NOX, RAS and PKC-δ as Key Players in Kidney Diseases: A Possible Role of Rottlerin

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Abstract

Renal injury is characterized by oxidative stress and inflammation. NADPH oxidase is the major superoxide radical generator in the kidney. It is activated by the renin angiotensin system and PKC-δ. Rottlerin is a plant product isolated from the fruit covering of Mallotus philipinensis and is an inhibitor of PKC-δ and other signaling molecules. This review discusses the role of NADPH oxidase, renin angiotensin system and PKC-δ in renal diseases and a potential role of rottlerin to study kidney disorders.

ABBREVIATIONS

ROS: Reactive Oxygen Species; RAS: Renin Angiotensin System; PKC-δ: Protein Kinase C-δ; NOX: NADPH Oxidase; BK Channels: Ca²⁺ Activated Potassium Channels; RAAS: Renin Angiotensin Aldosterone System; Cox: Calcium Oxalate; ACE2: Angiotensin-Converting Enzyme 2; Ang II: Angiotensin II; EMT: Epithelial To Mesenchymal Transition; AT1R: Ang II Type 1 Receptor; CKD: Chronic Kidney Disease; L-NAME: N⁴-Nitro-L-Arginine Methyl Ester; AGE: Advanced Glycation End Products; ESRD: End Stage Renal Disease; COX: Cylooxygenase; LO: Lipooxygenase

INTRODUCTION

Oxidative stress and inflammation play a crucial role in the pathogenesis of a number of kidney diseases. Sources of reactive oxygen species (ROS) include NADPH oxidase, heme oxygenase, lipooxygenase, nitric oxide synthase, renin angiotensin system (RAS) and mitochondrial dysfunction [1]. NADPH oxidase (NOX), in particular NOX4 isoform, is considered as the prime producer of ROS in kidney tissue. A series of events involving protein kinase C (PKC) proteins are known to regulate NADPH oxidase activation under stress conditions. Phosphorylation of PKC-δ is one of the important events of NOX enzyme activation. Other than activating NOX enzyme system, PKC-δ is implicated in a number of renal disorders. Renin angiotensin system (RAS) is another key player of renal diseases and it modulates various processes by activating protein kinase C/NADPH oxidase dependent signaling pathways. RAS is also involved in the production of ROS by activating NOX enzyme.

Rottlerin is a polyphenolic compound obtained from the red powder covering the fruit of Mallotus philipinensis. Traditionally, this red powder is used as a dye and has many uses in folklore medicine. Rottlerin is also identified as a PKC-δ inhibitor, a mitochondrial uncoupler and an activator of Ca²⁺ activated potassium channels (BK channels). Recently, rottlerin has emerged as a useful pharmacological drug moiety, and has presented promising results in various diseases including many types of cancers, neurological disorders, cardiac disorders and renal disorders. As rottlerin can interfere in signaling pathways, it has a tremendous potential to serve as a potential tool for understanding the mechanism of renal diseases and, therefore, aid in the treatment of these disorders.

The purpose of this review is to understand the involvement of NADPH oxidase, renin angiotensin system and protein kinase C-δ in kidney diseases and explore the potential role of rottlerin in inhibiting these regulators to have a better understanding of the mechanism of kidney diseases.

NADPH oxidase in renal complications

NOX enzyme plays a pivotal role in receptor-stimulated ROS generation in nonphagocytic cells [2]. Recent studies have suggested that PKCs activate NADPH oxidase by phosphorylating the NADPH oxidase subunit p47phox, and PKC-δ increases NADPH oxidase activity in diabetic glomeruli and HL60 cells [3,4]. PKC-δ has also been implicated as a regulator of NADPH oxidase and is required for an appropriate assembly of this complex enzyme's components [5]. From previous studies, it is known that in kidney tissue (vasculature, glomeruli, mesangial cells and nephron segments) the predominant isoform NADPH oxidase is NOX4. NOX1 is present in colon, NOX3 in fetal kidney, NOX5 in spleen and Duox1 and Duox2 are restricted to thyroid. NADPH oxidase, due to its role in production of superoxides and reactive oxygen species is targeted in various models of

renal diseases and its inhibitors decipher mechanism of ROS producing signaling pathways [6]. In oxalate induced renal injury, NADPH oxidase activity shows strong correlation with the expression of p22phox and p47phox subunits, production of superoxide and the release of lactate dehydrogenase, when cells are exposed to CaOx crystals. Activation of NADPH oxidase is mediated by mineral corticoid receptor Rac-1 and PKC-α and PKC-δ dependent activation of NADPH-oxidase [7]. Oxalate and Calcium oxalate monohydrate crystals also activate the renin-angiotensin-aldosterone system (RAAS); Aldosterone stimulates ROS production through activation of NOX with the involvement of Rac1 and mitogen-activated protein kinase. Hyperoxaluria-induced production of ROS, injury and inflammation are thus, in part associated with the activation of NOX through RAAS [8].

