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Review Article

THE ROLE OF NATURAL ANTIOXIDANTS AS POTENTIAL THERAPEUTIC AGENT IN NEPHROLITHIASIS

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ABSTRACT

Renal injury and inflammation caused by ROS play a major role in stone formation. Under the hyperoxaluric condition, crystal deposition results in angiotensin II (Ang II) activation. NADPH Oxidase is stimulated by activated Ang II, leading to ROS production, which can damage renal cells. Oxidative stress also results in mitochondrial dysfunction and release of pro-apoptotic factors from depolarized mitochondria that result in apoptosis that leads to renal injury. Crystal retention in the kidney requires tubular epithelial injury accompanied by luminal expression of HA, OPN, and CD44. The expression of these molecules turns a non–crystal-binding epithelium into a crystal-binding one, thereby setting the stage for crystal retention. Recently many antioxidants have been studied that prevent hyperoxaluria mediated nephrolithiasis. Antioxidant treatment significantly reduces CaOx crystal deposition in kidneys. Naturally occurring antioxidants such as Vitamin E, Apocynin, Phycocyanin, Fucoidin, Gallotannins, Rottlerin, Lupeol, Curcumin, etc. have shown significant effect in combating renal injury which is an early event in nephrolithiasis. These findings point towards a great potential for the therapeutic application of antioxidants and free radical scavengers to reduce stone occurrence particularly under hyperoxaluric conditions. This review article attempts to compile various naturally occurring antioxidants used in treatment of nephrolithiasis.

Keywords: Calcium oxalate, Oxidative stress, Hyperoxaluria, Reactive oxygen species, antioxidants

INTRODUCTION

Nephrolithiasis is a chronic disease involving imbalance between crystallization of largely calcium salts & inhibition of crystal formation or their dissolution [1]. Despite detection of urinary stones hundreds of years ago, their pathogenesis & prevention/cure are not fully understood. It is a multifactorial disease owing to multiple genetic or environmental factors that regulates calcium salt precipitation in the urinary system. With its multifactor etiology and high rate of recurrence, urinary tract stone disease provides a medical challenge [2].

Calcium oxalate (CaOx) is the most common type of human kidney stone, of which hyperoxaluria is the major risk factor. The mechanism by which a CaOx stone is formed is complex, and many factors are believed to be involved. However, the exact mechanism of renal stone formation is poorly understood [3]. Although it involves a cascade of events including one or more of the following: urinary super saturation, crystal nucleation, growth, and aggregation; retention of crystals in the renal tubules or interstitium and growth of a calculus upon a tubular plug or interstitial deposit so-called Randall's Plaque. Oxalate (Ox) is a naturally-occurring, highly oxidized organic compound with powerful chelating activity that can cause death at high concentrations in animals and occasionally humans due to its toxic corrosive effects on cells [4]. A higher concentration of Ox in human fluids can cause a variety of pathological disorders, including hyperoxaluria, cardiomyopathy, cardiac conductance disorders, renal failure and, in particular calcium oxalate (CaOx) nephrolithiasis.

Studies with animal models as well as tissue culture model systems, have demonstrated injury to the epithelial cells of the kidney in the presence of calcium oxalate crystals [5]. It has been confirmed that injury of renal epithelial cells is mediated by the overproduction of reactive oxygen species (ROS), produced mostly from mitochondria or nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. The interaction between injured renal tubular epithelium and CaOx crystals and/or oxalate ions is likely to play a critical role in the formation of urinary calculi [6].

