**DRUGS FOR DIABETIC NEPHROPATHY- FULL REVIEW**

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**ABSTRACT**

The present article highlights the molecules involved in development of diabetic nephropathy and the possible molecular target based on pathophysiological process which can mitigate the development of diabetic complication especially Diabetic nephropathy. In this review article summarizes the available evidence regarding novel drug/agents, Paths and targets under preclinical and clinical trial.

**KEYWORDS:** ACE, ARB, TGFβ, TNFα, VEGF, CTGF, AGEs, RAGE, Oxidative stress.

**INTRODUCTION**

Hyperglycemia is the hallmark and plays key-role in development of diabetic complication.

Diabetic nephropathy is one of the commonest complication in diabetic patient leading to chronic kidney disease and ultimately failure and death.

Drug used to prevent and treat diabetic nephropathy are angiotensin converting enzyme inhibitors ACE INHIBITORS and angiotensin receptor blocker (ARB).

It is a need of world since Diabetes is global disorder and the number is increasing day by day and it is very important to identify the early kidney damage by understanding the molecular pathophysiology of disease which also help us to identify the possible molecular target.
THERAPEUTIC MODALITY FOR TREATMENT OF DIABETIC NEPHROPATHY

Classification

Non pharmacological
1) Weight loss
2) Diet-protein restriction
3) Smoking cessation

PHARMACOLOGICAL
1) Glycaemic control
2) Blood pressure control

classification

| Investigational | 1)Antioxidant- NAC, Nox Inhibitors, Tempol, probucol |
|                 | ANTIOXIDANT-vitamins –C AND E, alpha lipoic acid |
|                 | Taurine, luteolin, D-saccharic 1,4 lactone, hemin |
|                 | Bardoxolone methyl (dh 404) |
|                 | 2) Nox1/4 inhibitor (GKT136901) |
|                 | 3) Nox4- pitavastatin (statin) |
|                 | 4) NADPH inhibitors apocynin |
|                 | 5) Nonspecific inhibitor of NADPH oxidase |
|                 | diphenyleneiodonium chloride. |
|                 | 6) NADPH SUBUNIT INHIBITORS |
|                 | Antisense oligonucleotides directed against p22(phox), a |
|                 | NADPH oxidase subunit. |
|                 | Selective NADPH subunit Nox 1 inhibitor- ML 171, NoxA1ds |
|                 | 7) kinases |
|                 | PKC INHIBITORS- ruboxistaurin, |
|                 | TYROSINE KINASE INHIBITOR- Imatinib, |
|                 | Rho kinase inhibitor –fasudil |
|                 | P3K INHIBITORS- WORTAMININ, IC87114 AND AS101 |
|                 | P38-MAPK-INHIBITORS- FR167653 |
|                 | 8) EGFR inhibitor AG1478 |
|                 | cannabinoid receptor type 1 (CB1) |
|                 | 9) PLCgamma inhibitor U73122 |
|                 | 10) VEGF INHIBITORS- Tempol. |
|                 | 11) Antifibrotic drug – ANTI-TGF –β –Ab, Pirfenidone, |
|                 | fesolimumab. |
|                 | 12) Xanthine oxidase inhibitors- allopurinol, febuxostat. (RCT) |
|                 | 13) Chemokine modulation -Anti-CCR2/5 |
|                 | 14) Matrix metalloproteinase inhibitor- TETRACYCLINE |
|                 | XL081, XL874 |
|                 | 15) Phosphoinositide-3-OH kinase inhibition. |
|                 | 14) MAP kinase/ERK kinase (MEK) inhibitors, -U0126 and |
|                 | PD98059. |
|                 | 15) Src kinase inhibitors, herbimycin A and PP2 |
16) Inhibition of osteopontin  
17) Anti TNFα- INFliximab  
18) mi RNA MODULATION- LNA –Anti-mi R-192  
19) Neuro-hormonal modification- D3-RA  
20) Anti CTGF- FG3019  
21) Endothelin receptor antagonism- Avosentan, Atrasentan(RCT)  
22) Glycosaminoglycans-Sulodexine  
23) Urotensin –II INHIBITION-Palosuran  
24) Chemokine inhibition-CCX140-B (RCT)  
25) Endogenous agents – Apelin ,activated protein C  
26) Aldose reductase inhibitors-  
27) Smad 2 phosphorylation – inhibition-IN1130, GW788388  
28) Multifactorial targeting – fluorofenidone,curcumin,pirfenidone  
29) Oral adsorbents – Kremezin (marketed in Japan)  
30) PDE INHIBITORS-Pentoxiphylline  
31) Direct renin inhibitors- aliskiren  
32) PARP INHIBITORS- PJ-34 AND IN 1001  
33) Hippo pathway inhibitors  
34) SOCS mimetics  
35) targeting ICAM-1  
36) Targeting MCP-1

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CLASSIFICATION BASED ON MECHANISM OF ACTION/ TARGET MOLECULE

1) GLP -1 AND DDP-4 INHIBITORS
2) PKC INHIBITOR
3) TK INHIBITOR
4) AGE PRODUCT INHIBITORS
5) TGF β INHIBITORS
6) PDGFβ inhibitors
7) MEK -1 INHIBITORS
8) RAS farnesylilation Inhibitors
9) Aldose Reductase inhibitors
10) Oligodonucleotide against p 22
11) EGFR Inhibitors
12) PLC gamma 1 inhibition
13) Inhibition of osteopontin
14) P38 INHIBITORS
15) NADPH OXIDASE INHIBITOR
16) Hippo pathway Inhibitors
17) Xanthine oxidase inhibitor
18) Src inhibitors
19) Matrix metalloproteinase inhibitors
20) Endothelium receptor antagonist
21) Smad 2 phosphorylation inhibitors
22) Chemokine inhibition
23) CTGF INHIBITORS
24) Urotensin inhibitors
25) PDE inhibitors
26) TNF α inhibitors
27) Rho kinase inhibitor
28) P38 MAPK INHIBITORS
29) Glycosaminoglycans
30) Renin inhibitors
31) AGE TARGETING
32) SGLT 2 INHIBITORS
33) VITAMIN D activators
34) JAK –STAT INHIBITORS
35) Endogenous Agents – Apelin,, Activated Protein C
36) MULTIFACTORIAL TARGET
37) Mineralocorticoid receptor antagonist
38) Antiplatelet drug- DilazepHCL
39) SOCS mimetics.
40) PARP inhibitors
41) Antioxidants

OXIDATIVE STRESS AND ANTIOXIDANT DRUGS FOR DIABETIC NEPHROPATHY

Hyperglycaemia generates oxidative stress which is responsible for renal damage. Several antioxidant and antioxidant vitamins are used to protect renal dysfunction from deleterious effect of hyperglycaemia.

Excessive production of ROS Free radical producing direct damage to cellular macromolecules such as DNA, PROTEIN, LIPIDS.

INDIRECT DAMAGES- through multiple pathway such as Activation of protein kinases, Tyrosine kinase, Protein kinase c, Rho kinase MAPK, Polyol pathway, Hexosamine pathway, AGEs formation, Expression of growth factors, NADPH ACTIVATION, Mitochondrial dysfunction. Along with this low availability of intracellular antioxidant further causing more damages.

The major source of ROS production in diabetic kidney is NADPH OXIDASES ACTIVATION and mitochondrial dysfunction.

