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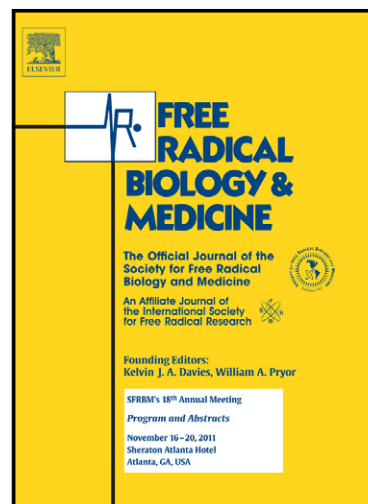
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**Dose-dependent deleterious and salutary actions of Nrf2 inducer, dh404, in chronic
kidney disease**

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Abstract

Oxidative stress and inflammation play a central role in progression and complications of CKD and are, in part, due to impairment of the Nrf2 system which regulates expression of antioxidant and detoxifying molecules. Natural Nrf2 inducing phytochemicals have been shown to ameliorate kidney disease in experimental animals. However due to adverse outcomes clinical trial of synthetic Nrf2 activator, bardoxolone methyl (BARD), in CKD patients was terminated. BARD activates Nrf2 via

covalent modification of reactive cysteine residues in Nrf2 repressor molecule, Keap-1. In addition to Nrf2, Keap1 suppresses IKKB, the positive regulator of NF κ B. Treatment with BARD analog, dh404, at 5-20 mg/kg/day in diabetic obese Zucker rats exacerbates whereas its use at 2 mg/kg/day in 5/6 nephrectomized rats attenuates CKD progression. We, therefore, hypothesized that deleterious effects of high dose BARD are mediated by activation of NF κ B.

CKD (5/6 nephrectomized) rats were randomized to receive dh404 (2 or 10mg/kg/day) or vehicle for 12 weeks. Vehicle-treated group exhibited glomerulosclerosis, interstitial fibrosis and inflammation, activation of NF κ B, upregulation of oxidative, inflammatory and fibrotic pathways, and suppression of Nrf2 activity and its key target gene products. Treatment with low dose dh404 restored Nrf2 activity and expression of its target genes, attenuated activation of NF κ B and fibrotic pathways, and reduced glomerulosclerosis, interstitial fibrosis and inflammation. In contrast treatment with high dh404 dosage intensified proteinuria, renal dysfunction and histological abnormalities, amplified up-regulations of NF κ B and fibrotic pathways, and suppression of Nrf2 system. Thus therapy with BARD analogs exerts a dose-dependent dimorphic impact on CKD progression.

Key words: Chronic kidney disease, CKD progression, Oxidative stress, Inflammation, Fibrosis, Nrf2, antioxidant system

Introduction

Chronic kidney disease (CKD) is invariably associated with oxidative stress and inflammation which play a major role in the pathogenesis and progression of kidney disease and its numerous complications [1-6]. Inflammation and oxidative stress are inseparably interconnected as they can trigger and intensify each other. Oxidative stress occurs as a result of either increased production of ROS,

depressed capacity of the antioxidant system, or both [7]. In presence of oxidative stress the uncontained or uncontrollable ROS attack, denature, and modify structural and functional molecules and activate redox-sensitive transcription factors and signal transduction pathways. These events lead to tissue damage and dysfunction by promoting necrosis, apoptosis, inflammation, fibrosis, and other disorders. Oxidative stress in CKD is due to a combination of increased production of ROS and impaired antioxidant capacity [8,9]. ROS production is markedly increased in the diseased kidney, as well as vascular and various other tissues. This is primarily driven by activation or upregulation of the ROS-producing enzymes (NAD(P)H oxidase (NOX) isoforms, cyclooxygenase-2, lipoxygenase, and uncoupled nitric oxide synthase), mitochondrial dysfunction, and endoplasmic reticulum stress [10-14]. Increased ROS production in CKD is compounded by the failure of the endogenous antioxidant defense system [4,15,16]. This is primarily due to the impaired activation of nuclear factor-erythroid-2-related factor 2 (Nrf2), a transcription factor that regulates basal activity and coordinated induction of numerous genes encoding phase-2 detoxifying and antioxidant enzymes and related proteins. The integrity of the Nrf2 function is essential for the maintenance of the redox balance under normal conditions and in mounting biological response to oxidative stress. Despite increased production of ROS which should provoke upregulation of endogenous antioxidant system, oxidative stress in CKD fails to elicit an appropriate antioxidant defense response. Instead, oxidative stress in animal models of CKD is associated with a diminished nuclear translocation (activation) of Nrf2, and significant reduction of its target gene products such as superoxide dismutase (SOD) isoforms, catalase, heme oxygenase-1 (HO-1, glutathione peroxidase, glutamate-cysteine ligase subunits, and NQO1 which are the major antioxidant and detoxifying enzymes [8,9]. In addition to promoting oxidative stress, impaired Nrf2 function contributes to intra-renal inflammation and progression of kidney disease. In fact ablation of Nrf2 gene results in intensification of oxidative stress, inflammation, and renal injury in

diabetic animals [17] and heightens the severity of ischemic and nephrotoxic acute kidney injury [18]. In contrast natural phytochemical Nrf2 activators such as sulforaphane, epigallocatechin-3-gallate, and curcumin, improve nephropathy in animal models of acute or chronic kidney diseases of diverse etiologies [19-23]. Together these observations support the role of Nrf2 deficiency in the pathogenesis of oxidative stress, inflammation, and progression of kidney disease.

The synthetic triterpenoid compounds belonging to the bardoxolone methyl (CDDO-Methyl ester) family are potent Nrf2 activators [24,25]. By interacting with the Keap1 molecule these compounds facilitate nuclear translocation of Nrf2 and expression of its target genes [26]. Although the phase IIb clinical trial of bardoxolone methyl in CKD patients with Type 2 diabetes showed improvement in the estimated glomerular filtration rate, it increased proteinuria, serum transaminase levels, and the incidence of adverse events [27]. Furthermore due to increased mortality from congestive heart failure the multi-center, randomized, placebo-controlled, Phase 3 clinical trial of a bardoxolone methyl analog in type 2 diabetic patients with stage 4 CKD (BEACON trial) was terminated [28]. Finally in a recent study Zoja et al [29] found substantial weight loss, anorexia, liver injury, exacerbation of proteinuria, dyslipidemia, glomerulosclerosis, and tubular damage with long term administration of the bardoxolone analogues, RTA 405 (50 and 100 mg/kg/day) and dh404 (5 and 25mg/kg/day) in obese Zucker rats with type-2 diabetes and nephropathy. These observations raised doubt about the potential efficacy of the bardoxolone methyl analogs in Type 2 diabetic nephropathy. In contrast, long term administration of BARD analog, dh404 at 2 mg/kg in rats with CKD induced by 5/6 nephrectomy resulted in amelioration of oxidative stress, inflammation, renal function and histology [30-32]. These observations point to the possible differences in the drug dosage as the potential cause of the divergent results of the reported studies. This assumption was confirmed by a recent elegant study by Tan et al. who showed that dh404, at lower but not higher doses, significantly lessens diabetes-associated

