Research Article

Phytochemical-Enriched Tragacanth Matrix for Fabricating Renal Organoids: A Novel Lipid- Hydrogel System for Enhanced Functionality and Survival

Dr. Sharadendu Bali

Professor General Surgery, Teerthanker Mahaveer University, Moradabad

Abstract

The development of physiologically relevant micro-physiological systems is a cornerstone of modern nanomedicine and traditional drug standardization. This study explores the fabrication of a functional renal organoid utilizing a hybrid scaffold composed of Tragacanth gel infused with herbal "Ghrita" (herbal clarified butter) containing Gokshura (Tribulus terrestris), Varuna (Crataeva nurvala), and Punarnava (Boerhavia diffusa). By formulating the Ghrita into solid lipid nanoparticles (SLNs) and nano-emulsions, a biomimetic interface is created, that supports the growth and polarization of human renal tubular cells. This lipid-hydrogel matrix can enhance organoid survivability beyond 14 days and provide a targeted environment for studying nephroprotection and lithotripsy in vitro. This model bridges the gap between ancient Ayurvedic formulations and contemporary organ-on-a-chip technology, offering a sustainable, high-fidelity platform for pharmaceutical screening.

1.Introduction

The kidney is a primary site for drug-induced toxicity and plays a central role in the elimination of metabolic waste, making it an essential organ for preclinical investigation. However, conventional two-dimensional (2D) cell culture systems frequently fail to mimic the intricate three-dimensional (3D) structure and lipid-rich microenvironment of the renal tubular network. To overcome these limitations, researchers are increasingly adopting organoid technology, in which stem cells or primary cells are used to generate miniature organ-like structures that closely resemble native kidney tissue (1).

A major challenge in organoid engineering is the selection of a scaffold that can mimic the native extracellular matrix (ECM). Natural polysaccharides, such as Tragacanth gel, have emerged as excellent candidates due to their biocompatibility and ability to form porous, 3D networks (2). Despite these structural benefits, simple hydrogels often lack the bioactive signalling required for long-term functional maintenance (3).

This article proposes the impregnation of herbal "*Ghrita*"—a Traditional Indian Medicine (Ayurvedic) lipid-based delivery system—into the Tragacanth matrix. By infusing the gel with Gokshura (Tribulus terrestris), Varuna (Crataeva nurvala), and Punarnava (Boerhavia diffusa), specific phytochemicals known for their nephroprotective and regenerative properties can be introduced (4). Further, by processing the lipid- based formulation (ghrita) into nanostructured lipid carriers (NLCs) or SLNs, a sophisticated bio-interface can be provided that can support the metabolic and structural demands of renal tubular cells (5). This approach can not only enhance the survivability of the organoid but also create a standardized platform for testing the efficacy of modern and traditional medicines within a rigorous scientific framework (6).

2. The Advantages of renal organoids over cell culture and animal studies

Use of rudimentary renal organoids, such as those stabilized in a bioactive Tragacanth-ghrita matrix, addresses critical gaps in current pharmacological testing by providing a middle ground between simplistic 2D cultures and complex animal models (7,8). While traditional "in-vitro"

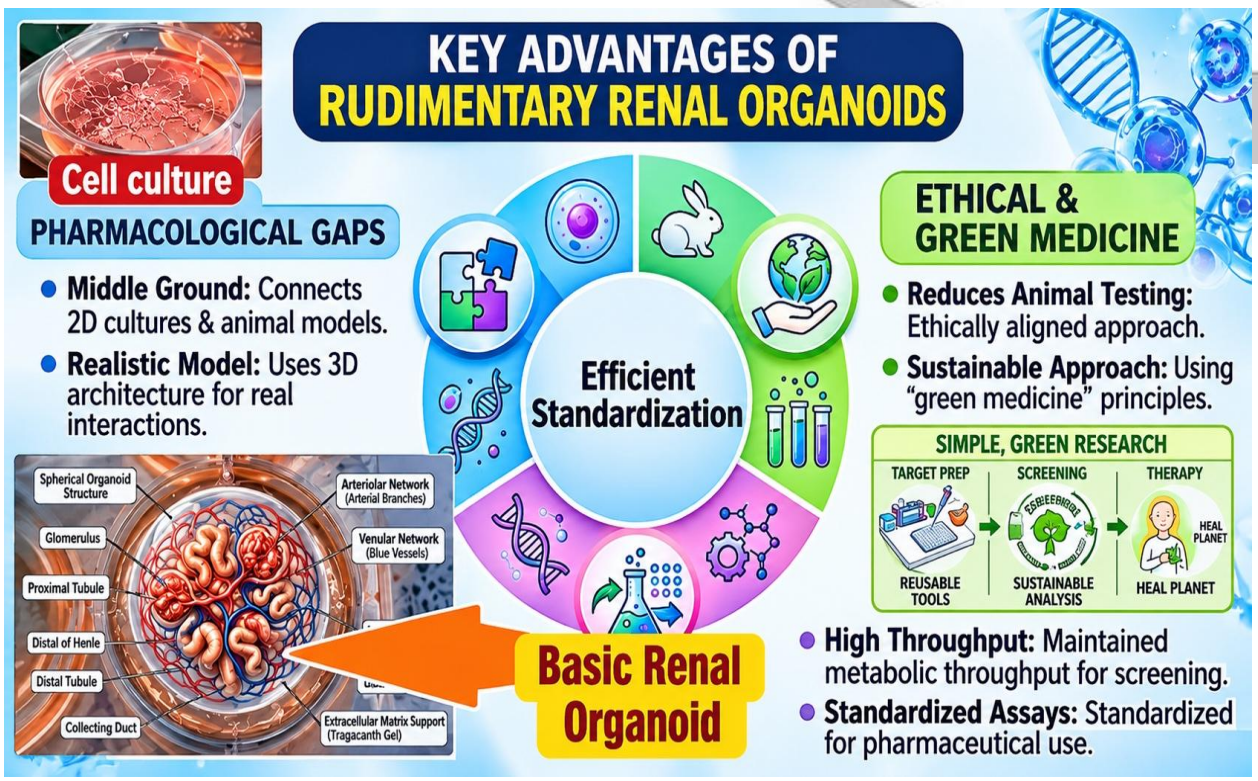


Figure 1. The Organoids are a better method for drug and toxicity testing than cell cultures.

assays lack the 3D architecture necessary for realistic cell-to-cell and cell-to-matrix interactions, animal testing is often limited by ethical concerns and inter-species physiological differences that can lead to inaccurate toxicity and drug-action predictions **(Figure 1).** The organoids offer a more ethically aligned, "green medicine" approach that reduces reliance on animal models, while maintaining the metabolic throughput required for pharmaceutical standardization (9). Furthermore, by **incorporating lipid-infused biological hydrogel scaffolds that mimic the renal extracellular matrix**, these models can facilitate high-resolution screening of drug partitioning and targeted tissue repair (7, 10).

3.THE INGREDIENTS USED IN THE LIPID HYDROGEL FUNCTIONAL SCAFFOLD FOR ORGANOIDS

3.a) Tragacanth Gel: The Structural Foundation

Tragacanth gum, a natural polysaccharide derived from Astragalus species, serves as an ideal base for renal organoids due to its unique physicochemical properties. Polysaccharide-based hydrogels are being increasingly favoured in tissue engineering because they naturally mimic the glycosaminoglycans found in the native ECM, providing a conducive microenvironment for cell encapsulation and viability (11, 12). The characteristics of Tragacanth gel (TG), which make it suitable in tissue engineering applications include the following:

a) Biocompatibility and Porosity: Tragacanth gel (TG) forms a highly porous 3-dimensional network that facilitates the exchange of oxygen, nutrients, and metabolites (13). This is critical for renal tubular cells, which require high metabolic throughput to maintain polarized transport functions.

b) Mechanical Tunability : The stiffness and elasticity of the gel can be adjusted to match the physiological modulus of renal cortical tissue, preventing premature cell death and promoting the formation of spherical, tubular structures (11).

c) Ability to stabilize the Tragacanth Gel structurally to maintain 3D architecture **(Figure 2).**

To maintain the 3D architecture under microfluidic flow, the **mechanical properties of Tragacanth can be stabilized** either by using Calcium Chloride ($CaCl_2$) for the ionic cross-linking of the polysaccharide chains to create a rigid, stable hydrogel, or by using Genipin, which is a natural, biocompatible cross-linker (14,15). Other methods include functionalization of the TG to MTG via methacrylation, using methacrylic anhydride (MA) or glycidyl methacrylate (GMA) to introduce vinyl functional groups onto the polysaccharide hydroxyl chains (16). The TG can also be Photo -crosslinked using Laponite (Lap), a synthetic 2D hectorite clay, under 405 nm visible light to seal the covalent network safely in the presence of cells (17,18).

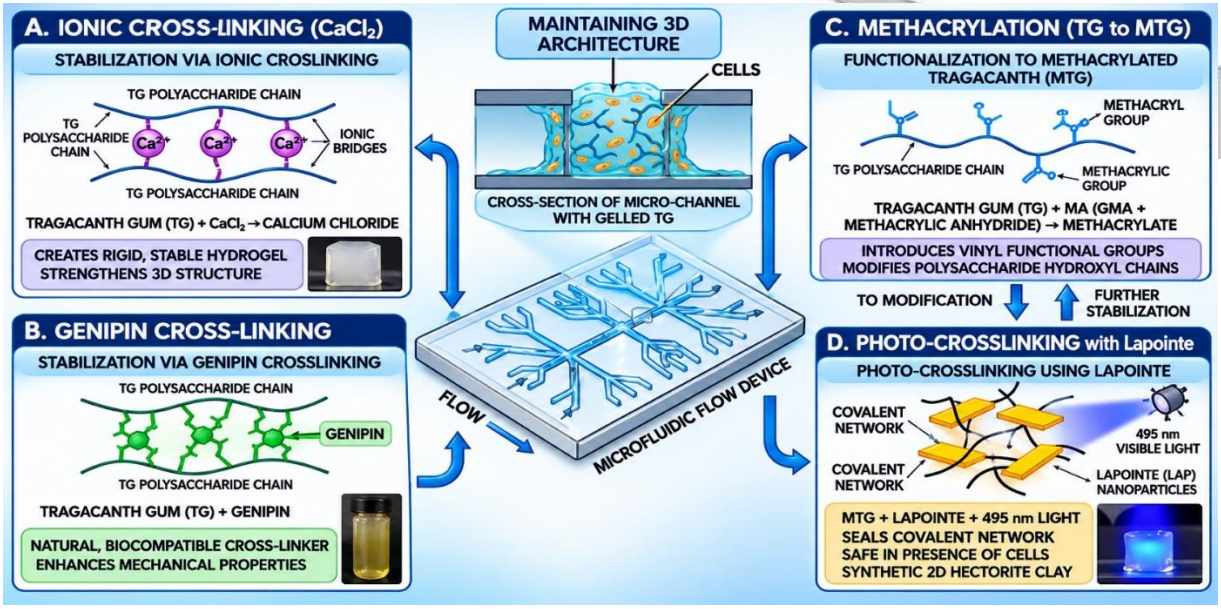


Figure 2. Depicts the various methods to stabilize and maintain the form of Tragacanth gel.