In a study on diabetic nephropathy, pharmacological inhibition of NOX1/4 by GKT137831 conferred nephroprotection in mice with pre-existing diabetes and established kidney disease. Thus rendering, GKT137831 as a potential compound for the treatment of diabetic nephoropathy by inhibiting NADPH oxidases induced ROS [9]. Resveratrol is another compound that inhibits NADPH oxidase and showed curative properties in high glucose induced epithelial to mesenchymal transition (EMT) in renal tubular epithelial cells which is involved in tubulointerstitial fibrosis in diabetic nephropathy [10]. Ischemia/reperfusion injury is one of the most common causes of acute renal failure in both native kidneys and allografts. Diphenyleneiodonium, a NADPH oxidase scavenger, inhibits apoptosis and hence is a potential therapeutic agent for acute renal ischemia [11]. Cisplatin induced nephrotoxicity is primarily caused by reactive oxygen species induced proximal tubular cell death. NADPH oxidase is major source of ROS production by cisplatin. Acetovanillone imparts protection towards renal injury by inhibiting cisplatin induced NOX2 and NOX4 isoforms of NADPH oxidase present in kidney (NOX4) and in infiltrating leukocytes particularly neutrophil (NOX2) [12]. Chronic kidney disease (CKD) is caused by inflammation and oxidative stress and simvastatin—another drug candidate, suppresses CKD induced inflammation and oxidative stress in angiotensin II stimulated human mesangial cells, by inhibiting signaling pathway involving NADPH oxidase and PKCs [13]. In dye-contrast induced kidney injury, pentoxifylline is able to reduce the renal injury by a mechanism which inhibits NADPH oxidase and decreases the levels of malondialdehyde in human mesangial cells [14]. Both in human and in animal models of focal segmental glomerulosclerosis, oxidative stress contributes to pathogenesis of glomerular lesions. In a mouse model of Adriamycin-induced glomerulosclerosis, circulating receptor for AGE (RAGE) ligands and podocyte-specific RAGE protein levels were linked to increased NOX activity and ROS, whereas RAGE-deficient mice were protected from glomerular lesions and podocyte damage [15].

In summary, PKC activates NOX in renal anomalies while inhibition of PKCs. NOX inhibition by a number of inhibitors reduces renal injury, apoptosis and EMT transition.

These studies put forth the role of NOX enzyme in a wide array of diseases and suggest that NOX inhibition can be a beneficial event, to counteract pathological kidney disorder.

**Renin Angiotensin system in renal complications**

Renin angiotensin system plays an important role in the pathogenesis of kidney disorders such as hypertension and renal injury. Angiotensin-converting enzyme 2 (ACE2) is highly expressed in the kidney and hydrolyzes angiotensin II to beneficial angiotensin (1–7) and thereby counterbalancing the ACE/angiotensin II actions. Since angiotensin II is a strong activator of oxidative stress, it promotes inflammatory response and ROS production via angiotensin II type 1 receptor (AT1R). Aldosterone induces excess production of ROS and oxidative stress in glomerular cells through activation of NOX. Activated mineralocorticoid receptors mediate the translocation of the cytosolic components of p47phox and p67phox to the cell membrane. Subsequently, ROS over-production elicits oxidative stress and triggers redox-sensitive cell signalling cascades that mediate mitochondrial dysfunction, cellular apoptosis, inflammatory response and fibrogenesis. These aldosterone-induced inflammatory, fibrotic and apoptotic pathways appear to be specifically activated in response to injury in both glomerular mesangial cells and podocytes [16].
As discussed above NOX4 is implicated in diabetic nephropathy and it is activated by glycation end-products, the renin-angiotensin system, TGF-β and protein kinase C [17]. In a study, genetic ablation of ACE2 in mice was found to be associated with increased NOX activity and the AT1 receptor blockade abrogated NOX over activity. It is suggested that observed increase in ROS was angiotensin II dependent [18]. In diabetic nephropathy, RAS is closely associated with ROS generation and the pathogenesis involves insulin resistance, renal lipid accumulation, inflammation, and activation of the RAS. Therefore, RAS blockers and AT1R antagonists are currently employed to hamper progression of diabetic nephropathy. Additionally, an activated RAS produces ROS via angiotensin II-mediated NOX activation. Another study showed that, ACE-2 by cleaving angiotensin II into angiotensin 1–7, counter regulates the ACE/angiotensin II/AT1R axis and angiotensin 1–7 treatment lowers ROS production in db/db kidneys, in association with reduced NOX activity and nitrosyrate levels [19]. Renal infusion of angiotensin II in rats reduces renal blood flow, glomerular filtration, sodium excretion, and NO levels, all of which are blunted by the AT1R antagonist valsartan, the superoxide scavenger tempol and the NOX inhibitor apocynin [20]. Unlike vascular smooth muscle cells, angiotensin II activation of NOX is less understood in renal cells. However, it is dependent on the production of arachidonic acid, PKC and Rac1. The multiple targets downstream of NOX activation by angiotensin II are Akt, EGFR, JNK, ERK1/2, ETS-1, COX-2 and PDK1 [21].