atherosclerosis and nephropathy in apolipoprotein E^{-/-} mice with streptozotocin-induced diabetes [33]. In an attempt to explore this possibility and to determine the optimal drug dosage, we conducted a series of preliminary experiments using a wide range of a bardoxolone methyl analog, dh404 dosage (0.5-20 mg/kg/day) in male Sprague Dawley rats. We found significant reduction in food intake, weight loss, intense proteinuria and deterioration renal lesions and the gross anatomy of the liver in animals treated with dh404 at doses between 5-20 mg/kg. However animals treated with 1-2 mg/kg/day dh404 showed favorable response. It is of note that, many natural Nrf2 inducing phytochemicals have hormetic properties with beneficial effects at nanomolar concentrations and toxic effects at higher concentrations [34,35]. It is, therefore, conceivable that the divergent response to bardoxolone analogues reported in the experimental animals [29-33] represents a shared hormetic property with their natural counterparts. The present study was undertaken to explore the impact of BARD analogue, dh404, dosage on kidney tissue Nrf2-Keap1, NFkB, fibrotic, and oxidative pathways as well as renal structure and function in rats with CKD induced by 5/6 nephrectomy.

Methods

Animals- Male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) weighing 225–250 g fed regular rat chow (Purina Mills, Brentwood, MO) and water were randomized to CKD and control groups. The CKD group underwent 5/6 nephrectomy as described previously [12] while the control group underwent sham operation. The CKD rats were further randomized to receive RTA dh404 2 mg/kg, 10 mg/kg, or vehicle (sesame oil) once daily for 12 weeks (n=7/group). Tail arterial pressure was determined by plethysmography as described previously [10]. The animals were then placed in metabolic cages for a 24-h urine collection and euthanized by exsanguination using cardiac puncture. The kidneys were harvested and immediately processed for histological evaluation and Western blot

analysis. Serum creatinine, urine protein and hematocrit were determined using standard laboratory methods. Serum malondialdehyde (MDA) was measured as described previously [8]. All experiments were approved by the University of California Irvine Institutional Committee for the Use and Care of Experimental Animals.

Histopathological procedure- Three-micron sections of the formalin-fixed and paraffin-embedded kidney tissues were cut and stained with hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) methods. The tissue sections were examined in a blinded fashion and the severity of the lesions quantified as described in in an earlier study [36].

Western blot analyses- Cytoplasmic and nuclear extracts of the kidney tissues were prepared as described previously [8]. The following antibodies were used to measure proteins of interest in the cytoplasmic or nuclear fractions by Western blot analysis: Rabbit antibodies against rat NF κ B p65, Nrf2, Keap1, modulatory and catalytic subunits of glutamate-cysteine ligase (GCLM and GCLC), cyclooxygenase-2 (COX-2) and heme oxygenase-1 (HO-1) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against phosphorylated inhibitor of NF κ B (pIKB), myeloperoxidase (MPO), TGF-beta, and NOX4, were purchased from Abcam Inc (Cambridge, Massachusetts, USA). Antibodies against catalase (EMD Chemicals, Gibbstown, NJ), inducible nitric oxide synthase (iNOS; Thermo Scientific, Rockford), MCP1 (Biovision, Milpitas, USA), eNOS (BD, Franklin Lakes), gp91^{phox} (BD Bioscience, San Jose, CA), Plasminogen activator inhibitor (PAI-1; BD, Franklin Lakes, USA), and alpha smooth muscle actin (Sigma-Aldrich, Saint Louis, MO, USA) were purchased from the cited sources. Antibodies against histone H1 (Santa Cruz Biotechnology), and actin were used for measurements of the housekeeping proteins for nuclear and cytosolic target proteins, respectively.

In brief, aliquots containing 50 µg proteins were fractionated on 8 and 4–20% Tris-glycine gel (Novex, San Diego, CA) at 120 V for 2 h and transferred to a Hybond-ECL membrane (Amersham Life Science, Arlington Height, IL). The membrane was incubated for 1 h in blocking buffer (1 × TBS, 0.05% Tween-20 and 5% nonfat milk) and then overnight in the same buffer containing the given antibodies. The membranes were washed three times for 5 min in 1 × TBS, 0.05% Tween-20 before a 2-h incubation in a buffer (1 × TBS, 0.05% Tween-20 and 3% nonfat milk) containing horseradish peroxidase-linked anti-rabbit IgG and anti-mouse IgG (Amersham Life Science) at 1:1,000 dilution. The membranes were washed four times and developed by autoluminography using the ECL chemiluminescent agents (Amersham Life Science).

Statistical analysis- Analysis of variance (ANOVA) was used in statistical evaluation of the data using SPSS software version 17.0 (SPSS, Chicago, IL). P values <0.05 were considered significant. Data are expressed as means ± SEM.

Results

General data- Data are shown in Table1. The vehicle-treated CKD rats exhibited proteinuria, hypertension, weight loss, anemia, and significant elevation of serum urea, creatinine, and MDA concentrations. Administration of high dose dh404 resulted in a significant increase in proteinuria, exacerbation of azotemia, and weight loss when compared with the vehicle-treated CKD group. This was associated with significant rise in serum MDA, denoting deterioration of oxidative stress. In contrast treatment with low dose dh404 resulted, amelioration of anemia and hypertension, modest reduction in serum creatinine and a significant reduction in MDA concentration. There was no significant difference in body weight between the vehicle-treated and low dose dh404-treated CKD groups.

Histological data- Remnant kidneys in the vehicle-treated CKD group showed interstitial fibrosis, leukocyte infiltration, tubular atrophy and dilatation, and glomerular sclerosis. Treatment with high dose dh404 resulted in dramatic exacerbation of glomerulosclerosis, interstitial inflammation and fibrosis. In contrast therapy with the low dose dh404 attenuated interstitial inflammation and fibrosis and glomerulosclerosis in the CKD rats (Figures 1).