1) Luteolin
   a) Various biological actions of luteolin are mediated by inhibiting oxidative stress and inflammation.\cite{1,2}
   b) luteolin has been reported to mediate its effects by modulating several important molecular targets, including transcription factors (NF-κB and activating protein-1).\cite{3}
   c) Inhibits inducible nitric oxide synthase.\cite{4}
   d) Tumour necrotic factor alpha interleukin- 1, interleukin-6, and chemokines\cite{2}
   e) Increases SOD level in kidney\cite{5}
f) Decreases cholesterol, triglycerides, phospholipid level[^5]

g) Increasing the HDL.[^5]

h) Increases the expression of heme oxygenase -1 which have antioxidant and cytoprotective role.[^5]

2) Taurine

a) Oxidants arising from puromycin- or adriamycin-induced renal injury in rats are diminished following administration of 1% taurine in the drinking water[^6]

b) Taurine reduces ischemic and reperfusion injury[^7]

c) Myeloperoxidase (MPO) located in neutrophils causes generation of radicals, including hypochlorous acid which acid activates tyrosine phosphorylation signal pathways, leading to calcium signaling and tumor necrosis factor α (TNF α) production and another component of glomerulonephritis is an increase in glomerular albumin permeability (GAP). In a model using isolated rat glomeruli, which are infiltrated by neutrophils, H₂O₂ alone does not increase GAP, but H₂O₂ MPO together do increase GAP. This increase can be inhibited by superoxide dismutase, catalase or taurine.[^7]

d) vitamin E and taurine is associated with a reduction in advanced glycosylation end products and the extent of lipid peroxidation. Taurine can also neutralize the aldehydes of glycation end products. The formation of Schiff’s base between taurine and the aldehydes may diminish glucose toxicity.[^7]

e) Trachtman et al. showed that culturing renal mesangial cells in the presence of high glucose concentration resulted in build-up of advanced glycosylation products that could limit cell growth. Addition of the antioxidants taurine and vitamin E reversed the growth inhibition[^6]

f) The elevated extracellular concentration of glucose disturbs cellular osmoregulation and sorbitol is synthesized intracellularly via the polyol pathway Intracellular accumulation of sorbitol crowds out other intracellular osmolytes, including taurine and myo-inositol. This disturbance of cell volume regulation might be altered by taurine supplementation, but this has not been tested[^8]

g) Taurine reduces the expression of ICAM-1[^8]

3) HEMIN/SILYMARIN/SILYBIN[^9]

CLINICAL TRIAL PHASE II completed result not posted

1) Direct free radical scavenging
2) Prevent free radical formation
3) Maintain integrity of electron transport chain of mitochondria in stress
4) Maintains optimal redox status
5) Activates antioxidant enzymes and non enzymatic antioxidants via transcription factors including Nrf2 and NFκB
6) Activates vitagenes responsible for synthesis of protection molecules.
7) Hsp, thioredoxin are also increased.

4) **Alpha lipoic acid**

Clinical trial phase II STUDY completed -result not posted.
1) ALA supplementation significantly reduced hsCRP levels, which is a risk factor for cardiovascular disease in HD patients.[10]
2) ALA administration led to regression of histopathology and morphologic lesions in extracted rat kidney. Also, positive effect of ALA on albuminuria was detected. However, no positive effect of ALA was found for antioxidant parameters at the tissue level[11]
3) Experimental study has demonstrated that intraperitoneal administration of α-lipoic acid to streptozotocin (STZ) diabetic Wistar rats normalises TBARS levels in plasma, and the retina, liver and pancreas[12]

5) **PROBUCOL**[13]

CLINICAL TRIAL PHASE II STUDY completed –result not posted
1) It has potent antioxidant property
2) Reduces glomerular $\text{O}_2^-$ to normal level
3) Decreases proteinuria
4) Decreases serum cholesterol
5) Nox 2 is suppressed and down regulated
6) Radical scavenger action
7) It is combined often with ARB and has advantage.[13]

6) **Bardoxolone methyl**[15]

Clinical trial phase II FOR Pulmonary Artery Hypertension Completed.
Phase III CLINICAL TRIAL FOR- Chronic kidney disease
1) Activator OF Nrf 2
2) NF-κB
3) Increases the level of antioxidant
4) Restores the balance of oxidant and antioxidant
5) Inhibits pro-inflammatory factors.
6) Improves renal function in type 2DM

7) **VITAMIN C AND VITAMIN E**[^16][^17]

**CLINICAL TRIAL PHASE II**

VITAMIN C AND VITAMIN E are well known antioxidant
Major target is ROS
Offer renal as well as cardiovascular protection
Delays the development of diabetic nephropathy

8) **Nox inhibitor**

Nox 4 plays important role in pathogenesis and development of diabetic nephropathy[^14] and targeting Nox 4 may have some important benefits.

Orally administrable small molecule inhibitors are pyrazole pyridine chemical GKT 136901 AND GKT 137831.

1) Nox inhibitor reduce albuminuria and prevent loss of podocyte and foot process
2) Prevent apoptotic death of podocyte in presence of high glucose.
3) It may restore the balance of oxidant and antioxidant in cell and thus reduce the oxidative stress
4) It also activate AMPK which is suppressor of oxidative stress.
5) AMPK activators, e.g. AICAR (5-aminimidazole-4-carboxamide-1-riboside) or adiponectin, significantly reduced Nox4 expression, oxidative stress and podocyte injury in vitro or in vivo. Other AMPK activator is metformin used for treatment of type 2 DM.
6) Nox 4 is upregulated by high glucose concentration and Targeting Nox 4 in tubular cells by oligonucleosides reduces ROS production in tubular cells
7) It decreases the extracellular matrix in cortex
8) Nox 4 is upregulated in fibroblast of kidney, lung, heart in response to oxidative stress and is responsible for interstitial cell injury inhibition of Nox4 reduces injury as well as fibrosis.
9) Statin Pitavastatin Inhibit Nox 4
10) Dual inhibitor Nox ¼ are also under investigation.
All the study carried are mostly in animal model and cell lines considering the role of Nox 4 in pathogenesis more focus is needed to develop and target Nox 4.

9) NADPH OXIDASE INHIBITORS

NADPH OXIDASE – plays important role in oxidative stress which is induced by hyperglycaemia in diabetic person contributing to development of diabetic nephropathy. NADPH OXIDASEs are present in each and every cell, Nox is the subunit of the NADPH Oxidase and has played important role in development of cardiovascular, renal, neuronal, cancer, organ failure and oxidative damage.

Inhibition can be done directly or indirectly or by directing oligonucleoside to specific NADPH subunit or specific drug to inhibit Nox 1/Nox 4.

Many natural compounds are found to be Inhibitors of NADPH Oxidases and synthetic compounds are also developed.

1) Specific – specific inhibitor Nox 4 isoform
2) Non specific – Apocynin, Diphenyleneiodnium chloride
3) Inhibiting NADPH oxidase subunit – p22(phox)
4) SELECTIVE NADPH subunit Nox 1 inhibitor - ML 171, NoxA1ds

5) OTHER NATURAL COMPOUNDS

EMODIN –active compound from rhubarb – Inhibits ROS generation.

FLAVONOIDS-Kaempferol, morin, quercetin etc inhibits neutrophil outburst
Ginkgo biloba – inhibit ROS generation.
Magnolia officinalis- magnolol and honokiol both inhibit superoxide generation by inhibiting NADPH OXIDASE.
Resveratol –can inhibit high glucose induce NADPH oxidase pathway and ROS generation.
Plumbagin inhibit NADPH DEPENDENT superoxide generation in cell lines that express Nox 4.
Celastrol derived from Tripterygium wilfordii –Is a potent inhibitor of several Nox enzymes and is used as anti-inflammatory, anticancer and in arthritis.

Apocynin
1) Orally active, blocks NADPH Oxidase assembly
2) Requires peroxidase for reaction
3) Reduces ROS
4) Prevents Endothelial Dysfunction
5) Increases Glutathione Synthesis
6) Direct ROS Scavenger
7) Activate AP-1 Transcription factor
8) Limits The Generation Of NO

Disadvantage
a) Does not work immediately
b) Not specific
c) It also interfere with arachidonic acid metabolism

ANTI SENSE OLIGONUCLEOTIDE DIRECTED p22 (phox)\textsuperscript{[19]}
Vascular Endothelial growth factor (VEGF) is implicated in development of proteinuria in diabetic nephropathy. Overexpression of p22 is associated with enhanced VEGF in Diabetes. High glucose level increased VEGF Expression is significantly increased in mesangial cells. Pathogenesis of increased level of VEGF is unclear.

High glucose induced VEGF can be blocked by antisense oligonucleotide directed towards p22(phox).

VEGF –can be also inhibited by TEMPOL which is used as investigational tool.

10) PROTEIN KINASE C INHIBITORS\textsuperscript{[20]}
PKC activation is well known in pathogenesis of diabetic nephropathy. The isoform of PKC are alpha, beta, zeta which can be targeted.

PKC beta is well know activated in renal tissue, studies till date done and report suggest that it can offer renal protection against diabetes induced renal hypertrophy, glomerular infiltration, reactive oxygen species and pro-fibrotic factors.

Targeting PKC alpha can reduce albuminuria and also decrease up regulated VEGF.

