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EXPERIMENTAL STUDY ON THE NEPHROPROTECTIVE POTENTIAL OF SHLESHMATAKADHYA AGAD IN PARACETAMOL-TREATED WISTAR RATS

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ABSTRACT

Introduction: Paracetamol overdose is a known cause of nephrotoxicity due to the accumulation of its toxic metabolite, N-acetyl-p-benzoquinone imine (NAPQI), which causes oxidative stress and renal damage. In Ayurvedic terms, this condition corresponds to the concept of *Dushi Visha*—a form of chronic, latent toxicity. **Material and Methods:** An experimental study was conducted on 18 Wistar rats divided into three groups (n = 6): Control (distilled water), Test *(Shleshmatakadhya Agad*, 12 gm/kg), and Standard (Mucinac, 54 mg/kg). Nephrotoxicity was induced by oral administration of paracetamol (1000 mg/kg) for seven days. Treatments continued for 30 days, followed by evaluation of Biochemical parameters (blood urea and serum creatinine) and kidney weight. Histopathological analysis of kidney tissues and acute toxicity testing (300mg/kg up to 2000 mg/kg) were also performed. **Result:** The results showed that the test group treated with *Shleshmatakadhya Agad* exhibited a significant reduction in blood urea (from 37.57 ± 2.10 to 24.71 ± 1.86 mg/dL, $p = 0.0012$) and serum creatinine levels (from 2.93 ± 0.28 to 1.21 ± 0.10 mg/dL, $p < 0.0001$), compared to the control group. Histopathological examination confirmed a marked improvement in renal architecture, showing only mild inflammation and necrosis in the test and standard groups, as opposed to moderate damage in the control group. No significant changes in kidney weight or no behavioural abnormalities were observed, indicating the formulation's safety. **Conclusion:** *Shleshmatakadhya Agad* significantly reduced nephrotoxicity markers and improved renal histology, supporting its traditional use as a *Vishaghna* and suggesting its potential as a safe, effective herbal nephroprotective formulation.

KEYWORD: Paracetamol, Nephrotoxicity, *Dushi visha*, *Shleshmatakadhya Agad.*

INTODUCTION

The kidneys play an important role in human physiology, maintaining fluid homeostasis, regulating blood pressure, erythrocyte production and bone density, regulating hormonal balance, and filtering and removing nitrogenous and other waste products. The kidney gets affected by various etiological factors which cause structural as well as functional damage to kidneys termed as Nephrotoxicity. "Nephrotoxicity is defined as the impairment of kidney function caused by exposure to toxic substances, including specific chemicals and medications. A wide range of therapeutic agents are known to adversely affect the kidneys, resulting in pathological conditions such as acute renal failure, chronic interstitial nephritis, and nephrotic syndrome.

Acetaminophen (N-acetyl-p-aminophenol; APAP) is also known as paracetamol, is generally accepted as a safe drug for analgesic and antipyretic action when administered within the therapeutic range. Its overdose can lead to hepatic and renal damage in both human and experimental animals.[1-3] Kidney is the second target organ of paracetamol toxicity and renal dysfunction.

In *Ayurveda*, poisons are classified as *Sthavar Visha*, *Jangam Visha* & *Kritrima Visha* (Artificial poison). "Dushi Visha is a latent form of poison derived from *Sthavara* (plant-based), *Jangama* (animal-based), or *Kritrima Visha* (artificial toxins) that cannot be completely eliminated from the body. Instead, it becomes attenuated or denatured due to prior exposure to anti-poisonous agents, as well as the effects of environmental factors such as heat and air. Despite its reduced potency, Dushi Visha does not cause immediate fatality; however, it remains latent in the body, encapsulated by *Kapha Dosha*, and can persist for years. Over time, this residual toxicity may contribute to the development of various chronic diseases and systemic imbalances. Prolonged use of any drug leading to chronic toxicity is also a type of *Dushi Visha.*[4-7]