Oxidative and inflammatory pathways- Compared with the control group, the kidney tissue in the vehicle-treated CKD rats showed a significant increase in phosphorylated IKB and a marked increase in the nuclear p65 translocation denoting NF_kB activation. This was associated with significant increase in the NAD(P)H oxidase subunits (gp91^{phox}, NOX4), MPO, iNOS, COX-2, and MCP1, and accumulation of nitrotyrosine, a biomarker of oxidative/nitrosative stress. Administration of high dose dh404 intensified whereas treatment with low dose attenuated these abnormalities in the remnant kidneys of the CKD animals (Figures 2 and 3).

Pro-fibrotic pathway- Compared to the normal control rats, kidney tissues in the vehicle-treated CKD rats exhibited significant upregulation of TGF beta, PAI-1 and alpha smooth muscle actin. Administration of high dose dh404 increased whereas treatment with low dose dh404 significantly lowered expression of these pro-fibrotic mediators in the remnant kidneys of the CKD animals (Figure 4).

Nrf2/Keap1 pathway- Compared with the control group nuclear Nrf2 content was markedly reduced and Keap1 abundance was significantly elevated in the kidneys of vehicle-treated CKD group. This was associated with significant down-regulations of CuZn-SOD, catalase, HO-1, and eNOS, in the kidneys of vehicle-treated CKD rats reflecting impaired Nrf2 activation. Administration of high dose dh404 intensified whereas treatment with low dose dh404 attenuated these abnormalities (Figure 5).

Discussion

In confirmation of earlier studies [8,9,14,30] oxidative stress and inflammation in the remnant kidney tissues in the vehicle-treated CKD rats were accompanied by impaired Nrf2 activity and downregulation of its target gene products. Long-term treatment with dh404 at 2 mg/kg/day restored Nrf2 activity and expression of its target gene products. This was associated with attenuation of oxidative stress, inflammation, and glomerular and tubulo-interstitial lesions, and partial improvement of renal function confirming the results of recent studies (30-32). In contrast, dh404 treatment at 10 mg/kg/day dosage resulted in intensification of proteinuria, deterioration of renal function and histological lesions, and amplification of oxidative stress and inflammation, confirming the effects observed in the Zucker rats with diabetic nephropathy and ApoE deficient mice with streptozotocine-induced diabetes [29, 33]. Taken together these findings indicate the dose dependent dimorphic actions of Bardoxolone methyl analogs on CKD progression with favorable effect at a low drug dosage and toxic effects at a high dosage. The dimorphic response to the Bardoxolone analogs which are potent synthetic Nrf2 inducers is consistent with the well-known hormetic properties of the natural plant-based Nrf2 activators [37,38]. These phytochemicals found in fruits and vegetables are known to reduce the risk of cancers, neurodegenerative disorders, and cardiovascular disease. Although the health benefits of these phytochemicals have been attributed to their antioxidant properties, the underlying mechanisms of their actions are actually based on their stress-inducing properties [39]. In fact many of these phytochemicals act as toxins protecting the plants against insects and parasites which consume huge quantities of the plant products relative to their body mass [40,41]. In contrast, when ingested by humans and other mammals at the relatively low amounts contained in the dietary fruits and vegetables, they can protect against a variety of adverse disorders by activating the adaptive stress response pathways. In this context several studies have demonstrated the hormetic mechanisms

of action of resveratrol, curcumin, sulforaphanes and catechins in cultured cells and in experimental animal [39,40]. Hormesis pathways activated by phytochemicals include Nrf-2 which stimulates transcription of genes controlled by the antioxidant response element, histone deacetylases of the sirtuin family, and FOXO transcription factors [40]. Activation of these pathways confers protection by up-regulating expression of antioxidant enzymes, detoxifying compounds, protein chaperones, and trophic factors. In fact these phytochemicals have been shown to prevent or ameliorate a variety of diseases including cardiovascular disease [42,43], neurodegenerative disorders [44-49], and acute and chronic kidney disease [19-23]. In contrast, excessive or unrestrained activity of Nrf2 system has been shown to have adverse consequences. For instance unrestrained activation of Nrf2 pathway leads to post-natal mortality in Keap1 knock out mice [50] and worsening of insulin resistance, adipose tissue contraction, weight loss, and hepatic steatosis in Keap1 knock down leptin-deficient mice [51]

Nrf2 is held in the cell cytoplasm as an inactive complex by Keap1 (Kelch-like ECH-associated protein 1) a repressor molecule which mediates Nrf2 degradation by serving as an adaptor for Cul3 Rbx1 E3 ligase complex. Keap1 molecule contains a number of reactive cysteine residues that serve as sensors of the intra-cellular redox state. Covalent or oxidative changes of thiols in these cysteine residues lead to conformational changes in Keap1 molecule which prevent its ability to bind Nrf2 [52]. This enables nuclear translocation and heterodimerization of Nrf2 with its co-activator, small Maf, leading to transcriptional upregulation of genes encoding antioxidant and phase 2 detoxifying molecules. Alternatively release and translocation of Nrf2 may occur via phosphorylation of its threonine or serine residues by protein kinase C, mitogen-activated protein kinases, phosphatidylinositol-3-kinase/Akt, casein kinase-2, or the endoplasmic reticulum enzyme PERK [53,54]. BARD analogues promote activation of Nrf2 via covalent modification of the specific thiols in the Keap1 molecules [26]. In addition to repression of Nrf2, Keap1 participates in repression of IKKB (the positive regulator of NF-

κ B) by facilitating its proteasomal degradation [55]. Accordingly oxidative or covalent modification of Keap1 can lead to release of IKKB, phosphorylation of IKB (the NF κ B inhibitor) by IKKB, dissociation of IKB-NF κ B complex, and nuclear translocation of NF κ B, events that trigger transcriptional upregulation of pro-inflammatory and pro-fibrotic mediators. In fact, our CKD animals treated with high dose of dh404 exhibited increased phosphorylated IKB and activation of NF κ B pathway. Activation of NF κ B, in turn, suppresses Nrf2 activity by preventing dissociation of Nrf2 from keap1 in the cytoplasm and by blocking Nrf2 binding to the antioxidant response elements of its target genes in the nucleus [56,57]. Thus excessive oxidative or covalent modification of keap1 leads to activation of inflammatory system and paradoxical inhibition of Nrf2, a scenario which is consistent with the divergent dose response to BARD analogs observed in CKD rats employed in the present study and in ApoE deficient mice with streptozotocin-induced diabetes. In this context the dimorphic dose response to the potent synthetic Nrf2 activator, bardoxolone analog dh404, observed in our CKD animals is consistent with the shared hormetic properties of their natural plant based counterparts.