PKC INHIBITORS- ruboxistaurin
Study report with ruboxstaurin mention about protection against in renal as well as retinal damage. Thus PKC targeting offer protection against micro vascular complication of diabetes.[44,45,46]

**11] TYROSINE KINASE INHIBITORS**[21,22,23]

EGFR – is found in glomerulus and tubules its expression is diabetic nephropathy, activation in response to hyperglycaemia. In Endoplasmic reticulum stress is increased in diabetes.

**EGFR PATHWAY IMPORTANCE**

EGFR pathway serves as docking sites for many intracellular signal molecules and in turn activates many other

JAK –STAT pathway

Phosphoinositide 3 kinase {PI3K}

Src kinase

Mitogen activated protein

Also activates and upregulates mTOR and decreases AMPK

INHIBITING EGFR –prevents downstream activation of other signalling molecules which play important role in pathogenesis.

Also the knowledge of this pathway helps to understand the role of other signalling molecules and new novel molecular target can be such as JAK –STAT pathway inhibitors, phosphoinositide 3 kinase {PI3K} inhibitors, src kinase inhibitors, Mitogen activated protein inhibitors, mTOR inhibitor can be used for treatment of diabetic nephropathy.

Also AMPK is reduced in EGFR activation AMPK ACTIVATOR are also novel molecular target for treatment of diabetic nephropathy.

**Erlotinib**

1) It is EGFR tyrosine receptor kinase inhibitor it decreases structural changes in diabetic nephropathy

2) Decreases mTOR activation

3) Increases AMPK activity and expression

4) Decreases endoplasmic reticulum stress in diabetes

Other EGFR inhibitor AG 1478, PKI 166, Gefitinib is also under investigation.
12] Src kinase inhibitor\[24\]
Src kinase – can be targeted by using herbimycin A and PP2
It can inhibit neovascularization and angiogenesis
Micro vascular complication are reduced with Src inhibitors

13] PP2\[25\]- IS NOVEL Src inhibitor

14] PI3K TARGETING –can offer protection against autophagy. The exact role of autophagy is unclear.

15] THE HIPPO SIGNALING PATHWAY INHIBITORS\[26-43\]
1) Control cell proliferation, differentiation and apoptosis. Tumorigenesis via phosphorylation and activarion of YAP (Yes associated protein)/TAZ (transcriptional co-activator with PDZ-binding motif)\[26,27\]
2) YAP - a adaptor protein modulate multiple transduction pathway.\[28\]
3]. Activation of TGF-β signaling is well-known to be implicated in DN development and progression, and Smad3 is a crucial mediator of TGF-β signaling in fibroblasts.\[29,30\]
4]. Interestingly, recent studies revealed association of YAP with Smad2/3 to activate CTGF gene expression and CTGF has been strongly associated with the development and progression of diabetic kidney injury via interaction with multiple ECM proteins.\[31-35\]
5] In addition, some studies have implicated YAP and TAZ in mechanical signaling and tissue remodeling independent of the canonical mammalian Hippo pathway under various stress conditions.\[37-40\]
6] Moreover, a recent study suggested that activation of YAP/TAZ in fibroblasts and subsequent activation of a renal profibrotic factor PAI-1 gene, SERPINE1, expression is involved in lung fibrogenesis.\[41\]
7] Interestingly, YAP was also found to be inactivated in response to energy stress via direct activation of AMPK or through AMPK dependent LATS activation, whereas release of energy stress by administration of glucose activated YAP, which increased the downstream target gene expression.\[42,43\]

IMPORTANCE OF TARGETTING YAP /HIPPO-PATHWAY
1) CTGF expression is decreased (CTGF is known for diabetic kidney injury)
2) Decrease fibrogenesis
3) Tissue remodelling is decreased
4) Since the activation of YAP occurs in stress inhibition of YAP may decrease stress associated tissue remodelling.
5) Hippo inhibitor can be novel target for treatment of lung fibrosis.
6) Hippo pathway inhibitor also novel molecular target for tumour and cancer.

16] JAK-STAT PATHWAY AND INHIBITOR \(^{[47-58]}\)
1) JAK–STAT PATHWAY- is activated in glomerular and tubular cells in humans with diabetic kidney and animal model
2) IL -6 AND Angiotensin II can activate the pathway. Jak 2 protein is widely distributed in renal and vascular tissue.
3) TGF β and fibronectin expression are increased through JAK-STAT SIGNALLING
4) JAK -2 is associated with diabetic complication
5) Elevated glucose level activate JAK2/STAT signalling.
6) elevated glucose level also increases gene expression for JAK-STAT.

JAK 2 INHIBITOR (AG 490,Baricitinib)
1) Experiments with JAK 2 Inhibitors in diabetic animal model have shown reduction in albuminuria and blood pressure.
2) JAK2 -novel target in treatment of diabetic nephropathy since it is selectively present in renal tissue and up regulated in response to hyperglycaemia.
3) It blocks the mitogenic and fibrotic action.
4) Both early and late changes can be treated with JAK 2 INHIBITORS
5) Antisense Oligonucleotide can be used to target JAK/STAT PATHWAY
6) Other drugs like captopril, statin and Rosiglitazones partly modulate JAK/STAT Pathway.
7) "Baricitinib, An Oral Janus Kinase (JAK)1/JAK2 Inhibitor, In Patients With Active Rheumatoid Arthritis (RA) And also used in diabetic nephropathy\(^{[66 .b]}\)
8) Dose dependent reduction in albuminuria with baricitinib\(^{[66 .c]}\)

17] SOCS MIMETIC\(^{[59-61]}\)
JAK STAT PATHWAY is controlled by different mechanism
1) Receptor internalization
2) Protein tyrosine phosphatases
3) Protein inhibitors of activated STAT
4) Suppressors of cytokine signalling. (SOCS)
SOCS – Is reported to have suppressor effect on JAK-STAT and has emerged as potential target. SOCS – protein expression are increased in patient of diabetic nephropathy and in animal model.

In vivo gene therapy with SOCS expressing adenovirus reduced JAK/STAT activation and thus ameliorated the early changes in diabetic rats.

Potential – for Research
SOCS mimetic or SOCS inducer are future therapeutics to prevent and retard the progression of diabetic nephropathy.

18] Rho Kinase Inhibitor\(^{62}\)
1) Rho A-ROCK is activated in renal cells in diabetic milieu.
2) Pathway contribute to progrowth, pro fibrotic /prosclerotic signalling
3) Enhanced ECM Production.
4) Plays important role in pathogenesis renal haemodynamic.

Rho A-ROCK can be targeted by
1) Fasudil
2) ROCK i Y 27632
3) siRNA

Rho A-ROCK is activated by high glucose level and Angiotensin II

REPORTS – other drugs
SIMVASTATIN- can inhibit Rho A-ROCK in mesangial cells' similar action with fluvastatin.
Study report with fasudil and statin suggest benefits.

Important benefits of Rho A-ROCK inhibition
1) Decreases fibrosis and sclerosis
2) Reduces extracellular matrix
3) Reduces VEGF
4) Increases microcirculation
5) Modulate renal haemodynamic
6) Decreases collagen
7) Blocks the effect of Angiotensin II Mediated signalling
8) Decreases expression of Nox 4
9) Decreases/reduces albuminuria
10) Stabilization GFR
11) Renal vasodilation
12) Decreases vascular resistance

19) Transcription factor NF-κB as molecular target
1) NF-κB is activated by oxidative stress, growth factors, cytokines, chemokine’s and adhesion molecules[63]
2) Activated NF-κB is found in diabetic human kidney and rodent[64,65]
3) T2 DM PATIENT proximal tubular cells also shows Activated NF-κB[66]
4) Activated NF-κB is pro-inflammatory[67]

NF-κB inhibitors[68]
Inhibitors can be divided into basic categories according to the step at which NF-κB is blocked: 1) upstream of IκB kinase complex; 2) IκB phosphorylation/degradation; 3) nuclear translocation; 4) DNA binding; and 5) gene transactivation.

NF-κB is inhibited by
1) Thiazolidinedione drugs in T2DM[69]
2) 1,25 dihydroxy vitamin D3 suppress hyperglycaemia induced NF-κB activation.[71]
3) Thiazolidinedione drugs Ameliorated renal injury in diabetic rats.[73]
4) Statin and fenofibrate down regulates NF-κB[72]
5) Plant alkaloid berberine inhibits NF-κB and ICAM-1 in diabetic rats and ameliorate renal dysfunction[65]

From the above study and pathological role it is clear that up regulation of NF-κB in diabetic kidney and plays important role in development of diabetes nephropathy. Thus targeting NF-κB may retard development of nephropathy.