3b) Incorporation of Additional Bioactive Molecules into Tragacanth Gel to Promote Stem Cell Differentiation into Renal Organoids

To grow stem cells into functional renal organoids within a Tragacanth hydrogel, the gel must be "functionalized." While Tragacanth gum provides the physical scaffold, it is biologically inert and lacks the specific **signalling molecules** required to guide stem cell differentiation into nephron structures (19,20). The following chemical and biological components need to be integrated into the Tragacanth matrix to facilitate renal organoid growth **(Chart 1).**

(i) Cell Attachment Factors

Stem cells and renal epithelial cells require specific "anchors" to survive and proliferate. The Addition of RGD (Arginylglycylaspartic acid) or small amounts of Type IV Collagen or Laminin into the Tragacanth backbone allows cellular integrins to bind to the gel, preventing "anoikis" (cell death caused by lack of attachment) and supporting the polarization necessary for tubular formation (21,22). RGD motifs function as the primary binding sites for integrins, which are transmembrane receptor proteins on the surface of cells. When an RGD motif binds to an integrin, it triggers cellular attachment and initiates bidirectional signals into the cell, directing processes like migration, growth, differentiation, and survival (23,24).

Component Category	Specific Examples	Role in Organoid Growth
Adhesion Molecules	RGD Peptides, Laminin	Facilitate cell-matrix attachment
Wnt Activators	CHIR99021	Induce renal lineage from stem cells
Stabilizers	$CaCl_2$, Genipin	Ensures structural integrity of gel under flow
Nutrient Support	ITS, L-Glutamine	Support cellular metabolism and bioenergetics

Chart 1. Summarizes The key Organoid Growth Components to be added to Tragacanth Gel to functionalize it for reno- organogenesis

(ii) Nephrogenesis Growth Factors

To direct undifferentiated stem cells (like iPSCs) toward a renal lineage, specific biochemical triggers are needed within the gel. These include the following :

• CHIR99021: A small molecule that activates the Wnt signalling pathway, essential for the initial induction of the intermediate mesoderm. (19,25).

• Fibroblast Growth Factor 9 (FGF9) & Heparin: These are critical for maintaining the nephron progenitor pool and preventing premature differentiation (19,26).

• BMP7 (Bone Morphogenetic Protein 7): This is specifically required to promote the maturation of the tubular structures and the glomerular podocytes (27,28).

(iii) Metabolic and Environmental Buffers

• L-Glutamine and Non-Essential Amino Acids: Required for high metabolic activity during the rapid cell division stages of organoid formation (29).

• Insulin-Transferrin-Selenium (ITS): This may be added to the gel to support long-term cell survival and stabilize mitochondrial function (30)

(iv) Mechanical Tuners for tragacanth gel

PEG (Polyethylene Glycol) can help tune the "stiffness" of the Tragacanth, which is important since renal stem cells differentiate differently depending on how "hard" or "soft" the matrix is. A stiffness of roughly 5-10 kP*a* is often ideal for mimicking the native kidney environment (31,32). Integrating PEG-diacrylate allows for the precise tuning of the hydrogel's elastic modulus to match the native kidney, ensuring proper organoid maturation (32). By adding these specific chemical cues, the Tragacanth gel transforms from being a simple vegetable gum, into a Bio-instructive Scaffold capable of nurturing complex, 3D renal tissues.

3c) Herbal *Ghritas*: Functionalization of TG via Lipids infused with phytochemicals

While Tragacanth gel (TG) provides the “skeleton”, the infusion of Herbal *Ghritas* provides the "biochemistry." ***Ghee* (clarified butter), when infused with herbal extracts, is known as *Ghrita*.** Ghee acts as a lipid-based carrier, transforming the hydrogel into a nanostructured lipid carrier (NLC) system. Hence, the specific benefits of using *ghrita* either as such, or as LBDDS, results in extra benefits as given below.

I) Enhanced Drug Delivery : Lipids are known to improve the penetration of bioactive molecules into cellular membranes. By infusing the gel with Ghrita, the organoid becomes a more accurate model for testing lipid-soluble drugs and phytochemicals.

ii) Nutritional Signalling : The fatty acids in Ghrita can serve as a long-term energy source and signalling molecules for the developing organoid, enhancing the longevity of the renal tubular cells beyond the typical 14-day limit seen in rudimentary models.

iii) Phytochemical Synergy: The herbs Gokshura, Varuna, and Punarnava contain phytochemicals that provide a targeted nephroprotective environment, especially the bioactives present in Punarnava **(Figure 3**). These herbs have been traditionally utilized in Indian Medicine for treatment of renal stones and chronic kidney diseases due to their antioxidant and diuretic properties (33). According to Ayurvedic principles, there is no incompatibility if these three herbs are used together. The most significant beneficial actions of these three herbs are summarized below, and these benefits are discussed in great detail in a later section

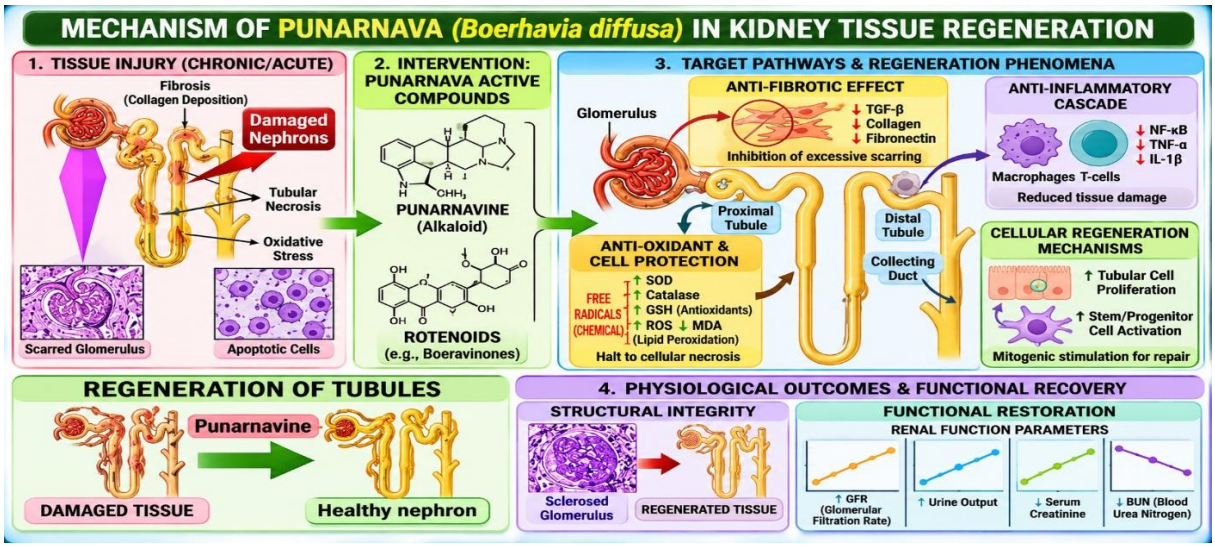


Figure 3. Beneficial actions of B. diffusa on diseased nephrons and glomeruli

Gokshura (Tribulus terrestris) : Known for its ability to prevent the formation of calcium oxalate crystals, its presence in the scaffold allows researchers to study lithotripsy (stone-breaking) mechanisms in vitro (34).

Varuna (Crataeva nurvala): Acts as a natural anti-inflammatory agent that protects the tubular basement membrane from oxidative stress (35,36)

Punarnava (Boerhavia diffusa): Promotes renal detoxification and supports the regenerative capacity of the tubular epithelium (37,38).

4. Actions of each herb in promoting Renal Organoid Development and Longevity

4a. Role of Punarnava root extract

Cytoprotective Microenvironment

Punarnava (B diffusa) serves as a valuable phytotherapeutic supplement in the culture medium used for renal organoid development. Tubular epithelial organoids, especially Kidney organoids.

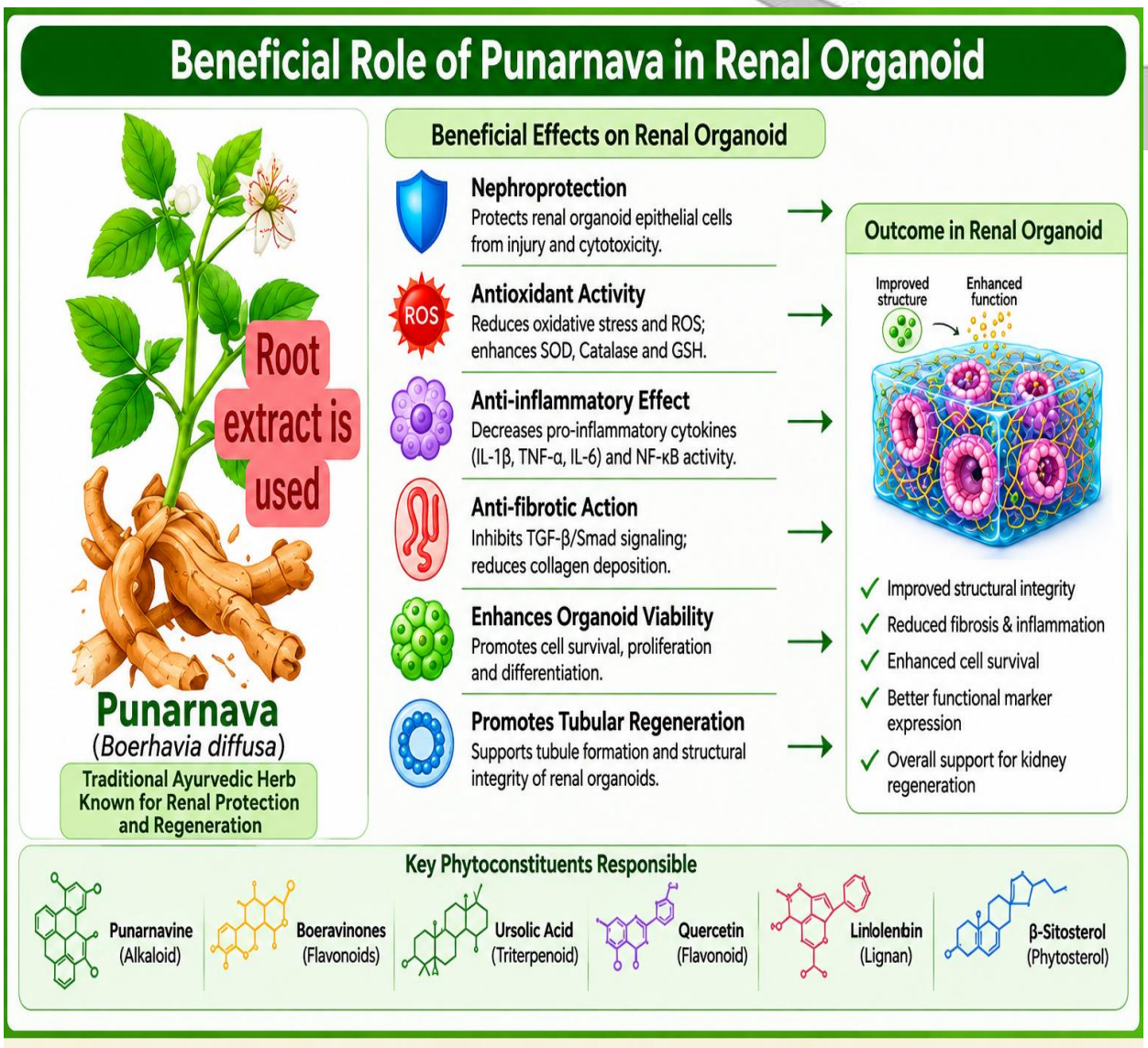


Figure 4. Protective effects of Punarnava on Renal Organoid development.