It should be noted that in an earlier in vitro study of human leukemia cell line Ahmad et. al. [58] found that Triterpenoid CDDO-Me can block the NF κ B pathway by directly interacting with Cys-179 residue of IKK β . The finding of reduced IKB phosphorylation and NF κ B activation in our CKD animals treated with low dose dh404 is consistent with the results of the above in vitro study. However, remnant kidney tissues of CKD rats treated with high dose dh404 for an extended period showed heightened IKB phosphorylation and NF κ B activation. This observation likely points the dominant action of the extended in vivo exposure to high levels of these compounds on Keap-1 enabling release of IKKB and activation of NF κ B.

In conclusion, administration of low dose dh404 restored Nrf2 activity and expression of its target genes, attenuated activation of NF κ B and TGF- β pathway, and reduced glomerulosclerosis, interstitial

fibrosis and inflammation in rats with CKD. In contrast the CKD animals given high dh404 dosage showed massive weight loss, intense proteinuria, exacerbation of kidney lesions and dysfunction. These findings point to the dose-dependent dimorphic impact of the BARD analogs on CKD progression.

References

1. Himmelfarb J, Hakim RM. Oxidative stress in uremia. *Curr Opin Nephrol Hypertens* 2003; 12: 593-598
2. Himmelfarb J, Stenvinkel P, Ikizler TA, Hakim RM. The elephant in uremia: oxidant stress as a unifying concept of cardiovascular disease in uremia. *Kidney Int* 2002; 62: 1524-1538
3. Vaziri ND. Roles of oxidative stress and antioxidant therapy in chronic kidney disease and hypertension. *Curr Opin Nephrol Hypertens* 2004; 13:93-99
4. Vaziri ND. Oxidative stress in uremia: nature, mechanisms, and potential consequences. *Semin Nephrol* 2004;24 (5):469-73
5. Cachofeiro V, Goicochea M, de Vinuesa SG, Oubina P, Lahera V, Luno J. Oxidative stress and inflammation, a link between chronic kidney disease and cardiovascular disease. *Kidney Int* 2008; 111: S4-S9
6. Shah SV, Baliga R, Rajapurkar M, Fonseca VA. Oxidants in chronic kidney disease. *J Am Soc Nephrol* 2007; 18: 16-28.
7. Halliwell B. Biochemistry of oxidative stress. *Biochem Soc Trans* 2007; 35: 1147-1150
8. Kim HJ, Vaziri ND. Contribution of impaired Nrf2-Keap1 pathway to oxidative stress and inflammation in chronic renal failure. *Am J Physiol Renal Physiol* 2010; 298: F662-671

9. Aminzadeh MA, Nicholas SB, Norris KC, Vaziri ND. Role of impaired Nrf2 activation in the pathogenesis of oxidative stress and inflammation in chronic tubulo-interstitial nephropathy. *Nephrol Dial Transplant* 2013; 28: 2038-2045
10. Vaziri ND, Ni Z, Oveisi F, Liang K, Pandian R. Enhanced nitric oxide inactivation and protein nitration by reactive oxygen species in renal insufficiency. *Hypertension* 2002; 39: 135-141
11. Vaziri ND, Oveisi F, Ding Y. Role of increased oxygen free radical activity in the pathogenesis of uremic hypertension. *Kidney Int* 1998; 53: 1748-1754
12. Vaziri ND, Dicus M, Ho ND, Boroujerdi-Rad L, Sindhu RK. Oxidative stress and dysregulation of superoxide dismutase and NADPH oxidase in renal insufficiency. *Kidney Int* 2003; 63: 179-185
13. Vaziri ND, Bai Y, Ni Z, Quiroz Y, Rodriguez-Iturbe B. Intra-renal angiotensin II/AT1 receptor, oxidative stress, inflammation and progressive injury in renal mass reduction. *J Pharmacol Exp Ther* 2007; 323: 85-93
14. Aminzadeh AM, Sato T, Vaziri ND. Participation of endoplasmic reticulum stress in the pathogenesis of spontaneous glomerulosclerosis—Role of intra-renal angiotensin system. *Translational Res* 2012; 160: 309-318
15. Sindhu RK, Ehdaie A, Farmand F *et al.* Expression of catalase and glutathione peroxidase in renal insufficiency. *Biochim Biophys Acta* 2005; 1743: 86-92
16. Vaziri ND, Moradi H, Pahl MV, Fogelman AM, Navab M. In vitro stimulation of HDL anti-inflammatory activity and inhibition of LDL pro-inflammatory activity in the plasma of patients with end-stage renal disease by an apoA-1 mimetic peptide. *Kidney Int* 2009; 76: 437-44
17. Yoh K, Hirayama A, Ishizaki K *et al.* Hyperglycemia induces oxidative and nitrosative stress and increases renal functional impairment in Nrf2-deficient mice. *Genes to Cells* 2005; 13: 1159-1170

18. Liu M, Grigoryev DN, Crow MT, Haas M, Yamamoto M, Reddy SP, Rabb H. Transcription factor Nrf2 is protective during ischemic and nephrotoxic acute kidney injury in mice. *Kidney Int* 2009; 76: 277-285
19. Zheng H, Whitman SA, Wu W *et al.* Therapeutic potential of Nrf2 activators in streptozotocin-induced diabetic nephropathy. *Diabetes* 2011; 60: 3055-3066
20. Tsai PY, Ka SM, Chang JM *et al.* Epigallocatechin-3-gallate prevents lupus nephritis development in mice via enhancing the Nrf2 antioxidant pathway and inhibiting NLRP3 inflammasome activation. *Free Radic Biol Med* 2011; 51: 744-754
21. Peng A, Ye T, Rakheja D *et al.* The green tea polyphenol (-)-epigallocatechin-3-gallate ameliorates experimental immune-mediated glomerulonephritis. *Kidney Int* 2011; 80: 601-611
22. Sahin K, Tuzcu M, Gencoglu H *et al.* Epigallocatechin-3-gallate activates Nrf2/HO-1 signaling pathway in cisplatin-induced nephrotoxicity in rats. *Life Sci* 2010; 87: 240-245
23. Molina-Jijon E, Tapia E, Zazueta C *et al.* Curcumin prevents Cr(VI)-induced renal oxidant damage by a mitochondrial pathway. *Free Radic Biol Med* 2011; 51: 1543-1557
24. Liby K, Hock T, Yore MM *et al.* The synthetic triterpenoids, CDDO and CDDO-imidazolide, are potent inducers of heme oxygenase-1 and Nrf2/ARE signaling. *Cancer Res* 2005; 65: 4789-4798
25. Sporn MB, Liby KT, Yore MM, Fu L, Lopchuk JM, Gribble GW. New synthetic triterpenoids: potent agents for prevention and treatment of tissue injury caused by inflammation and oxidative stress. *J Nat Prod* 2011; 74: 537-545
26. Yates MS, Tauch M, Katsuoka F *et al.* Pharmacodynamic characterization of chemopreventive triterpenoids as exceptionally potent inducers of Nrf2-regulated genes. *Mol Cancer Ther* 2007; 6: 154-162