Oxidative stress in hyperoxaluria

Oxidative stress develops due to overproduction of ROS and/or a reduction in cellular antioxidant capacity with down regulation of the expression of antioxidant enzymes [7]. As most ROSs are short-lived and do not travel long distances so OS is generally manifested as abundance of by-products of ROS interaction with lipids, amino acids, proteins, carbohydrates and nucleic acids. Malondialdehyde, isoprostanes, and oxidized lipids are among the most common by-products of ROS induced OS [8]. Previous studies have demonstrated that both oxalate and CaOx crystals directly induce renal epithelial cell injury through lipid per oxidation and involve free radicals [9]. Recently obtained human data are also suggestive of the development of oxidative stress in hyperoxaluric kidney stone patients [10]. Tissue culture and animal model studies have provided evidence of development of OS that leads to renal epithelial cells injury in the presence of high levels of oxalate and CaOx crystals [11-13].

Mechanisms of development of OS

Studies have revealed that mitochondria are the major contributors of free radicals in oxalate toxicity, and the contribution of other non - mitochondrial sources is minimal [14]. Mitochondrial dysfunction is the primary event in oxalate toxicity [15]. *In vitro* and *in vivo* studies have demonstrated that oxalate disrupts the electron transport chain in mitochondria and induces the leak of free radicals [16]. Cells have adapted to aerobic environment through development of antioxidant defense system which limits the free radical generation within the cell's threshold. Dysfunction of these antioxidant systems during pathological conditions as in urolithiasis increases oxidative stress [17]. With decrease in respiratory complex components there is an energy deficit state and also an oxidative environment. One cause of the decrease in enzymes of respiratory chain is presence of oxalate and increase in the production of lipid signalling molecules like arachidonic acid. The latter can uncouple electron transport and increase the univalent reduction of oxygen and the production of highly reactive intermediates, by complex I and II of mitochondrial respiratory chain. It has been proposed that these reactive intermediates can damage the critical active sites of the respiratory complex enzymes which indicate that mitochondria might serve as a source as well target for reactive species [18]. Also there is evidence

for the activation of Renin–angiotensin system and NADPH Oxidase when cells are exposed to high Ox and CaOx/CaP crystals [19].Under the hyperoxaluric condition, crystal deposition results in angiotensin II (Ang II) activation [20]. As depicted in Fig. 1, NADPH Oxidase is stimulated by activated Ang II, and through phosphorylation of the former's cytosolic subunit p47phox and translocation to the membrane assembling the catalytic complex of active Oxidase leading to ROS production, which can damage renal cells [21]. The Ox and CaOx crystal exposures to renal epithelial cells in culture cause changes in the expression of various subunits which in turn effect activation of NADPH Oxidase. Significant correlation was seen between CaOx crystal-induced up regulation of p22phox and p47phox and NADPH Oxidase activation and associated cell injury [6]. These are two basic mechanisms that have been proposed for development of OS that leads to apoptosis and renal injury.

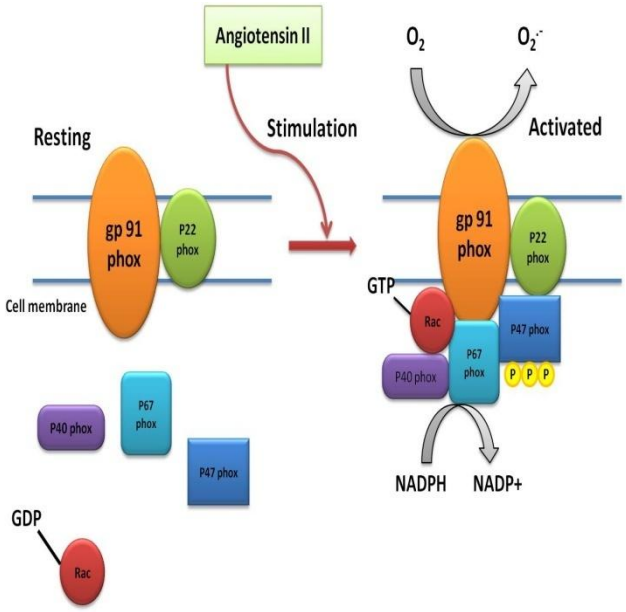

Figure 1: Angiotensin II stimulated NADPH oxidase activation

One of the major findings was that there is no crystal retention in the absence of tubular injury/ regeneration. The cell or tissue injury leads to exposure of molecules on cell surface which are not normally accessible to crystals resulting in crystal attachment [22].

