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NEPHROPROTECTIVE POTENTIAL OF HINGULAD RASA SINDOORA AGAINST GENTAMICIN-INDUCED NEPHROTOXICITY IN WISTAR ALBINO RATS

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ABSTRACT

Background: Rasashastra, a specialized branch of Ayurveda, focuses on potent mineral formulations, with Kupipakwa Rasayana valued for stability and rapid action. The rising burden of chronic kidney disease necessitates safer nephroprotective agents. Hingulad Rasa Sindoora is one such classical formulation with potential renal protective action. **Objectives:** The present study aimed to evaluate the nephroprotective potential of Hingulad Rasa Sindoora against gentamicin-induced renal toxicity in Wistar albino rats through experimental assessment of renal biochemical and organ parameters. **Methodology:** Hingulad Rasa Sindoora was prepared and standardized prior to experimentation. Nephroprotective activity was evaluated in Wistar albino rats using a gentamicin-induced nephrotoxicity model. Animals were divided into normal control, gentamicin control, and treatment groups receiving Hingulad Rasa Sindoora and Hingulad Rasa Sindoora with Guduchi Swarasa. Renal toxicity was induced by gentamicin administration, followed by oral treatment with the test drugs. Nephroprotective efficacy was assessed by evaluating renal biochemical parameters, along with changes in body weight and organ weights. Data were statistically analyzed to determine the protective effect of Hingulad Rasa Sindoora. **Results:** Hingulad Rasa Sindoora was successfully prepared and analytically confirmed as HgS with characteristic physicochemical properties. Gentamicin induced significant nephrotoxicity, evidenced by elevated serum urea and creatinine levels. Treatment with Hingulad Rasa Sindoora significantly reduced these parameters ($p < 0.05$), while the combination with Guduchi Swarasa showed moderate improvement, **Conclusion:** *Hingulad Rasa Sindoora* is a safe, stable, and potent *Kupipakwa Rasayana*. demonstrated significant nephroprotective activity against gentamicin-induced renal toxicity by improving renal biochemical parameters in Wistar albino rats. The findings support its potential role as a renoprotective agent, warranting further experimental and clinical evaluation.

KEYWORDS: *Hingulad Rasa Sindoora, Kupipakwa Rasayana, Gentamicin nephrotoxicity, Nephroprotective activity.*

INTRODUCTION

Rasashastra is a vital branch of Ayurveda emphasizing the purification and therapeutic use of mercury, metals, and minerals to prepare potent formulations. Among the *Chaturvidha Rasayana*, *Kupipakwa Rasayana* holds special significance due to its stability, rapid action, and broad therapeutic potential.

Chronic kidney disease (CKD) is a major global health concern, mainly associated with diabetes, hypertension, obesity, and long-term drug use. Recent data show that CKD affects around 8–9% of the global population (≈673 million people), with a rising trend in India.[1] The condition leads to quality of life, emphasizing the need for safer nephroprotective agents.

Hence, interest has increased in Ayurvedic drugs with antioxidant and anti-inflammatory properties that can protect renal tissues naturally.

Hingulad Rasa Sindoora[2] (HRS), a classical Kupipakwa Rasayana prepared from Shuddha Hingula and Shuddha Gandhaka in equal proportion, is Sagandha, Sagni,

Kanthastha, and Bahirdhuma, known for its Rasayana, Yogavahi, and Sarvarogahara properties. Shuddha Hingula is Deepana, Pachana, Tridoshahara, and Rasayana,[3] while Shuddha Gandhaka is Rasayana, Vishahara, and Agnivardhaka,[4] also acting as an antidote to Hingula. Classically indicated in Prameha, Shotha, and Mutrakrucchra[5], HRS acts on Mutravaha Srotas, exhibiting diuretic and nephron-revitalizing actions.

Considering safety concerns about mercurial formulations, the present study was undertaken to evaluate the nephroprotective potential of *Hingulad Rasa Sindoora* against gentamicin-induced nephrotoxicity in Wistar albino rats, correlating its classical and modern perspectives.