20] PARP INHIBITORS[74,75,76,77]
PARP enzyme activity is increased and has shown to participate in pathogenesis of diabetic complication.

ACTIVATION – by High glucose level, oxidative stress
Inhibitors of PARP – PJ-34 AND INO -1001
**Benefits of PARP inhibition**

1) Decreases diabetes induced Podocyte loss
2) Blocks hyperglycaemia induced apoptosis
3) Blocks hyperglycaemia induced ROS generation
4) Blocks hyperglycaemia induced NF-κB in podocytes
5) Decreases hypertrophy of kidney in diabetic mice\(^{[74]}\)
6) NF-κB p50 nuclear translocation is decreased\(^{[75]}\)
7) PARP-1 Inhibition prevents NF-KB/AP-1 binding at MMP-9 promoter. Hyperglycaemia induced PARP activation plays important role in pathogenesis of glomerulopathy associated with T2DM and could serve as novel therapeutic target.

**21] ICAM-1 AS MOLECULAR TARGET**\(^{[65,78,79,80,81,82]}\)

ICAM-1- is associated with diabetes and diabetic nephropathy.\(^{[80]}\)

**INDUCERS OF ICAM-1**\(^{[81,82]}\)

1) Hyperglycaemia
2) AGEs
3) Oxidative stress
4) Hyperlipidemia
5) Hyperinsulinemia
6) Elevated level of TNFα

**ICAM-1 LEVEL CAN BE REDUCED BY**

1) GLP-1 AGONIST\(^{[78]}\) extra pancreatic action of GLP-1 -ameliorate albuminuria, hypertrophy, hyperfiltration and mesangial expansion
2) Calcium channel blocker nifedipine blocks AGE product induced damage in tubular cells and reduces ICAM-1 expresion.\(^{[79]}\)
3) Plant derived alkaloid berberine reduces ICAM-1 gene expression and NF-κB\(^{[65]}\)
4) Anti ICAM-1 monoclonal Ab prevents mononuclear infiltration into diabetic glomeruli\(^{[83]}\)
5) Taurine also decreases over expression of ICAM-1 and provides renoprotection.\(^{[84]}\)

Taking together the above facts Inhibition Of ICAM-1 activity, the study suggest ICAM-1 is good candidate for development of drug for diabetes and diabetic nephropathy.
22] Chemokine MCP-1 AS MOLECULAR TARGET\(^{[85-97]}\)

**MCP-1 IS INDUCED BY**

1) High glucose concentration  
2) AGEs  
3) Cytokines  
4) ACTIVATION OF RAAS\(^{[95]}\)  
5) TGFβ\(^{[95]}\)

**Role** – recruits monocytes, T cells, macrophages and dendritic cells at site of injury, infection and inflammation.

**MCP-1 PRODUCTION**

**Cells** – renal cells like podocyte and mesangial cell produce MCP-1. Also epithelial, endothelial and smooth muscle.

**SIGNALLING** - autocrine and paracrine activation by MCP-1 with interaction with CCR2  
**CCR2** – is main receptor for MCP-1\(^{[89,93]}\)

**MCP-1 AS BIOMARKER\(^{[89,93]}\)**

MCP-1 urinary level correlates with albuminuria, therefore being considered as marker of renal function decline.

**MCP-1/CCR2** – is involved in cytoskeleton reorganization, motility, mesangial expression of fibronectin and TYPE IV collagen.\(^{[92,94]}\)

**MCP-1** – is up regulated in human diabetic kidney injury.

**DRUGS TARGETING MCP-1/CCR2 SYSTEM**

1) **DIRECT INHIBITOR OF MCP-1** - Emapticap pegol (NOX-E36)\(^{[87]}\)  
2) **CCR2 ANTAGONIST** – RO 5234444\(^{[89,93]},\) CCX-140 B\(^{[88]}\)  
3) **Bindarit ( AF-2838)**\(^{[90]}\)

Bindarit reduces urinary MCP-1 level and albumin excretion rate, clinical trial of bindarit with RAAS blockade therapy is on-going in type 2 diabetic patient with micro-albuminuria and macro albuminuria\(^{[90]}\)
ADVANTAGE OF TARGETING MCP-1/CCR2 SYSTEM
1) Alleviate pro-inflammatory state\textsuperscript{[85]}
2) Preserve Podocyte function\textsuperscript{[85]}
3) Preserve renal function\textsuperscript{[85]}
4) Reduces albuminuria\textsuperscript{[85]}
5) Improves GFR\textsuperscript{[89,93]}
6) Reduces Glomerulosclerosis\textsuperscript{[89,93]}
7) Dose dependent reduction of albuminuria occur with use of CCX-140B\textsuperscript{[88]}

OTHER DRUGS THAT REDUCE MCP-1 ACTIVITY
Local reduction in MCP-1 level and provide renoprotection
1) Pioglitazone\textsuperscript{[91]}
2) Clarithromycin\textsuperscript{[96]}
3) Exenatide\textsuperscript{[97]}

CYTOKINE INHIBITORS IL 1, IL 6 AND TNF $\alpha$
First time the role of cytokine was proposed in diabetic nephropathy\textsuperscript{[98]}

23] IL-1
Cytokine and growth factor play important role in renal disease\textsuperscript{[104]}

Cells that secrete cytokine-Tubular cells, endothelialcell, mesangial cell and epithelial cell secrete cytokine which act in autocrine or paracrine manner\textsuperscript{[104]}

Experimental model with diabetes shows increase expression of IL-1\textsuperscript{[99,100]}

IL-1 expression in increased diabetic nephropathy in several studies\textsuperscript{[98,100]}

Increase level of renal IL-1 correlates with albuminuria and macrophage content\textsuperscript{[99,100,104]}

IL-1 Increases ICAM-1 ALSO ACTIVATE DOWNSTREAM NF-κB

EFFECTS OF IL-1
1) Facilitate Leukocyte Endothelium Adhesion
2) Increases the production and expression of ICAM-1
3) Infiltration of macrophage
4) Expansion of extracellular matrix
5) Glomerulosclerosis

**DRUG THAT INHIBIT IL-1 RECEPTOR**

**IL-1 receptor antagonist** – Anakinra[^101] It reduce release of cytokine and chemokine’s, reduces hyperglycaemia, improves insulin sensitivity, markers of systemic inflammation reduces, improves beta cell function.[^102] Further studies are needed to demonstrate the beneficial effect in diabetic kidney.

**24] IL-6[^103,105-112]**

IL-6- secreted by renal cell in response to diabetic milieu[^105]

Significant association between severity of diabetic glomerulopathy and IL-6 level[^107] IL-6 level are increased in diabetic patient as compared to normal[^106]

High level of IL-6 are reported in diabetic patient and correlates with urinary albumin excretion and High sensitive CRP level[^108] same result were found in other study in type 1 diabetes patient IL-6 level were high and correlated with urinary albumin excretion[^109]

**IL-6 SIGNALLING** – IL-6 activate its receptor and brings about activation of JAK/STAT pathway and STAT 3 protein is translocate in nucleus and enhance transcription factors[^110]

**EFFECTS OF IL-6[^111]**

1) Promote Growth And Proliferation Of Mesangial Cells
2) Extracellular Matrix Accumulation
3) Glomerular Basement Membrane (GBM) thickening
4) Glomerulosclerosis

Thus the data indicate IL-6 is involved in pathogenesis and development of diabetic nephropathy.

**TARGETING IL-6 /IL-6 RECEPTOR**

Anti- IL-6 Are Currently Used In Rheumatoid Arthritis, As Novel Anticancer, tocilizumab, siltuximab, clazakizumab, olokizumab, sarilumab, sirukumab. Application of these drugs in diabetic model and further study should be done and the data suggest IL-6 plays important role in pathogenesis.
PENTOXYIFYLLINE – AND IL-6\textsuperscript{[98,99,100]}

1) It is non-selective phosphodiesterase inhibitor (methylxanthine derivative)
2) It decreases urinary albumin secretion
3) Reduces IL-6 level

25| TNF α AS MOLECULAR TARGET\textsuperscript{[103,113-124]}

TNF α – is produced by monocyte and macrophage involved in systemic inflammation\textsuperscript{[83]}

It exert cytotoxic effect.\textsuperscript{[105,114]}

Cells that produce TNF α- mesangial, tubular, endothelial, glomerular and epithelial cells.