27. Pergola PE, Raskin P, Toto RD *et al.* Bardoxolone methyl and kidney function in CKD with type 2 diabetes. *N Engl J Med* 2011; 365: 327-336
28. De Zeeuw D, Akizawa T, Audhya P *et al.* The Effect of Bardoxolone Methyl in Patients with Type 2 Diabetes Mellitus and Stage 4 Chronic Kidney Disease: Bardoxolone methyl Evaluation in patients with Chronic kidney disease and type 2 diabetes mellitus: the Occurrence of renal events (BEACON) Trial. *N Engl J Med* 2004; 369: 2492-2503
29. Zoja C, Corna D, Nava V *et al.* Analogs of bardoxolone methyl worsen diabetic nephropathy in rats with additional adverse effects. *Am J Physiol Renal Physiol* 2013; 304: F808-F819
30. Aminzadeh MA, Reisman SA, Vaziri ND *et al.* Role of impaired Nrf2 activity in Chronic Kidney Disease-induced endothelial dysfunction and its restoration with the Synthetic Triterpenoid RTA dh404. *Redox Biol* 2013; 1: 527-531
31. Aminzadeh MA, Reisman SA, Vaziri ND, Yuan J, Khazaeli M, Meyer CJ. The Synthetic Triterpenoid RTA dh404 (CDDO-dhTFEA) Restores Nrf2 Activity and Attenuates Oxidative Stress, Inflammation, and Fibrosis in Rats with Chronic Renal Failure. *Xenobiotica* 2014; 44: 570-578
32. Son YK, Liu S, Farzaneh SH, Nazertehrani S, Khazaeli K, Vaziri ND. Nrf2 activator, dh404, restores renal Klotho expression and attenuates oxidative stress and inflammation in rats with chronic kidney disease. *J Applied Health Sci Int* 2015; 2: 22-34
33. Tan SM, Sharma A, Stefanovic N, Yuen DY, Karagiannis TC, Meyer C, Ward KW, Cooper ME, de Haan JB. Derivative of bardoxolone methyl, dh404, in an inverse dose-dependent manner lessens diabetes-associated atherosclerosis and improves diabetic kidney disease. *Diabetes*. 2014 (9):3091-103.

34. Mann GE, Bonacasa B, Ishii T & Siow RC. Targeting the redox sensitive Nrf2-Keap1 defense pathway in cardiovascular disease: protection afforded by dietary isoflavones. *Curr Opin Pharmacol* 2009; 9: 139-145
35. Siow RC, Mann GE. Dietary isoflavones and vascular protection: activation of cellular antioxidant defenses by SERMs or hormesis? *Mol Aspects Med* 2010; 31, 468-477
36. Vaziri ND, Liu S, Lau WL *et al.* High amylose resistant starch diet ameliorates oxidative stress, inflammation, and progression of chronic kidney disease. *PLoS One* 2014; 9: e114881
37. Speciale A, Chirafisi J, Saija A, Cimino F. Nutritional antioxidants and adaptive cell responses: an update. *Curr Mol Med* 2011; 11: 770-789
38. Mattson MP, Son TG, Camandola S. Viewpoint: mechanisms of action and therapeutic potential of neurohormetic phytochemicals. *Dose Response* 2007; 5:174-186
39. Maher J, Yamamoto M. The rise of antioxidant signaling-The evolution and hormetic actions of Nrf2. *Toxicol Appl Pharmacol* 2010; 244: 4-15
40. Son TG, Camandola S, Mattson MP. Hormetic Dietary Phytochemicals. *Neuromolecular Med* 2008; 10: 236-246
41. Sudakin DL. Biopesticides. *Toxicol Rev* 2003; 22: 83-90
42. Miura Y, Chiba T, Tomita I *et al.* Tea catechins prevent the development of atherosclerosis in apoprotein E-deficient mice. *J Nutr* 2001; 131:27-32
43. Hayek T, Fuhrman B, Vaya J *et al.* Reduced progression of atherosclerosis in apolipoprotein E-deficient mice following consumption of red wine, or its polyphenols quercetin or catechin, is associated with reduced susceptibility of LDL to oxidation and aggregation. *Arterioscler Thromb Vasc Biol* 1997; 17: 2744-2752.

44. Joseph JA, Shukitt-Hale B, Casadesus G. Reversing the deleterious effects of aging on neuronal communication and behavior: beneficial properties of fruit polyphenolic compounds. *Am J Clin Nutr* 2005; 81: S313-S316
45. Guo S, Yan J, Yang T, Yang X, Bezdard E, Zhao B. Protective effects of green tea polyphenols in the 6-OHDA rat model of Parkinson's disease through inhibition of ROS-NO pathway. *Biol Psychiatry* 2007; 62: 1353-1362
46. Ehrnhoefer DE, Duennwald M, Markovic P *et al.* Green tea (-)-epigallocatechin-gallate modulates early events in huntingtin misfolding and reduces toxicity in Huntington's disease models. *Hum Mol Genet* 2006; 15: 2743-2751
47. Zhang B, Safa R, Rusciano D, Osborne NN. Epigallocatechin gallate, an active ingredient from green tea, attenuates damaging influences to the retina caused by ischemia/reperfusion. *Brain Res* 2007; 1159: 40-53
48. Wang Q, Sun AY, Simonyi A *et al.* Neuroprotective mechanisms of curcumin against cerebral ischemia-induced neuronal apoptosis and behavioral deficits. *J Neurosci Res* 2005; 82: 138-148
49. Parker JA, Arango M, Abderrahmane S *et al.* Resveratrol rescues mutant polyglutamine cytotoxicity in nematode and mammalian neurons. *Nat Genet* 2005; 37: 349-350
50. Wakabayashi N, Itoh K, Wakabayashi J *et al.* Keap1-null mutation leads to postnatal lethality due to constitutive Nrf2 activation. *Nat Genet* 2003; 35: 238-245
51. Xu J, Kulkarni SR, Donepudi AC, More VR, Slitt AL. Enhanced Nrf2 activity worsens insulin resistance, impairs lipid accumulation in adipose tissue, and increases hepatic steatosis in leptin-deficient mice. *Diabetes* 2012; 61: 3208-3218.
52. Uruno A, Motohashi H. The Keap1-Nrf2 system as an in vivo sensor for electrophiles. *Nitric Oxide* 2011; 25: 153-160