So, here Nephrotoxicity due to Paracetamol can be correlated with this concept of *Dushi Visha*. Looking at this, nephroprotective drugs may play an important role in this area. *Shleshmatakadhya Agad* is the herbal medication stated by *Yogaratnakara* under the *Visha Chikitsa* chapter for its detoxifying effects (*Sarva Visha Nashanam*).[8] It contains *Shleshmataka* (*Cordia dichotoma*), *Guduchi* (*Tinospora cordifolia*), *Apamarga* (*Achyranthes aspera*), *Nripdruma* (*Cassia fistula*), *Kantakari* (*Solanum surattense*), and *Brihati* (*Solanum indicum*). The ingredients of *Shleshmatakadhya Agad* possess anti-inflammatory, immunomodulatory, anti-toxic, nephroprotective and anti-oxidant properties.[9-12]

Therefore, the present experimental study was conducted to evaluate the nephroprotective activity of *Shleshmatakadhya Agad* in Paracetamol induced nephrotoxicity animal model.

MATERIALS AND METHODS

PREPARATION OF DRUGS AND SOLUTIONS

Test Drug - In *Shleshmatakadhya Agad*, no specific proportions (*pramana*) of the ingredients are mentioned. Therefore, in accordance with *Sharangadhara Samhita*, "when proportions are not specified, all ingredients in a formulation should be taken in equal quantities.[13]" Accordingly, all the ingredients were taken in equal proportions, powdered individually, and then thoroughly mixed to form a homogeneous fine powder.

Dose calculation of *Shleshmatakadhya Agad*: Dose of *Churna* in *Sharangadhara Samhita* was given as 12 gm.[14]
So, Human dose of *Shleshmatakadhya Agad* = 12gm
Conversion factor of rat (200gm) = 0.018 X 5
Dose of *Shleshmatakadhya Agad* in rat = 12mg X 0.018 X 5 i.e. dose of rat = 1.08 gm/200g body weight with distilled water to form a uniform 20% solution

Toxicant (Paracetamol): Paracetamol was used to induce nephro toxicity as per the established protocol, at a dosage of 1000 mg/kg body weight.

Dose: Paracetamol- Rat dose= 1000 mg/kg Body wt.

Standard Drug: Mucinac (N-acetylcysteine) was used as the standard nephroprotective agent and was administered orally at a dose of 54 mg/kg body weight.

Dose: Mucinac- Rat dose = 54 mg/kg Body wt. mixed in distilled water to prepare a 10 % solultion.

ADMINISTRATION OF DOSES

The test drug (*Shleshmatakadhya Agad*), Standard drug (Mucinac) and toxicity drug (paracetamol) was administered orally by using oral gavage feeding tube fixed to the syringe.

ANIMALS

The experimental animal room had a temperature of 22°C ± 3°C. Artificial lighting was used, with 12 hours of light and 12 hours of darkness. Regular laboratory diets and supply of drinking water was allowed for feeding.

The Wistar rats were randomly selected, marked with Picric acid as H (marking on head), B (marking on the back), T (marking on the tail), HB (marking on the head and tail), BT (marking on the back tail), HT (marking on the head tail) for individual identification and kept in their cages 7 days prior to dosing to allow acclimatization to the laboratory conditions.

STUDY PROTOCOL

IEC: The experimental protocol was approved by Rishikul Campus, Uttarakhand Ayurved University Registration NO- UAU/RC/IEC/2024.PG.189.

IAEC: The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Drug Innovative Centre, A unit of Bilwal Medchem and Research Laboratory Pvt. Ltd., Jaipur, Rajasthan, on 18/07/2024 (Reference No.: BMRL/DIC/CCSEA/IAEC/2024/I/06). The study was conducted in accordance with the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA), under registration number 2304/PO/Rc/S/2024/CCSEA.

The acute oral toxicity study was conducted in accordance with OECD Guideline 423 (Annexure 2c) and the evaluation of hepatoprotective activity was performed following the protocols outlined in OECD Guideline 407.

STUDY DESIGN

Acute oral toxicity study: 6 Wister rats were divided in two groups each group have 3 Wistar rats.
Group 1 - 3 Wister rats had received *Shleshmatakadhya Agad* at dose 300mg/kg orally.
Group 2 - 3 Wister rats had received *Shleshmatakadhya Agad* at dose 2000mg/kg orally.

