All Kerala Govt. Ayurveda College Teacher's Association
KERALA JOURNAL OF AYURVEDA
An International Journal of Ayurveda Specialties

ISSN 2583-8555 (ONLINE)
Vol 3(3): 36-44 (2024)
https://doi.org/10.55718/kja.308

REVIEW ARTICLE

Drosophila: Translational Renal Model for Ayurvedic Research

Kounaina Khan & Subrahmanya Padyana*

*Department of Dravyaguna Vignana, ATMA Research Centre, A Unit of Alva's Ayurveda Medical College and Hospital, Affiliated to Rajiv Gandhi University of Health Sciences (RGUHS), Vidyagiri, Moodubidire, Karnataka - 574 227, India

*Email: papersresearch08@gmail.com

ARTICLE HISTORY

Received: 28 August 2024
Accepted: 13 September 2024

Available online
Version 1.0 : 31 September 2024

Keywords

Drosophila melanogaster, Human biomimic, Malpighian tubules, Nephrocytes, Podocyte

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://keralajournalofayurveda.org/index.php/kja/open-access-policy

Publisher's Note: All Kerala Govt. Ayurveda College Teacher's Association remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

CITE THIS ARTICLE

Khan K, Padyana S. Drosophila: Translational Renal Model for Ayurvedic Research. Kerala Journal of Ayurveda. 2024; 3(3):36-44. https://doi.org/10.55718/kja.308

Abstract

Ayurveda, an ancient Indian system of medicine, emphasizes holistic and natural approaches to healthcare. However, its integration into modern medical research faces challenges due to the lack of systematic, evidence-based studies. With the global rise in renal disorders, there is a critical need for innovative and reliable preclinical models to study kidney related diseases. *Drosophila melanogaster* (fruit fly), a well-established model organism in biomedical research, offers a unique opportunity to bridge this gap due to its genetic similarities to humans and its potential as a biomimic for human renal physiology. The current review explores the utility of fly as a translational renal model in Ayurvedic research, particularly in studying renal physiology, toxicity, genetic and acquired renal disorders. With the parallels between drosophila and human renal systems and the use of nephrocytes as analogs to human podocytes and malpighian tubules as functional equivalents to human kidneys. Further, the review highlights methodologies for studying renal physiology and toxicity in drosophila, as well as the generation of renal disease phenotypes through genetic manipulation, diet and chemical induction. The current review emphasizes on drosophila as an experimental model to enhance the scientific validation of Ayurvedic treatments, particularly in the context of renal health, offering a promising avenue for the integrating traditional and modern medicine in developing better drugs and establishing the mechanism of action for renal care.

Introduction

The global resurgence of Ayurveda, termed the 'Ayurvedic boom' reflects a profound shift in healthcare preferences towards natural and holistic modalities. This ancient Indian system of medicine is rooted in a deep understanding of the body's interconnectedness with nature, has captured the interest of individuals and healthcare practitioners worldwide. The rise in popularity can be attributed to increasing awareness of sustainable and preventive healthcare practices. Comprehensive approach of Ayurveda, integrates with herbal medicine, dietary guidelines and lifestyle adjustments, which aligns with modern trends toward personalized and integrative medicine[1]. Despite its ancient roots and widespread practice, Ayurveda faces challenges due to the lacunae in robust, evidence-based research. The traditional knowledge, passed down through generations, often undergoes modifications and adaptations, leading to regional practices that are not well

documented in terms of Standard Operating Procedures (SOPs). This variability complicates the establishment of consistent scientific evidence[2]. Moreover the demoralization of traditional datasets, coupled with a fragmented documentation process, further hampers efforts to validate and assimilate Ayurvedic practices within contemporary healthcare frameworks[3].The reverse pharmacology approach, which starts with traditional knowledge and works backward to understand the scientific basis, offers a promising pathway, to enhance the quality outcomes[4].

Ayurveda dependence on crude formulations that contain both lead molecule and promoters, which are believed to maintain the homeostasis of *Doshas*. These complex mixtures allow for a natural reorientation of phytochemicals based on the specific *Dosha* that is vitiated, aligning with traditional Ayurvedic principles. However, this approach poses challenges in scientific validation, as the inherent complexity and variability of these formulations make it difficult to standardize treatments, mode of action and isolate specific therapeutic effects[5]. The practices in Ayurveda are highly effective against various disease conditions[6]. However, a gap exists in scientific studies that focus on identifying and purifying active ingredients, removing antagonistic molecules and isolating single phytochemical moieties. Such studies are key for elucidating the mechanism of action and specifying the biochemical and molecular interactions that determine therapeutic efficacy[7]. While Ayurveda includes the concept of *Shodhana*, this method has traditionally been used for purification rather than for the identification and isolation of lead molecules[8]. Consequently, the reproducibility of results becomes questionable, as procedures are manually performed, leading to variations in biophysical parameters such as absorption, adsorption, polarity, solubility, miscibility, lipophilicity, temperature, pH and ionic and non-ionic (-inter, -intra and -trans) interactions[9]. It makes challenging to reliably assess the efficacy, mode of action, toxicity, and target-oriented therapy (through a "one drug, one target" or "one drug, multitarget" approach) in Ayurveda. The standardized preclinical models further exacerbate these issues. Therefore, it is imperative for Ayurvedic researchers to adopt interdisciplinary approach, embracing a ring-side perspective with minimal bias and prejudice. By promoting trans-disciplinary research, the Ayurvedic community can bridge these gaps and enhance the scientific rigor and credibility of Ayurveda practices[10,11]. A larger emphasis has to be laid on rapid, reliable and reproducible experimental methods. In preclinical research invertebrate (insects, nematode, zebra fish etc.) and vertebrate (mouse, rat, rabbit etc.) models are extensively used both under wild and genetic variation models. However, utilization of invertebrate models is not extensively explored in Ayurveda.