The hyaluronic acid (HA) has been identified as a major crystal binding molecule at the surface of human renal tubular cells in primary culture [23]. In addition, it was found that crystal-binding

cells expressed not only HA at their apical surface but also osteopontin (OPN) and CD44 [24]. The transmembrane protein CD44 is a cell surface receptor for HA and OPN and it is up regulated during inflammation in the kidney. Although the expression of HA, OPN, and CD44 by injured/regenerating tubular epithelial cells most likely is aimed at reestablishment of the epithelial barrier integrity and restoration of renal function but a negative side effect could be that it turns a non–crystal-binding epithelium into a crystal-binding one, thereby setting the stage for crystal retention. Cell injury provokes the retention of calcium oxalate crystals, which forms the nidus and grows by a cascade of events to stone formation.

Role of antioxidants in treatment of hyperoxaluria

Management of urolithiasis mainly involves techniques like extracorporeal shock wave lithotripsy (ESWL) and percutaneous nephrolithotomy (PCNL). However, the recurrence of stone formation is quite common. Besides, these treatments cause undesirable side effects such as haemorrhage, hypertension, tubular necrosis and subsequent fibrosis of the kidney leading to cell injury and recurrence of renal stone formation [7]. Experiments have shown that supplementation of agents which could decrease oxidative stress was able to rescue the cells from oxalate-induced toxic effects.

Natural antioxidants

Several recent studies have highlighted the potential efficacy of several Oriental medicinal herbs or natural compounds for the treatment of nephrolithiasis. Now a day various phytotherapeutic agents have been proposed as useful alternative or complementary therapies for the management of urolithiasis, in part due to their anti-oxidative effects. There are various antioxidants which have been shown to reduce oxidative stress. The present review includes natural antioxidants with their chemical structure (Figure 2) and proven beneficial effects (Table 1).

Table 1: Anti-oxidant compounds and their mechanism of action

S. No.	Compound	Systematic name/ Functional group	Mechanism of action	Reference
1.	Vitamin E	(2R)-2,5,7,8-Tetramethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]-6-chromanol /Tocopherols and Tocotrienols	Major lipid per oxidation chain-breaking antioxidant	[25]
2.	Phycocyanin	Tetrapyrrole chromophore	Free radical scavenger & antioxidant activity	[26]
3.	Lupeol	(3β,13ξ)-Lup-20(29)-en-3-ol/ Pentacyclic triterpene	Antioxidant activity	[27]
4.	PGG	1,2,3,4,6-Pentakis-O-(3,4,5-trihydroxybenzoyl)-β-D-glucopyranose/1,2,3,4,6-Penta-O-galloyl-beta-D-glucose	Protect against ROS induced renal cell injury and reduce renal hyaluron expression	[28]
5.	Gallotannin	1,3,6-Tris-O-(3,4,5-trihydroxybenzoyl)-β-D-glucopyranose/ Polyphenolic hydrolysable tannin	Inhibit COM crystal growth and adhesion to renal epithelial cells	[29]
6.	Berberine	9,10-Dimethoxy-5,6-dihydro[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-7-ium/ Isoquinoline alkaloid	Antioxidant activity	[30]
7.	Apocynin	4-hydroxy-3-methoxy-acetophenone	NADPH oxidase inhibitor	[4]
8.	Rottlerin	(2E)-1-[6-(3-Acetyl-2,4,6-trihydroxy-5-methylbenzyl)-5,7-dihydroxy-2,2-dimethyl-2H-chromen-8-yl]-3-phenyl-2-propen-1-one/ Polyphenol	PKC-δ inhibitor	[31]
9.	Curcumin	(1E,6E)-1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione/ Polyphenol	PKC-δ inhibitor	[32]
10.	Thymoquinone	2-Isopropyl-5-methyl-1,4-benzoquinone	Antioxidant and antibacterial activity	[33]
11.	Fucoidans	Sulphated polysaccharides	Normalize the redox status	[34]
12.	Atorvastatin	(3R,5R)-7-[2-(4-Fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid/ Statins	Inhibit renal crystal retention	[35]
13.	Taurine	2-aminoethanesulfonic acid	Antioxidant activity	[5]