METHODOLOGY

Preparation of *HRS*

The present work focuses on the preparation of a good-quality formulation *of HRS*, undertaken in the Department of PG Studies in *Rasashastra* and *Bhaishajya Kalpana*, TGAMC, Bellary,

Place of Procurement

Hingula, *Gandhaka*- Bharat Trading Company, Mumbai.

Method of preparation[6]

- *Shuddha Gandhaka*, *Shuddha Hingula* taken in *khalva yantra* and triturate till it becomes homogenous mixture and attain *sukshmatva*.
- This homogenous mixture will be filled in *kachakupi* and *Hingulad Rasasindoora* is prepared by *kupipakwa* method-*kramagnipaka* till *siddhi lakshanas* will be seen.
- After *swangasheeta Hingulad Rasasindoora* will be collected and triturate in *khalva yantra* to powder form.

Methodology for Analytical Study

Analytical evaluation of the two samples, homogenous mixture of Hingula and gandhaka and HRS, was carried out by adopting both classical Ayurvedic and modern analytical parameters to ensure standardization and quality assessment. Classical analysis included organoleptic evaluation and classical tests such as *Rasa*, *Varṇa, Sparśa, Gandha, Rekhāpūrṇatva, Sūkṣmatva*, and *Ślakṣṇatā*.

Physico-chemical parameters including pH, total ash, acid-insoluble ash, water-soluble ash, and loss on drying were assessed to determine purity, stability, and inorganic content. Modern instrumental analyses such as X-ray diffraction (XRD), scanning electron microscopy with energy dispersive spectroscopy (SEM-EDS), and Fourier transform infrared spectroscopy (FTIR) were performed to evaluate crystallographic nature, elemental composition, and chemical bonding. Particle size and distribution were analyzed using Zeta PALS to assess the impact of pharmaceutical processing on drug fineness and potential bioavailability.

EXPERIMENTAL STUDY

The study was conducted at the SDM Centre for Research in Ayurveda and Allied Sciences, Udupi. Animals were acclimatized and maintained under standard laboratory conditions (24 ± 2 °C, 70% RH, 12 h light/dark cycle) in the animal house, Department of Pharmacology. The experiment was carried out in accordance with CPCSEA guidelines after approval from the Institutional Animal Ethics Committee (IAEC Approval No: SDMCRA/IAEC/TB-R-13).

24 healthy Wistar albino rats were selected and grouped into 4 groups. Selected animal was grouped by randomisation method. 6 animals in each group. The individual rat was weighed and rats with weight around 200-250 only taken for study and marked with picric acid for identification.

Experimental design

Wistar albino rats were randomly divided into 4 groups.
Group 1-normal control
Group 2-disease control
Group 3-test drug group 1
Group 4-test drug group 2

Experimental induction of nephrotoxicity

Nephrotoxicity was induced to rats by oral administration of Gentamycin oraly from 8th to 16th day daily basis for 8 days. Dose was calculated according to individual rat weight. PTU was given by diluted in distilled water.

DOSE CALCULATION

- The dose for Rats was calculated by referring the table of Paget and Barnes.

i.e Rat Dose =Human Dose mg/kgbw X Km ratio/kgbw.

- (Rat Dose = Human dose(mg/kgbw) X $K_m$ratio/ kgbw, it gives per kg body weight dose)
- The therapeutic Human dose of *Hingulad Rsasindoora* 125mg per day.

There for Rat dose= human dose X 6.2=12.9/kg bw. Hence Rat dose of HRS- 12.9mg/kg bwpo.

Dose of Gentamycin: 40mg/kg

DOSAGE FORM

The Dosage of *HRS* (12.9mg/kgbw) was administered in the form of liquid, by adding powdered drug into distilled water.

DRUG DOSING SCHEDULE

In Genramycin induced nephrototoxicity model, test drug was administered between 8 to 10 am and toxicant was administered 1 hour after drug administration on 9th day to 16th day.

♦ **RANDOMISATION AND GROUPING**

Animals are randomly selected and grouped into 4 groups of six animal each.

♦ **STATISTICAL ANALYSIS**

The data obtained was analysed by using analysis of variance (ANOVA) followed by Dunnett's 't' test for determining the level of significance of the observed effects. A 'P' value of less than 0.05 was considered statistically significant.