Renal disorders are increasingly prevalent worldwide, affecting millions of people across diverse populations. Chronic kidney disease (CKD) is one of the major public health concern, with an estimated >10% of the global population suffering from some form of kidney impairment[12]. The incidence of end-stage renal disease (ESRD), requiring dialysis or transplantation, has also been rising steadily, particularly in low- and middle-income countries where access to early detection and treatment is limited[13]. Risk factors such as hypertension, diabetes and obesity contribute to the growing burden of kidney diseases, exacerbated by aging populations and lifestyle changes[14]. Despite advances in medical care, disparities in healthcare access and awareness persist, leading to high morbidity and mortality rates associated with renal disorders globally. Addressing these challenges requires coordinated efforts in prevention, early diagnosis and equitable access to treatment to mitigate the global impact of renal diseases[15]. Ayurveda can play an important role in the prevention, management and treatment of renal disorders through its holistic approach to health and wellness without altering the basic principles. In Ayurveda, kidney health is closely linked to the balance of *Doshas* and the functioning of the body's filtration and detoxification processes[16].Which can offer promising adjuncts to conventional care through various forms of treatment. However, further scientific research is necessary to validate their efficacy. As there is a huge similarity and variations with multifaceted complex system a proper preclinical system is required which can be used as an experimental model at genetic level and induction level to differentiate between physiological and pathological condition[17].Hence the major responsibility lies on researchers to establish experimental models which is also evident and aligns with the vision and mission of ministry of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homeopathy (AYUSH) and National Commission for Indian System of Medicine (NCISM) wherein the changes are been brought at grassroot level[18].

The *Drosophila melanogaster* model has been instrumental in advancing biological research, particularly in genetics and developmental biology. The importance of drosophila as a model organism is emphasizes by the multiple Nobel prizes awarded for research using this species, which has significantly contributed to understanding of fundamental biological processes. Its genetic similarity to humans and its simplicity as an invertebrate model make drosophila an invaluable tool for studying complex human diseases. This has led to its recognition as a human biomimic, allowing researchers to explore disease mechanisms and treatments in a cost-effective and efficient manner[19]. Hence the current review with a trans disciplinary, exploratory and conceptual framework designed to explore drosophila as a model organism for renal research in Ayurveda.

Drosophila as a Model Organism

The fruit fly *Drosophila melanogaster* has been a cornerstone in biomedical research for over a century, offering numerous advantages over vertebrate models, such as ease of culturing, short life cycles, easy and inexpensive to maintain, no ethical concern, genome sequenced and the ability to produce large numbers of offspring allows for the efficient generation of experimental data. These features make drosophila an important model for studying genetics, embryonic development, behavior, regenerative and aging[20]. Due to the ethical and practical limitations clinical experiments on humans, much of what we know about cell and tissue biology has come from model organisms like mice, rat and drosophila. Though vastly different in appearance, many of the essential biological mechanisms and pathways that govern development and survival are conserved across species, including humans and fruit flies[21]. Moreover, the sequencing of the drosophila genome, completed in the year 2000, provided crucial insights into the similarities between fruit flies and humans, revealing about 75% of known human disease genes have counterparts in drosophila[22]. The genome sequencing project also led to the development of *FlyBase* an online database (https://flybase.org) that provides extensive information on gene sequences, phenotypes, genetic interactions, and other data related to drosophila[23].

The history of drosophila genetics is rich with discoveries that have provided deeper understanding into biological processes. The organisms' short life cycle and the vast array of genetic tools available make it an ideal subject for genetic experiments. Beyond genetics, drosophila life cycle has also made it a model for studying developmental biology[24]. Which undergoes into a four-stage life cycle-egg, larva, pupa and adult fly (Figure 1). This process of metamorphosis, during which most embryonic and larval tissues are destroyed and adult tissues develop from imaginal discs, helps to understand the tissue regeneration[25].

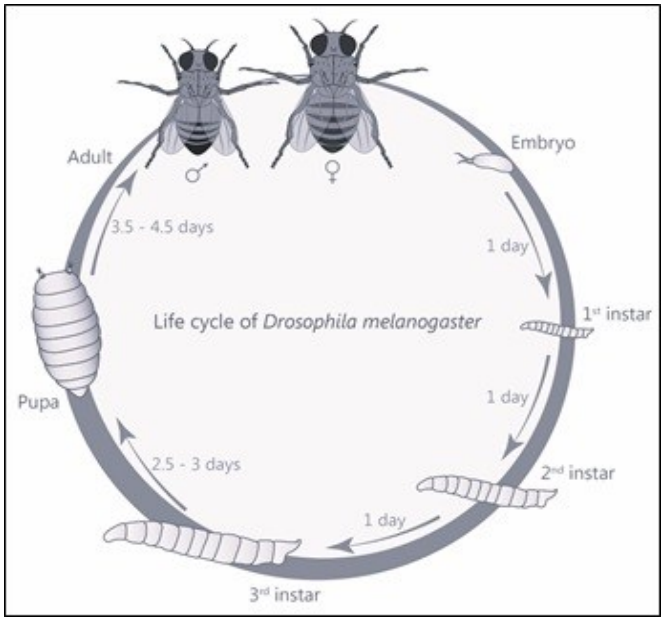

Figure 1: Life cycle of drosophila (Courtesy: The Walter Lab)