14.	Losartan	2-butyl-4-chloro-1-{[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-1*H*-imidazol-5-yl)methanol	Competitive Angiotensin II type 1 receptor antagonist	[36]
15.	N-Acetyl cysteine (NAC)	2-Acetamido-3-sulfanylpropanoic acid	Antioxidant activity	[37]

Vitamin E

It refers to a group of eight fat-soluble compounds that include both tocopherols and tocotrienols. Tocopherols have a saturated phytyl tail attached to their chromanol ring, whereas tocotrienols have an unsaturated aliphatic tail. The structure is shown in Fig. 2A. Naturally occurring alpha- tocopherol is found in lipid rich plant products and vegetable oils, while rice bran and palm oil have high concentrations of tocotrienols. It is the major lipid peroxidation chain-breaking antioxidant in lipid domains and thought to be an effective radical scavenger. It also has immunostimulatory activity [25]. The beneficial effect of vitamin E in reducing CaOx accumulation is due to attenuation of tubular cell death and enhancement of the defensive roles of OPN and Tamm–Horsfall protein (THP). It can also preserve renal function and reduce levels of free radicals, vasoconstrictive thromboxanes, and tubulointerstitial fibrosis in nephrotoxicity model in rats [38].

Phycocyanin

The main source of phycocyanin is Spirulina, a cyanobacteria . Phycocyanins function as a highly potent hydroxyl or peroxyl free radical scavenger. Due to its free radical quenching capacity against oxalate mediated tissue injury it acts as a neuroprotective agent [26]. A biliprotein pigment containing an open chain tetrapyrrole chromophore is known as phycocyanobilin (Fig. 2B) [39]. Administration of phycocyanin significantly reduces the levels of urinary risk factors such as oxalate, creatinine, calcium and protein. Phycocyanin was found to be diuretic and it also regulated the excretion of calcium. Under hyperoxaluric conditions, the activity of brush border enzymes such as ALP, ACP and γGT increased but in case of phycocyanin pretreated rats, their levels remain unaltered. Histological studies indicated no signs of tissue damage in phycocyanin pretreated rats [40]. Phycocyanin administration resulted in a significant improvement in the thiol content of renal tissue and RBC lysate via increasing glutathione and reducing malondialdehyde levels in the plasma of oxalate induced rats [41]. This effect might be helpful in reduction in OS and prevention of stone formation.

Lupeol & Lupeol linoleate

Lupeol is a pentacyclic triterpene (Fig. 2C). It has been isolated from stem bark of *Creataeva nurvala.* It has diuretic properties. Treatment with this triterpene significantly reduced the risk of calcium oxalate nephrolithiasis by increasing the urinary volume, which results in reduction in the calcium oxalate supersaturation in the urine [22]. Lupeol is also able to normalize the increased excretion of renal enzyme in urine during lithogenesis and thus shows its renoprotective effect. The esterified derivative of lupeol has been proved to be more effective than lupeol due to its more bioavailability, penetration and retention ability into the cell member and these compounds act by inhibiting some steps of oxalate synthesis from glycolic acid. The possible mechanism behind the stabilizing effect of these compounds may be due to increase in surface area/volume ratio of cells which is achieved by an expansion of membrane or shrinkage of the cell through an interaction of these compounds with membrane protein. Hyperoxaluric conditions lead to increased conversion of xanthine dehydrogenase to xanthine oxidase. This abnormal elevation in enzyme activity is normalized with both lupeol and its derivative treatment [27]. They also found to enhance the activities of SOD, CAT etc and ester of Lupeol is found to be more effective as compared to lupeol. They also protect from oxidative stress caused by ADP/Fe^{2+} and CCl_4 which might be due to reduction in overall concentration of oxalate [42].