GENTAMYCIN INDUCED NEPHROTOXICITY

Nephroprotective study using gentamycin induced nephrotoxicity model in rats was carried out. Wistar albino rats of either sex was selected and divided into four groups of six animals in each group (n = 6). Treatment was given as described below for 16 days.

Treatment protocol

Table no. 42: Showing grouping of aninmals.

Group	Group name	Treatment	Dose	Frequency	Duration
i.	Normal control	Vehicle	Saline	Daily	10 days
ii.	Positive control	Gentamycin	40mg/kg BW	Daily	8 Days
iii.	Induced nephrotoxicity Gentamycin + Test drug: HRS with Guduchi swarasa anupana	Gentamycin	40mg/kg BW	9^{th} -16^{th} day	8Days
		HRS with guduchiswarasa as Anupana	12.9mg/kg BW 2.16 ml B W	Daily once upto 16 Days	16Days
iv.	Induced nephrotoxicity gentamycin + test drug: HRS	Gentamycin	40mg/kg BW	9^{th} -16^{th} day	8 Days
		HRS	12.9mg/kg BW	Daily once upto 16 Days	16 Days

Group II (Gentamicin control) received gentamicin (40 mg/kg, i.p.) along with distilled water, while **Group III (Test drug)** received gentamicin with Hingulad Rasa Sindoora administered orally with Guduchi Swarasa as anupāna. The test drug was given for 8 consecutive days. Gentamicin was administered intraperitoneally from day 9 to day 16, one hour after test drug administration, to all groups except the normal control.

On day 16, one hour after gentamicin administration, animals were weighted and sacrificed by cervical dislocation. Blood samples were collected for biochemical analysis. Kidneys and hearts were dissected, cleaned, blotted, weighed, and preserved in 10% formalin for histopathological examination.

Serum was separated and biochemical parameters including serum urea, creatinine, uric acid, and ALP were estimated using standard diagnostic kits (Agappe Diagnostics Ltd., Kerala, and Span Diagnostics Ltd., Surat, India).

Effect of Test Drug on Biochemical and Organ Parameters in Wistar Albino Rats.

Parameter	Normal Control (Mean ± SEM) Percentage Change	Positive Control (Mean ± SEM) Percentage Change	Test Drug HRS (Mean ± SEM) Percentage Change	HRS + Guduchi Swarasa (Mean ± SEM) Percentage Change
Blood Urea (mg/dl)	**43 ± 0.93**	**91 ± 17.21 ↑ ****	**54.56 ± 8.43 ↓ ***	**79.66 ± 5.89 ↓**
		111.63 ↑	**40.04 ↓**	**12.46 ↓**
Serum Creatinine(mg/dl)	**0.46 ± 0.019**	**1.816 ± 0.603 ↑ ***	**0.671 ± 0.107 ↓ ***	**0.87 ± 0.110 ↓**
		294.7 ↑	**61.56 ↓**	**52.09 ↓**
Serum Uric Acid (mg/dl)	**1.183 ± 0.159**	**0.916 ± 0.132 ↓**	**1.035 ± 0.093 ↑**	**1.106 ± 0.164 ↑**
		22.57 ↓	**12.99 ↑**	**20.74 ↑**
Direct Bilirubin (mg/dl)	**0.07 ± 0.029**	**0.086 ± 0.042 ↑**	**0.045 ± 0.008 ↓**	**0.036 ± 0.010 ↓**
		22.86 ↑	**47.67 ↓**	**58.13 ↓**
Total Bilirubin (mg/dl)	**0.19 ± 0.03**	**0.27 ± 0.05 ↑**	**0.17 ± 0.03 ↓**	**0.29 ± 0.05 ↑**
		42.11 ↑	**83 ↓**	**7.41 ↑**
ALP (IU/L)	**482.83 ± 76.47**	**707.66 ± 53.17 ↑**	**870.83 ± 102.47 ↑**	**1130.83 ± 120.65 ↑ ***
		46.56 ↑	**23.06 ↑**	**59.80 ↑**
Body Weight (g)	**16.83 ± 2.586**	**7.026 ± 2.567 ↓**	**9.89 ± 2.441 ↑**	**18.51 ± 4.317 ↑ ***
		58.25 ↓	**40.34 ↑**	**163.45 ↑**
Kidney Weight (g)	**1.955 ± 0.089**	**1.776 ± 0.099 ↓**	**1.285 ± 0.021 ↓ ****	**1.315 ± 0.096 ↓ ****
		9.15 ↓	**27.65 ↓**	**25.95 ↓**
Heart Weight (g)	**0.94 ± 0.020**	**0.675 ± 0.025 ↓ ****	**0.527 ± 0.021 ↓ ****	**0.477 ± 0.026 ↓ ****
		28.19 ↓	**21.93 ↓**	**25.62 ↓**