Drosophila: a human biomimic

Fruit fly, has been a classical model organism in biological research. Despite its small size and the evolutionary distance from humans, drosophila offers remarkable insights into human biology, particularly in genetics, development, and disease. This is largely due to the surprising degree of genetic, cellular and even organ-level conservation between fruit flies and humans, making it an exceptional biomimic for human organs[26]. One of the most striking areas of similarity between drosophila and humans is in the nervous system. Although the fruit fly's nervous system is simpler, it includes a brain, ventral nerve cord, and peripheral nerves, similar to those found in humans. The fly brain has regions that are analogous to parts of the human brain, including areas involved in learning, memory and sensory processing. This structural and functional similarity has made it an essential model for studying neurological functions and diseases. For example, research on drosophila has contributed to understanding of neurodegenerative diseases such as Parkinson's, Alzheimer's and Huntington's, as well as basic neurological processes like sleep, learning, memory, anxiety and depression[27,28]. Further, the drosophila heart, or dorsal vessel, is a simple linear tube that pumps hemolymph (the insect equivalent of blood) throughout the body. Despite its simplicity, the drosophila heart shares many genetic and functional similarities with the human heart. Genes involved in heart development and function are conserved between fly and humans, making the fruit fly a model organism for studying cardiovascular diseases. Research using drosophila have helped to understand congenital heart defects, cardiomyopathies and the role of specific genes in heart development and function[29].

The digestive system of fly also mirrors the human gastrointestinal tract in several key aspects. The fruit fly digestive system includes a foregut, midgut and hindgut, with the midgut being particularly analogous to the human small intestine, where nutrient absorption takes place. This similarity has allowed researchers to use fly to study various aspects of metabolism, gut microbiota and gastrointestinal disorders[30]. In terms of liver function, the drosophila fat body serves a role similar to that of the human liver. The fat body is a key component for metabolism, energy storage, and detoxification, paralleling the functions of the human liver. This organ in fly has been used extensively to model human metabolic diseases, including fatty liver disease, obesity and diabetes. Furthermore, the fat body is also a key site for studying the effects of toxins, drugs and liver-related conditions[31].

The kidneys in humans have their counterpart in the malpighian tubules of drosophila. These tubules are responsible for osmoregulation, waste excretion and maintaining ion balance, much like the human kidneys. Podocytes are specialized cells that play key role in the filtration processes of the kidneys in human, wherein nephrocytes, are equivalent of podocytes, found in drosophila. These cells are part of the fly's excretory and circulatory systems, functioning similarly to mammalian podocytes by filtering hemolymph. Despite their simpler structure compared to podocytes, nephrocytes share key functional characteristics, such as forming slit diaphragms that regulate filtration. The study of nephrocytes in insects helps to understand the mechanisms of kidney function and disease in humans, highlighting the evolutionary conservation of these critical filtration cells. This helps in studying kidney function, fluid balance and related diseases[32]. Further, drosophila immune system, though simpler than that of humans, shares key components with the human innate immune system. The fruit fly's immune system includes pathways like the Toll and Imd pathways, which are analogous to the human Toll-like receptor (TLR) pathways. These similarities have enabled researchers to use fly to study the innate immune response, infection, inflammation, and immune-related diseases, that are directly applicable to understanding human immunity[33]. Further, the reproductive system of fly mirrors human reproductive organs in function. The processes of gametogenesis and early embryonic development are highly conserved between fly and humans. As a result, fly has been used to study fertility, reproductive biology and developmental genetics, shedding light on human reproductive health and related disorders[34] (Figure 2).

Further, our previous research work has made significant strides in understanding the parallels between human and *Drosophila melanogaster* by identifying and characterizing hypothetical models that demonstrate the biomimicry potential of the fruit fly. Specifically, the work was focused on claudin proteins like, claudin-19[35], claudin-16[36] and claudin-14[37] in humans and their counterparts in drosophila, including Kune-kune[38], Sinuous[39], Megatrachea[40] and dual oxidase[41]. These proteins play a key role in regulating paracellular transport in both species, with claudins in humans contributing to kidney function and tight junction integrity and their fly analogs functioning within septate junctions to maintain epithelial barriers. Furthermore, the role of dual oxidase, a protein involved in the immune response, particularly in generating reactive oxygen species (ROS) as a defense mechanism against intestinal bacterial infections was explored. By modeling and analyzing dual oxidase in both humans and drosophila, our research highlights the evolutionary conservation of these proteins and their functions for studying human biology in fly. The current review emphasizes on exploration of *Drosophila melanogaster* as a model for studying renal health, focusing on various aspects including normal renal physiology, nephrotoxicity, genetic renal disorders and induced renal diseases.

Renal physiology and toxicity study in drosophila model

To study normal renal physiology in *Drosophila melanogaster*, the procedure includes maintenance of flies under standard conditions and dissect the malpighian tubules to estimate various assays[42]. Fluid secretion assays are used to measure the rate of fluid production and osmoregulation. Ion transport assays with ion-specific fluorescent dyes assess the movement of ions such as sodium, potassium and calcium.