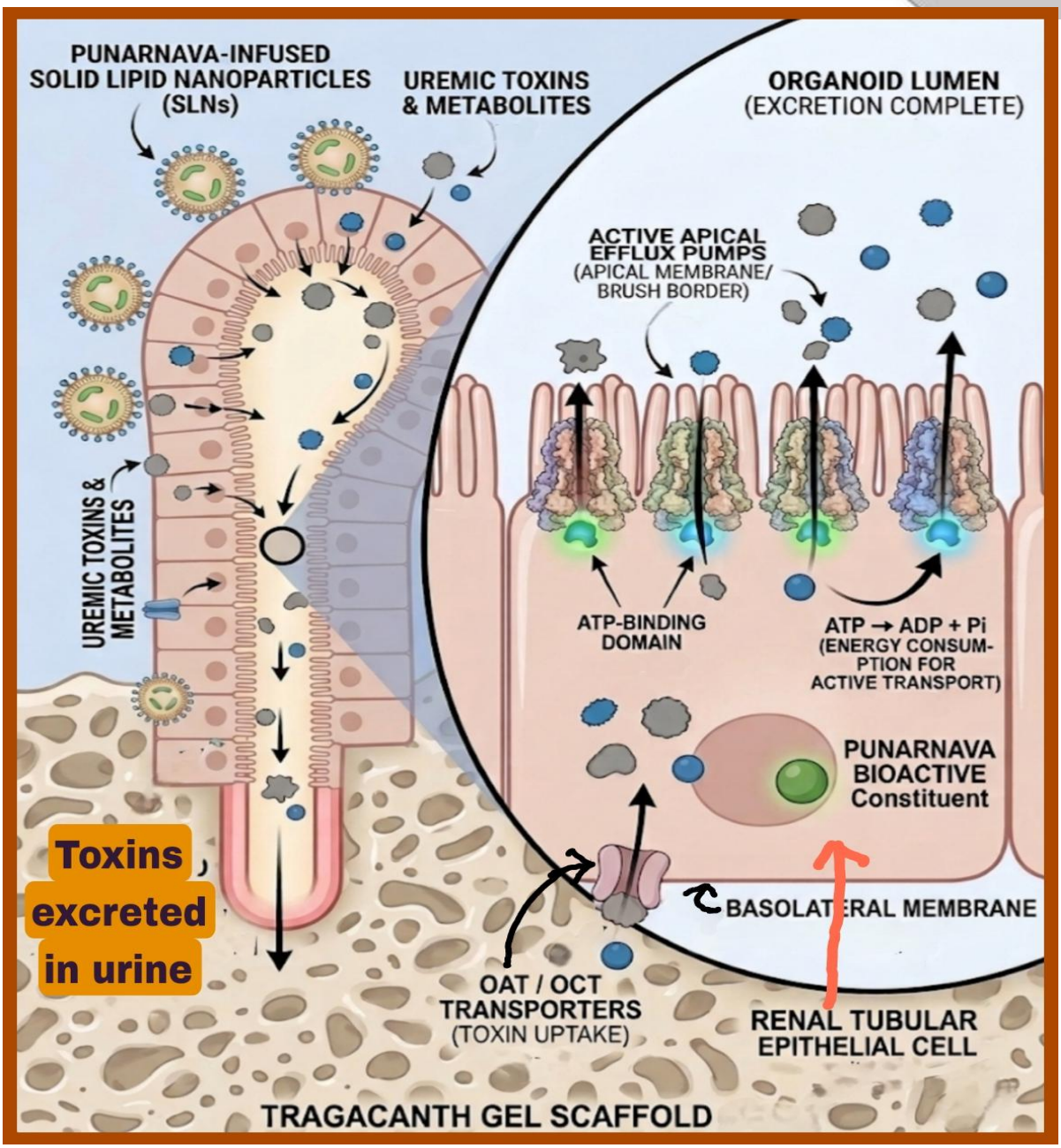
are highly susceptible to oxidative stress, inflammatory injury, and mitochondrial dysfunction during in-vitro growth (39,40). Punarnava contains flavonoids, boeravinones, alkaloids, and glycosides that can neutralize reactive oxygen species and stabilize cellular membranes (41,42). These antioxidant effects may improve survival of renal progenitor cells and developing tubular epithelium
(**Figure 4).**

i) Anti-inflammatory and Anti-fibrotic Effects

Inflammatory cytokines and fibrotic signalling can impair maturation of renal organoids. Punarnava has demonstrated suppression of NF-κB activation and inflammatory mediators in experimental models (43). Studies have also reported nephroprotective and anti-fibrotic actions through reduction of oxidative injury and modulation of TGF-β related pathways (44). Such effects could help preserve nephron-like architecture and reduce degeneration during prolonged organoid culture.

ii).Restoration of Tubular Transport Systems

Ion Channel Protection: Nephrotoxicity typically impairs the Na/K-ATPase pump located on the basolateral membrane of renal tubular epithelial cells. Punarnava directly mitigates oxidative stress within the renal cortex, restoring the activity of these vital ATP-dependent transport systems, which are the primary engines that power the OAT and OCT transporters. Punarnava bioactives also enhance the activity of the efflux pumps (that actively excrete toxins) located on the brush border of epithelial cells (see facing diagram).



Aquaporin-1 Upregulation: AQP-1 is a vital water channel protein located in the apical and basolateral membranes of the proximal convoluted tubules and the descending thin limbs of the loop of Henle. It reabsorbs massive amounts of water matching solute reabsorption in the nephron. Recent molecular evaluations indicate that Punarnava plays a role in the up-regulation of aquaporin-1 (AQP-1) expression. This optimization of water-channel transport supports balanced osmotic diuresis without inducing the acute electrolyte depletion often associated with synthetic loop diuretics (45).

iii).Enhancement of Cellular Repair

Punarnava supports mitochondrial ATP production and reduces apoptosis in renal tubular cells by decreasing oxidative stress and improving intracellular antioxidant defence systems (46,47). Improved cellular energetics may enhance proliferation and differentiation of epithelial cells within the organoid microenvironment.

4b. Role of Varuna (Crataeva nurvala) bark in Renal Organoid Culture

Varuna bark has been used for ages by traditional Indian medical system practitioners to provide relief from kidney ailments. The actions are summarized in **Figure 5.**