Evaluation of Nephroprotective Activity of *Shleshmatakadhya Agad*

Eighteen healthy Wistar rats had been selected in this animal model and divided in three groups each group contain six rats. All rats orally received 1000 mg/kg of PCM for 7 days to induce nephrotoxicity.

Control Group - 1: Six Nephrotoxicity induced Wistar rats received distilled water 5 ml/kg/P.O. for 30 days.

Test Group - 2: Six Nephrotoxicity induced Wistar rats received Test sample (*Shleshmatakadhay Agad*) 12 mg/kg/P.O. for 30 days

Standard Group - 3: Six Nephrotoxicity induced Wistar rats received Standard drug (Mucinac) 54 mg/kg/P.O. for 30 days

ASSESMENT PARAMETERS

Acute oral toxicity study includes changes in Behavioural, Haematological parameters.

Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter for 14 days.

- **Behavioural changes**- Skin and fur, eyes, mucous membranes, salivation, diarrhoea, lethargy, sleep, coma, convulsions, tremors, mortality, morbidity were observed these finding are shown in table no 1 and 2.
- **Haematological parameters-** Finding of complete blood count shown in table no 3.

  Nephroprotective study includes changes in Biochemical parameters, Each Kidney weight and histopathological changes.
- **Biochemical Parameters** – kidney function tests - including Serum Creatinine and Blood Urea was conducted with the help of automatic biochemistry analyser. Blood samples were collected from animals through retro orbital plexus under aseptic conditions. The biochemical finding is presented in Table no 4 and 5
- **Collection of organs** - At the end of the experimental period, three animals of each group were sacrificed by cervical dislocation and each kidney was carefully dissected out, cleaned to remove extraneous tissues, blotted to eliminate blood stain and weighed these finding are presented in table no 6 and 7.
- **Histopathological studies** - A portion of kidney tissue was preserved in 10% buffered formalin for histopathological examination finding are presented in figure no 1.

STATISTICAL ANALYSIS

All the observation were analysed statistically. Mean, standard error for each group was calculated. Statistical evaluation of the data was done by Two-way ANOVA and Dunnett's Multiple Comparison Tests. All the groups were compared with Control group, Test group (*Shleshmatakadhya Agad*) and Standard group (Mucinac). The Multiple Comparison Test was performed only if the P value obtained from ANOVA was less than 0.0001. (P value < 0.0001 was considered significant).

OBSERVATIONS

Acute oral toxicity study- According to OECD Guideline 423 (Annexure 2c)

Table no 1: Behavioral Observation.

			Group 1			
Observation	**30min.**	**4hr.**	**24hr.**	**48hr.**	**1w**	**2w**
Skin, Fur and Eyes	Normal	Normal	Normal	Normal	Normal	Normal
Mucous Membrane	Normal	Normal	Normal	Normal	Normal	Normal
Salivation, Lethargy	Absent	Absent	Absent	Absent	Absent	Absent
Sleep	Normal	Normal	Normal	Normal	Normal	Normal
Coma	Absent	Absent	Absent	Absent	Absent	Absent
Convulsions and Tremors	Absent	Absent	Absent	Absent	Absent	Absent
Diarrhea	Absent	Absent	Absent	Absent	Absent	Absent
Morbidity and Mortality	Absent	Absent	Absent	Absent	Absent	Absent

Table No 2: Behavioral Observation.

			Group 2			
Observation	**30min.**	**4hr.**	**24hr.**	**48hr.**	**1w**	**2w**
Skin, Fur and Eyes	Normal	Normal	Normal	Normal	Normal	Normal
Mucous Membrane	Normal	Normal	Normal	Normal	Normal	Normal
Salivation, Lethargy	Absent	Absent	Absent	Absent	Absent	Absent
Sleep	Normal	Normal	Normal	Normal	Normal	Normal
Coma	Absent	Absent	Absent	Absent	Absent	Absent
Convulsions and Tremors	Absent	Absent	Absent	Absent	Absent	Absent
Diarrhea	Absent	Absent	Absent	Absent	Absent	Absent
Morbidity and Mortality	Absent	Absent	Absent	Absent	Absent	Absent

Table 3: Showing haematological parameter in Acute oral toxicity study.

