as the average signal of four different blots. (B) Relative intensity of cleaved and uncleaved bands. Columns and vertical bars represent means ± SEM. CTL: control group; A: adenine treated group; Chry 10: 10 mg/kg chrysin treated group; Chry 10+A: 10 mg/kg chrysin with adenine; Chry 50: 50 mg/kg chrysin treated group; Chry 50+A: 50 mg/kg chrysin with adenine; Chry 250: 250 mg/kg chrysin treated group; Chry 250+A: 250 mg/kg chrysin with adenine.

dehydrogenase (LDH). The three doses of chrysin alone exerted no significant effect on any of the enzymatic activities. In the adenine co-treated groups, the three doses of chrysin were effective in ameliorating the action of adenine on these enzymes.

As shown in Fig. 4, adenine treatment increased the plasma concentrations of endothelin, adiponectin, TNF-α, IL - 1ß, and clusterin, and decreased that of sclerostin. Treatment of rats with any of the three doses of chrysin alone did not significantly affect any of the above measured biomarkers. Co-administration of chrysin (50 and 25 mg/kg) and adenine produced dose – dependent decrease in clusterin levels when compared to control rats and rats treated with chrysin alone. Chrysin at the highest dose (250 mg/kg) decreased IL - 1ß concentrations in adenine treated rats. Chrysin, at the three doses used, also markedly decreased the TNF- α levels when compared with that in adenine – treated rats. The effect of chrysin treatment in the latter animals was less marked, and was only significant in case of endothelin – 1, and at the two highest dose of chrysin. Adenine decreased the concentration of sclerostin, and co- treatment with chrysin antagonized this action in a dose –dependent fashion.

Molecular Analysis of Apoptosis

As shown in Fig. 5, adenine induced the expression and cleavage of Caspase-3 in those rats treated with adenine as a single agent as well as those treated with 10 mg/kg chrysin. However, the addition of extra chrysin (50 mg/kg and 250 mg/kg) inhibited the apoptosis

Table 3. Effect of chrysin on kidney morphology in rats with adenine-induced kidney chronic disease. Values in the tables are mean ± SEM (n=6). Chronic kidney disease (CKD) was induced by the inclusion of adenine in the feed at a concentration of 0.25 % w/w for 35 days and then chrysin (10, 50, and 250mg/kg) was given orally by gavage for 10 days after induction of CKD. Histopathology was evaluated from kidney slices stained with hematoxylin, Masson trichrome and periodic schiff acid. Values with superscripts are statistically different p value a vs control; b vs. Chrysin (10 mg/kg); c vs. Chrysin (50 mg/kg); d vs. Chrysin (250 mg/kg); e vs. Chrysin (10 mg/kg) + Adenine; f vs. Chrysin (50 mg/kg) + Adenine

Group	Inflammation	Fibrosis	Atrophy	Dilatation
Control	0.08±0.02	0	0	0
Adenine	2.52±0.25^{a}	3.03±0.18^{a}	2.73±0.40^{a}	0.20±0.05
Chrysin (10mg/Kg)	0.32±0.05	0	0.24±0.07	0.07±0.04
Chrysin (50mg/Kg)	0.07±0.07	0.17±0.04	0.33±0.09	0
Chrysin (250mg/Kg)	0	0	0.47±0.13	0
Chrysin (10mg/Kg)+Adenine	2.48±0.26^{ab}	3.26±0.17^{ab}	1.35±0.28^{ab}	0.42±0.03^{ab}
Chrysin (50mg/Kg)+Adenine	2.2±0.15^{ac}	2.06±0.19^{ade}	1.43±0.15^{ad}	0.69±0.05^{ade}
Chrysin (250mg/Kg)+Adenine	2.03±0.13^{ad}	2.40±0.27^{ade}	1.58±0.16^{ad}	0.47±0.06^{adf}