1,2,3,4,6-Penta-O-galloyl-beta-D-glucose

1,2,3,4,6-Penta-O-galloyl-beta-D-glucose (PGG) is a water soluble gallotannin (Fig. 2D). It has been isolated from a gallnut of *Rhus chinensis* MILL or *Paeonia lactiflora* . It can affect the surface of CaOx crystal and renal cells that ultimately decrease their propensity to adhere and hence is more efficient at higher concentration. It effectively decreased the calcium and oxalate excretion and proved to be diuretic [28]. PGG significantly reduced the urinary oxalate crystal excretion and also reduced the ROS production in EG induced human primary renal epithelial cells (HRCs). It also restored the SOD expression, CAT and glutathione peroxidase activity. It has been found to decrease the OPN expression and hyaluronan expression in dose dependent manner. This suggests that it can prevent renal cell injury under hyperoxaluric conditions [43].

Gallotannin

It is polyphenolic hydrolysable tannin (Fig 2E). It is commonly found in green tea. It has antioxidant properties and it effectively blocks renal calcification. It reduces the production of ROS and MDA and also enhanced activity of the antioxidant enzyme SOD in oxalate-treated HRCs. Gallotannin inhibited COM crystal growth and adhesion to renal epithelial cells. RT-PCR analysis revealed that it also attenuated the mRNA and protein expressions of MCP-1, OPN, NADPH oxidase subunit p22phox and p47phox after oxalate treatment in HRCs [29].

Berberine

It is an isoquinoline alkaloid (Fig. 2F). It is widely distributed in nature and exists as main constituent of several plants including *Hydrastis canadensis* (goldenseal), *Coptis chinensis* (coptis or goldenthread), *Berberis aquifolium* (Oregon grape), *Berberis aristata* (tree turmeric) and *Berberis vulgaris* (barberry) [44]. Berberine is therapeutically effective for both prevention as well as treatment of calcium oxalate urolithiasis, exhibiting these effects through a combination of antioxidant, diuretic, hypocalciuric and urine alkalanizing activities. Berberine has been tested *in vitro* for the antioxidant effect and *in vivo* for diuretic and antiurolithic effects on an animal model of calcium oxalate urolithiasis [45]. It exhibited concentration-dependent (50–150 μg/ml) antioxidant effect and increased the urine output accompanied by increased pH and Na^+ and K^+ excretion and decreased Ca^{2+} excretion. It prevented as well as eliminated calcium oxalate crystal deposition in renal tubules and protected against deleterious effects of lithogenic treatment including weight loss, impaired renal function and oxidative stress, manifested as increased malondialdehyde and protein carbonyl content, depleted GSH and decreased antioxidant enzyme activities of the kidneys. These findings suggest berberine as active principle of the plants used in urolithiasis [30].

Apocynin

It is 4-hydroxy-3-methoxy-acetophenone (Fig. 2G). It is a natural nontoxic compound isolated from a medicinal plant, *Picrorhiza kurroa* [6]. It prevents activation of NADPH oxidase by blocking the association of cytosolic units with the membrane complex. Oral treatment with apocynin, reversed not only the transcriptome profile of the NADPH Oxidase-associated genes, but also multiple molecular pathways involving numerous cell components. These data raise the possibility that apocynin is a broad-spectrum antioxidant [4, 36].

Rottlerin

It is (5, 7-dihydroxy-2,2-dimethyl-6-(2,4,6-trihydroxy-3-methyl-5-acetylbenzyl)-8-cinnamoyl-1,2-chromine), also called mallotoxin (Fig. 2H). 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 [31]. It displays specificity as an inhibitor of PKC-δ activity *in vitro*, and on this basis it has been a component of patent applications to consider it for therapeutic use [46]. PKC-δ is reportedly a key signaling molecule in the ROS-induced apoptotic pathway through generation of active catalytic fragments by proteolytic cleavage and also inhibits translocation of PKC-δ.