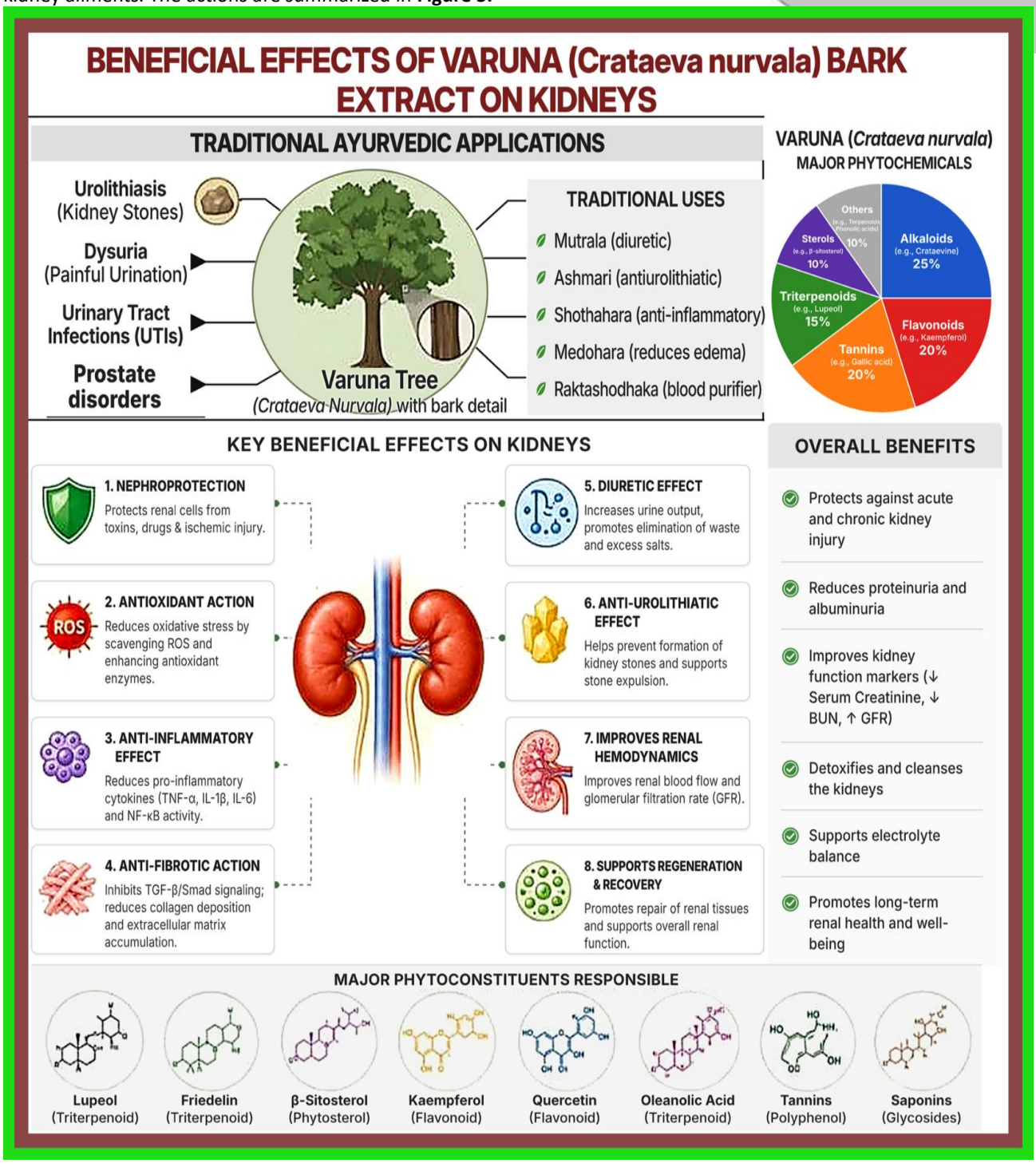


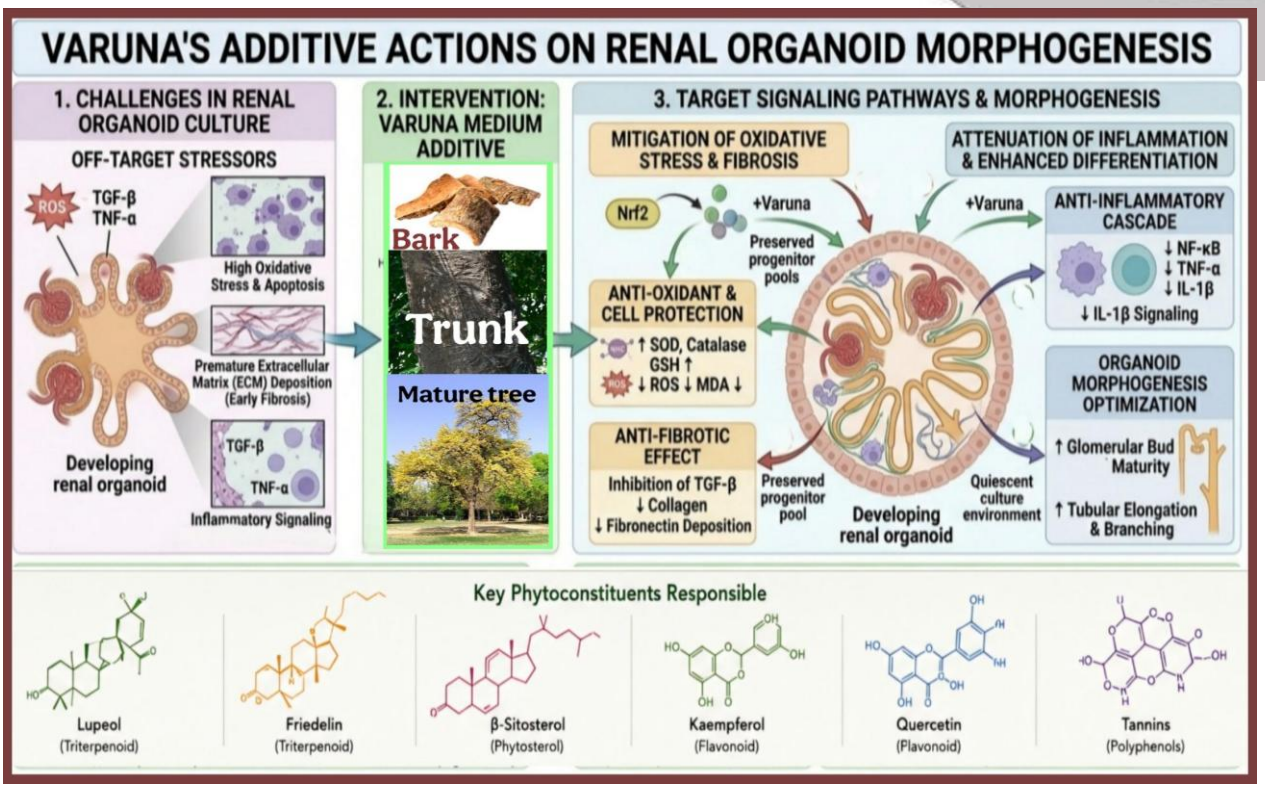
Figure 5. Beneficial actions of Varuna bark on normal kidneys.

Enhancing Renal Organoid Morphogenesis with Varuna

Integrating the bioactive fractions of Varuna (Crataeva nurvala) into the culture medium helps to optimize the differentiation, survival, and structural integrity of 3D renal organoids (Figure 6). Renowned for its nephroprotective properties, *Varuna's* phytochemical profile—rich in the pentacyclic triterpene *lupeol* and unique polyphenols—directly addresses severe cellular stress vulnerabilities encountered during "in vitro" nephrogenesis (48,49).

i). Mitigation of Oxidative Stress and Apoptosis

During the intensive mesenchymal-to-epithelial transition, emerging renal progenitor cells are highly vulnerable to radical-induced apoptosis. *Varuna* extracts modulate oxidative stress-related gene expression and restore



crucial antioxidant enzymes, including superoxide dismutase (SOD) and catalase, within the fluid medium (Jahan et al., 2023). This safeguards nascent nephron lineages by elevating reduced glutathione levels and decreasing lipid peroxidation, effectively preventing the premature necrosis of developing epithelial tissues (48).

Figure 6. The bark of Varuna tree is highly beneficial for renal tissue

ii). Varuna as Nrf2 activator

Studies suggests that Varuna helps protect organs—particularly the kidneys and heart—by acting as an **Nrf2 activator** (50). By replenishing the glutathione levels and Nrf2 factor levels, Varuna bark modulates oxidative stress by helping restore the Nrf2-HO-1 pathway (51). Studies on animal models also show that Varuna, by increasing antioxidant levels and decreasing inflammation in the kidneys by replenishing Nrf2, exerts a renal protection action.

iii). Suppression of Off-Target Fibrosis

A significant challenge in long-term organoid development is the abnormal proliferation of myofibroblasts, which leads to premature formation of scar-like structures. *Varuna* helps limit excessive extracellular matrix accumulation and collagen deposition within renal tissues (50). Through suppression of TGF-β-driven pro-fibrotic pathways, Varuna extract may help maintain the structural openness required for proper tubular growth, elongation, and branching, thereby supporting organoid architecture (50, 52).

iv). Attenuation of Inflammatory Cascades

By suppressing localized inflammatory gene expression, Varuna extract maintains a quiescent, homeostatic culture environment (50,53). This baseline protection limits inflammatory cell degradation, successfully steering the organoid towards developing into a highly organized and functionally mature 3D- tissue model, with glomerular and tubular segmentation (49).

4c. Role of Tribulus terrestris in Tragacanth-Based Renal Organoid Matrix

Tribulus terrestris (*Gokshura or Gokhru*) is an Ayurvedic medicinal herb known for its nephroprotective and anti-inflammatory properties (Figure 7). When incorporated into a tragacanth-based renal organoid matrix, it may help create a biologically supportive microenvironment for renal cell growth and differentiation.

Beneficial Effects of Gokhru (Tribulus terrestris) on the Kidneys in General

Gokhru (Ayurvedic: Gokshura; botanical name *Tribulus terrestris*) is a well-known medicinal herb, mentioned in all ancient Ayurvedic treatises, which has been used for maintaining urinary and renal health. Experimental studies have demonstrated significant nephro- protective effects of Gokhru, through its antioxidant, anti-

inflammatory, and diuretic properties. The herb reduces oxidative stress in renal tissues by enhancing endogenous antioxidant enzyme production, consequently decreasing lipid peroxidation, thereby protecting kidney cells from injury (54,55). It has also been shown to ameliorate gentamicin-induced nephrotoxicity, improving renal histology and preserving kidney function through reduction of oxidative damage and inflammation (56).

Gokhru has also exhibited renoprotective effects in hypertensive renal injury by protecting glomerular endothelial cells and modulating inflammatory signalling pathways (57). Additionally, it has demonstrated antiurolithiatic activity by reducing urinary stone formation.

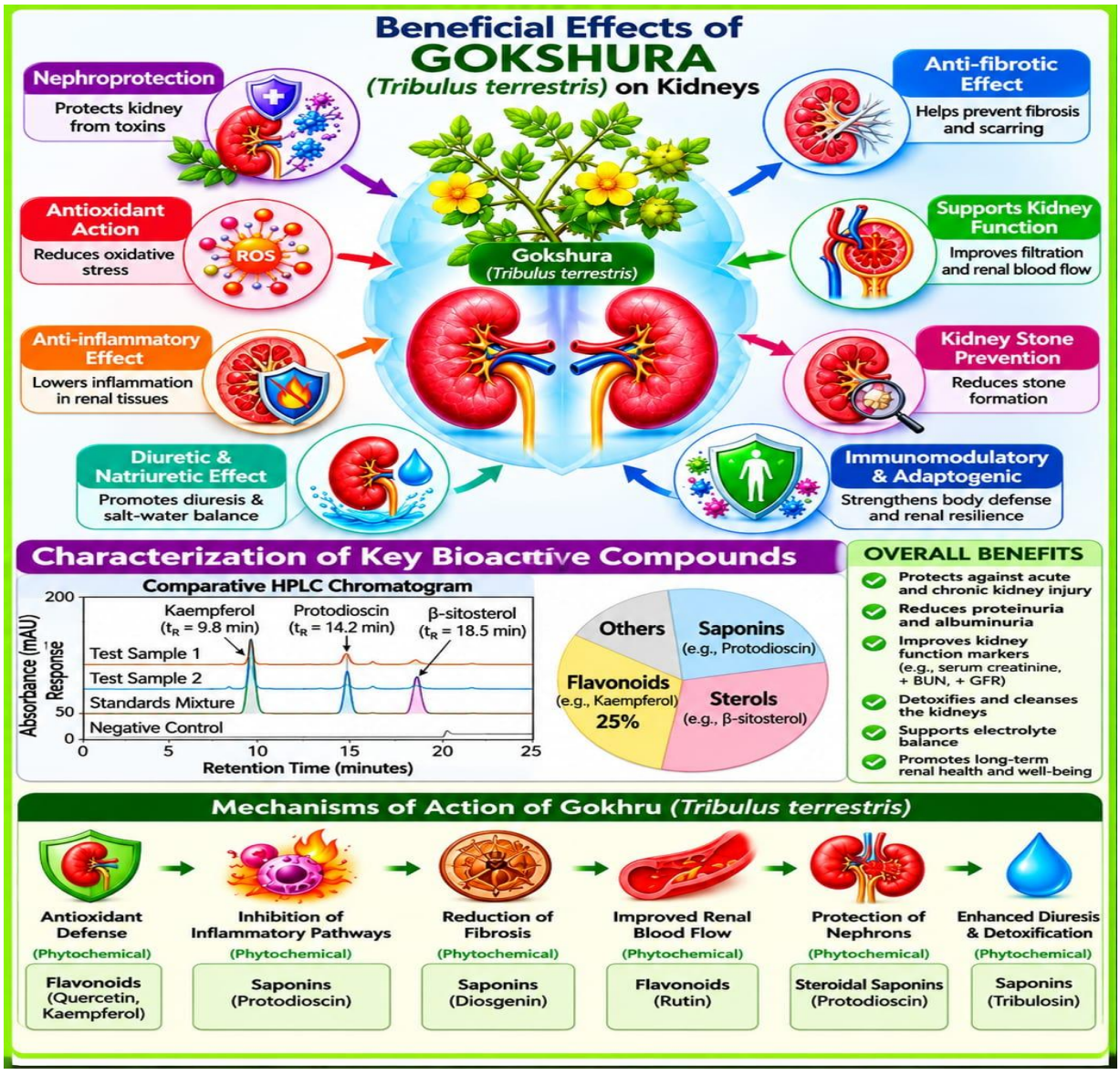


Figure 7. Beneficial effects of T. terrestris (*Gokhru*) on the renal tissues

restoring normal renal architecture, and improving renal function in experimental models of urolithiasis (58). Its mild diuretic action promotes urine flow and aids in the elimination of urinary toxins and crystals, supporting overall kidney health (59). These findings support the traditional Ayurvedic use of Gokhru as a valuable nephroprotective and urinary tract-supportive herb.