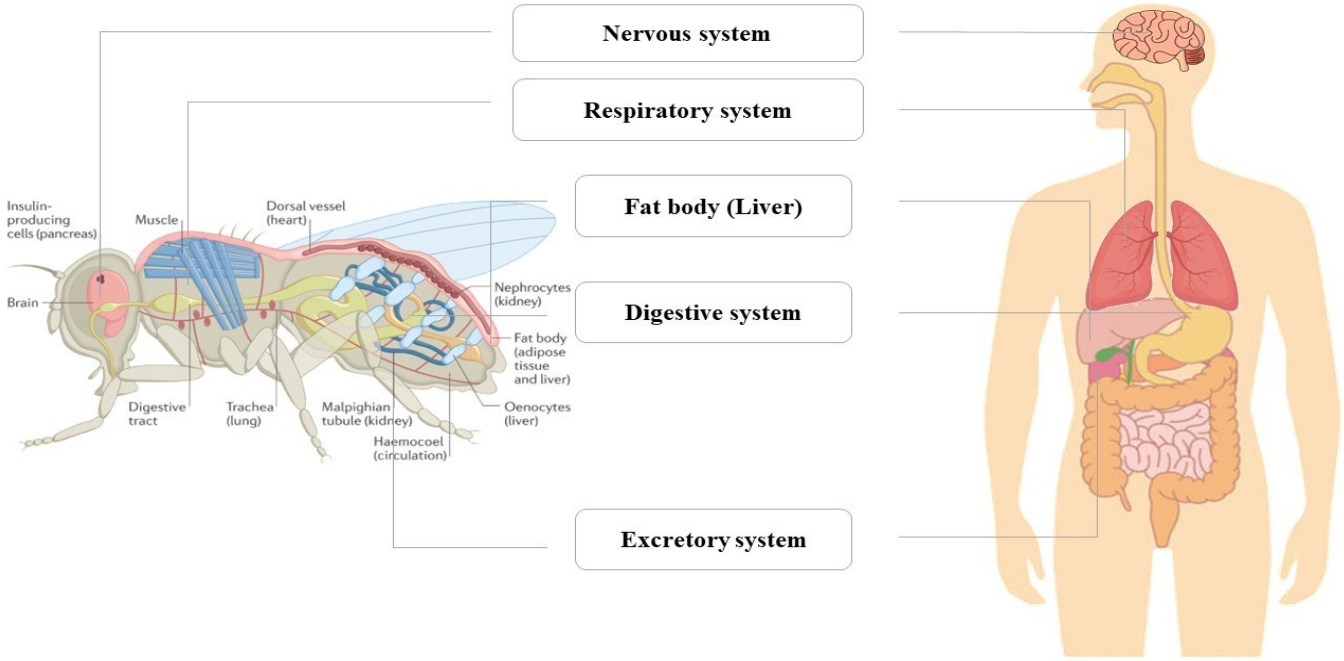

Figure 2: Organ match between human and drosophila

pH regulation assays using pH-sensitive dyes to study acid-base balance in the tubules[43]. Further, metabolic activity measurements through ATP quantification or mitochondrial function assessments, which give clarity on the energy requirements of renal processes. Imaging techniques like confocal microscopy allow for the observation of tubule structure and protein localization. Histological staining, such as Hematoxylin and Eosin (H&E), is used to examine the tubule morphology. Reactive oxygen species (ROS) assays to detect oxidative stress levels, while apoptosis assays, like the TUNEL assay, are done to evaluate cell death. Further, protein quantification through Western blotting and localization through immunohistochemistry contribute to understand the normal functioning of the renal system in fly. These comprehensive approaches enable a detailed analysis of renal physiology under normal conditions in the fly model[44,45].

For renal toxicity study nephrotoxic compounds, such as cisplatin, cadmium or any drug of interest which are incorporated into the fly food at specific concentrations, with cisplatin, for example, being used at 10-50 µM. Adult flies are exposed to this treated media for a set period, ranging from 24 hours to several days based on acute and chronic toxicity study. After exposure, the malpighian tubules, are dissected for further analysis. Further various biochemical assays are performed for toxicity analysis[46].

Drosophila as a model organism for genetic renal disorders

Using *Drosophila melanogaster* as a genetic model to study renal diseases such as cystic kidney disease, hereditary renal carcinoma, nephrotic syndrome, hereditary nephrolithiasis, and human glomerulopathies involves several key steps to replicate these conditions and understand their various underlying mechanisms (Figure 3). First, identify drosophila genes that are homologous to human genes implicated in these renal diseases. For cystic kidney disease, homologous genes such as PKD1 and PKD2 are identified; for hereditary renal carcinoma, genes like VHL and MET are studied; nephrotic syndrome involves genes like NPHS1, NPHS2, WT1, and LAMB2; hereditary nephrolithiasis includes genes such as SLC26A1, CLDN16 and CLDN19; and for human glomerulopathies, genes like COL4A3, COL4A4, COL4A5 and PODXL are of interest[47]. Second either use available mutants or employ genome editing techniques like CRISPR-Cas9 to generate knockouts of these genes in drosophila[48]. Genetic manipulation follows, where gene expression can be knocked down using RNA interference (RNAi) or overexpressed using the UAS-GAL4 system, enabling the modeling of both loss-of-function and gain-of-function scenarios[49]. Flies are then genetically crossed to create specific mutations or combinations of mutations relevant to these diseases, such as mimicking the development of hereditary renal carcinoma or the formation of kidney stones (nephrolithiasis).

Further to validate the model, the drosophila malpighian tubules, analogous to human renal tubules, are examined for relevant changes, including cystic formations, neoplastic growth or other morphological alterations through phenotypic analysis and histological staining. Functional assays are conducted to measure fluid secretion rates and study ion transport defects, as these are often disrupted in renal diseases like nephrotic syndrome and glomerulopathies. At the molecular level, expression profiling through qPCR, Western blotting or RNA sequencing is performed to analyze key genes involved in disease pathways, including those regulating ciliary function, cell proliferation, apoptosis, glomerular function and kidney