Curcumin

It is a polyphenol obtained from the spice turmeric (*Curcuma longa*, Zingiberaceae) and is responsible for the yellow colour of turmeric (Fig. 2I). Curcumin is a dietary antioxidant and has been known since ancient times to possess therapeutic properties. It has been reported to scavenge oxygen free radicals, to inhibit lipid peroxidation, and has anticarcinogenic activities in experimental models [32]. The possible nephroprotective effect of curcumin is inhibition of lipid peroxidation. Recent studies reported that curcumin decreases PKC protein levels by forming tight complexes with the enzyme and generating tyrosine phosphorylated PKC fragments. It has been found that by forming hydrogen bonds with the activator binding domain of the enzyme, curcumin and its analogues could change PKC-δ conformation and influence activation and membrane translocation properties [31].

Thymoquinone

Thymoquinone is a phytochemical compound found in the plant *Nigella sativa* (Fig 2J). It is active quinone, has antioxidant effect, scavenges free radicals and superoxide anions, and inhibits cyclooxygenase and 5-lipoxygenase pathways; therefore, it inhibits inflammatory products [47]. Thymoquinone has an antibacterial effect, and, therefore, calculi with a bacterial origin such as struvite calculi may be prevented by thymoquinone [48]. Urine oxalate concentration was also decreased by thymoquinone which is in agreement with its preventive effects on the CaOx kidney calculi [33].

Fucoidans

These are sulfated polysaccharides from brown algae (Fig. 2K). They are rich in the sugar, fucose. These naturally occurring glycosaminoglycans from edible seaweeds were able to modulate the altered redox status in hyperoxaluric rats [49]. They are reported to have blood anticoagulant, anti-tumour, anti-mutagenic, anti-complementary, immunomodulating, hypoglycaemic, antiviral, hypolipidemic and anti-inflammatory activities [50]. It possesses antioxidant potential which is unique to polysaccharides of marine origin. Fucoidans administration is able to normalize the redox status of the renal cells under hyperoxaluria, and also prevent the mitochondrial damage as was evident from biochemical investigations and electron microscopic analysis [34].

Atorvastatin

Atorvastatin is a 3-hydroxy-3-methylglutaryl coenzyme A reduction inhibitor (Fig. 2L). It is a high cholesterol lowering drug with anti-inflammatory and antioxidant activities [51]. It has an inhibitory effect on renal tubular cell injury and on oxidative stress by ROS. Atorvastatin treatment also decreases the apoptosis of renal tubular cells and the formation of renal crystal deposits in kidney tissue. It stimulates the production of SOD which converts super oxide ions into H_2O_2, and catalase which converts H_2O_2 into H_2O and O_2. The possible mechanism of action behind its antioxidant activity is that it decreases the NADPH Oxidase (NOX) activity by reducing the expression of NADPH oxidase subunits in a rat stone forming kidney model. Therefore, one of the mechanisms by which it inhibits the renal tubular cell injury and oxidative stress caused by ROS, is the inhibition of NOX-1 [52]. It has been found that atorvastatin inhibited the expression of TGF- β. TGF-β increases the activity of NADPH oxidase and leads to the production of ROS ultimately resulting in oxidative stress. The suppression of TGF- β in kidney tissue was thought to be additional mechanism to inhibit renal crystal retention by atorvastatin[35].