Pathogenesis of hypertension involves activation of RAS, renal inflammation and oxidative stress. It has been found that genetic hypertension in rats is associated with renal up-regulation of AT1R. The molecular mechanisms of the activation of angiotensin II during hypertension involve the stimulation of NOX, the resulting overproduction of ROS may lead to over expression of AT1R and vice-versa. NO inhibition using Nω-nitro-L-arginine methyl ester (L-NAME) induced-hypertension is associated with up-regulation of renal AT1R. NADPH oxidase activation and increased intra renal oxidative or inflammatory pathways. AT1R blockade decreases blood pressure, reduces renal damage and attenuates up-regulations of the pro-oxidative/inflammatory pathways in L-NAME-induced hypertension. Losartan reduces the renal oxidative stress, NF-κB activation and IL-6 expression after L-NAME administration [22].

Podocyte injury has been considered as the most important early event initiating glomerulosclerosis in many proteinuric kidney diseases. Podocytes express a functional intrinsic rennin angiotensin- aldosterone system (RAAS) [23]. Angiotensin II activation of the AT1R leads to oxidative stress [24], which is linked to glomerular injury. NOX is also associated with RAAS-induced podocyte and filtration barrier injury [25]. TG (mRen2)27 (Ren2) transgenic rat model over expresses the mouse renin gene, exhibits increased RAAS activity, elevated angiotensin II levels, and oxidative stress [26,27]. Direct renin inhibition, AT1R blockade, and MR antagonism attenuates increased NOX activity and subunit expression, accompanied by restored podocyte slit diaphragm protein nephrin expression and ultrastructural changes in Ren2 rats [25]. Proteinuria has been shown to elicit the renal activation of RAS. High levels of albumin trigger the production of intracellular reactive oxygen species by a PKC-NOX-dependent pathway and this, in turn, leads to activation of nuclear factor-κB and activation protein-1. Inhibition of PKC or NOX abolishes albumin-induced activation of RAS [28]. Chronic kidney disease is characterized by inflammation and oxidative stress. Simvastatin suppresses the increased mRNA expression of monocyte chemoattractant protein-1, tumor necrosis factor-α, interleukin -1β and IL-6 and ROS induced by angiotensin II in a dose-dependent manner. Simvastatin also suppresses inflammation and oxidative stress in angiotensin II-stimulated HMCs via COX-2, PPARγ, NF-κB, NOX and PKCs, thereby exerting a protective effect on CKD [29].

To summarise, RAS is involved in activation of NOX and induction of oxidative stress in a number of renal diseases, and this activation is thought to be mediated through PKC dependent pathway.