Stimuli for TNF α- AGEs, angioII

It is involved in renal damage in experimental model\textsuperscript{[114,115]}

Serum TNF α level are increased in diabetic patient\textsuperscript{[118]}

Urinary TNF α level and renal TNF α correlates with urinary albumin excretion\textsuperscript{[118]}

ROLE OF TNF α

1) Mediate inflammatory reaction
2) Reduction GFR
3) Reduction in glomerular blood flow
4) Damage to glomerular permeability barrier
5) Development of albuminuria
6) Recruitment of Polymorph- nuclear leukocyte and monocyte
7) Induction of apoptosis
8) Increase pro-coagulant activity.

ANIMAL STUDY WITH TNF α INHIBITORS\textsuperscript{[124]}

In diabetic rat model study anti-TNF α reduces urinary protein, prevented sodium retention and renal hypertrophy\textsuperscript{[124]}

Infliximab – a chimeric mmonoclonal antibody directed against TNF α reduces albuminuria and urinary TNF α level.\textsuperscript{[117]}

Also anti-TNF α improved glucose tolerance and control in type 2 diabetic patient\textsuperscript{[116]}

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Thus TNF $\alpha$ is potential for future drug development.

Limitation
The study carried out till date lacks the experimental data regarding structural changes, haemodynamic parameter and other biomarkers of kidney damage. Future study is needed with TNF $\alpha$ inhibitors on early and late Structural changes in diabetic kidney with solid support.

TARGETING GROWTH FACTORS:- TGFβ, CTGF, VEGF

$^{26]}$ TGFβ$^{[75, 90, 95, 125-132]}$ - TRANSFORMING GROWTH FACTOR-BETA

STIMULI FOR TGFβ$^{[75]}$

1) Hyperglycaemia
2) AGEs
3) Endothelin
4) Lipids
5) Oxidative stress
6) RASS

ROLE IN PATHOGENESIS $^{[75]}$

1) Tubuloglomerular Sclerosis $^{[75]}$
2) Podocyte apoptosis
3) Produces fibrosis
4) Increases the production of extracellular matrix
5) Albuminuria

TGFβ- gene and protein level are increased in glomeruli and tubulointerstitium of T1DM and T2 DM patient and animals $^{[95]}$

Drugs that modify TGFβ expression or reduce TGFβ level

1) ACE inhibitor Perindopril reduces intrarenal TGFβ expression and activity $^{[125]}$
2) ACE inhibitor reduces serum and urinary level of TGFβ $^{[131]}$
3) Antifibrotic agent N-acetyl-seryl-aspartyl-lysyl-proline reduced extracellular matrix production, prevented fibrosis and albuminuria in mice model. Also additional renoprotection can be offered when combined with angiotensin II receptor antagonist losartan $^{[130,140]}$
Targeting TGFβ\textsuperscript{[75,90,129,132]}

1) Many novel compounds are under investigation to inhibit TGFβ and TGFβ pathways in diabetes.

2) Several blocking Abs against TGFβ reduce mesangial matrix accumulation and glomerulosclerosis in diabetic mouse model\textsuperscript{[75,132]}

3) TGF-AY1Ab is in clinical development for the treatment of chronic kidney diseases with focus on diabetic nephropathy.\textsuperscript{[90]}

**DRUGS**- Pirfenidone\textsuperscript{[133]}, Tranilast\textsuperscript{[134,135]}, fresolimumab.\textsuperscript{[136,137]}

**TGFβ BLOCKERS**- have been tested in animal models but human study yet to be tested [138,139]

Fresolimumab, tranilast- reduces proteinuria. Pirfenidone – pilot study shows beneficial renal effect but were halted due to adverse effect.

**27] CTGF- CONNECTIVE TISSUE GROWTH FACTOR**\textsuperscript{[90,140-145]}

It is downstream mediator of profibrotic activity like TGFβ.

Its expression is increased in diabetic kidney and is considered as marker and mediator of disease.

**Synthesis**- of CTGF is stimulated by-

1) Hyperglycaemia
2) AGEs
3) CTGF itself

**Role**\textsuperscript{[140]}

1) Mesangial cell hypertrophy
2) Cytoskeleton disassembling
3) Up regulated fibronectin and collagens
4) Profibrotic – thus this profibrotic factor may be more attractive target for development of renoprotective therapies.

**CTGF CAN BE DECREASES BY FOLLOWING DRUGS**

1) AGEs inhibitor-aminoguanidine, XL-III-43\textsuperscript{[140]}
2) Aldosterone Receptor Blocker- Spironolactone\textsuperscript{[145]}
3) Flavonoid compounds- astilbin\textsuperscript{[144]}
4) FG-3019 Humanized monoclonal Ab that neutralizes the effect of CTGF in diabetic animal.\textsuperscript{[90]}- it has shown to reduce albuminuria in diabetic nephropathy

Further investigation on this line is necessary.

28] VEGF – VASCULAR ENDOTHELIAL GROWTH FACTOR\textsuperscript{[147-151]}
VEGF –plays important role in development of diabetic nephropathy, inducer of vasopermeability and angiogenesis.

Expression of VEGF and its receptor are modulated by-\textsuperscript{[147,148]}
1) High glucose level
2) AGEs
3) Endothelin 1
4) Angiotensin II
5) Stretch
6) TGFβ

Serum level of VEGF correlates with albuminuria and increase with Diabetic nephropathy stage in patients with type 1 and type 2 diabetes.\textsuperscript{[146]}

VEGF affects podocyte function and involved in influx of macrophage.\textsuperscript{[147]}

TARGETING VEGF
1) Antibodies against VEGF improves renal dysfunction, ameliorate early and late changes in experimental diabetic animals.\textsuperscript{[149,150]}
2) Pan –VEGF TYROSINE KINASE INHIBITOR SU 5416- ameliorate diabetic albuminuria.\textsuperscript{[151]}
3) VEGF can be blocked by using TEMPO\textsuperscript{[19]}
4) Overexpression p22 (phox) enhances VEGF directing antisense oligonucleotide will decrease VEGF.\textsuperscript{[19]}

Several studies are carried out with VEGF inhibitor in diabetic retinopathy and more studies are needed to determine the potency of VEGF inhibitors in renal disease.
29] VITAMIN D RECEPTOR ACTIVATOR\textsuperscript{[152-159]}

It is well known vitamin D Deficiency can trigger immune, inflammation and cardiovascular event.\textsuperscript{[152]}

Low level of vitamin D are linked to diabetic nephropathy\textsuperscript{[153]}

Vitamin D receptor activator such as paricalcitol is found to be interesting.\textsuperscript{[154]}

Paricalcitol

1) Reduces proteinuria in diabetic patient\textsuperscript{[155]}
2) Reconfirmed in VITAL study- albuminuria was reduced instead of proteinuria\textsuperscript{[157]}
3) Combination with aliskiren can reduce progression of diabetic nephropathy\textsuperscript{[158]}
4) Addition of adequate RAAS blocker is necessary
5) Calcitriol also have same effect\textsuperscript{[159]}

30] ENDOTHELIN RECEPTOR ANTAGONIST\textsuperscript{[160-165]}

ENDOTHELIN- vasoactive peptide plays important role in hypertension and chronic kidney disease.\textsuperscript{[160,161]}

Receptor

ETA and ETB – are endothelin receptor,
ETB- is in proximal tubule enhance sodium excretion.

Endothelin dysfunction- lead to vasoconstriction, cell damage and albuminuria\textsuperscript{[160,161]}

Endothelin inhibitors

Effect of inhibiting ETB- inappropriate sodium retention, peripheral oedema, congestive heart failure and cardiovascular event.\textsuperscript{[162-164]}

ETB INHIBITORS- sitaxsentan, avosentan trial has terminated because of adverse effect.
All inhibitors shows renoprotective effect and reduce proteinuria.

Currently SONAR study is in trial with ATRASENTAN in type 2 Diabetic patient.\textsuperscript{[165]}

Drug –bosentan has higher affinity ETA than ETB and used in pulmonary hypertension.