Statistical Laegend
Data expressed as **Mean ± SEM**, ↑ / ↓ = Increase / Decrease, **@** Compared with Normal Control, **#** Compared with Positive Control, $P < 0.05$ = Significant, **P** < 0.01 / 0.001 = Highly significant, NS = non-significant.

A significant elevation in serum urea and creatinine was observed in the positive control group compared to normal. Treatment with Hingulad Rasa Sindoora (HRS) significantly reduced both parameters, while the HRS–Guduchi Swarasa combination showed a lesser, non-significant reduction. Uric acid levels decreased in the positive control but showed a non-significant rise in both treatment groups.

Direct and total bilirubin levels exhibited mild, non-significant elevations in the positive control; HRS reduced these values, whereas the combination group showed minimal, non-significant changes. ALP levels increased across all treated groups, with a statistically significant rise noted in the combination group.

Gentamicin-induced body weight loss was improved by both treatments, with significant recovery in the combination group. Kidney and heart weights decreased in all experimental groups, with reductions in HRS and combination groups being statistically highly significant, and the greatest heart weight reduction observed in the combination group.


Preparing Homogenous mixture of hingula and gandhaka


Kpi bharana


Shalaka incertion


Hingulad Rasa Sindoora

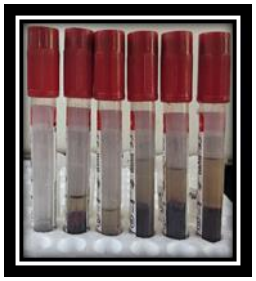

Hingulad rasa Sindoora sample ptreparation