Protein Kinase C-δ in renal disorders

PKC proteins are a family of serine/threonine kinases, and differences in their structural domains determine their activation dependence on Ca²⁺ ions and/or lipids. Conventional PKC (cPKC) family members (PKCa, βI, βII, γ) are calcium-dependent and are activated by diacylglycerol (DAG) and phospholipids (eg. Phosphatidylserine). Novel PKC (nPKC) members (δ, ε, θ, η) do not bind Ca²⁺ ions but are activated by DAG and phospholipids. The activities of the atypical PKCs (λ, ζ) do not depend on Ca²⁺ ions or DAG. PKC-δ is ubiquitously expressed in many cells and tissues [30-32]. As a member of the novel PKC subfamily, PKC-δ can be activated by diacylglycerol and phospholipid esters in the absence of Ca²⁺ ions. Recent studies have further revealed additional mechanisms of PKC-δ activation, which involve tyrosine phosphorylation and subcellular translocation [33]. Functionally, PKC-δ has been implicated in the regulation of a variety of cellular processes, ranging from signal transduction to apoptosis [34-35]. PKC-δ is reportedly a key signaling molecule in the ROS-induced apoptotic pathway through generation of active catalytic fragments by proteolytic cleavage [36]. In a number of kidney diseases, a proximal tubular cell undergoes activation of PKC-δ which promotes intrinsic mitochondrial pathway of apoptosis. As discussed above, even though PKC activation under hyperglycemia is largely related to an increase in de novo synthesis of diacylglycerol (DAG), activation of PKC can also be regulated by oxidative stress. Activation of PKC-δ occurs even before the cell displays any apoptotic features [37]. PKC-δ and PKC-ε are sensitively activated by hyperglycemia-induced oxidative stress in diabetic rat kidney [38]. The tight junction, or zonula occludens, which encircles each epithelial cell, has a role in maintaining epithelial polarity and in selectively sealing the paracellular pathway and, thereby, maintaining the barrier. Any change in permeability of tight junctions will have significant effect on cell physiology. In LLC-PK1 cells, over expression of PKC-δ is capable of inhibiting tight junction leakiness without the requirement of phorbol esters [39]. Purinergic regulation of intestinal oxalate transport plays an important role in overall oxalate homeostasis and, thereby, affects urinary oxalate excretion and risk of stone formation. ATP and UTP negatively regulate oxalate transport by lowering anion exchanger Slc26a6 surface expression in C2 cells by the signaling mechanisms that likely involve PKC-δ through the purinergic receptor P2Y2 and phospholipase C. Mice
lacking the anion exchanger Slc26a6 have a critical defect in intestinal oxalate secretion, resulting in enhanced net absorption of ingested oxalate, hyperoxalemia, hyperoxaluria, and a high incidence of calcium oxalate kidney stones [40]. Migration and adhesion of tumor cells are essential prerequisites for the formation of metastases in malignant diseases. PKC has been shown to regulate cell migration, adhesion and proliferation. PKC-δ is found to have a critical role in the regulation of tumor cell migration, via altering the expression and activity of β 1-integrins and focal adhesion kinase in renal cell carcinomas [41].

Taken together, above in-vitro and in-vivo studies assert the role of PKC-δ in kidney diseases, involving either direct intervention by PKC-δ or PKC-δ mediated activation of other signaling molecules. Inhibition of PKC-δ may cause reversal of the deleterious effects of PKC-δ in kidney functioning.

**Mallotus philippinensis**

*Mallotus philippinensis* Muell. (commonly called Kamala and Kampillaka) is a very common perennial shrub or small tree found in outer Himalayas ascending to 1500 meters. The red powder of fruits when mixed with some oil is good remedy for ulcers (Figure 2). The decoction of bark is used in abdominal pain. Among the tribe of chhota Nagpur the root, well ground is rubbed on the painful parts in articular rheumatism. In Burma, the seeds are grounded to paste and applied to wounds and cuts. The powdered seeds are mixed with sulphur sandalwood oil and the mixture is very effective when applied externally in rheumatic joints and also in dermatitis [42]. Some of the pharmacological effects of the plant are listed in table 1.

**Rottlerin and its effects**

Rottlerin, unlike other polyphenols, is not present in edible vegetables and in common beverages; instead, it is primarily present in the gland hair covering the fruit of *Mallotus philippinensis* (Euphorbiaceae), an evergreen rain forest tree that is inedible and only used by indigenous populations of Southeast Asian tropical regions. Rottlerin is used as a dye for coloring textiles and as an old folk remedy against tapeworm (when taken orally) and scabies and ringworm (when applied topically) [43]. Rottlerin has capabilities to inhibit members of PKC family with varying efficacy, it inhibits PKC-δ (IC$_{50}$=3-6µM), PKC-α,β,γ (IC$_{50}$=30-42µM) and PKC-ε,η (IC$_{50}$=80-100 µM). It also inhibits p38 regulated activated protein kinase, MAPK activated protein kinase, JNKα1, cAMP dependent protein kinase 2, JNKα1, cAMP dependent protein kinase, 3-phosphoinositide dependent protein kinase 1, protein kinase B-α, GSK-3-β and non kinase enzymes like β-lactamase, α-chymotrypsin and malate dehydrogenase [44]. Rottlerin, potently activates the large conductance voltage gated potassium channels (BK) expressed in a heterologous expression system and human vascular smooth muscle cells, shifting the conductance/voltage relationship by >100 mV. BK channels can be activated in the absence of divalent cations (Ca$^{2+}$, Mg$^{2+}$), suggesting that mechanism of action involves the voltage gating of the channels [45-46]. The general mechanism of action of rottlerin is depicted in figure 3.