ET receptor are also good molecular target and further study with safety is needed.
31] XANTHINE OXIDASE INHIBITOR\(^{166-169}\)

Allopurinol has shown its efficacy in slowing down the progress of kidney disease as well as shown cardiovascular benefits.\(^{166,167}\)

PEARL AND FEATHER STUDY- are under current investigation / study to prove usefulness of allopurinol and febuxostat in type 2 Diabetes (T2DM).\(^{168,169}\)

TARGETING MICRO RNA [miRNA]\(^{170,171,172,173}\)

32] miRNA

miRNA can be targeted, the small RNA are involved in gene expression, regulation and many of them are identified as pathogenic and protective.\(^{170,172}\)

Targeting

1. By silencing the pathogenic miRNA by drugs or anti-miRNA oligonucleotide OR
2. By enhancing renoprotective miRNA by mimics/vector or exosomes.

Till date only in animal model oligonucleotide has been tested.\(^{172}\)

Further study with all other parameter should be done to find out Reno protective efficacy.

miRNA -21, miRNA-195, let-7 are possible target for diabetic nephropathy the exact role of let-7 is not clear and need further studies.\(^{173}\)

Natural agents inhibiting\(^{173}\)

miRNA -21 contribute to diabetic nephropathy and curcumin and resveratrol may ameliorate by inhibiting miRNA-21

33] UROTENSIN–II RECEPTOR AS MOLECULAR TARGET\(^{174}\)

Urotensin II a peptide plays important role in Kidney like sodium transport, lipid and glucose metabolism and natriuretic effect. It is present in other tissue brain, heart adrenal, placenta, Spleen and thymus.

Dysfunction is found in diabetes result in hypertrophy and fibrosis.

Receptor Antagonist

Palosuran - its arrival in phase II or III clinical trial the result are not up to mark.
34] Glycosaminoglycan’s- sulodexide\textsuperscript{[175-181]}
Sulodexide ameliorate diabetic nephropathy\textsuperscript{[175,176]}
Decreases albuminuria and extracellular matrix accumulation\textsuperscript{[179]}
Sulodexide fail to offer renoprotection in type 2 diabetes\textsuperscript{[181]}

Mechanism\textsuperscript{[178]}
1. Restoration of glomerular ionic permeability
2. Inhibition of PKC-BETA-III,ERK,FGF-2 AND HEPARANASE-1
3. Improves endothelial dysfunction
4. Reduces vascular growth factor

35] Endogenous agents Apelin And Activated Protein C

APELIN\textsuperscript{[182,183]}
1] Apelin retards the progression of diabetes nephropathy\textsuperscript{[182]}
2] Apelin promotes diabetic nephropathy by inducing podocyte dysfunction via inhibiting proteasome.\textsuperscript{[183]}

Study have created confusion apelin retard progress of diabetic nephropathy or promote podocyte dysfunction need to studied further.

Activated Protein C\textsuperscript{[184]}

Study reported following action of activated protein C
1) It inhibit redox sensitive transcription facor NF-Kb.
2) Suppress SOD-1
3) Direct antioxidant effect.
4) Suppress glucose induced release of cytochrome c and smac /Diablo from mitochondria and mitochondrial apoptosis
5) p66\textsuperscript{Shc} is potential target of activated protein C
6) Activated protein C Reduces ROS formation in podocytes.
7) Suppress glomerular p66\textsuperscript{Shc} expression in diabetic mice.
8) Inhibits glucose induced expression p66\textsuperscript{Shc} in podocyte via PAR-1 and PAR-3 in vitro.
9) p66Shc expression in podocyte is epigenetically inhibited by activated protein c
10) maintains mitochondrial membrane potential and inhibit ROS generation.
This study as given insight of cytoprotective effect in vitro and in vivo further trial need to be done to prove the efficacy in diabetic patient with nephropathy.

36] ORAL ADSORBENTS
KREMEZIN/AST 120\textsuperscript{[185-189]}

MECHANISM
It adsorbs uremic toxins, reduces systemic inflammation and immune activation.\textsuperscript{[185]}
Hopeful result in early CKD.\textsuperscript{[186,187]}
Disappointing result in moderate to severe CKD in clinical trial.\textsuperscript{[188]}
Prevents glomerulosclerosis in rats.

Niaoduqing keli – a herbal combination marketed tea, also granules and tablet which is widely used in china claims are made its reduces BUN, creatinine level, cytoprotective, diuretic and anaemia of CKD. The mechanism remains unclear.\textsuperscript{[189]}

37] MATRIX METALLOPROTEASE INHIBITORS (MMP Inhibitors)\textsuperscript{[190-196]}
MMP-plays important role in development of diabetic nephropathy.\textsuperscript{[193,194]}
Dysregulation in NGAL, MMP-9 aand MMP2 is noted in type1 diabetes.\textsuperscript{[195,196]}
MMP9 MMP2 –can be biomarkers for early kidney damage.

Inhibitors
Doxycycline, XL081, XL874 are known to inhibit MMP still further testing is needed.\textsuperscript{[191,192]}
Limitation – results in humans are not satisfactory.

NEUROHORMONAL MODIFICATION\textsuperscript{[197-199]}
38] Sarpogrelate\textsuperscript{[197,198]}
5HT receptor antagonist, antiplatelet action. It has demonstrated anti-inflammtory and anti-proteinuric effect.

Further study with sch molecule are needed to be evaluated.
39] ACTH[^199]

It Has Shown To Reduce Proteinuria In Diabetic Kidney Disease.

MINERALOCORTICOID RECEPTOR TARGETING[^200-207]

40] Spironolactone and eplerenone

Both drugs showed slight superior renoprotection as compared to ACE and ARB therapy.[^203]

Proteinuria was reduced with same adverse profile.[^201]

One clinical trial documented reduction of proteinuria with Eplerenone.[^202]

However potential adverse reaction must be monitored like hypotension, hyperkalemia and renal function.[^205,206]

41] FINERENONE[^207]

Reduces albuminuria without adverse effect on serum potassium level or renal function in Japanese patient with T2DM and diabetic nephropathy.

IRON CHELATOR

42] Deferiprone[^208]

Animal model- diabetic rat.

Deferiprone – inhibited the expression of NF-κB MCP-1, COX-2 and nitrotyrosine which are overexpressed in diabetic nephropathy rats.

Decrease level of mmp-9 and increased TIMP-1 expression in DN rats.

It has nephroprotetive effect by reliving oxidative stress, inflammation and fibrosis.

Chronic kidney disease is associated with Anaemia it may aggravate anaemia and produce Iron deficiency in diabetic patient. Further testing of drugs in human subject is needed.

Serum iron, serum ferritin, TIBC parameter should be added in study for CKD if deferiprone is to be used.

43] Exogenous klotho[^209-211]

KLOTHO – is anti-senescence protein favours epithelial regeneration and inhibits fibroblast
Exogenous Klotho decreases the TGFβ bioactivity, expression, receptor expression, fibronectin induced by hyperglycaemia.

Intravenously delivered to diabetic rat, klotho gene was able to prevent progression of renal hypertrophy and fibrosis.

Further studies are needed with such therapy.

44] Interleukin -17[212]

Animal study has data suggest- IL-17A Treated diabetic mice reversed diabetic nephropathy in low dose, decreased urinary albumin excretion(UAE), Kidney size, mesangial matrix expansion, interstitial fibrosis.

Urinary MCP-1, TNF α, IL-6 and serum urea level were decreased in diabetic mice as compared to control.

Weighing risk benefit and adverse effect IL-17A should be tested in human subject.

ORAL ANTIDIABETIC DRUG

45] METFORMIN[213]

1. It activates AMPK
2. Inhibits mTOR
3. Inhibits apoptosis of podocyte
4. Metformin thus act by AMPK activation and mTOR inhibition.

46] THIAZOLIDINEDIONES[214]

Pioglitazone

1. Decreases systolic and diastolic blood pressure
2. Reduces urinary podocalyxin
3. Reduces urinary albumin secretion
4. Suppress diabetes induced local inflammation
5. Decreases urinary MCP-1 LEVEL

Since it causes fluid retention, precipitate heart failure, oedema use becomes limited in CKD.
47] Glucagon like peptide -1 agonist\cite{215,216}

GLP-1
1. Has antioxidant property
2. Decreases urinary albumin excretion
3. Decreases glomerular ROS
4. Down regulates NADPH OXIDASE
5. Inhibit mesangial cell expansion
6. Inhibit expression of TGFβ1 and CCN2

So far no study with GLP-1 in human subject with diabetic nephropathy.