53. Surh YJ, Kundu JK, Na HK. Nrf2 as a master redox switch in turning on the cellular signaling involved in the induction of cytoprotective genes by some chemopreventive phytochemicals. *Planta Med* 2008; 74: 1526-1539
54. Cullinan SB, Diehl JA. PERK-dependent activation of Nrf2 contributes to redox homeostasis and cell survival following endoplasmic reticulum stress. *J Biol Chem* 2004; 279: 20108-20117
55. Lee DF, Kuo HP, Liu M *et al.* KEAP1 E3 ligase-mediated down-regulation of NF-kappaB signaling by targeting IKKB, *Mol. Cell* 2009; 36: 131-140
56. Faraonio R, Vergara P, Di Marzo D *et al.* p53 suppresses the Nrf2-dependent transcription of antioxidant response genes. *J Biol Chem* 2006; 281: 39776-39784
57. Yu M, Li H, Liu Q *et al.* Nuclear factor p65 interacts with Keap1 to repress the Nrf2-ARE pathway. *Cell signal* 2011; 23: 883-892
58. Ahmad R, Raina D, Meyer C, Kharbanda S, Kufe D. Triterpenoid CDDO-Me blocks the NF- κ B pathway by direct inhibition of IKK β on Cys-179. *J Biol Chem* 2006; 281: 35764-35769

Table1- Body weight (BW), arterial pressure (BP), Serum urea (SUr), serum creatinine (SCr), serum Malondialdehyde (MDA), 24-hr urine volume (UV), and 24 hour urine protein (UP) in sham- operated control (CTL), untreated CKD, CKD rats treated with low and high dose dh404.

	BW (g)	BP (mmHg)	Urea (mg/dl)	Cr (mg/dl)	MDA (μ M/L)	UV (ml)	UP (mcg/mg cr)
CTL	416 \pm 9	118 \pm 2.7	19.3 \pm 1.09	0.39 \pm 0.09	0.61 \pm 0.05	8.3 \pm 2.1	12.7 \pm 1.6
CKD	380 \pm 5.1*	152 \pm 5.1*	72.98 \pm 10.2*	1.19 \pm 0.08*	1.41 \pm 0.09*	23.7 \pm 1.7*	39.4 \pm 9*
CKD+L	385 \pm 10.2*	123 \pm 1.5	41.58 \pm 2.96*#	0.73 \pm 0.05*#	0.84 \pm 0.07#	21.3 \pm 2.4*	41.6 \pm 3.4*
CKD+H	366 \pm 8.8*	136 \pm 4.2	99.8 \pm 4.9*&	1.07 \pm 0.04*&	2.33 \pm 0.2*&	29.9 \pm 3.1*	80.0 \pm 4.0*&

* $p < 0.05$ compared to CTL, # $p < 0.05$ compared to CKD, & $p < 0.05$ compared to CKD + Rx Low;
n=6; (Mean \pm SEM)

Figure 1- Representative Photomicrographs of the HE and PAS stained kidney sections and bar graphs depicting the extent of tubulo-interstitial injury (upper panel) and glomerulosclerosis (lower panel) in the normal control rats and vehicle treated, low dose dh404-treated, and high dose dh404-treated CKD rats.

** P<0.01, CKD+VEHICLE vs CKD+Rx Lo

* P<0.05, CKD+VEHICLE vs CKD+Rx Hi

P<0.001, CKD+Rx Lo vs CKD+Rx Hi

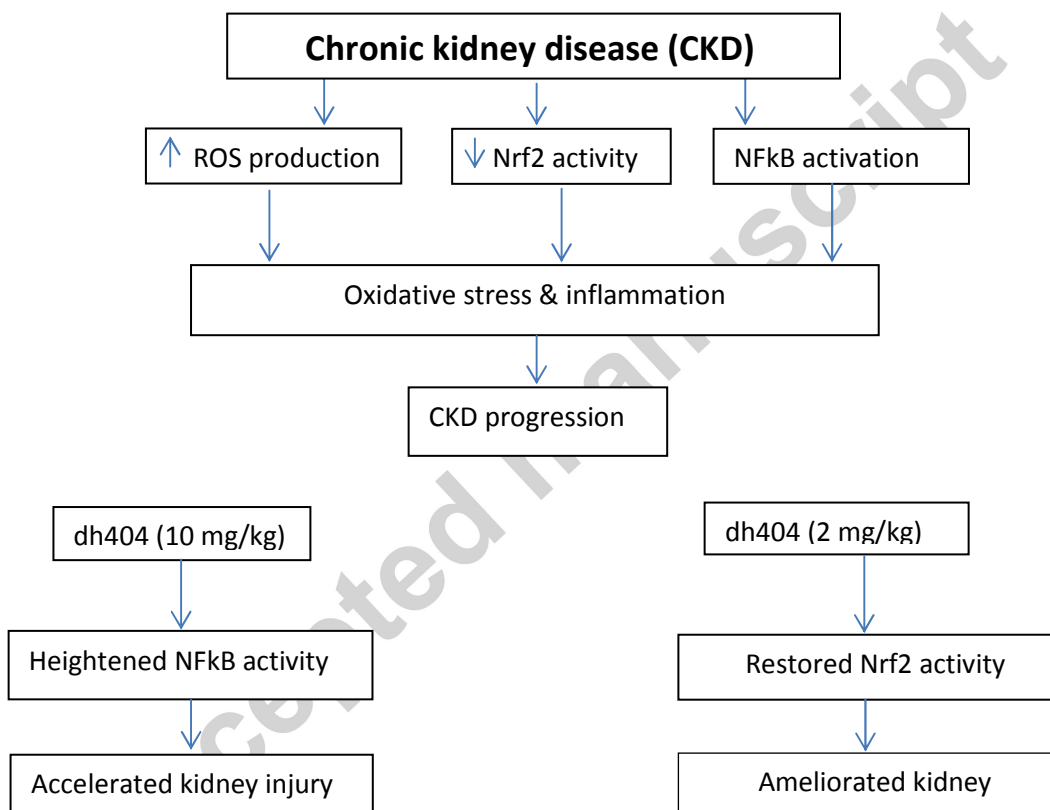
Figure 2 – Representative Western blots and group data depicting phosphorylated IKB, nuclear content of p65 active subunit of NFκB and protein abundance of MCP-1, iNOS, COX-1, COX-2, and myeloperoxidase (MPO) in the renal tissues of the normal control rats (n=6) and CKD rats treated with vehicle (CKD), low dose dh404 (CKD-L, n=7) or high dose dh404 (CKD-H, n=7). Data are means ± SE.