### Table 1: General pharmacological effects of part/phytochemical of *M.phillipinensis* [42].

<table>
<thead>
<tr>
<th>Pharmacological effect</th>
<th>Part/phytochemical of plant used</th>
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<tbody>
<tr>
<td>1. Anti-filarial activity</td>
<td>Aqueous and alcoholic leaf extract</td>
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<tr>
<td>2. Anti-fertility activity</td>
<td>Seeds extract</td>
</tr>
<tr>
<td>3. Anti-bacterial activity</td>
<td>Glandular hair of fruits</td>
</tr>
<tr>
<td>4. Anti-inflammatory and immunoregulatory activities</td>
<td>Chalcone derivatives from fruits</td>
</tr>
<tr>
<td>5. Anti-oxidant and anti-radical activity</td>
<td>Ethanol extract of fruit and bark fraction</td>
</tr>
<tr>
<td>6. Hepatoprotective effect</td>
<td>Methanolic extract of leaves</td>
</tr>
<tr>
<td>7. Cytotoxic activity</td>
<td>Glandular hair extract</td>
</tr>
<tr>
<td>8. Anti-cestodal activity</td>
<td>Fruit extract</td>
</tr>
<tr>
<td>9. Purgative effect</td>
<td>Resins isolated from plant</td>
</tr>
<tr>
<td>10. Anti-tuberculosis effect</td>
<td>Ethanolic extract of leaves</td>
</tr>
<tr>
<td>11. Anti-allergic activity</td>
<td>Phloroglucinol derivatives from plant</td>
</tr>
<tr>
<td>12. Anti-proliferative effect</td>
<td>Fruit extract</td>
</tr>
<tr>
<td>13. Anti-HIV effect</td>
<td>Phloroglucinol derivatives</td>
</tr>
<tr>
<td>14. Anti-carcinogenic effect</td>
<td>Triterpenoids from stem bark</td>
</tr>
<tr>
<td>15. Anti-leukemic activity</td>
<td>Root extract</td>
</tr>
<tr>
<td>16. Wound healing activity</td>
<td>Bark extract</td>
</tr>
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</table>

i. **Effect on PKC-δ**: Rottlerin inhibits PKC-δ translocation and activity by acting as a mitochondrial uncoupler that, by lowering ATP levels, can prevent PKC-δ tyrosine phosphorylation and activation (Figure 3). In addition, rottlerin can cause PKC-δ cleavage via caspase-3 activation, thereby preventing PKC-δ membrane translocation and signaling. These indirect inhibitory effects, as well as the eventual lack of inhibition, are likely cell specific. Additionally, rottlerin acting as a direct inhibitor would block constitutively active PKC-δ fragment. Inhibition of apoptosis by rottlerin in *in vivo* and in cultured cells is related to reductions in the levels of PKC-δ mRNA expression and protein expression. Rottlerin (10µM) alone promotes apoptosis but 5 µM rottlerin blocks the etoposide induced apoptosis in C6 glioma cells. This narrow dosage range may be attributed to concentration dependence of rottlerin to uncouple mitochondrial oxidative phosphorylation. Rottlerin can also inhibit ROS synthesis by various NOX isoforms [44].

ii. **Antioxidant effect**: The reaction between rottlerin and
that complex assembly of subunits of NADPH oxidase requires especially PKC-δ, rottlerin is used to study signaling pathways in renal complications.


DPPH as measured by UV and electron paramagnetic resonance spectroscopy showed that two molecules of DPPH are reduced by one molecule of rottlerin, indicating that rottlerin can donate two hydrogens from the five phenolic hydroxyl groups. Subsequently, the antioxidant properties of rottlerin were also confirmed in cultured cells [MCF-7] [47]. According to Longpre’s study, rottlerin prevents deoxycholate-induced ROS generation in human colon epithelial cells, it was demonstrated that rottlerin neutralized hydrogen peroxide added to the MCF-7 cells and prevented free radical generation [48].