Nephroprotective Effects of T. terrestris when used in development of renal organoid

T. terrestris contains steroidal saponins, flavonoids, and polyphenols that reduce oxidative stress and inflammatory injury in renal tissues, thus protecting tubular epithelial cells from nephrotoxic damage (60,61). It has been shown that Calcium oxalate crystals damage renal tubular epithelial cells by adhering to the surface of these epithelial cells, triggering endocytosis, mitochondrial dysfunction, and oxidative stress, which ultimately kills the cell. Since Gokshura saponins directly inhibit the nucleation, growth, and aggregation of calcium oxalate

crystals, crystal-cell adhesion is minimized, thereby mitigating downstream oxidative stress and preservation of cell viability (62,63)

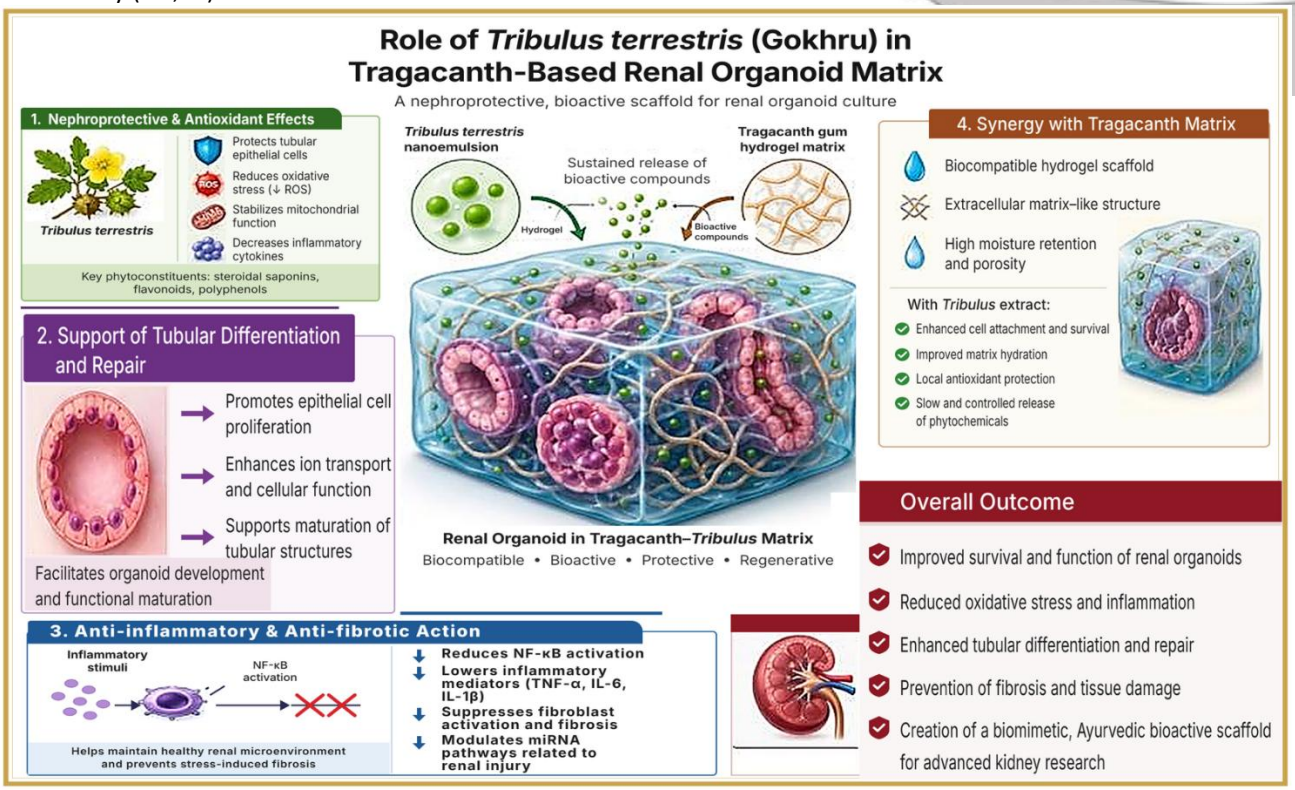


Figure 8. Reno-protective and Tubular differentiation–promoting activity of T. terrestris

These effects hold promise in improving survival and maturation of renal organoid cells cultured within the tragacanth matrix (**Figure 8).** Incorporation of nanoemulsified Tribulus extract into tragacanth hydrogel enables sustained phytochemical release and enhanced extracellular signalling. Gokshura-derived phytochemicals may also help maintain a pro-regenerative microenvironment by reducing oxidative stress and fibrosis-associated TGF-β signalling, thereby supporting renal tubular epithelial survival, differentiation, and matrix–cell interactions during organoid maturation (64,65).

5. Discussion

Biological gels, both animal cell and plant based, are being increasingly used for organoid culture (66). Out of these, plant-derived gels, such as agarose gel, gellan gums, nanocellulose hydrogels, offer a low-cost, chemically defined alternative to animal tumour extracts like Matrigel for 3D organoid culture (67,68). These sustainable plant-based matrices mimic the extracellular mechanics of Matrigel, feature tuneable porosity, and can be functionalized with adhesion peptides to support mini-organ growth (68,69). The further benefits of Plant-Derived Gels include low production cost, defined composition (eliminates risk of unknown animal pathogens), uncomplicated tuning capacity and eco-friendly attributes (66,67).

Tragacanth gum is a plant derived gel, derived from the Sterculia urens tree, and is called *Katira goond* in the vernacular. It acts as a natural hydrogel scaffold with excellent biocompatibility and moisture-retaining properties (70). It can be structurally stabilized by various cross-linking processes, as described in Section 3 above. The integration of herbal extract-infused Ghritas with tragacanth gel presents a significant advantage in the development of functional bio interfaces. This hybrid scaffold offers the unique gain of mimicking the biochemical interactions between bioactive phytochemicals and tissue cells within a lipid-enriched biological matrix, thereby providing a physiologically relevant microenvironment for cellular growth, signalling, and tissue regeneration.

5a. Conversion of traditional *ghrita* into nano- lipid particles (LBDDS) for enhanced Bioavailability and Absorption

Ghritas (clarified butter infused with herbal extracts) can be converted into nano-emulsions and solid lipid nanoparticles (SLNs) using modern lipid nanotechnology techniques (Figure 9). Conversion of the *Ghrita* into Nano forms significantly improves the delivery and bioavailability
of herbal phytochemicals to the developing cells of the tissue organoid (71,72). For preparing a **nano-emulsion**, medicated *ghrita* (containing herbal extracts) is heated and mixed with an aqueous phase containing surfactants like Tween-80 and co-surfactants such as PEG-400 (73,74). High-speed homogenization and ultra sonication reduce the lipid droplets into nanosized particles, usually between 50–200 nm, producing a stable nano-emulsion with enhanced membrane permeability, improved phytochemical dispersion, and better cellular uptake (75).

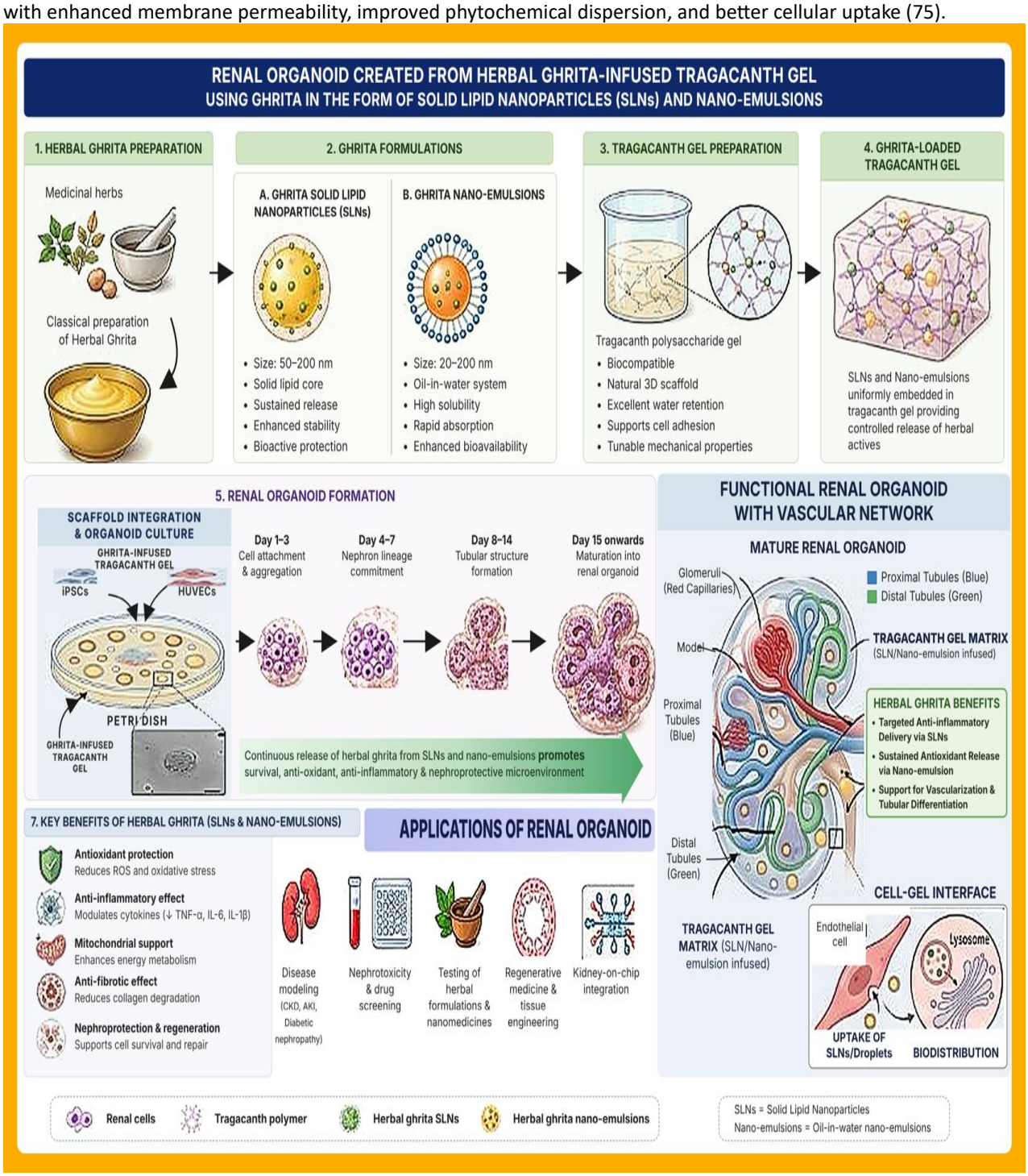


Figure 9. Diagram of renal organoid created using ghrita -infused tragacanth gel, showing the *ghrita* (*Ghee* infused with herbal extracts) used in the form of SLNs and nano-emulsions.

For **SLN** preparation, the ghrita is combined with solid lipids such as stearic acid or glyceryl monostearate and heated above the lipid melting point (72). The molten lipid phase is then dispersed into a heated aqueous surfactant solution using high-pressure homogenization followed by ultrasonication (76). Upon cooling, the lipid recrystallizes to form solid lipid nanoparticles encapsulating the herbal phytochemicals. These SLNs provide sustained release, protection from oxidation, and prolonged biological activity, making them useful for regenerative medicine, renal organoid culture, and targeted nephroprotective delivery systems (77).

5b. Advantages of a Renal Organoid grown in Bioactive medium, in Testing of new drugs and Research

The principal usefulness of this hybrid organoid platform is its capacity to evaluate bioactive compounds derived from traditional medicinal systems in a physiologically relevant three-dimensional microenvironment. Unlike conventional in vitro cell cultures, the organoid architecture facilitates the cellular uptake, metabolism, and biological interaction of these compounds in a manner that more closely resembles native tissue. This enables comprehensive assessment of their pharmacological efficacy, cytoprotective effects and mechanisms of action. Furthermore, the platform is highly amenable to *high-throughput screening*, allowing the rapid evaluation of large libraries of traditional medicinal formulations, herbal extracts, purified phytochemicals, or nanoparticle-based delivery systems. Consequently, this hybrid organoid serves as a robust translational model for identifying promising therapeutic candidates and accelerating the scientific validation of traditional or modern medicines prior to animal studies and clinical investigations.