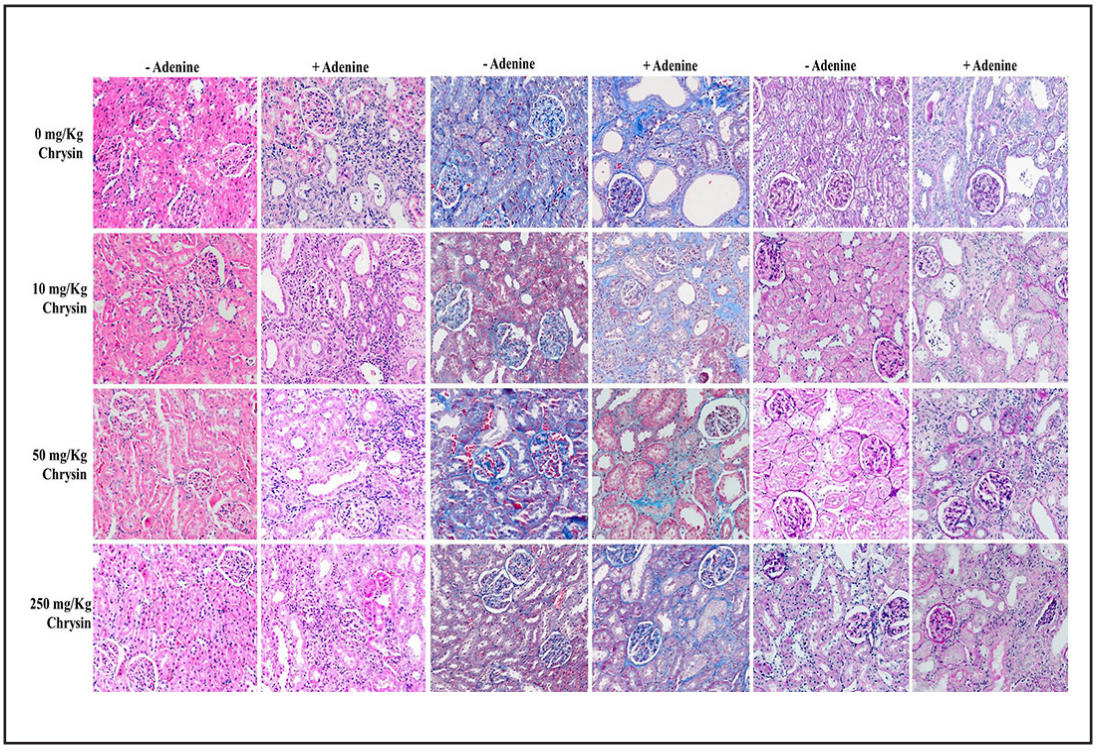

Fig. 6. Morphological changes in the kidney following treatment with chrysin at 0, 10, 50 and 250mg/Kg to rats with (+ Adenine) or without (- Adenine) adenine. (A) hematoxylin and eosin stain used for the identification and semi-quantitative scoring of inflammation. (B) Masson Trichome stain used for identification and semi-quantitative scoring of fibrosis. (C) periodic acid-Schiff stain, used for the identification and semi-quantitative scoring of atrophy of the basal membrane and dilatations.

signal and brought the levels of Caspase-3 to those levels detected in the control group. Chrysin did not show any apoptotic activity when it was used alone at the three different doses.

Histopathology

The results of the histopathological examination of kidney sections of all the groups used (representing the degree of inflammation, fibrosis, atrophy and dilatation) are summarized in Table 3. Sections from control rats showed no signs of damage. The kidneys of adenine-treated animals showed several signs of extensive damage, inflammation, as well as fibrosis. Chrysin alone did not alter the histological appearance of the kidneys. Chrysin, given after end of adenine treatment slightly ameliorated, the pathological changes seen, mostly at the highest doses of chrysin (Fig. 6).

Discussion

Recently, we studied the preventive action of chrysin in rats given chrysin concomitantly with adenine in the feed to induce CKD, and have found that it was effective in ameliorating several of the biochemical and physiological changes induced by adenine [13]. The results of the present work have also broadly shown that chrysin produces variable therapeutic salutary effects in rats with the established adenine -induced CKD. It lessened, mostly in a dose – dependent fashion, the physiological, biochemical and histopathological changes induced by adenine.

Chrysin is known to have a strong antioxidant activity, mainly due to hydrogen atom transfer, single electron transfer or metal chelation [17, 18]. The latter has been shown to be responsible for the amelioration of hepatic damage in diabetic mice and several other experimental organ damages [18, 19]. The molecular basis for a connection between oxidative damage and the uremic toxin indoxyl sulfate, which have been shown to significantly rise in adenine –induced CKD [20, 21], has recently been reviewed [22].

Adenine –induced CKD was associated with a significant rise in several pro-inflammatory cytokines and a reduction in pro-antiinflammatory cytokines, as was reported before [20, 23, 24]. The three graded doses of chrysin used have not affected the levels of the measured cytokines in control rats. However, when chrysin was given after the end of the adenine treatment most of the measured inflammatory indices returned to normal, especially the TNF-α. It is well –known that TNF–α is an important pro-inflammatory cytokine with well - established actions in several conditions including adenine – induced CKD [23, 25]. The inhibitory effect of chrysin on TNF–α suggests that this phytochemical has exerted its therapeutic effect on adenine – induced CKD, at least partly, by an anti-inflammatory action. Recently the anti-inflammatory action of chrysin has been confirmed in a mouse model of focal cerebral ischemia/ reperfusion injury by inhibiting the NF –kB signaling pathway, which is a regulator of iNOS and COX-2 expression [26].

Sclerostin, which is a novel marker of bone remodeling and vascular calcification, was depressed in rats with adenine – induced CKD. It has recently been shown that a high circulating level of this protein is associated with better survival in CKD patients undergoing dialysis [27, 28].

In this work, the three doses of chrysin caused only slight improvement in the morphological damage induced by adenine in the kidney. Using molecular quantification of apoptosis, chrysin did not cause any significant increase in caspase-3 levels indicating in part its lack of toxicity on the kidneys, and this was also confirmed by light histopathological examination. On the other hand, it caused only slight improvement in kidneys of rats treated with adenine.

The adenine-induced CKD model can either be reversible or irreversible depending on the duration of the treatment. Adenine fed for more than two weeks usually result in a reversible renal failure while a four weeks or a longer fed can result in a non-progressive or progressive, irreversible renal failure [29]. In this study, adenine was given for 35 days. The irreversibility of the occurred renal damage might explain the insignificant differences seen in some of the measured biomarkers and the observed histopathological changes among the chrysin treated CKD rats.

The present results also showed an elevation of some liver enzymes in plasma of rats treated with chrysin alone. However, these were not statistically significant from control. Furthermore, chrysin ameliorated the elevation of these enzymes in adenine treated groups. Such effect of chrysin on the liver activities warrants further pharmacological and toxicological investigations.

Conclusion

This work has shown that the three graded doses of chrysin given after the induction of CDK by feeding adenine were moderately effective in abating some of the actions of adenine–induced CKD in rats. Further studies to confirm the safety and therapeutic usefulness of chrysin in this condition are warranted.

Acknowledgments

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Disclosure Statement

None.

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