iii. Anti-inflammatory effect: In a study of endothelial cells, rottlerin decreased acrolein-induced COX-2 protein levels. Rottlerin also inhibited manganese-induced COX-2 expression in A549 human lung epithelial cells, and COX-2 expression and PGE2 biosynthesis induced by the 5-lipoxygenase inhibitor Zileuton in cardiac myogenic H9c2 cells. In all of these studies, rottlerin was used as a PKC-δ inhibitor and the observed effects were ascribed to PKC-δ blockage. Rottlerin, like curcumin, has been demonstrated to prevent NF-κB nuclear migration and transcriptional activity in several cell types. Thus, it can reasonably downregulate the NF-κB target gene COX-2 through a PKC-δ independent pathway also. Rottlerin has also been reported to modulate 15-lipoxygenase (15-LO) expression. Lipoxigenases, like COX, are inflammatory enzymes implicated in the arachidonic acid metabolism that catalyzes the insertion of oxygen into various positions in arachidonic acid, resulting in the production of leukotrienes. Rottlerin exhibits anti-inflammatory properties by inhibiting arachidonic acid metabolism through some pathways, such as interference with PKC-δ signaling, inhibition of NF-κB and STAT transcriptional activity, and/or direct binding to COX and LOX [44].

Rottlerin in renal complications

Owing to its inhibition of various signaling molecules, especially PKC-δ, rottlerin is used to study signaling pathways in many renal complications. Previous studies have demonstrated that complex assembly of subunits of NADPH oxidase requires phosphorylation by PKC-δ. Rottlerin has been used by various researchers to study the complications related to kidney diseases which are tabulated in Table 1. Using renal proximal tubular cells and mouse kidneys, it is identified that PKC-δ plays a critical role in cisplatin induced nephrotoxicity and its inhibition by rottlerin proved to be nephroprotective by attenuating kidney cell apoptosis and tissue damage. It also preserves renal function during cisplatin treatment without affecting anti-cancer efficacy of cisplatin [49].

Angiotensin has emerged as a key player in initiation and progression of fibrogenic progression in kidney under hypertension. Angiotensin II promotes fibrosis by exerting several prominent nonhemodynamic effects including proliferative, proinflammatory, and profibrotic activities. Nucleo-cytoplasmic HuR (RNA-binding protein human-antigen R) shuttling and subsequent stabilization of target mRNAs, viz. serine protease inhibitor plasminogen activator inhibitor-1 and COX-2, by angiotensin II is a long-lasting process that critically depends on PKC-δ which is blocked by rottlerin. Thus, rottlerin provides insights into mechanisms of renal inflammation and tissue fibrosis [50]. Intestinal oxalate secretion mediated by anion exchanger SLC26A6 plays a major constitutive role in limiting net absorption of ingested oxalate, thereby preventing hyperoxaluria and calcium oxalate urolithiasis. It is found that PKC-δ activation inhibits Slc26a6 activity in mouse duodenal tissue. Knockdown studies in T84 cells also demonstrated that endogenous SLC26A6 mediated most of the oxalate transport. Cholinergic stimulation with carbachol modulated intestinal ion transport through signaling pathways including PKC activation. Carbachol significantly inhibited oxalate transport by T84 cells too, an effect that was blocked by rottlerin [51]. The primary cause of vascular disease in individuals undergoing hemodialysis is the inadequate removal of uremic toxins and AGE (advanced glycation end products). Human serum albumin-AGE (HSA-AGE), prepared in vitro, elicits phosphatidylserine externalization in a small subpopulation of human platelets, which leads to vascular damage and inflammation. This response is completely blocked by both the 5-hydroxytryptamine (5-HT) 2A/2C receptor antagonist ritanserin and rottlerin [52]. The most active estrogen, 17β-estradiol (E2), stimulated a female sex-specific antisecretory response in the intestine. This effect was thought to contribute to the increase in whole body