Taurine

It is 2-aminoethanesulfonic acid, one of the few known naturally occurring sulfonic acids (Fig. 2M). Taurine is a derivative of cysteine, an amino acid which contains a thiol group so it is an amino sulphonic acid [53]. It occurs naturally in food, especially in seafood and meat. It is a major constituent of bile and can be found in the large intestine and in the tissues of many animals, including humans [54, 55]. Taurine is known to have antioxidant activity and shows renoprotective effect. It has been found to localize in mitochondria where it serves as mitochondrial matrix buffer so it has been proposed that by stabilizing the environment in the mitochondria, it would prevent leakage of the reactive compounds formed in the reactive mitochondrial environment and thus indirectly acts as an antioxidant. Taurine treatment repairs the oxidative injury of the kidney, improved SOD and GSH-Px activities, as well as the mitochondrial membrane injury, with lesser crystal depositions in the kidney [36]. In this way, it protects the kidney from oxidative injury through mitochondrial-linked pathway.

Losartan

Losartan is a selective, competitive angiotensin II type 1 (AT1) receptor antagonist (Fig. 2N). Treatment with Losartan resulted in reduction of urinary 8-IP and a decrease in renal p47phox expression. It has been shown that administration of AT 1 receptor blockers or ACE inhibitors significantly reduced hyperoxaluria-induced production of renal lipid peroxides [4]. OS is a key component of both oxalate and angiotensin-induced renal injury. Also, losartan treatmet may act by lowering the Bax and caspase-3 expression and decrease the apoptotic cell numbers in hyperoxaluric rat model [36].

Our group is also actively involved in identification of novel calcium oxalate inhibitor molecules from various natural sources like, a novel dimeric protein DAP (98 kDa) from *Dolichos biflorus* seeds has been purified. Based on its ability to inhibit calcium oxalate crystallization *in vitro* it is postulated to have antilithiatic activity. We proposed that DAP, which is CNX, Calnexin like protein has a calcium binding site, which might also be responsible for its ability to inhibit CaOx crystallization [56]. Recently, our group has also purified an anticalcifying protein from the seeds of *Trachyspermum ammi* (TAP).The antilithiatic potential of TAP was confirmed by its ability to maintain renal functioning, reduce renal injury and decrease crystal excretion in urine and retention in renal tissues [57]. Also N-acetylcysteine (NAC) has been extensively studied, with some reports indicating its outstanding efficacy in the renal protection. NAC has been shown to reverse hyperoxaluric manifestations in rat liver. The thiol group of NAC reduces the level of free radicals responsible for the lipid peroxidation and thus decreases the level of malondialdehyde, the end product of peroxidation. This shows that NAC is a potential free radical quencher in renal tissue and it reduces oxalate induced free radical damage which is evident from histological analysis [37]. In one more study *Achyranthes aspera* extract has been checked for its ability to maintain renal functioning and reduced renal injury [58]. This treatment reduced changes in the architecture of renal tissue and also decreased the size of crystals thereby helping in quick expulsion of the crystals [59]. The antilithiatic potency of the protein biomolecules of *Tribulus terrestris* has been checked by various biochemical methods and was tested on the oxalate induced injury on renal epithelial cell lines (NRK 52E) [60]. The protective potency of TTP on NRK 52E was quite comparable to the aqueous extract of cystone. These findings suggest that this purified protein biomolecule from *Tribulus terrestris* could open new vista in medical management of urolithiasis [61]. Coconut water has also potential to inhibit the genes of oxidative stress to push the activity of these enzymes towards normal. The rebalancing of elevated antioxidant enzyme gene expression by coconut water treatment, reduced mineral deposits in kidney tissue further substantiated the prophylactic nature of coconut water in nephrolithiasis.