Nephrotoxicity Screening : Because the scaffold contains protective herbal compounds, it can be used to determine the "protective threshold" of traditional medicines against nephrotoxic drugs like cisplatin or gentamicin.

Disease Modelling : The presence of Varuna and Gokshura within the matrix allows for the creation of "calculi-prone" models to test how effectively these herbs prevent crystal adhesion to tubular walls (78,79).

Sustainable Bioengineering : Using natural polymers like Tragacanth aligns with "green medicine" approaches, offering a low-cost, biodegradable alternative to expensive synthetic matrices like Matrigel (80).

Increased longevity of Organoid **:** An additional advantage of the cross-linked tragacanth gum (TG) hydrogel is its ability to improve the structural stability and functional longevity of the organoid. The reinforced hydrogel network slows degradation, preserves a hydrated microenvironment, and supports long-term cell viability and tissue architecture. As a result, the organoids remain viable for extended culture periods, enabling prolonged drug exposure, repeated dosing, and more reliable **high-throughput screening of traditional and modern medicinal compounds.**

5c. Challenges in Renal Organoid Development and the Potential of Tragacanth Gel–Based Lipid Drug Delivery Systems Containing Phytochemicals to Overcome Them

The generation of renal organoids from stem cells is often limited by several biological and matrix structural challenges. One major problem is poor cellular survival due to oxidative stress, inflammatory injury, and nutrient diffusion limitations within the three-dimensional organoid matrix (81). Renal epithelial and podocyte-like cells are highly sensitive to reactive oxygen species (ROS), hypoxia, and shear stress, leading to premature degeneration of nephron-like structures (82). Another major stumbling block is inadequate extracellular matrix (ECM) support, resulting in poor cellular attachment, abnormal differentiation, fibrosis-like changes, and limited maturation of renal tubules (83). Synthetic matrix scaffolds may also fail to adequately create the hydrated microenvironment of native kidney tissue.

A lipid-based drug delivery system (LBDDS) formulated with these phytochemicals and incorporated into a tragacanth gel may help overcome several of these limitations. *Punarnava* possesses antioxidant and anti-inflammatory phytochemicals that help reduce ROS-mediated tubular injury and cytokine-induced degeneration (84). *Gokshura* may improve cellular resilience and membrane stability through its flavonoids and steroidal saponins, while *Varuna* may reduce inflammatory and fibrotic responses within the organoid microenvironment (85). Incorporation of these phytoconstituents into ghrita-based solid lipid nanoparticles (SLNs) can improve sustained release and membrane penetration of lipophilic bioactive compounds (86).

6. Conclusion

Herbal *Ghrita*-infused Tragacanth gel model represents a paradigm shift in renal organoid engineering by integrating the structural reliability of a natural polysaccharide scaffold with the functional pharmacological benefits of lipid-based Ayurvedic formulations (87). Tragacanth gel functionalized with Adhesion Molecules, Wnt Activators, Stabilizers and Nutrient Support,

provides a hydrated, biocompatible extracellular matrix (ECM)-like environment that supports cell adhesion, nutrient diffusion, and structural integrity while reducing shear stress during microfluidic perfusion. In combination with the herbal lipid-based drug delivery systems (LBDDS), it creates a biomimetic and

cytoprotective microenvironment that promotes long-term organoid viability, maturation, and functional stability (**Figure 10**).

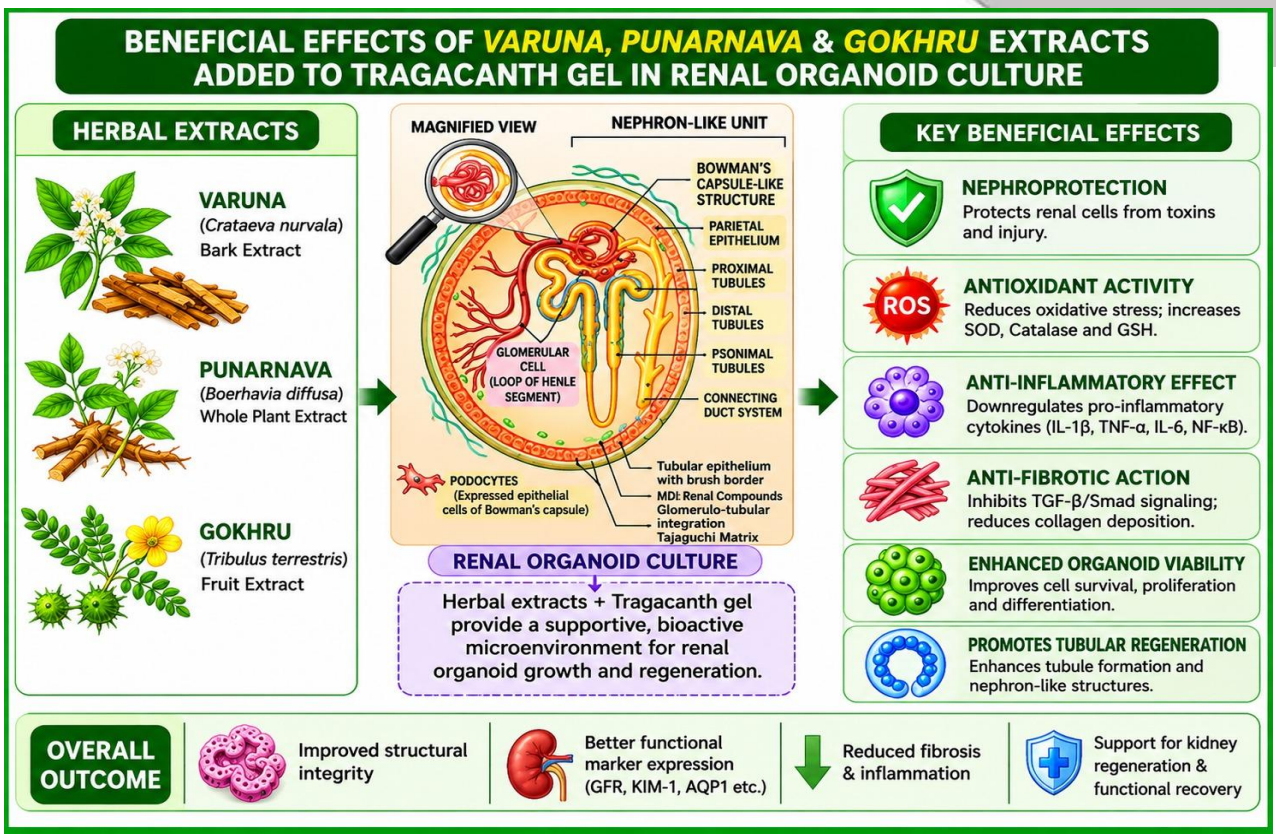


Figure 10 : A diagram showing how the herbs improve the survivability and functionality of the renal organoid.

This hybrid platform also serves as a "green medicine" alternative to synthetic matrices like Matrigel (88). It aligns with the current shift toward laboratory-based evaluations that prioritize ethical standards by reducing animal testing. Furthermore, the ability to standardize the concentration of phytochemicals within the SLN-infused gel provides a high level of reproducibility, which is essential for the pharmaceutical validation of traditional Ayurvedic formulations. In conclusion, the *Ghrita*-infused Tragacanth organoid offers a high-fidelity model for investigating renal physiology and disease, evaluating nephrotoxicity, and assessing the therapeutic efficacy of phytochemicals and new drug molecules. Further research can be based upon the concepts included herein, using a mixture of cross-linked plant hydrogels to increase the degradation time, and using nanolipid delivered phytochemicals to increase functional capacity and survivability of the organoids.

References

1. Takasato, M., Er, P. X., Chiu, H. S., & Little, M. H. (2016). Generation of kidney organoids from human pluripotent stem cells. Nature Protocols, 11(9), 1681–1692. DOI: 10.1038/nprot.2016.098
2. Dogra S, Yadav D, Koul B, Rabbee MF. Plant-Based Polysaccharide Gums as Sustainable Bio-Polymers: Focus on Tragacanth Gum and Its Emerging Applications. Polymers (Basel). 2025 Nov 27;17(23):3163. doi: 10.3390/polym17233163.
3. Murphy, S. V., Skardal, A., & Atala, A. (2013). Evaluation of hydrogels for bioprinting applications. Journal of Biomedical Materials Research Part A, 101(1), 272–284. DOI: 10.1002/jbm.a.34326
4. Mishra S, Aeri V, Gaur PK, Jachak SM. Phytochemical, therapeutic, and ethnopharmacological overview for a traditionally important herb: Boerhavia diffusa Linn. Biomed Res Int. 2014;2014:808302. doi: 10.1155/2014/808302.
5. Naseri, N., Valizadeh, H., & Zakeri-Milani, P. (2015). Solid lipid nanoparticles and nanostructured lipid carriers: Structure, preparation and application. Advanced Pharmaceutical Bulletin, 5(3), 305–313. DOI: 10.15171/apb.2015.043
6. Zhou Z, Cong L, Cong X. Patient-Derived Organoids in Precision Medicine: Drug Screening, Organoid-on-a-Chip and Living Organoid Biobank. Front Oncol. 2021 Dec 30;11:762184. doi: 10.3389/fonc.2021.762184.