Figure 3 – Representative Western blots and group data depicting the NAD(P)H oxidase subunits (NOX-4, gp91phox), iNOS, and nitrotyrosine abundance in the normal control rats (n=6) and CKD rats treated with vehicle (CKD), low dose dh404 (CKD-L, n=7) or high dose dh404 (CKD-H, n=7). Data are means ± SE.

Figure 4 – Representative Western blots and group data depicting TGF-β, α-SM actin, and PAI-1 abundance in the kidney tissues of normal control rats (n=6) and CKD rats treated with vehicle (CKD), low dose dh404 (CKD-L, n=7) or high dose dh404 (CKD-H, n=7). Data are means ± SE.

Figure 5– Representative Western blots and group data depicting nuclear translocation of Nrf2 and protein abundances of its downstream gene products, CuZn-SOD, catalase, heme oxygenase-1 (HO-1)

and eNOS in the renal tissues of the normal control rats (n=6) and CKD rats treated with vehicle (CKD), low dose dh404 (CKD-L, n=7) or high dose dh404 (CKD-H, n=7).



I-Oxidative stress plays a central role in progression and complications of CKD

II- Oxidative stress in CKD is in part due to impaired Nrf2 activity

III- However recent clinical trial of Nrf2 inducer bardoxolone (BARD) in CKD patients failed

IV- Given Nrf2 activators' hormetic properties this could be due to drug overdose.

V- This assumption was confirmed in our CKD rats treated with high vs low BARD dosages