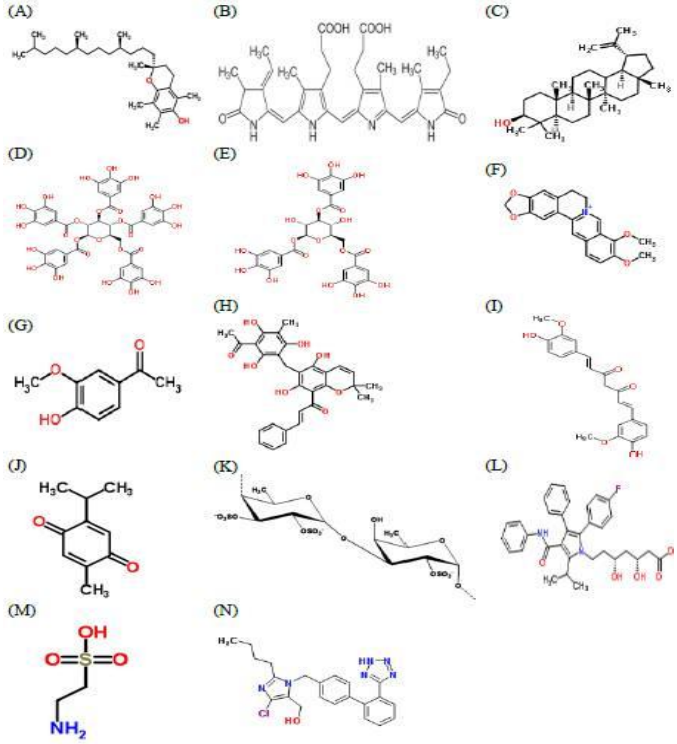

Figure 2: Chemical structure of natural antioxidants (http://www.chemspider.com) (A) Vitamin E; (B) Phycocyanin; (C) Lupeol; (D) 1,2,3,4,6-Penta-O-galloyl-beta-D-glucose (PGG); (E) Gallotannin; (F) Berberine; (G) Apocynin; (H) Rottlerin; (I) Curcumin; (J) Thymoquinone; (K) Fucoidans; (L) Atorvastatin; (M) Taurine; (N) Losartan

CONCLUSION

There is plenty of experimental and clinical indication for the production of ROS, development of oxidative stress and associated cellular injury when renal cells are exposed to Ox and/or CaOx crystals. Antioxidant treatments reduce CaOx crystal deposition in the kidneys of experimental animals. Therefore, it will be useful to evaluate the therapeutic application of antioxidants on reducing stone recurrence. The development of oxidative stress involves many sources and a variety of signaling pathways so it is essential to identify all enzymes and pathways active in Ox and CaOx crystal induced generation of ROS. A combination of anti-oxidants and free radical scavengers may provide superior renal protection. There are a number of antioxidants known having antiurolithiatic activity. Recent studies demonstrated that treatment with antioxidants and free radical scavenger reduced CaOx crystal induced renal injury. The studies have suggested that most of them restore the SOD expression, CAT activity and glutathione peroxidase in renal cells and reduce the risk of stone formation. Vitamin E supplement in diet can reduce urinary risk factors, prevent the tissue of lipid peroxidation of the tissue, inhibit oxalate synthesis and enhance enzymatic and non-enzymatic antioxidant status in liver and kidney under lithogenic environment. Other antioxidants like lupeol and its esterified derivative are also able to normalize the increased excretion of renal enzyme in urine during lithogenesis and thus show their renoprotective effect. Similarly PGG and Gallotannins can affect the surface of CaOx crystal and renal cells that ultimately decrease their propensity to adhere. Berberine has been found to be effective through a combination of antioxidant, diuretic, hypocalciuric and urine alkalanizing activities. Fucoidan prevents mitochondrial damage and normalizes the redox status of the renal cells under hyperoxaluria. These antioxidants may have other specific pathways also, like rottlerin and curcumin decrease the PKC-δ activity while apocynin is an inhibitor of NADPH oxidase. Losartan is ACE inhibitor which indirectly inhibits NADPH oxidase activity. Therefore, treatment with natural antioxidants and free radical scavengers, seems to be possible therapeutic strategy for ameliorating hyperoxaluria induced oxidative stress and renal cell injury in urolithiasis. Further research on these molecules is required to explore their potential and confirm their candidature as an antiurolithiatic drug.

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