7. Low, J. H., Li, P., Chew, E. G. Y., Zhou, B., Suzuki, K., Zhang, T., et al (2019). Generation of human PSC-derived kidney organoids with patterned nephron segments and a de novo vascular network. Cell Stem Cell, 25(3), 373-387. DOI: 10.1016/j.stem.2019.06.009
8. National Academies of Sciences, Engineering, and Medicine; Division on Earth and Life Studies; Institute for Laboratory Animal Research; Rose Li and Associates, Inc.. Microphysiological Systems: Bridging Human and Animal Research: Proceedings of a Workshop—in Brief. Washington (DC): National Academies Press (US); 2021 Aug 30. DOI: 10.17226/26124
9. Astashkina, A., Mann, B., & Grainger, D. W. (2012). A critical evaluation of in vitro cell culture models for high-throughput drug screening and toxicity. Pharmacology & Therapeutics, 134(1), 82-106. Doi: 10.1016/j.pharmthera.2012.01.001
10. Kim JW, Nam SA, Yi J, et al. Kidney decellularized extracellular matrix enhanced the vascularization and maturation of human kidney organoids. Adv Sci (Weinh). 2022;9(15):e2103526. doi:10.1002/advs.202103526.
11. Tayeb, S. (2026). A Comprehensive Review of Polysaccharide-Based Bioinks for 3D Bioprinting: Analyzing Structure–Property–Processing Relationships and Addressing Translational Challenges. Preprints. https://doi.org/10.20944/preprints202602.1845.v1
12. Yazdi T, M.E, Nazarnezhad, S.; Mousavi, S.H.; Sadegh Amiri, M, Darroudi, M.; Baino, F.; Kargozar, S. Gum Tragacanth (GT): A Versatile Biocompatible Material beyond Borders. Molecules 2021, 26, 1510. https://doi.org/10.3390/molecules26061510
13. Apablaza, J. A., Lezcano, M. F., Lopez Marquez, A., Godoy Sánchez, K., Oporto, G. H., & Dias, F. J. (2021). Main Morphological Characteristics of Tubular Polymeric Scaffolds to Promote Peripheral Nerve Regeneration—A Scoping Review. Polymers, 13(15), 2563. https://doi.org/10.3390/polym13152563
14. Abdi G, Jain M, Patil N, Tariq M, Choudhary S, Kumar P, Raj NS, Mohsen Ali SS and Uthappa UT (2024) Tragacanth gum-based hydrogels for drug delivery and tissue engineering applications. Front. Mater. 11:1296399. doi: 10.3389/fmats.2024.1296399
15. Glasman JD, Rubinstein AJ, Alaimo A, Pérez OE. Recent advances in genipin-crosslinked biopolymer nanosystems for biomedical and food engineering applications. J Drug Deliv Sci Technol. 2026;122:108383. doi:10.1016/j.jddst.2026.108383
16. Khadijeh Hemmati, Arameh Masoumi, Mousa Ghaemy; Synthesis and characterization of pH-responsive nanohydrogels as biocompatible drug carriers based on chemically modified tragacanth gum polysaccharide. *RSC Adv.* 2015; 5 (104): 85310–85318. https://doi.org/10.1039/c5ra14356j
17. Codispoti G, Trucco D, Carniato M, et al. Visible light-mediated crosslinked methacrylated gellan gum nanocomposite hydrogel: physicochemical characterization and in vivo safety. Mater Des. 2026;267:116244. doi:10.1016/j.matdes.2026.116244.
18. Silva-Correia J, Zavan B, Vindigni V, et al. Biocompatibility evaluation of ionic- and photo-crosslinked methacrylated gellan gum hydrogels: in vitro and in vivo study. Adv Healthc Mater. 2013;2(4):568-575. doi:10.1002/adhm.201200256
19. Takasato, M., Er, P. X., Becroft, M., Vanslambrouck, J. M., Stanley, E. G., Elefanty, A. G., & Little, M. H. (2015). Directing human embryonic stem cell differentiation towards a renal lineage generates a self-organizing kidney. Nature Cell Biology, 16(1), 118–126. DOI : 10.1038/ncb2894
20. Freedman, B. S., Brooks, C. R., Lam, A. Q., Fu, H., Morizane, R., Agrawal, V., Saad, A. F., Li, M. K., Hughes, M. R., et al.(2015). Modelling kidney disease with CRISPR-mutant kidney organoids derived from human pluripotent epiblast spheroids. Nature Communications, 6, 8715. DOI: 10.1038/ncomms9715
21. Hynes, R. O. (2002). Integrins: bidirectional, allosteric signaling machines. Cell, 110(6), 673–687. DOI: 10.1016/s0092-8674(02)00971-6
22. Kleinman, H. K., Philp, D., & Hoffman, M. P. (2003). Role of the extracellular matrix in morphogenesis. Current Opinion in Biotechnology, 14(5), 526–532. DOI: 10.1016/j.copbio.2003.08.002
23. Ruoslahti, E. (1996). RGD and other recognition sequences for integrins. Annual Review of Cell and Developmental Biology, 12, 697–715. DOI: 10.1146/annurev.cellbio.12.1.697
24. Hersel, U., Dahmen, C., & Kessler, H. (2003). RGD modified polymers: biomaterials for stimulated cell adhesion and beyond. Biomaterials, 24(24), 4385–4415. DOI: 10.1016/s0142-9612(03)00343-0
25. Morizane, R., Lam, A. Q., Freedman, B. S., Kishi, S., Valerius, M. T., & Bonventre, J. V. (2015). Nephron organoids derived from human pluripotent stem cells model kidney development and injury. Nature Biotechnology, 33(11), 1193–1200. DOI: 10.1038/nbt.3392
26. Barak, H., Huh, S. H., Chen, S., Jeanpierre, C., Martinovic, J., Parisot, M., Bole-Feysot, C., Nitschké, P., Salomon, R., Antignac, C., & Ornitz, D. M. (2012). FGF9 and FGF20 maintain the stemness of nephron progenitors in mice and man. Developmental Cell, 22(6), 1191–1207. DOI: 10.1016/j.devcel.2012.04.018

27. Tsujimura T, Idei M, Yoshikawa M, Takase O, Hishikawa K. Roles and regulation of bone morphogenetic protein-7 in kidney development and diseases. World J Stem Cells. 2016 Sep 26;8(9):288-96. doi: 10.4252/wjsc.v8.i9.288.
28. Oxburgh, L., Dudley, A. T., Godin, R. E., Koonce, C. H., Islam, A., Anderson, D. C., Bikoff, E. K., & Robertson, E. J. (2005). BMP4 substitutes for loss of BMP7 during kidney development. Developmental Biology, 286(2), 637–646. DOI: 10.1016/j.ydbio.2005.08.024
29. Zhang, J., Nuebel, E., Daley, G. Q., Koehler, C. M., & Teitell, M. A. (2016). Metabolic regulation in pluripotent stem cells during reprogramming and self-renewal. Cell Stem Cell, 11(5), 589–595. DOI : 10.1016/j.stem.2012.10.005
30. Barnes, D., & Sato, G. (1980). Serum-free cell culture: a unifying approach. Cell, 22(3), 649–655. DOI: 10.1016/0092-8674(80)90540-1
31. Engler, A. J., Sen, S., Sweeney, H. L., & Discher, D. E. (2006). Matrix elasticity directs stem cell lineage specification. Cell, 126(4), 677–689. DOI : 10.1016/j.cell.2006.06.044
32. Gjorevski, N., Sachs, N., Manfrin, A., Giger, S., Bragina, M. E., Ordóñez-Morán, P., Clevers, H., & Lutolf, M. P. (2016). Designer matrices for intestinal stem cell and organoid culture. Nature, 539(7630), 560–564. DOI: 10.1038/nature20168
33. Bagdi P. Medicinal Plants in the Management of Urolithiasis: Phytochemical Constituents and Pharmacological Evidence. Journal of Drug Delivery and Therapeutics. 2026; 16(5):139-144. DOI: https://dx.doi.org/10.22270/jddt.v16i5.7715
34. Mahboubi M, Tribulus Terrestris and its Efficacy in the Treatment of Urinary Calculi, The Natural Products Journal; Volume 12, Issue 7, Year 2022, e310322202907. DOI:10.2174/2210315512666220331153916
35. Agarwal S, Gupta SJ, Saxena AK, Gupta N, Agarwal S. Urolithic property of Varuna (Crataeva nurvala): An experimental study. Ayu. 2010 Jul;31(3):361-6. doi: 10.4103/0974-8520.77161.
36. Sudhanshu Kumar Meher, P K Mukherjee, S K Banarjee Chaudhury, Bani Marjit, B PShaw. Experimental studies on the Renal Protective effect of Gokshura (Tribulus terrestris Linn) and Varuna (Crataeva nurvala). Research J. Pharmacology & Pharmacodynamics. 2016; 8(2): 75-82. doi: 10.5958/2321-5836.2016.00014.8
37. Sawardekar SB, Patel TC. Evaluation of the effect of Boerhavia diffusa on gentamicin-induced nephrotoxicity in rats. J Ayurveda Integr Med. 2015 Apr-Jun;6(2):95-103. doi: 10.4103/0975-9476.146545.
38. Oburai NL, Rao VV, Bonath RB. Comparative clinical evaluation of Boerhavia diffusa root extract with standard Enalapril treatment in Canine chronic renal failure. J Ayurveda Integr Med. 2015 Jul-Sep;6(3):150-7. doi: 10.4103/0975-9476.166390.
39. Takasato M, Er PX, Chiu HS, Maier B, Baillie GJ, Ferguson C, Parton RG, Wolvetang EJ. Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis. Nature. 2015 Oct 22;526(7574):564-8. doi: 10.1038/nature15695.
40. Kim YK, Refaeli I, Brooks CR, Jing P, Gulieva RE, Hughes MR, Cruz NM, Liu Y, Churchill AJ, Wang Y, Fu H, Pippin JW, Lin LY, Shankland SJ, Vogl AW, McNagny KM, Freedman BS. Gene-Edited Human Kidney Organoids Reveal Mechanisms of Disease in Podocyte Development. Stem Cells. 2017 Dec;35(12):2366-2378. doi: 10.1002/stem.2707.
41. Chandan BK, Sharma AK, Anand KK. Boerhaavia diffusa: a study of its hepatoprotective activity. J Ethnopharmacol. 1991 Mar;31(3):299-307. doi: 10.1016/0378-8741(91)90015-6.
42. Beegum GRJ, Beevy S S, V S Sugunan V S (2016). Natural antioxidant activity of Boerhavia diffusa International Journal of Pharmacognosy and Phytochemical Research 2016; 8(1); 8-13
43. Jalan, R. S., Arivarasu, L., Rajeshkumar, S., & Thangavelu, L. (2021). Anti-Inflammatory Activity of Boerhavia diffusa Zinc Oxide Nanoparticle. Journal of Pharmaceutical Research International, 33(61B), 343–353. https://doi.org/10.9734/jpri/2021/v33i61B35607
44. Kumar, Rajesh & Singh, K D & Kumar, Pramod & Haque, Nilufar & Yadav, Vibha & Kumar, Rabindra & Kumar, S. (2018). Pharmacological properties of Boerhavia diffusa: A review. International Journal of Chemical Studies 2018; SP4: 72. DOI : 10.13140/RG.2.2.13517.69608.
45. Juneja K, Mishra R, Chauhan S, Gupta S, Roy P, Sircar D. Metabolite profiling and wound-healing activity of *Boerhavia diffusa* leaf extracts using *in vitro* and *in vivo* models. J Tradit Complement Med. 2019 Feb 19;10(1):52-59. doi: 10.1016/j.jtcme.2019.02.002.
46. Lee OYA, Wong ANN, Ho CY, Tse KW, Chan AZ, Leung GP, Kwan YW, Yeung MHY. Potentials of Natural Antioxidants in Reducing Inflammation and Oxidative Stress in Chronic Kidney Disease. Antioxidants (Basel). 2024 Jun 20;13(6):751. doi: 10.3390/antiox13060751.
47. Prathapan A, Vineetha VP, Raghu KG. Protective effect of Boerhaavia diffusa L. against mitochondrial dysfunction in angiotensin II induced hypertrophy in H9c2 cardiomyoblast cells. PLoS One. 2014 Apr 30;9(4):e96220. doi: 10.1371/journal.pone.0096220.