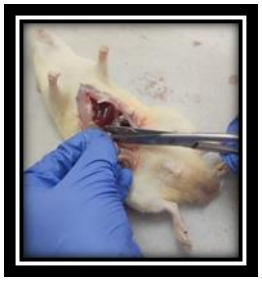

Guduchi swarasa


Administration of HRS


Administration of guduchi swarasa

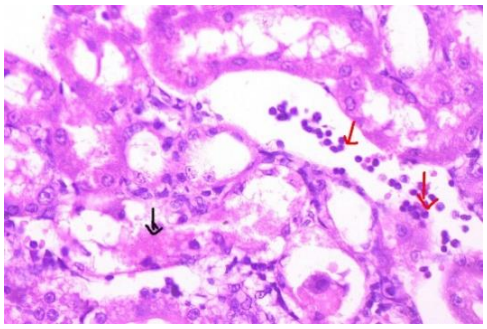

Blood collection


Centrifuged blood sample

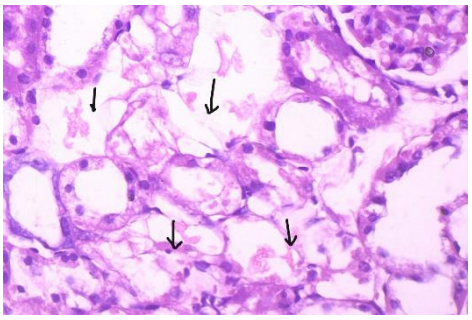

Kidney dissection


Dissected kidneys

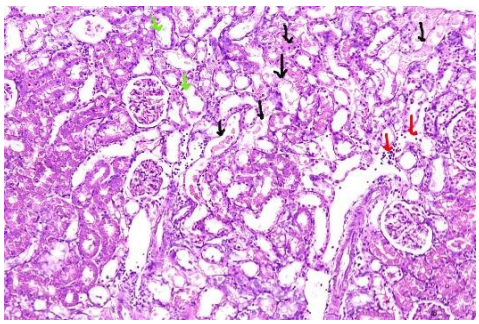
Positive control group change



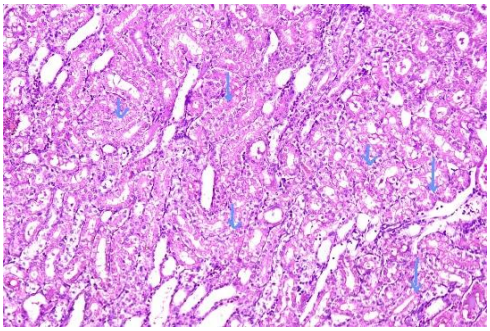
Necrosed tubuele and inflammatory infiltrate



Necrosed tubele in cortex 1

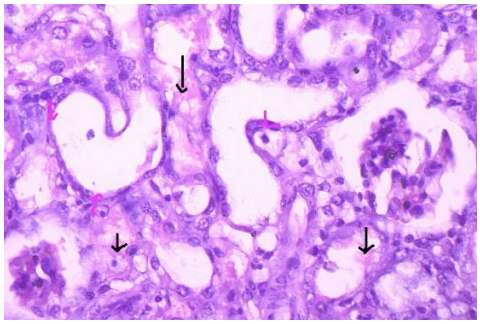


Degenerated tubules in cortex

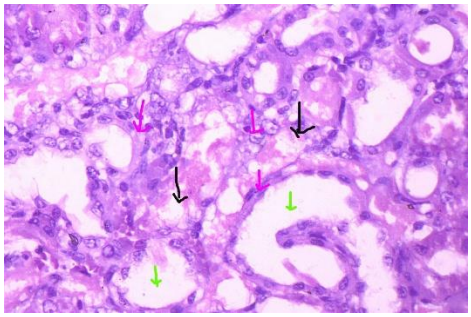


Necrosed tubules, dilated tubules, imflamatory infiltrates

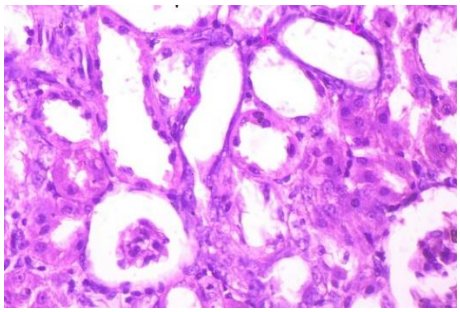
Group B



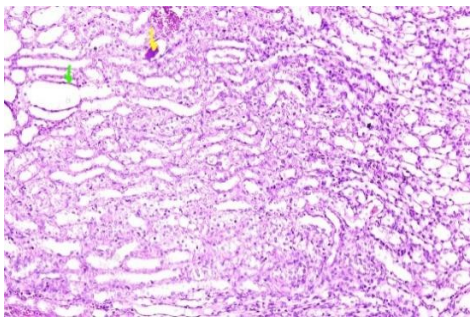
Area showing regeneration around the



Area of regeneration around dilated and necrosed tubules 2 necrosed tubules



Area showing regeneration around dilated tubules



Area showing dilated tubules and cast

HISTOPATHOLOGICAL STUDIES

Kidney

Table: Showing histological changes.

Slide No	Necrosis	Inflammatory infiltrate	Degenerated tubules	Dilated tubules	Cast	Fibrosis
C1	++	+	++	+	++	-
C2	-	-	-	++	+	-
A-1	+	-	+	-	+	-
A-2	-	+	+	-	+	-
B-1	++	-	+	++	-	-
B-2	+++	-	++	++	++	-

-Nil, + Mild, ++ Moderate, +++ severe

Histopathological Studies – Kidney

All slides showed kidney tissue comprising cortex and medulla. The cortex contained renal corpuscles along with tubules of varying morphology, including proximal and distal convoluted tubules.