S.no	Parameters	Group 1	Group 2
1.	**WBC**	4.53±1.335	4.33±0.895
2.	**LYM%**	72.53±1.991	82.43±1.737
3.	**MID%**	7.33±0.533	6.27±1.299
4.	**NEUT%**	20.13±2.146	8.97±3.028
5.	**RBC**	6.48±0.610	5.82±0.496
6.	**HGB**	11.57±1.477	11.67±0.953
7.	**HCT**	38.10±4.813	26.40±6.102
8.	**MCV**	58.53±1.832	60.27±0.617
9.	**MCH**	17.73±0.601	20.00±0.208
10.	**MCHC**	30.33±0.612	33.27±0.296
11.	**PLT**	323.33±56.846	607.33±25.129
12.	**MPV**	12.60±2.804	8.90±0.551
13.	**PDW**	16.53±0.797	15.43±0.318
14.	**PCT**	0.39±0.068	0.54±0.057
15.	**P-LCR**	40.50±12.492	21.20±5.292

Evaluation of Nephroprotective Activity of *Shleshmatakadhya Agad*

- Present study was aimed to check the Nephroprotective activity of *Shleshmatakadhya Agad* on Paracetamol induced Nephrotoxicity.
- Nephrotoxicity was induced by oral administration of PCM 1000 mg/kg for 7 consecutive days. Therefore, all the groups received their respective treatment for a period of 30 days. On the 31st day, Kidney function tests and histopathology examination were conducted to evaluate the Renal changes.

Kidney Function Test

Table no 4: Effects of *Shleshmatakadhya Agad* on biochemical parameters in Paracetamol induced nephrotoxicity.

Parameters	Group 1	Group 2	Group 3
Blood Urea	37.57±2.100	24.71±1.866	24.39±1.976
Serum Creatinine	2.93±0.281	1.21±0.102	0.93±0.105

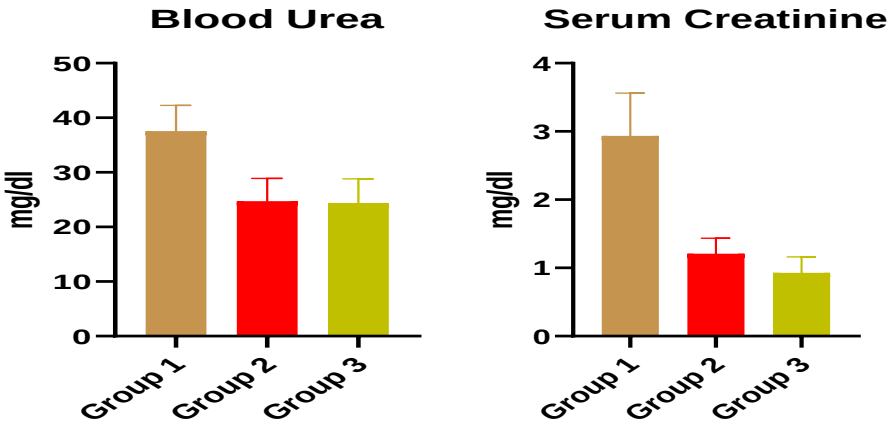
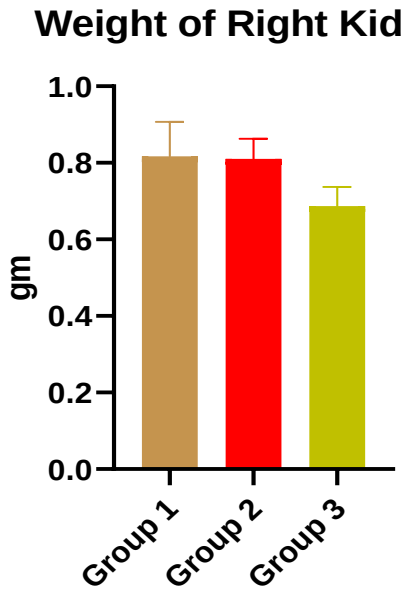


Table no 5: Two-way ANOVA followed by Dunnett's Multiple Comparisons Test finding on biochemical parameters.