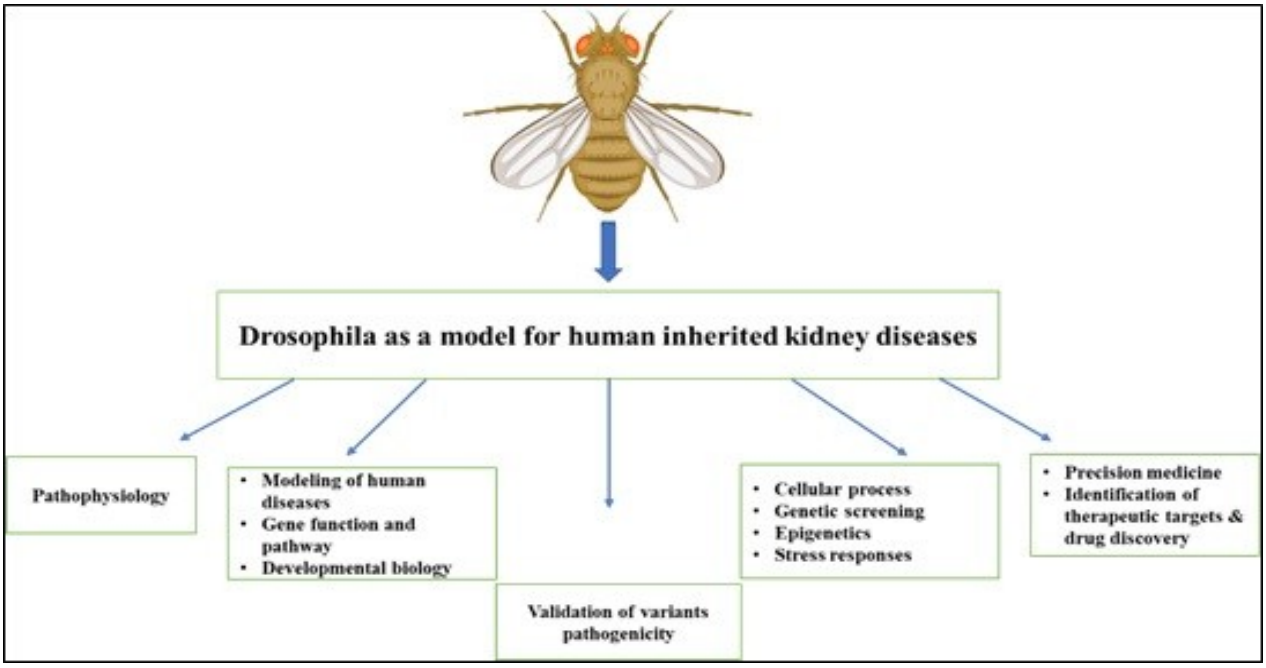

Figure 3: Different mechanisms which could be studied using drosophila as a genetic model for renal research

stone formation. Immunostaining is also used to localize proteins within the tubule cells, which is particularly important for studying glomerulopathies and nephrotic syndrome. Finally, the drosophila model serves as a platform for drug screening, where therapeutic compounds are tested for their effect on reducing cyst formation, preventing tumor growth, or mitigating nephrotic symptoms. Additionally, genetic screens may be conducted to identify other genes or pathways that could modify the disease phenotype, potentially revealing new therapeutic targets[50,51,52]. This comprehensive approach allows for detailed study across a range of renal diseases and aids in the development of treatments.

Generation of renal disease phenotypes in drosophila models through diet or chemical-induced methods

Drosophila melanogaster could be used as a model for studying kidney diseases via dietary or chemical induction, through the selection of appropriate dietary components or chemical agents capable of specifically inducing the kidney disease of interest. For chronic kidney disease (CKD), use chemicals like nephrotoxins that mimic the effects of prolonged kidney damage. For diabetic nephropathy, introduce high-fat diet or sucrose or streptozotocin to induce metabolic conditions similar to diabetes. To model kidney stones, expose flies to elevated calcium or oxalate levels, which can lead to stone formation in the renal-like malpighian tubules. For acute kidney injury (AKI), administer acute nephrotoxins or stressors that cause acute damage to renal structures. To induce hypertensive nephrosclerosis, use of chemicals that induce hypertension or elevate blood pressure in the flies[53,54].

Further, upon chemical exposure, carefully monitor the drosophila for structural changes in malpighian tubules such as cyst formation, tubular dilation, or cellular damage. Further perform histological staining techniques (Hematoxylin and Eosin), to assess structural abnormalities. To measure alterations in fluid secretion rates and ion transport, functional assays are done. Additionally, gene expression and protein localization could be analyzed to the impact of chemicals on molecular pathways associated with kidney disease. Finally, therapeutic compounds should be tested to evaluate their effectiveness in mitigating disease symptoms, providing insights into possible treatments for human kidney disorders[55,56,57] (Figure 4).

Conclusion

The use of drosophila as a model organism in Ayurvedic renal research offers a promising avenue for understanding the molecular and genetic underpinnings of kidney function and disease. The evolutionary conservation of key physiological processes between flies and humans makes drosophila an effective and efficient model for studying renal physiology, toxicity, and various disease conditions. By integrating this model with Ayurvedic principles, researchers can bridge traditional and modern medicine, enhancing the validation and scientific credibility of Ayurvedic treatments for renal pathology.