Accepted manuscript

Fig. 1

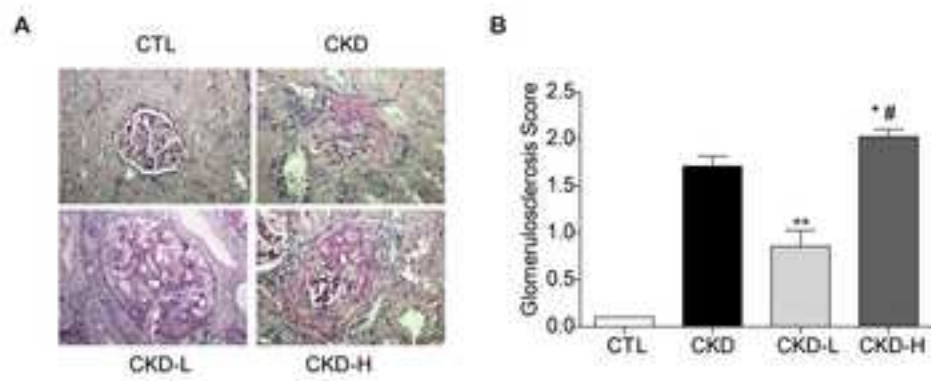


Fig. 4

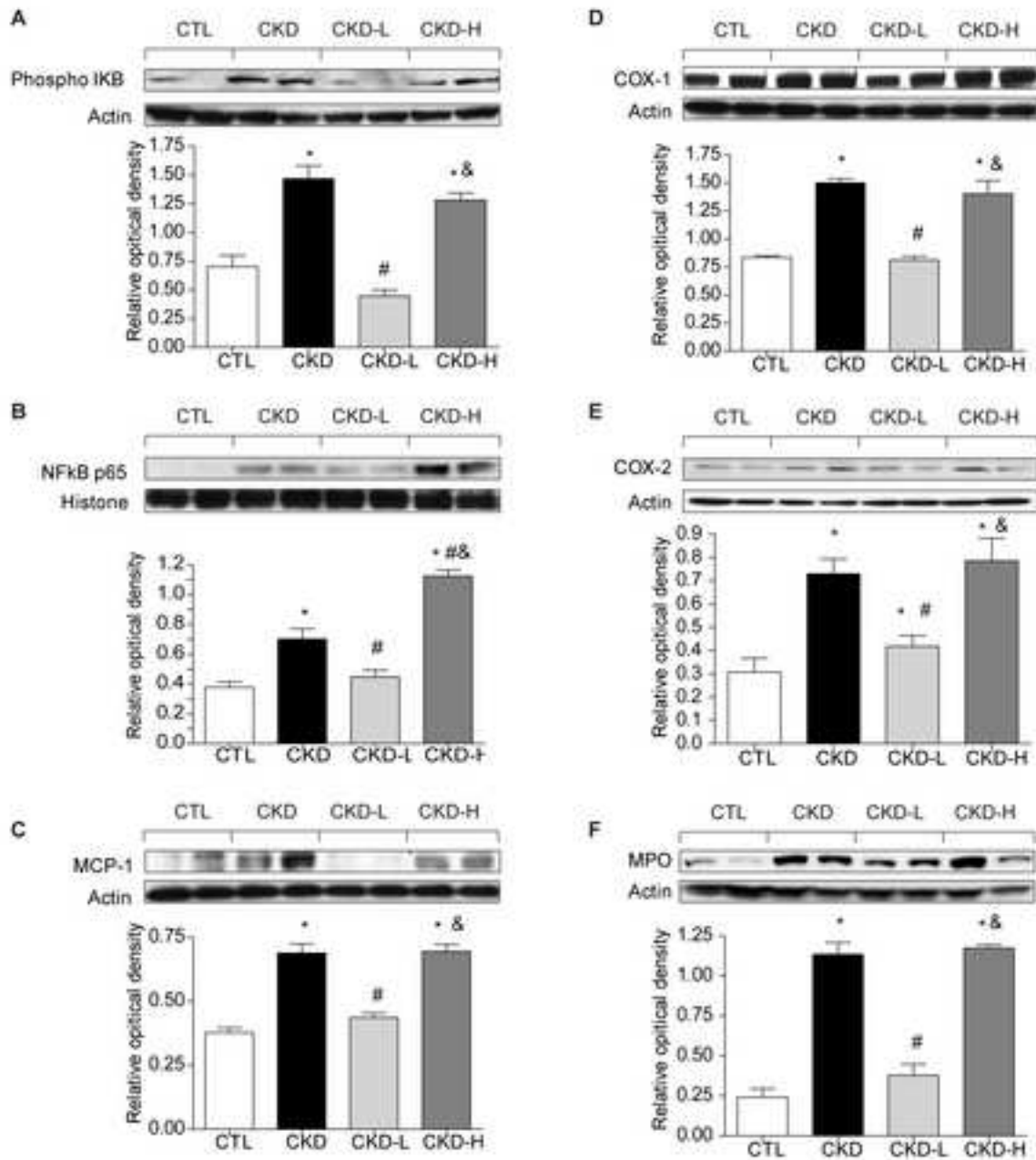


Fig. 3

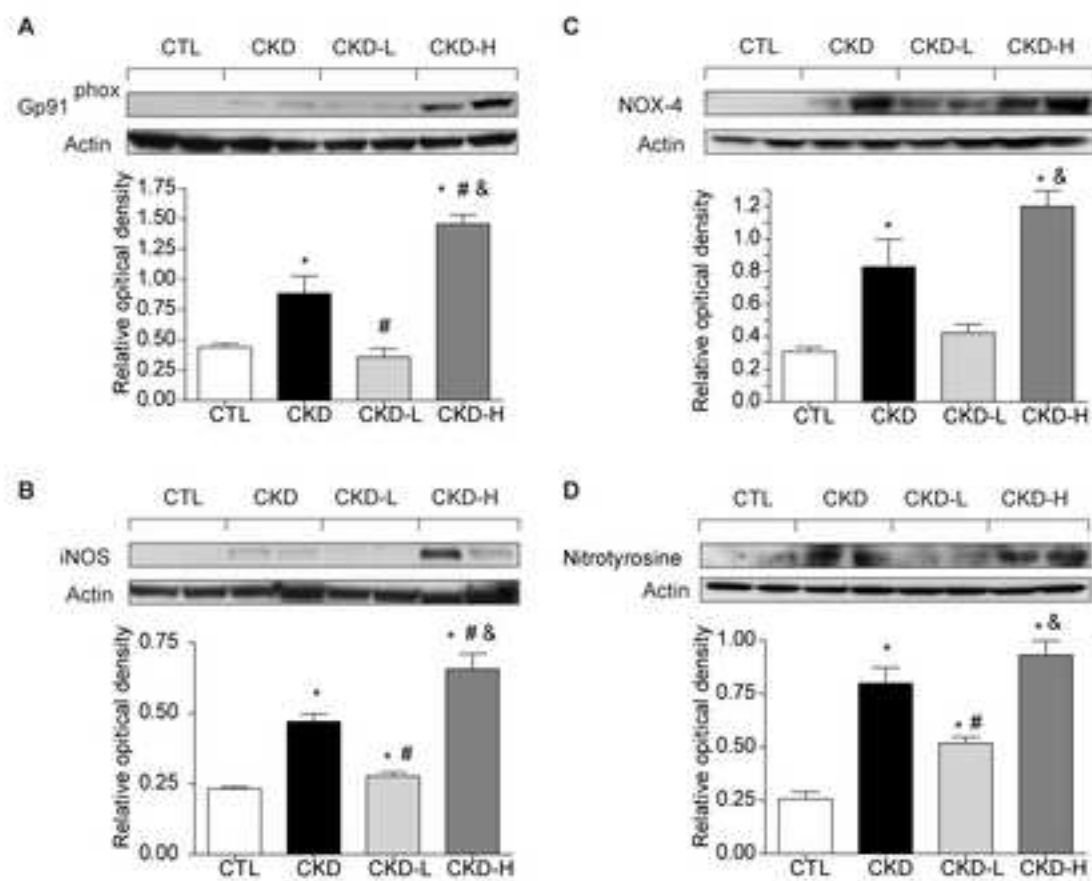


Fig. 6

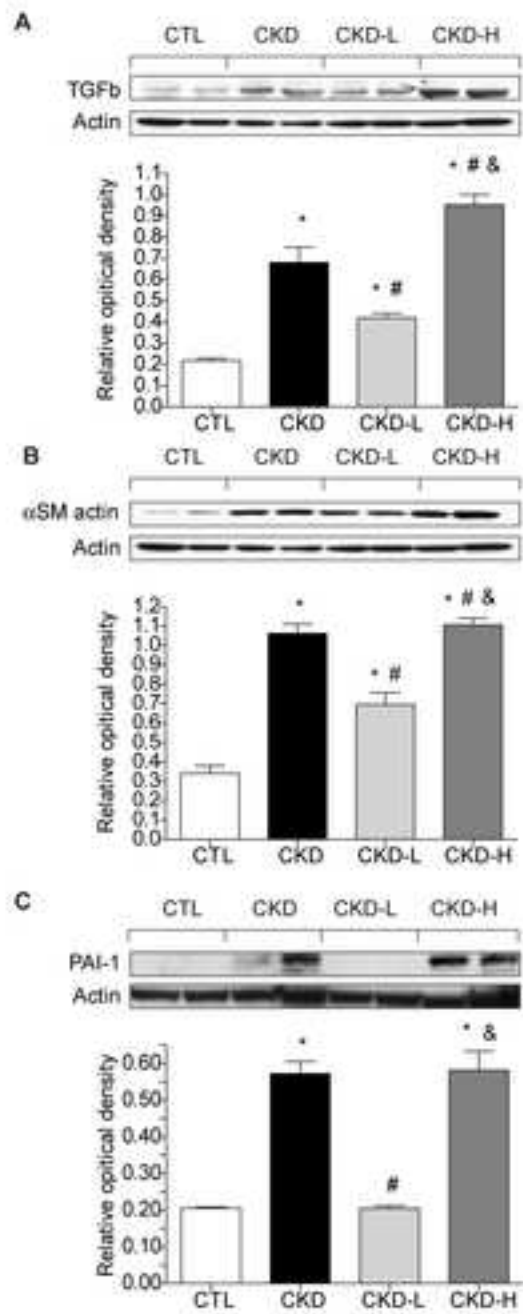


Fig. 5