extracellular fluid (ECF) volume which occurred in high estrogen states, such as in the implantation window during estrous cycle. The increased ECF volume may be short-circuited by a renal compensation unless estrogen exerted a proabsorptive effect in the nephron. Thus, the effect of E2 on epithelial Na+ channel (ENaC) in kidney cortical collecting duct (CCD) cells is of interest to understand estrogen regulation of ECF volume. This process depended on the activation of PKC-δ which played a role in the intracellular trafficking of γ-ENaC to the apical plasma membrane following E2 treatment. It was found that rottlerin inhibited the E2-induced PKC-δ autophosphorylation and activation in CCD cells. Rottlerin inhibitory effect on secretion and absorption may be interpreted as specifically involving PKC-δ [53]. Annexin A1 (ANX-1), a calcium-dependent, phospholipid binding protein, is known to be involved in diverse cellular processes, including regulation of cell growth and differentiation, apoptosis, and inflammation.
The mitogen phorbol 12-myristate 13-acetate (PMA) induced expression and phosphorylation of ANX-1: PMA induced cleavage of ANX-1 in human embryonic kidney (HEK) 293 cells, and cleaved form of ANX-1 translocates to the nucleus. The PMA induced nuclear translocation of ANX-1 was inhibited by rottlerin, indicating that PKC-δ played a role in nuclear translocation of the cleaved ANX-1. Hence, a novel mechanism of PMA-induced translocation of ANX-1 to the nucleus that may participate in the regulation of cell proliferation and differentiation was deduced [55]. Cephaloridine (CER), a β-lactam antibiotic, is nephrotoxic and has been reported to cause acute renal failure as a side effect in humans as well as experimental animals. Pretreatment of rats with rottlerin, suppressed the early translocation of PKC-δ into mitochondria and inhibited the CER-derived development of renal dysfunction. These results suggested that the CER-derived early translocation of PKC-δ into mitochondria probably led to the enhanced production of free radicals through the mitochondrial respiratory chain during the development of the nephrotoxicity caused by CER [55]. Axl receptor tyrosine kinase (Axl) is a 140-kDa protein expressed in various cell types, including endothelial cells, vascular smooth muscle cells, and mesangial cells. Axl plays a role in the pathogenesis of vascular and diabetic diseases. Gas6, a ligand for Axl, stimulates mesangial cell proliferation and hypertrophy through binding to its cell-surface Axl receptor. Axl and Gas6 expression were increased in the glomeruli of rats with type 1 diabetes and experimental glomerulonephritis. Axl gene expression and Axl protein expression was significantly increased in MMCs treated with GO-LDL and in the glomeruli of diabetic rats. Moreover, Axl expression is also increased in MMCs cultured under diabetic conditions (high glucose or Methylglyoxal treatment). In addition, Gas6 induces TGF-β1 secretion, and this increases TGF-β1 expression induced Axl expression, suggesting that GO-LDL can increase Axl expression via Gas6-induced TGF-β1 up regulation. The PKC-δ inhibitor, rottlerin, completely blocks Gas6-induced TGF-β1 expression, suggesting the involvement of a PKC-δ mediated signaling mechanism [56]. Renal medullary thick ascending limb of type I diabetic rats shows increased superoxide production in these rats, which is due to increased activity of NADPH oxidase. Rottlerin treatment significantly reduced PKC-δ activity and hence activation of NADPH oxidase in these rats [57]. Under antidiuretic conditions, the increase in toxicity may be instrumental in increasing urea permeability through PKC-signaling pathways, in addition to the actions of vasopressin. Rottlerin along with another PKC blocker chelerythrine was found to decrease hypertonicity stimulated urea permeability in rat inner medullary collecting ducts [58]. Angiotensin II is a mediator of renal injury in diabetic nephropathy and other chronic kidney diseases. Angiotensin II rapidly increased vascular endothelial