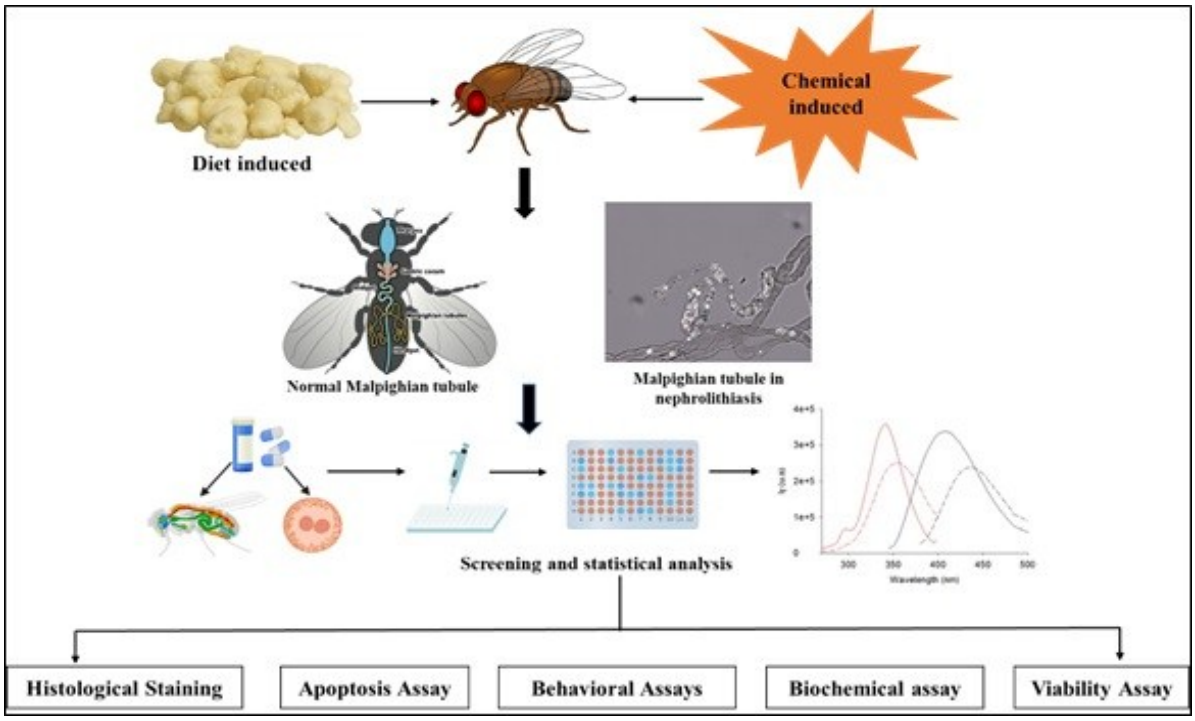

Figure 4: Work flow for drosophila as a model to study diet or chemical induced renal diseases

Limitations

Ayurvedic formulations, known for their multi-herbal compositions present a challenge in isolating specific active principles due to the synergistic nature of their ingredients. The complex interaction between these bioactive compounds and the antagonistic effects of certain phytochemicals can complicate the translation of findings from the simplified drosophila Malpighian tubule system, which lacks the structural and functional complexity of human nephrons. Moreover, variability in methodology and dosage in Ayurveda limits the reproducibility and standardization for scientific validation, making it difficult to correlate results with human renal pathophysiology.

Future Prospects

The future integration of *Rasayana* with drosophila genetic models can provide a promising avenue for mimicking human kidney conditions and exploring their therapeutics in kidney disorders. Advanced tools like CRISPR can help simulate *Dosha* imbalances in drosophila, allowing for targeted Ayurvedic interventions. By combining fly-based studies with more complex in vivo models and human clinical data, and by focusing on the synergistic effects of formulations researchers can develop evidence-based, personalized treatments that align with both Ayurvedic principles and modern medicine, paving the way for a smoother preclinical-to-clinical transition.

Acknowledgement

KK is thankful to Rajiv Gandhi University of Health Sciences (RGUHS), Bangalore for the support extended for this research work. All authors thank the Dr. M. Mohan Alva, Chairman, Dr. Vinay Alva, Managing trustee, Alva's Education Foundation (AEF), Dr. Sajith M., Principal, Alva's Ayurveda Medical College for their constant support and motivation.

Conflict of interest

All authors declare no conflict of interest

References

1. Gupta R. Integrating Ayurveda with modern medicine for enhanced patient care: Analysis of realities. *The Physician*. 2024; 9:1-6. https://doi.org/10.38192/1.9.1.3
2. Vyas M. A view point into framework of Ayurvedic research. Ayu. 2016; 37(1):1-2. https://doi.org/10.4103/ayu.AYU_26_17
3. Rastogi S. Poor quality and improper use: A review of common reasons of possible adversity in Ayurvedic practice. Int J Risk Saf Med. 2008; 21: https://doi.org/10.3233/JRS-2009-0470
4. Sidhic J, Athira CK, Narayanankutty A, George S. Ayurveda-inspired reverse pharmacology in drug discovery. Drugs from nature: Targets, assay systems and leads. Singapore: Springer; 2024. https://doi.org/10.1007/978-981-99-9183-9_5
5. Jyothi Raga PM, Vivek P, Harinarayanan CM. Need of Standardization of Ayurveda Formulations. Int J Ayurveda Pharma Res. 2023; 11(10):40-45. https://doi.org/10.47070/ijapr.v11i10.2856
6. Sen S, Chakraborty R. Revival, modernization and integration of Indian traditional herbal medicine in clinical practice: Importance, challenges and future. J Tradit Complement Med. 2017; 7(2):234-44. https://doi.org/10.1016/j.jtcme.2016.05.006
7. Vaidya VN, Tatiya AU, Elango A, Kukkupuni SK, Vishnuprasad CN. Need for comprehensive standardization strategies for marketed Ayurveda formulations. J Ayurveda Integr Med. 2018; 9(4):312-15. https://doi.org/10.1016/j.jaim.2018.09.002
8. Maurya SK, Seth A, Laloo D, Singh NK, Gautam DN, Singh AK. Śodhana: An Ayurvedic process for detoxification and modification of therapeutic activities of poisonous medicinal plants. Anc Sci Life. 2015; 34(4):188-97. https://doi.org/10.4103/0257-7941.160862
9. Manohar PR. Toxicity of Ayurveda medicines and safety concerns: The need to revive the branch of toxicology in Ayurveda. Anc Sci Life. 2014; 34(1):1-2. https://doi.org/10.4103/0257-7941.150761
10. Kore SA. Need of Interdisciplinary Researches and Publications with Ayurvedic Scientist: Valuable for Community and Sciences. Mod Appl Pharm Pharmacol. 2017; 1(4). https://doi.org/10.31031/MAPP.2017.01.000505
11. Chauhan A, Semwal DK, Mishra SP, Semwal RB. Ayurvedic research and methodology: Present status and future strategies. Ayu. 2015; 36(4):364-69. https://doi.org/10.4103/0974-8520.190699
12. Kovesdy CP. Epidemiology of chronic kidney disease: an update 2022. Kidney Int Suppl (2011). 2022; 12(1):7-11. https://doi.org/10.1016/j.kisu.2021.11.003
13. Thurlow JS, Joshi M, Yan G, et al. Global Epidemiology of End-Stage Kidney Disease and Disparities in Kidney Replacement Therapy. Am J Nephrol. 2021; 52(2):98-107. https://doi.org/10.1159/000514550
14. Mallamaci F, Tripepi G. Risk Factors of Chronic Kidney Disease Progression: Between Old and New Concepts. Journal of Clinical Medicine. 2024; 13(3):678. https://doi.org/10.3390/jcm13030678
15. Khan K, Padyana S. Diabetic nephropathy: Ayur-nutri-pharmaco approach. Food Safety and Health. 2024; 2(3):344-61. https://doi.org/10.1002/fsh3.12048
16. Patel MV, Gupta SN, Patel NG. Effects of Ayurvedic treatment on 100 patients of chronic renal failure (other than diabetic nephropathy). Ayu. 2011; 32(4):483-86. https://doi.org/10.4103/0974-8520.96120
17. Huang W, Percie du Sert N, Vollert J, Rice ASC. General Principles of Preclinical Study Design. Handb Exp Pharmacol. 2020; 257:55-69. https://doi.org/10.1007/164_2019_277
18. Shankar D, Patwardhan B. AYUSH for New India: Vision and strategy. J Ayurveda Integr Med. 2017; 8(3):137-39. https://doi.org/10.1016/j.jaim.2017.09.001