Positive control group (C)

C1 showed necrosed tubules with loss of nuclear and cellular details. Degenerated tubules with pale cytoplasm and sloughed cellular remnants within the lumen were observed in most areas. Mild chronic inflammatory infiltrate was present. Dilated tubules and intratubular casts were noted in both cortex and medulla.

HRS group (A)

Very few necrosed tubules were observed in A1. Some tubules showed degeneration with sloughing of epithelial cells and occasional casts. Mild chronic lymphocytic infiltrate was seen in one slide, with no evidence of tubular dilatation. Compared to the positive control, there was a marked reduction in necrosis, tubular degeneration, and overall histological damage.

HRS + Guduchi Swarasa group (B)

Both slides showed necrosed tubules with loss of nuclear and cellular details, along with degenerated and dilated tubules containing casts in certain areas. Notably, areas of regeneration characterized by cytoplasmic basophilia, karyomegaly, and nuclear crowding were observed adjacent to necrosed and dilated tubules. Compared to the positive control, regenerative changes were evident in both cortex and medulla.

DISCUSSION

Pharmaceutical and analytical

The preparation of Hingulad Rasa Sindoora was carried out through a systematic pharmaceutical process involving shodhana, mardana, and Kupipaka, which ensured effective detoxification, uniformity, and formation of a stable final product. Controlled application of Mridu, Madhyama, and Teevra agni facilitated appropriate mercury–sulfur interaction and sublimation, resulting in the formation of purified HgS with minimal material loss. Classical parameters confirmed fineness and absence of free mercury, while modern analytical evaluations (pH, ash values, XRD, SEM-EDS, FTIR, and particle size analysis) substantiated the purity, crystalline nature of HgS, nanoscale particle size, organo-metallic character, and overall pharmaceutical stability of the formulation.

The study assessed the nephroprotective effects of Hingulad Rasa Sindoora (HRS), alone and with Guduchi Swarasa, against gentamicin-induced nephrotoxicity in Wistar albino rats using physical, biochemical, and histopathological evaluations.

Biochemical Parameters

Blood Urea

Gentamicin markedly increased serum urea, indicating renal insufficiency. Treatment with *Hingulad Rasasindoora* (HRS) significantly reduced urea levels, reflecting improved glomerular filtration and cortical perfusion. The effect is attributed to HRS's antioxidant and microcirculatory enhancing actions that stabilize nephron membranes and promote nitrogenous waste excretion.

Serum Creatinine

A significant rise in creatinine after gentamicin confirmed glomerular and tubular dysfunction. HRS normalized creatinine, indicating restoration of tubular transport, mitochondrial metabolism, and Na^+/K^+ ATPase activity through antioxidant protection.

Serum Uric Acid

Gentamicin lowered uric acid due to impaired purine metabolism. HRS partially restored levels, suggesting improved oxidative balance and tubular transport, while the combination with *Guduchi swarasa* showed additional though non-significant improvement.

Bilirubin (Direct and Total)

Gentamicin induced mild hepatic stress reflected by increased bilirubin levels. HRS reduced both direct and total bilirubin, indicating hepato-renal functional recovery. Guduchi further improved hepatic parameters, showing synergistic hepatoprotective but limited nephroprotective benefit.

Alkaline Phosphatase (ALP)

ALP elevation in HRS-treated groups likely represents tissue regeneration rather than injury. The rise suggests activation of renal prostaglandin and GAG-mediated

repair pathways, confirming cellular regeneration and metabolic reactivation under HRS therapy.

Physical Changes

Gentamicin-induced nephrotoxicity led to marked reductions in body, kidney, and heart weights, indicating systemic catabolism, renal tissue damage, and circulatory imbalance. Treatment with *Hingulad Rasa Sindoora* (HRS) improved all parameters, while its combination with *Guduchi swarasa* produced a highly significant increase in body weight ($P<0.0457$), demonstrating enhanced metabolic restoration through Rasayana and immunomodulatory effects.