48] DIPEPTIDYL PEPTIDASE -4 INHIBITORS (DPP-4 INHIBITORS)\cite{217-223}

Vildagliptin -decreased urinary albumin excretion by 44.6% in single arm clinical study with T2DM Patient.\cite{217}

Sitagliptin –signigicantly reduced (UAE) Urinary albumin excretion and HBA1c as compared to other oral hypglycaemic drugs.\cite{218}

Linagliptin- is unique does not need dose adjustment with decline in GFR\cite{219} It blunts TGFβ signalling and restores the physiological balance of VEGF. All these response inhibit Endothelial mesenchymal transition (EndMT) and subsequently renal fibrosis.\cite{220}

Saxagliptin- in rat model with T1DM – limit renal hypertrophy\cite{221} it also decreased UAE.\cite{222}

ALOGLIPTIN- reduced UAE\cite{223}

Studies suggest DPP-4 interfere with TGFβ signalling which plays role in pathogenesis of diabetic nephropathy. Linagliptin should be tried in patient with early, moderate to severe diabetic nephropathy.

49] SODIUM GLUCOSE TRANSPORTER -2 INHIBITORS

Hyperglycaemia causes increased expression of SGLT2 which leads to increase sodium absorption by PCT decreases the sodium delivery in DCT. The ATP consumption by DCT decreases and macula densa decrease level of AMP, decrease level of adenosine in macula dense which leads to vasodilation of afferent arteriole hyperfiltrations and renal injury.
SGLT 2 – INHIBITOR–\textsuperscript{[224,225]}

Empagliflozin- reduces albuminuria, creatinine level, bodyweight and blood pressure.

SGLT 2 –INHIBITION- has no renoprotective effect in non-diabetic kidney disease.\textsuperscript{[226]}

50] NUCLEAR FACTOR ERYTHROID 2-RELATED FACTOR 2 ACTIVATION (Nrf2)\textsuperscript{[227-228]}

Nrf 2 is potential emerging target for diabetes nephropathy it activation promote antioxidant genes and restores the depleted antioxidant by ROS. Thus intracellular anti-oxidant are restored back.

Natural compounds\textsuperscript{[227]}

1. Sulforaphane –present in cruciferous vegetables
2. Cinnamic aldehyde- present in cinnamon oil
3. Curcumin- in turmeric
4. Resveratrol – found in grapes
5. Sinomenine- found in roots sinomenium acutum
6. Rutin- found in black tea,citrus fruit and apple peel
7. Berberin- found in berberis vulgaris, garlic, bitter melon

Curcumin – in T2DM patient curcumin decreased uae, lipid peroxidation and inflammatory markers.\textsuperscript{[227]}

RESVERATROL- antioxidant, scavenges free radical, acts on NADPH and alleviates epithelial mesenchmal transition [EMT] induced by NADPH OXIDASE\textsuperscript{[228]} No single trial in diabetic nephropathy.

Bardoxolone methyl- antioxidant, activates Nrf-2 but causes hypomagnesemia\textsuperscript{[15,229]}

Silybin- antioxidant and activator of Nrf 2\textsuperscript{[9]}

Sodium butyrate\textsuperscript{[230]}- activates Nrf2 to ameliorate diabetes nephropathy possibly via inhibition of HDCA. (HISTONE DEACETYLASE).
51] DIRECT RENIN INHIBITOR\textsuperscript{[231]}

ALISKIREN- combined with paricalcitol can reduce the progression of diabetic nephropathy in rats. It also reduces proteinuria. Human testing with such combination in various stages of diabetic nephropathy is lacking.

52] TARGETING AGEs AND RAGE\textsuperscript{[232-238]}

Hyperglycaemia derived advanced end product of glycation (AGEs) are known to play important role in pathogenesis of diabetic complication and their interaction with receptor RAGE causing protein dysfunction and altered collagen turnover\textsuperscript{[232,233]} AGE also activate NAPDH OXIDASE and also help in ROS generation.

Targeting RAGE-\textsuperscript{[234]}- Inhibiting RAGE with neutralizing antibodies reversed pathogenic effect.

Several molecules known to inhibit age formation like pimagedine or pyridoxamine\textsuperscript{[235]} showed benefit in animal model.

Pimagedine stopped due to adverse effect and pyridoxamine is ineffective in many study setup.\textsuperscript{[236-238]}

Berberine exert renoprotective effects by regulating the AGEs –RAGE Signalling pathway in Mesangial cells during diabetic nephropathy.\textsuperscript{[253]}

53] CARNITINE SUPPLEMENT\textsuperscript{[239]}

Carnitine could prevent contrast media induced injury.

Oral L-carnitine was able to prevent an increase in NGAL following contrast medium administration in patients undergoing PCI. More studies should be performed to fully elucidate the nephroprotective effects of L-carnitine.\textsuperscript{[239]}

54] COENZYME Q10\textsuperscript{[240,241,242]}

CoQ10 –Deficiency precipitates diabetic kidney disease and also plays important role in pathogenesis of T2DM.\textsuperscript{[240]}

Action\textsuperscript{[241]}

1. It improves glycaemic control
2. Reduces HbA1c level
3. Protect beta cell
4. Antioxidant
5. Improves insulin action prevents down regulation of insulin receptor
6. Scavenger of ROS
7. Protect the cell from oxidative damage especially from mitochondrial ROS
8. Nephroprotection

**Combination with DPP-4 INHIBITORS**

**SITAGLIPTIN combined with CoQ10**[^242]- Reduces oxidative stress, TNFα, TGFβ, MPO Activity.

Such combination therapy should put in to trial in human subject, instead of sitagliptin linagliptin combined with CoQ10 Should be tried since study suggest linagliptin does not require dose adjustment and also restore the physiological balance of VEGF.[^219,220]

**55] ALDOSE REDUCTASE [AR] INHIBITORS**[^243]

ALDOSE REDUCTASE- enzyme plays key role in pathogenesis of diabetic nephropathy as well as neuropathy.

In vitro study inhibition of AR – Epalrestat prevented the signalling and renal cell injury indced by high glucose, thus AR inhibition could be good potential target for treatment of diabetic nephropathy.[^243] Further testing in animal model and human subject is necessary.

**56] TARGETING LPA-1 RECEPTOR**[^244]

LPA receptor 1 siRNA treatment inhibited LPA induced TGF β expression, whereas cells overexpressing LPA receptor1 showed LPA – induced TGF β expression.[^244]

LPA receptor1 signalling increasd TGFβ expression via GSK3β phosphorylation and SREBP1 activation contributing to the development of diabetic nephropathy.[^244]

Also GSK3β Could be targeted so that TGF β expression will decrease which plays key role in development of diabetic nephropathy.
ANTAGONIST OF LPA RECEPTOR: –Ki6425.

57] PLC γ AS MOLECULAR TARGET

Increase level of angiotensin II will cause podocyte dysfunction through PLCγ mediation causing changes in α actinin distribution.

PLCγ INHIBITOR U-73122 prevented

1) Actin changes
2) Podocyte dysfunction
3) Preserve PIP2 and regulate function of filtrations slits

Unfortunately no more progress is made on such inhibitors.

58] NLRP-3 AS MOLECULAR TARGET

In experimental study curcumin diminished

1) Renal hypertrophy
2) Reduced mesangial matrix expansion
3) Decreased collagen IV and fibronectin level
4) Reduction in interleukin 1β and NLRP 3 level
5) Antifibrotic activity
6) Curcumin inhibits NLRP3 Inflammasome activity.

Role of NLRP 3

1) Hyperuricemia induced NLRP 3 activation of macrophages contributes to progression of diabetic nephropathy

2) ATP-P2X4 signaling mediates high glucose-induced activation of the NLRP3 inflammasome, regulates IL-1 family cytokine secretion and causes the development of tubulointerstitial inflammation in Diabetic Nephropathy.

NLRP 3 is activated in many inflammatory and autoimmune disease.

Inhibitors of NLRP 3-

1) MCC 950 AND β Hydroxybutyrate.
2) Type I interferon (IFN) and IFN β
3) CB2R agonist similar to HU 308 for treating NLRP3 inflammasome related disease by inducing autophagy
4) Plant polyphenolic compound Resveratrol induce autophagy, suppresses mitochondrial damage in macrophage and thereby inhibits NLRP3 inflammasome activation.-mediated by IL-1βsecretion.