Table 2: Effect of rottlerin in various renal complications.

<table>
<thead>
<tr>
<th>Condition studied</th>
<th>Cell line/animal model</th>
<th>Effect</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxyplatin induced nephrotoxicity</td>
<td>RPTCs and mouse kidneys</td>
<td>Nephroprotective</td>
<td>Pabla et al., 2011 [48]</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Rat kidneys</td>
<td>Blocking nucleo-cytoplasmic HuR (RNA-binding protein-human-antigen R) shunting</td>
<td>Doller et al., 2009 [49]</td>
</tr>
<tr>
<td>Hyperoxaluria</td>
<td>a) Mouse duodenal tissue and T84 cells b) LLC-PK1 cells</td>
<td>a) Blocks inhibition of anion exchanger inhibits SlC26a6 b) Prevents activation of NADPH oxidase and renal injury</td>
<td>Hassan et al., 2011 [50]</td>
</tr>
<tr>
<td>Hemodialysis complications</td>
<td>Human subjects</td>
<td>Blocks Phosphatidylserine externalization</td>
<td>Wang et al., 2008 [51]</td>
</tr>
<tr>
<td>Increase in ECF volume</td>
<td>Renal cortical collecting duct cells</td>
<td>Inhibits epithelial Na+ channel (ENaC)</td>
<td>Yusef et al., 2014 [52]</td>
</tr>
<tr>
<td>Cell proliferation and differentiation</td>
<td>Human Embryonic Kidney (HEK-293) cells</td>
<td>Inhibits PMA induced nuclear translocation of Annexin A1</td>
<td>Kim et al., 2003 [53]</td>
</tr>
<tr>
<td>Cephaloridine induced nephrotoxicity</td>
<td>Rat kidneys</td>
<td>Prevents renal dysfunction</td>
<td>Kohda et al., 2005 [54]</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>a) mouse glomerular mesangial cells b) Murine proximal tubular epithelial (MCT) cells and mouse kidney</td>
<td>a) Blocks Gas6-induced TGF-β1 expression b) Prevents binding of hnRNK to VEGF mRNA</td>
<td>Kim et al., 2012 [55]</td>
</tr>
<tr>
<td>Diabevtes</td>
<td>a) Renal medullary thick ascending limb of type I diabetic rats b) Human renal tubular cells (HRTC)</td>
<td>a) Decreased superoxide production b) Abolishes the C-peptide effect on ERK1/2 phosphorylation.</td>
<td>Yang et al., 2010 [56]</td>
</tr>
<tr>
<td>Hypertonicity stimulated urea permeability</td>
<td>Rat inner medullary collecting ducts</td>
<td>Decreased urea permeability</td>
<td>Wang et al., 2013 [57]</td>
</tr>
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growth factor (VEGF) protein synthesis in proximal tubular epithelial (MCT) cells by augmenting mRNA translation, which is partly dependent on activation and binding of heterogeneous ribonucleoprotein K (hnRNP K) to 3’ untranslated region (UTR) of VEGF mRNA. Rottlerin prevented binding of hnRNP K to VEGF mRNA and reduced the efficiency of VEGF mRNA translation [59]. Urolithiasis involves nucleation, epitaxy, growth and aggregation of crystals in the urinary tract and imbalance in the promoters and inhibitors of crystallization [60]. Oxidative stress is thought to play a crucial role in pathogenesis of stone formation process. Oxalate-induced free radical production involves activation of NADPH oxidase in renal tubular cells and is mediated by PKC signaling. PKC-dependent activation of NADPH oxidase may be one of the essential mechanisms responsible for peroxidative cell injury in hyperoxaluria. As a result of oxalate exposure, the injured renal tubular membrane plays a significant role in calcium oxalate adhesion, aggregation, and growth of kidney stones. Rottlerin treatment decreased oxalate-induced NADPH oxidase activity, superoxide and H₂O₂ apoposis, necrosis, and peroxidative injury in LLC-PK1 cells [61] (Table 2).

**DISCUSSION AND CONCLUSION**

Kidneys play a vital role in keeping the blood composition constant. As discussed in this review, oxidative stress and inflammation can give rise to a number of kidney diseases which in turn produce more reactive oxygen species. NADPH oxidase system, renin angiotensin system and PKC-δ are important mediators of ROS and inflammation in the kidneys. They play central role in pathogenesis of a number of renal complications. Hence, regulating their activities is one of the strategies to reduce oxidative stress mediated renal injury. Phytochemicals have found an outstanding role in medicine either directly or by chemical modifications. Many polyphenolic compounds are used in medicine, but full potential of rottlerin is yet to be realised. It is a lesser studied phytochemical isolated from kamala tree. Rottlerin is found to have antioxidant, anti-inflammatory, antimyeloid and anticarcinogenic properties. It is also an inhibitor of a few signaling molecules. Hence, it has tremendous potential in exploring the mechanism of elusive renal diseases. Owing to its antioxidant and anti-inflammatory properties, it can develop into a promising drug for the treatment of various renal complications involving oxidative stress and inflammation [62]. Most of the studies that used rottlerin involved cell cultures and in-vitro systems. The need of the hour is to study the potential of rottlerin in animal models and elucidate its mechanism and function in an in-vivo scenario. Rottlerin is a pharmacological inhibitor of PKC-δ and hence, it indirectly inhibits NADPH oxidase. Rottlerin has not been studied for its effects on the renin angiotensin system. As RAS and PKC-δ have been shown to activate NADPH oxidase, it should be interesting to study the effect of rottlerin on RAS and establish a link between three important players involved in the pathogenesis of renal injury i.e. NADPH oxidase, Renin angiotensin system and PKC-δ.

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**REFERENCES**

17. Thalas-Bonke V, Jandeleit-Dahm KA, Cooper ME. Nox-4 and progressive kidney disease. Curr Opin Nephrol Hypertens. 2015; 24: 89-

Zhang F, Sun D, Chen J, Guan N, Huo X, Xi H. Simvastatin attenuates angiotensin II-induced inflammation and oxidative stress in human


Steinberg SF. Structural basis of protein kinase C isoform function. Physiol Rev 2008; 88: 1341-1378.