48. Shirwaikar A, Manjunath S M, Bommu P & Krishnanand B (2004) Ethanol Extract of Crataeva nurvala Stem Bark Reverses Cisplatin-induced Nephrotoxicity, Pharmaceutical Biology, 42:7, 559-564, DOI: 10.3109/13880200490901249
49. Dalimunthe A, Carensia Gunawan M, Dhiya Utari Z, Dinata MR, Halim P, Estherina S Pakpahan N, Sitohang AI, Sukarno MA, Yuandani, Harahap Y, Setyowati EP, Park MN, Yusoff SD, Zainalabidin S, Prananda AT, Mahadi MK, Kim B, Harahap U, Syahputra RA. In-depth analysis of lupeol: delving into the diverse pharmacological profile. Front Pharmacol. 2024 Nov 13;15:1461478. doi: 10.3389/fphar.2024.1461478.
50. Jahan I, Saha P, Eysha Chisty TT, Mitu KF, Chowdhury FI, Ahmed KS, Hossain H, Khan F, Subhan N, Alam MA. *Crataeva nurvala* Bark (Capparidaceae) Extract Modulates Oxidative Stress-Related Gene Expression, Restores Antioxidant Enzymes, and Prevents Oxidative Stress in the Kidney and Heart of 2K1C Rats. Evid Based Complement Alternat Med. 2023 Aug 9;2023:4720727. doi: 10.1155/2023/4720727.
51. Choucry MA, Khalil MNA, El Awdan SA. Protective action of Crateva nurvala Buch. Ham extracts against renal ischaemia reperfusion injury in rats via antioxidant and anti-inflammatory activities. J Ethnopharmacol. 2018 Mar 25;214:47-57. doi: 10.1016/j.jep.2017.11.034. Epub 2017 Dec 5. PMID: 29217496.
52. Meng XM, Nikolic-Paterson DJ, Lan HY. TGF-β: the master regulator of fibrosis. Nat Rev Nephrol. 2016 Jun;12(6):325-38. doi: 10.1038/nrneph.2016.48. DOI: 10.1038/nrneph.2016.48
53. Huang S, Mo C, Zeng T, Lai Y, Zhou C, Xie S, Chen L, Wang Y, Chen Y, Huang S, Gao L, Lv Z. Lupeol ameliorates LPS/D-GalN induced acute hepatic damage by suppressing inflammation and oxidative stress through TGFβ1-Nrf2 signal pathway. Aging (Albany NY). 2021 Mar 11;13(5):6592-6605. doi: 10.18632/aging.202409.
54. Najafi H, et al. (2014). *Protective effects of Tribulus terrestris L extract against acute kidney injury induced by reperfusion in rats*. Iranian Journal of Kidney Diseases, 8(4), 292–298.
55. Singh K, Yadav KS, Singh A, Bisen AC, Yadav G, Bhatta RS, Khan F, Mugale MN, Dev K. Tribulus terrestris L. extract alleviates diabetic nephropathy through regulation of oxidative stress and inflammation: insights from in silico, in vivo and LC-MS/MS-based metabolite profiling studies. J Ethnopharmacol. 2026 Apr 6;360:121180. doi: 10.1016/j.jep.2026.121180.
56. Al-Mohamadi A, Eldin IMT, Ibrahim D, Albadani R. Ameliorative Effects of *Tribulus Terrestris* (Qutayb) Extracts Against Gentamicin-Induced Nephrotoxicity in Wistar Rats. Int J Nephrol. 2025 Oct 29;2025:7620700. doi: 10.1155/ijne/7620700.
57. Pei HJ, Yang J, Hu FX, Chen YZ, Yang CH. *Tribulus terrestris L*. protects glomerular endothelial cells via the miR155-H2AC6 interaction network in hypertensive renal injury. Ann Transl Med. 2021 Nov;9(21):1626. doi: 10.21037/atm-21-5641.
58. Kaushik J, Tandon S, Bhardwaj R, Kaur T, Singla SK, Kumar J, Tandon C. Delving into the Antiurolithiatic Potential of Tribulus terrestris Extract Through -In Vivo Efficacy and Preclinical Safety Investigations in Wistar Rats. Sci Rep. 2019 Nov 4;9(1):15969. doi: 10.1038/s41598-019-52398-w.
59. Kiran, A., Kumar, S. E. P., Dash, D., Suresh, G., Pai, V. R., Amuthan, A., & Shenoy, K. G. (2025). Tribulus terrestris: A Revisit to a Promising Herbal Diuretic. *Pharmacognosy Journal*, *17*(5), 653-661. https://doi.org/10.5530/pj.2025.17.8
60. Raoofi A, Khazaei M, Ghanbari A. Protective effect of hydroalcoholic extract of Tribulus terrestris on cisplatin-induced renal tissue damage in male mice. Int J Prev Med. 2015;6:11. doi:10.4103/2008-7802.151817
61. Farokhi f, peeri m, azarbayjani ma, hosseini sa. the effects of tribulus terrestris extract and endurance training on oxidative stress and apoptotic markers in the kidney tissue of rats, poisoned with arsenic. gene cell tissue. 2023;10(3):e131298. doi: https://doi.org/10.5812/gct-131298
62. Kamboj P, Aggarwal M, Puri S, Singla SK. Effect of aqueous extract of Tribulus terrestris on oxalate-induced oxidative stress in rats. Indian J Nephrol. 2011 Jul;21(3):154-9. doi: 10.4103/0971-4065.83727.
63. Aggarwal A, Tandon S, Singla SK, Tandon C. Diminution of oxalate induced renal tubular epithelial cell injury and inhibition of calcium oxalate crystallization in vitro by aqueous extract of Tribulus terrestris. Int Braz J Urol. 2010 Jul-Aug;36(4):480-8; discussion 488, 489. doi: 10.1590/s1677-55382010000400011.
64. Sharma US, et al. Hydroalcoholic extract of Tribulus terrestris protects against cisplatin-induced nephrotoxicity in rats. International Journal of Pharmacotherapy. 2019
65. Chhatre S, Nesari T, Somani G, Kanchan D, Sathaye S. Phytopharmacological overview of Tribulus terrestris. Pharmacogn Rev. 2014 Jan;8(15):45-51. doi: 10.4103/0973-7847.125530. PMID: 24600195; PMCID: PMC3931200.
66. Jo, Y. K. (2023). Natural biopolymer-based hydrogels as designer matrices for organoid cultures. Organoid, 3, e13. https://doi.org/10.51335/organoid.2023.3.e13
67. Curvello, R., & Garnier, G. (2020). Cationic cross-linked nanocellulose-based matrices for the growth and recovery of intestinal organoids. Biomacromolecules, 22(2), 701-709. https://doi.org/10.1021/acs.biomac.0c01510

68. Curvello, R., Kerr, G., Micati, D. J., Chan, W. H., Raghuwanshi, V. S., Rosenbluh, J., Abud, H. E., & Garnier, G. (2020). Engineered plant-based nanocellulose hydrogel for small intestinal organoid growth. Advanced Science, 8(2), 2002135. https://doi.org/10.1002/advs.202002135
69. Poudel, H., Sanford, K., Szwedo, P. K., Pathak, R., & Ghosh, A. (2021). Synthetic matrices for intestinal organoid culture: Implications for better performance. ACS Omega, 7(1), 38-47. https://doi.org/10.1021/acsomega.1c05136
70. Priytama pawar, Sarika nikam, Ifat Shaikh, Anisha Nagar, Dhanraj Kande, Amol sapkal, Nisha Choudhary , Unveiling The Secrets of Gum Karaya – An Excipient , Int. J. of Pharm. Sci., 2026, Vol 4, Issue 4, 4056-4067, https://doi.org/10.5281/zenodo.19729963
71. Müller RH, Mäder K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery – a review of the state of the art. *Eur J Pharm Biopharm.* 2000;50(1):161-177. doi:10.1016/S0939-6411(00)00087
72. Mehnert W, Mäder K. Solid lipid nanoparticles: production, characterization and applications. *Adv Drug Deliv Rev.* 2001;47(2-3):165-196. doi:10.1016/S0169-409X(01)00105-3
73. Solans C, Izquierdo P, Nolla J, Azemar N, Garcia-Celma MJ. Nano-emulsions. *Curr Opin Colloid Interface Sci.* 2005;10(3-4):102-110. doi:10.1016/j.cocis.2005.06.004
74. Jaiswal M, Dudhe R, Sharma PK. Nanoemulsion: an advanced mode of drug delivery system. *3 Biotech.* 2015;5(2):123-127. doi:10.1007/s13205-014-0214-
75. Gupta A, Eral HB, Hatton TA, Doyle PS. Nanoemulsions: formation, properties and applications. *Soft Matter.* 2016;12(11):2826-2841. doi:10.1039/C5SM02958A
76. Pardeike J, Hommoss A, Müller RH. Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. *Int J Pharm.* 2009;366(1-2):170-184. doi:10.1016/j.ijpharm.2008.10.003
77. Ganesan P, Narayanasamy D. Lipid nanoparticles: different preparation techniques, characterization, hurdles, and strategies for the production of solid lipid nanoparticles and nanostructured lipid carriers for oral drug delivery. *Sustain Chem Pharm.* 2017;6:37-56. doi:10.1016/j.scp.2017.07.002
78. Aggarwal A, Tandon S, Singla SK, Tandon C. A novel antilithiatic protein from Tribulus terrestris having cytoprotective potency. Protein Pept Lett. 2012 Aug;19(8):812-9. doi: 10.2174/092986612801619552. PMID: 22702898.
79. Atanu Bhattacharjee, Shastry Chakrakodi Shashidhara, Aswathanarayana, Phytochemical and ethno-pharmacological profile of Crataeva nurvala Buch-Hum (Varuna): A review.Asian Pacific Journal of Tropical Biomedicine, Volume 2, Issue 2, Supplement, 2012, Pages S1162-S1168, https://doi.org/10.1016/S2221-1691(12)60379-7
80. Ng JY, Obuobi S, Chua ML, Zhang C, Hong S, Kumar Y, Gokhale R, Ee PLR. Biomimicry of microbial polysaccharide hydrogels for tissue engineering and regenerative medicine—A review. Carbohydr Polym. 2020;241:116345. doi:10.1016/j.carbpol.2020.116345.
81. Kim S, Takayama S. Organ-on-a-chip and the kidney. *Kidney Research and Clinical Practice*. 2016;35(1):11–22. DOI: 10.1016/j.krcp.2015.08.001
82. Homan KA, Gupta N, Kroll KT, et al. Flow-enhanced vascularization and maturation of kidney organoids in vitro. *Nature Methods*. 2019;16:255–262. DOI: 10.1038/s41592-019-0325-y
83. Garreta E, Prado P, Tarantino C, et al. Fine tuning the extracellular environment accelerates the derivation of kidney organoids from human pluripotent stem cells. *Nature Materials*. 2019;18:397–405. DOI: 10.1038/s41563-019-0287-6
84. Chaudhary, D. (2021). Boerhavia Diffusa (Punarnava): A Review Based on Its Ayurvedic and Modern Therapeutic Uses. International Journal of Research in Ayurveda and Pharmacy. https://doi.org/10.7897/2277-4343.120262
85. Ștefănescu R, Tero-Vescan A, Negroiu A, Aurică E, Vari CE. A Comprehensive Review of the Phytochemical, Pharmacological, and Toxicological Properties of *Tribulus terrestris* L. Biomolecules. 2020 May 12;10(5):752. doi: 10.3390/biom10050752.
86. Wissing SA, Kayser O, Müller RH. Solid lipid nanoparticles for parenteral drug delivery. Adv Drug Deliv Rev. 2004;56(9):1257–1272. doi:10.1016/j.addr.2003.12.002
87. Berradi A, Aziz F, Achaby ME, Ouazzani N, Mandi L. A Comprehensive Review of Polysaccharide-Based Hydrogels as Promising Biomaterials. Polymers (Basel). 2023 Jun 30;15(13):2908. doi: 10.3390/polym15132908.
88. Premjit Y, Lawrence M, Goyal A, Ferreira C, Jones EA, Ganguly P. Biomimetic Three-Dimensional (3D) Scaffolds from Sustainable Biomaterials: Innovative Green Medicine Approach to Bone Regeneration. J Funct Biomater. 2025 Jun 29;16(7):238. doi: 10.3390/jfb16070238.