Targeting NLRP -3 would be promising approach for diabetic nephropathy\textsuperscript{[250]}

59] PGE2 ANALOGUE\textsuperscript{[251-252]}
1) PGE2 –produces natriuretic effect\textsuperscript{[251]}
2) PGE2 - acts through EP-4 receptor activates protein kinase A(PKA) which directly inhibit NLRP-3,\textsuperscript{[252]}
3) PKA directly phosphorylate cytoplasmic receptor NLRP-3 and attenuated its ATPase function.\textsuperscript{[252]}

Unfortunately the no more study is done with PGE2 analogue in diabetic nephropathy.

60] Natural Plant Products /traditional medicine in current research\textsuperscript{[253-255]}
BERBERINE\textsuperscript{[253]} Berberine exert renoprotective effects by regulating the AGEs –RAGE Signalling pathway in mesangial cells during diabetic nephropathy.\textsuperscript{[253]}

Prunella Vulgaris\textsuperscript{[254]}
1) Suppress ICAM-1 and MCP-1
2) Inhibits activation and translocation of NF-Kb
3) Decreases blood sugar, BUN, plama creatinine
4) Collagens IV and TGF β 1Decreased
5) CTGF decreased
6) Prevents fibrosis
7) Reno protective action is due to disruption of TGFβ/Smad signalling

Tangzhijing Granules\textsuperscript{[255]}- In diabetic rat model
1) Increase nephrin level
2) Reduces TGF β and Smad
3) Protects podocytes
4) Prevents structural damage
5) Restores function
**Rhein**\(^{[256]}\)

1) Decreases blood sugar level  
2) Decreases lipid level  
3) Antioxidant  
4) Renal protection  

Needs further study in human subjects

**Apigenin**\(^{[257]}\) - A flavone found in plants parsley, celery and chamomile plant.

1) Decreases TGF β, fibronectin and type IV collagen  
2) Reduces TNFα,IL-6,NF-κB level  
3) Apoptosis is inhibited-caspase 3 level are reduced  
4) Inhibits MAPK pathway  
5) Relieves oxidative stress.

**Jiangtang**\(^{[258]}\)

**Jiangtang**- activates the PI3K/Akt signaling pathway and inhibits NF-κB signaling pathways.\(^{[258]}\)

61] **Smad 2 phosphorylation inhibition**\(^{[259]}\)

Smad signalling role in pathogenesis of diabetic nephropathy is well understood, herbal drugs like Prunella Vulgaris\(^{[254]}\) inhibit TGFβ/Smad pathway.

62] **Notch -1 signalling modulation**\(^{[260]}\)

Notch-1 signalling get Up-regulated in high glucose podocyte and induces VEGF expression and subsequent repression and apoptosis. Modulation of NOTCH-1 signalling may hold promise as a novel therapeutic strategy for treatment of diabetic nephropathy.

63] **p38MAPK Pathway Inhibitors**\(^{[261]}\)

**ANIMAL MODEL STUDY**

**BERAPROST SODIUM** – in experimental study BPS improved lipid profile, blood glucose, 24 hour urine protein and p38MAPK signalling pathway in diabetic kidney attenuated.

T2DM-It prevented renal dysfunction and pathological change the protective mechanism is complicated but attributed to inhibition of p38MAPK signalling pathway.

Further study are needed to understand the beneficial effect of inhibiting pathway.
64] **mTOR as molecular Target**\[^{262}\]**

High glucose level activate mTOR via PI3K OR AKT, increase expression mTOR causes
1. Tubular injury and apoptosis
2. Mesangial cell expansion
3. Thickening of basement membrane
4. Epithelial mesenchymal transition (EMT)
5. Fibroblast Proliferation
6. Increase CCN2 activity

Leading to fibrosis
Targeting mTOR would be beneficial
At present no clinical study with mTOR inhibitor in diabetic nephropathy is available.

65] **DILAZEP HCL**\[^{263}\]**

Dilazep hydrochloride, an antiplatelet agent, on the development and progression of diabetic nephropathy in Otsuka Long-Evans Tokushima fatty (OLETF) rats, a type 2 diabetes mellitus animal model.\[^{263}\]

**DILAZEP HCL**
1. Reduces Urinary Protein Excretion
2. Prevented glomerulosclerosis in rats
3. Reduces collagen type IV
4. Prevented Tubular Atrophy
5. Delays the progression of diabetic nephropathy,

Only a single study till date no study trial in humans reported.

66] **AGE CROSS LINK BREAKER- Alagebrium**\[^{264,265}\]**

Alagebrium /ALT-711 DEVELOPED by Alteon and cloically tested for breaking the crosslinks caused by AGEs and thereby reversing aging. It has being tested for hypertension and heart failure. It entered clinical phase II Unfortunately the company terminated all trial.
No clinical study or animal study with biomarker parameter of kidney damage in diabetic nephropathy is available.

67] **Anti-Angiogenic therapy for Diabetes Nephropathy**\[^{266}\]**

A novel endogenous inhibitor Vasohibin -1 (VASH1)-
Protect endothelial cells from oxidative stress and helps in survival of endothelial cells.

VASH-1 is associated with progression of kidney disease, up regulated in oxidative stress and local inflammation.

Anti-angiogenic and anti-inflammatory effect.

Protect mesangial cells and podocytes.

Protects the endothelial cells from hyperglycaemia.

**Animal model T1DM AND T2DM Diabetic nephropathy evaluation**

*Vector target- adenovirus encoding (Ad-VASH1) human VASH1-* used in diabetic animal the result reported are it significantly reduced glomerular hypertrophy, hyper filtration and albuminuria. Also Type IV collagen was reduced. In T1DM model enhanced phosphorylation of VEGFR2 was prevented.

*Recombinant human VASH1 (rhVASH1)*

It suppressed angiogenic response in glomeruli, activation of VEGFR2, Suppressed TGFβ, MCP-1 induced by high glucose level in mesangial cells.

Taken together all results suggest therapeutic potential of VASH1 for early diabetic nephropathy and eliminate the risk of endothelial injury which is often seen with ANTI – VEGF antibodies.

Anti-angiogenic effect mechanism unclear and need to be studied. Future trial may be helpful based on available study data.

**VITAMINS IN DIABETIC NEPHROPATHY**

**68] THIAMINE AND BENFOTHIAMINE**

High dose thiamine and benfothiamine increases transketolase expression in renal glomeruli, increased the conversion of triosephosphates to ribose -5-phosphate and strongly inhibits the development of microalbuminuria. This is associated with decrease activation of protein kinase C and decreased glycation and oxidative stress –three major pathways of biochemical dysfunction in hyperglycaemia. Benfothiamine also inhibits diabetes induced hyper-filtration High-dose thiamine and benfothiamine therapy is a potential novel strategy for the prevention of clinical diabetic nephropathy. 

[267]
69] CAVEOLIN-1 AS POTENTIAL TARGET[268,269]
Caveolin-1 plays important role in MMP2 activation, interaction of MT1-MMP with caveolin is well known. MMP2 is up regulated in oxidative stress, diabetic nephropathy. It is known to be up-regulated in cancer of bladder.

Caveolin-1 can play important role of endocytosis of MT1-MMP Targeting caveolin-1 may be promising molecular target in future for treatment of diabetic nephropathy.

70] MAP Kinase/ERK Kinase inhibitor (MEK inhibitors)[270]
BMK-1 is activated in glomeruli of diabetic rats and mesangial cells by high glucose level.

High glucose causes renal cell injury through various signal transduction, including mitogen activated protein (MAP) Kinases cascade. Big MAP KINASE 1(BMK1) also known as extracellular signal regulated kinase 5(ERK5) is recently identified MAP kinase family member and is reported to be sensitive in oxidative and osmotic stress. However the role of BMK1 in diabetic nephropathy is not clear.

Inhibitors
U0126 and PD98059 both inhibited activation of BMK1 by high glucose in a concentration dependent manner. Unfortunately the further study with such inhibitors is not available.

71] N-ACETYL CYSTEINE (NAC)[271]
It has antioxidant property, antidote to Paracetamol poisoning, used to overcome nitrate tolerance.

In many clinical trial tested for prevention of contrast induced nephropathy with controversial results and not be effective in diabetic nephropathy however coming to conclusion is difficult because only small studies are conducted.

72] MULTIFACTORIAL TARGETING
Since so many molecules, enzymes, are up-regulated and play important role in pathogenesis along with oxidative stress and free radical, AGEs product generation leads to development of diabetic nephropathy. Natural or synthetic compounds which will inhibit multiple target are core area of research.

Natural source-curcumin and drugs like pirfenidone, fluorofenidone act on multiple targets.
Conflict of Interest- Author declares there is no conflict of interest.

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