19. Lopez-Ortiz C, Gracia-Rodriguez C, Belcher S, et al. Drosophila melanogaster as a Translational Model System to Explore the Impact of Phytochemicals on Human Health. Int J Mol Sci. 2023; 24(17). Published 2023 Aug 29. https://doi.org/10.3390/ijms241713365

20. Wangler M, Yamamoto S, Bellen H. Fruit Flies in Biomedical Research. Genetics. 2015; 199(3):639-53. https://doi.org/10.1534/genetics.114.171785

21. Cheng L, Baonza A, Grifoni D. Drosophila Models of Human Disease. Biomed Res Int. 2018. Published 2018 Aug 30. https://doi.org/10.1155/2018/7214974

22. Banerjee S, Benji S, Liberow S, Steinhauer J. Using Drosophila melanogaster To Discover Human Disease Genes: An Educational Primer for Use with "Amyotrophic Lateral Sclerosis Modifiers in Drosophila Reveal the Phospholipase D Pathway as a Potential Therapeutic Target". Genetics. 2020; 216(3):633-41. https://doi.org/10.1534/genetics.120.303495

23. Marygold S, Crosby M, Goodman J. Using Fly Base, a Database of Drosophila Genes and Genomes. In: Methods in Molecular Biology. 2016; 1478:1-31. https://doi.org/10.1007/978-1-4939-6371-3_1

24. Bharucha K. The Epicurean Fly: Using Drosophila melanogaster to Study Metabolism. Pediatr Res. 2009; 65(2):132-37. https://doi.org/10.1203/PDR.0b013e318191fc68

25. Landis GN, Doherty D, Tower J. Analysis of Drosophila melanogaster Lifespan. Methods Mol Biol. 2020; 2144:47-56. https://doi.org/10.1007/978-1-0716-0592-9_4

26. Yamaguchi M, Yoshida H. Drosophila as a Model Organism. Adv Exp Med Biol. 2018; 1076:1-10. https://doi.org/10.1007/978-981-13-0529-0_1

27. Kim T, Song B, Lee IS. Drosophila Glia: Models for Human Neurodevelopmental and Neurodegenerative Disorders. Int J Mol Sci. 2020; 21(14). Published 2020 Jul 9. https://doi.org/10.3390/ijms21144859

28. Satapathy P, Prakash JK, Gowda VC et al,. Targeting Imd Pathway Receptor in Drosophila melanogaster and Repurposing of Phyto-Inhibitors: Structural Modulation and Molecular Dynamics. J Biomol Struct Dyn. 2020; 40(4):1659-670. https://doi.org/10.1080/07391102.2020.1831611

29. Souidi A, Jagla K. Drosophila Heart as a Model for Cardiac Development and Diseases. Cells. 2021; 10(11). Published 2021 Nov 8. https://doi.org/10.3390/cells10113078

30. Miguel-Aliaga I, Jasper H, Lemaitre B. Anatomy and Physiology of the Digestive Tract of Drosophila melanogaster. Genetics. 2018; 210(2):357-96. https://doi.org/10.1534/genetics.118.300224

31. Sondergaard L. Homology between the mammalian liver and the Drosophila fat body. Trends Genet. 1993; 9(6):193. https://doi.org/10.1016/0168-9525(93)90113-V

32. Cohen E, Sawyer JK, Peterson NG, Dow JAT, Fox DT. Physiology, Development, and Disease Modeling in the Drosophila Excretory System. Genetics. 2020; 214(2):235-64. https://doi.org/10.1534/genetics.119.302289

33. Kuo CJ, Hansen M, Troemel E. Autophagy and innate immunity: Insights from invertebrate model organisms. Autophagy. 2018; 14 (2):233-42. https://doi.org/10.1080/15548627.2017.1389824

34. Giedt MS, Tootle TL. The Vast Utility of Drosophila Oogenesis. Methods Mol Biol. 2023; 2626:1-36. https://doi.org/10.1007/978-1-0716-2970-3_1

35. Patil JAG, Prakash, Prasad N, et al. Hypothetical model of Claudin-19 in Human. Model Archive, Swiss Institute of Bioinformatics, University of Basel. 2021.