Although kidney and heart weights remained lower than normal, significant improvement was observed in the HRS and combination groups ($P<0.0002$ and $P<0.0001$, respectively), suggesting reduction in edema, inflammation, and necrosis. Overall, HRS, particularly when combined with *Guduchi swarasa*, effectively restored systemic balance and supported structural and functional recovery during nephrotoxic stress.

Discussion on Histopathological Study

The gentamicin-treated group exhibited classical features of proximal tubular necrosis—cellular degeneration, tubular dilation, proteinaceous casts, and mild inflammation—indicative of acute nephrotoxic injury due to oxidative and mitochondrial damage.

In contrast, kidneys from the HRS-treated group showed near-normal architecture with minimal necrosis, rare casts, and absence of fibrosis, suggesting effective protection and membrane stabilization. These findings reflect the antioxidant and Rasayana properties of HRS that preserve renal cellular integrity and enhance recovery.

The HRS + *Guduchi swarasa* group demonstrated evidence of active tubular regeneration with basophilic cytoplasm and nuclear proliferation, signifying repair and rejuvenation. Guduchi's adaptogenic and antioxidant effects appeared to complement HRS action, promoting faster tissue recovery and functional restoration this may aligns with rasayana property of hrs and Guduchi swarsa.

Probable Mode of Action of Hingulad Rasa Sindoora (HRS)

Hingulad Rasa Sindoora (HRS), a Kupipakwa Rasayana prepared from Shuddha Hingula and Shuddha Gandhaka forming a stable mercuric sulfide (HgS) compound, possesses Guru, Snigdha, and Ushna qualities with Rasayana and Tridoshahara properties. Hingula acts as Kapha-Pittahara and Garavishahara, aiding toxin elimination, while Gandhaka contributes Deepana, Pachana, Vishahara, and Rasayana actions, together promoting Deepana, Pachana, Mutrala, and tissue regeneration across Srotasas. Its indication with Guduchi Swarasa in Prameha suggests a targeted action on Mutravaha Srotas with cytoprotective and nephroregenerative potential, further enhanced by Guduchi's nephroprotective, hepatoprotective, and antioxidant effects.

From a modern perspective, HRS exhibits potent antioxidant and cytoprotective activity by neutralizing free radicals and supporting glutathione regeneration. The sulfur–mercury (HgS) lattice maintains mitochondrial and tubular integrity, reflected by reduced serum urea and creatinine. Its *Guru-Snigdha* nature stabilizes glomerular membranes and maintains electrolyte–water balance, while As a Rasayana, HRS reduces inflammatory cytokines, alleviates edema, and restores vitality of Rasa, Rakta, Mamsa, and Meda Dhatus, ensuring epithelial regeneration and normalized renal morphology. Enhanced microcirculation and prostaglandin-mediated perfusion preserve nephron oxygenation, aligning with improved ALP activity and tissue repair. Additionally, HRS chelates reactive ions and toxins, reducing oxidative burden and enhancing renal detoxification. Acting as a *Yogavahi*, it improves gastrointestinal absorption and potentiates the bioactivity of *Guduchi Swarasa*.

Thus, the synergistic *Rasayana* and *Shodhana* actions of *Hingula* and *Gandhaka* in HRS confer potent nephroprotective, antioxidant, and rejuvenative effects, effectively restoring renal function and maintaining systemic homeostasis.

CONCLUSION

The study scientifically validated *Hingulad Rasa Sindoora* (HRS) as a safe, stable, and potent *Kupipakwa Rasayana* with significant nephroprotective activity. Prepared from *Shuddha Hingula* and *Shuddha Gandhaka*, analytical tests confirmed formation of detoxified nanosized HgS particles (~465 nm). Experimentally, HRS markedly reduced serum urea and creatinine ($p < 0.05$) and restored renal architecture. Its *Deepana*, *Pachana*, *Mutrala*, *Rasayana*, and *Tridoshahara* properties improved *Agni*, cleared *Aama*, and rejuvenated *Mutravaha Srotas*. *Guduchi Swarasa Anupana* enhanced *Rasayana* and *Vishahara* effects. Thus, HRS exerts nephroprotective action through antioxidant, cytoprotective, and classical Ayurvedic mechanisms, substantiating its therapeutic relevance in protecting renal function.

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