36. Patil JAG, Prakash, Prasad N, et al. Hypothetical model of Claudin-16 in Human. Model Archive, Swiss Institute of Bioinformatics, University of Basel. 2021.

37. Patil JAG, Prakash, Prasad N, et al. Hypothetical model of Claudin-14 in Human. Model Archive, Swiss Institute of Bioinformatics, University of Basel. 2021.

38. Patil AG, Prakash JK, Zameer F, et al. Claudin like Protein Kune-Kune (Kune) in Drosophila. Model Archive, Swiss Institute of Bioinformatics, University of Basel. 2020.

39. Patil AG, Prakash JK, Zameer F, et al. Claudin like Protein Sinuous (Sinu) in Drosophila. Model Archive, Swiss Institute of Bioinformatics, University of Basel. 2020.

40. Satapathy P, Skariyachan S, Khan K, et al. Hypothetical Model of Dual Oxidase. Model Archive - SIB, Swiss Institute of Bioinformatics and Biozentrum, University of Basel. April 1, 2019.

41. Patil AG, Prakash JK, Zameer F, et al. Claudin like Protein Megatrachea (Mega) in Drosophila. Model Archive, Swiss Institute of Bioinformatics, University of Basel. 2020.

42. Rodan AR. The Drosophila Malpighian tubule as a model for mammalian tubule function. Curr Opin Nephrol Hypertens. 2019; 28(5): 455-64. https://doi.org/10.1097/MNH.0000000000000521

43. Koehler S, Huber TB. Insights into human kidney function from the study of Drosophila. Pediatr Nephrol. 2023; 38(12):3875-887. https://doi.org/10.1007/s00467-023-05996-w

44. Cohen E, Sawyer JK, Peterson NG, Dow JAT, Fox DT. Physiology, Development and Disease Modeling in the Drosophila Excretory System. Genetics. 2020; 214(2):235-64. https://doi.org/10.1534/genetics.119.302289

45. Gautam NK, Verma P, Tapadia MG. Drosophila Malpighian Tubules: A Model for Understanding Kidney Development, Function and Disease. Results Probl Cell Differ. 2017; 60: 3-25. https://doi.org/10.1007/978-3-319-51436-9_1

46. Saini S, Rani L, Shukla N, Banerjee M, Chowdhuri DK, Gautam NK. Development of a Drosophila melanogaster based model for the assessment of cadmium and mercury mediated renal tubular toxicity. Ecotoxicol Environ Saf. 2020; 201. https://doi.org/10.1016/j.ecoenv.2020.110811

47. UniProt Consortium. UniProt: a worldwide hub of protein knowledge. Nucleic Acids Res. 2019; 47(D1):D506-D515. https://doi.org/10.1093/nar/gky1049

48. Gratz SJ, Rubinstein CD, Harrison MM, Wildonger J, O'Connor-Giles KM. CRISPR-Cas9 Genome Editing in Drosophila. Curr Protoc Mol Biol. 2015; 111:31.2.1-31.2.20. Published 2015 Jul 1. https://doi.org/10.1002/0471142727.mb3102s111

49. Homem RA, Davies TGE. An overview of functional genomic tools in deciphering insecticide resistance. Curr Opin Insect Sci. 2018; 27: 103-10. https://doi.org/10.1016/j.cois.2018.04.004

50. Millet-Boureima C, Porras Marroquin J, Gamberi C. Modeling Renal Disease "On the Fly". Biomed Res Int. 2018: Published 2018 May 31. https://doi.org/10.1155/2018/5697436

51. Rani L, Gautam NK. Drosophila Renal Organ as a Model for Identification of Targets and Screening of Potential Therapeutic Agents for Diabetic Nephropathy. Curr Drug Targets. 2018; 19 (16):1980-990. https://doi.org/10.2174/1389450119666180808114533

52. Millet-Boureima C, He S, Le TBU, Gamberi C. Modeling Neoplastic Growth in Renal Cell Carcinoma and Polycystic Kidney Disease. Int J Mol Sci. 2021; 22(8):3918. Published 2021 Apr 10. https://doi.org/10.3390/ijms22083918

53. Rani L, Saini S, Thakur RS, Patel DK, Chowdhuri DK, Gautam NK. Single and combined effect of bisphenol A with high sucrose diet on the diabetic and renal tubular dysfunction phenotypes in Drosophila melanogaster. Environ Toxicol Pharmacol. 2022; 96: https://doi.org/10.1016/j.etap.2022.103977

54. Hudeda SP, Khan K. Drosophila: An Emerging Translational Model for Diabetic Nephropathy. Alochana Chakra Journal. 2020; 9 (6):9304-312.

55. Patil AG, Prakash JK, More SS, Chandramohan V, Zameer F. Exploring Banana Phytosterols (Beta-Sitosterol) on Tight Junction Protein (Claudin) as Anti-Urolithiasis Contributor in Drosophila: A Phyto-Lithomic Approach. Informatics in Medicine Unlocked. 2022; 29: https://doi.org/10.1016/j.imu.2022.100905

56. Devi AT, Nagaraj R, Prasad A, Lakkappa DB, Zameer F, Nagalingaswamy NP. Nephrolithiasis: Insights into Biomimics, Pathogenesis and Pharmacology. Clin Complement Med Pharmacol. 2023; 3(2). https://doi.org/10.1016/j.ccmp.2022.100077

57. Zhang F, Chen X. The Drosophila nephrocyte has a glomerular filtration system. Nat Rev Nephrol. 2014; 10(9):491. https://doi.org/10.1038/nrneph.2012.290-c1

§§§