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
		Blood Urea			
Group 1 vs. Group 2	12.86	5.840 to 19.88	Yes	**	0.0012
Group 1 vs. Group 3	13.18	6.166 to 20.20	Yes	***	0.0010
		Serum Creatinine			
Group 1 vs. Group 2	1.726	1.079 to 2.373	Yes	****	<0.0001
Group 1 vs. Group 3	2.006	1.359 to 2.653	Yes	****	<0.0001

Table no 6: Effects of *Shleshmatakadhya Agad* on changes in kidney weight in paracetamol induced nephrotoxicity.

Organ Weight (gm)	Group 1 Mean±SEM	Group 2 Mean±SEM	Group 3 Mean±SEM
Right Kidney	0.82±0.040	0.81±0.023	0.69±0.022
Left Kidney	0.85±0.034	0.83±0.013	0.75±0.036



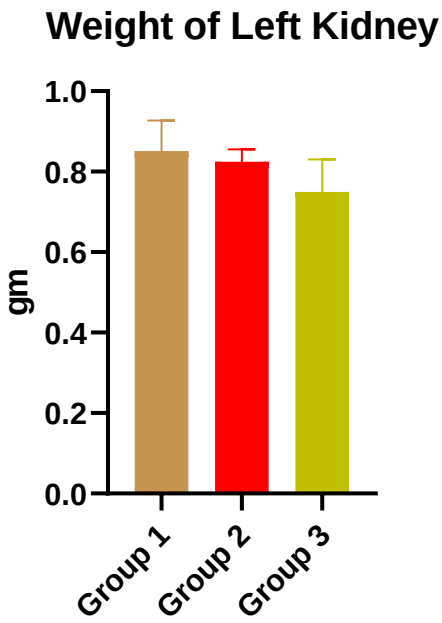


Table no 7: Two-way ANOVA followed by Dunnett's Multiple Comparisons Test finding on weight of kidney.

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
		Right Kidney			
Group 1 vs. Group 2	0.006000	-0.09951 to 0.1115	No	ns	0.9853
Group 1 vs. Group 3	0.1298	0.02429 to 0.2353	Yes	*	0.0176
		Left Kidney			
Group 1 vs. Group 2	0.02660	-0.07779 to 0.1310	No	ns	0.7537
Group 1 vs. Group 3	0.1018	-0.002588 to 0.2062	No	ns	0.0558

RESULTS

Blood Urea

Distilled water administration in Group1(control group) resulted in a significant elevation of blood urea level (37.57±2.100), indicating impaired renal function. Treatment with *Shleshmatakadhya Agad* (Group 2) significantly reduced blood urea to 24.71±1.866(p=0.0012), while the standard drug group receiving Mucinac (Group 3) showed a comparable decrease to 24.39±1.976 (p=0.0010).

Serum Creatinine

A marked increase in serum creatinine was observed in the distilled water control group 1(2.93 ± 0.28 mg/dL), consistent with reduced glomerular filtration rate and renal impairment. Treatment with *Shleshmatakadhya Agad* Group 2 significantly lowered creatinine levels to 1.21 ± 0.10 mg/dL ($p < 0.0001$), and the standard group receiving Mucinac exhibited a further reduction to 0.93 ± 0.11 mg/dL ($p < 0.0001$). These findings suggest effective preservation and restoration of renal function by both the test and standard drugs.

Right Kidney Weight

The mean right kidney weight in the control group (Group 1 treated with distilled water) was 0.82 ± 0.040 g. Group 2 (treated with *Shleshmatakadhya Agad*) showed a slight, non-significant reduction to 0.81 ± 0.023 g ($p = 0.9853$ vs. Group 1). In contrast, Group 3 (standard drug, Mucinac) exhibited a statistically significant decrease in

right kidney weight to 0.69 ± 0.022 g (p = 0.0176 vs. Group 1), indicating a potential reversal of inflammatory swelling or hypertrophy.

Left Kidney Weight

The mean left kidney weight in control Group 1(treated with distilled water) was 0.85 ± 0.034 g. Group 2 (treated with *Shleshmatakadhya Agad)* recorded 0.83 ± 0.013 g (p = 0.7537 vs. Group 1), and Group 3 (standard drug, Mucinac) recorded 0.75 ± 0.036 g (p = 0.0558 vs. Group 1). Although Group 3 showed a downward trend, the reduction was not statistically significant.

Histopathology changes

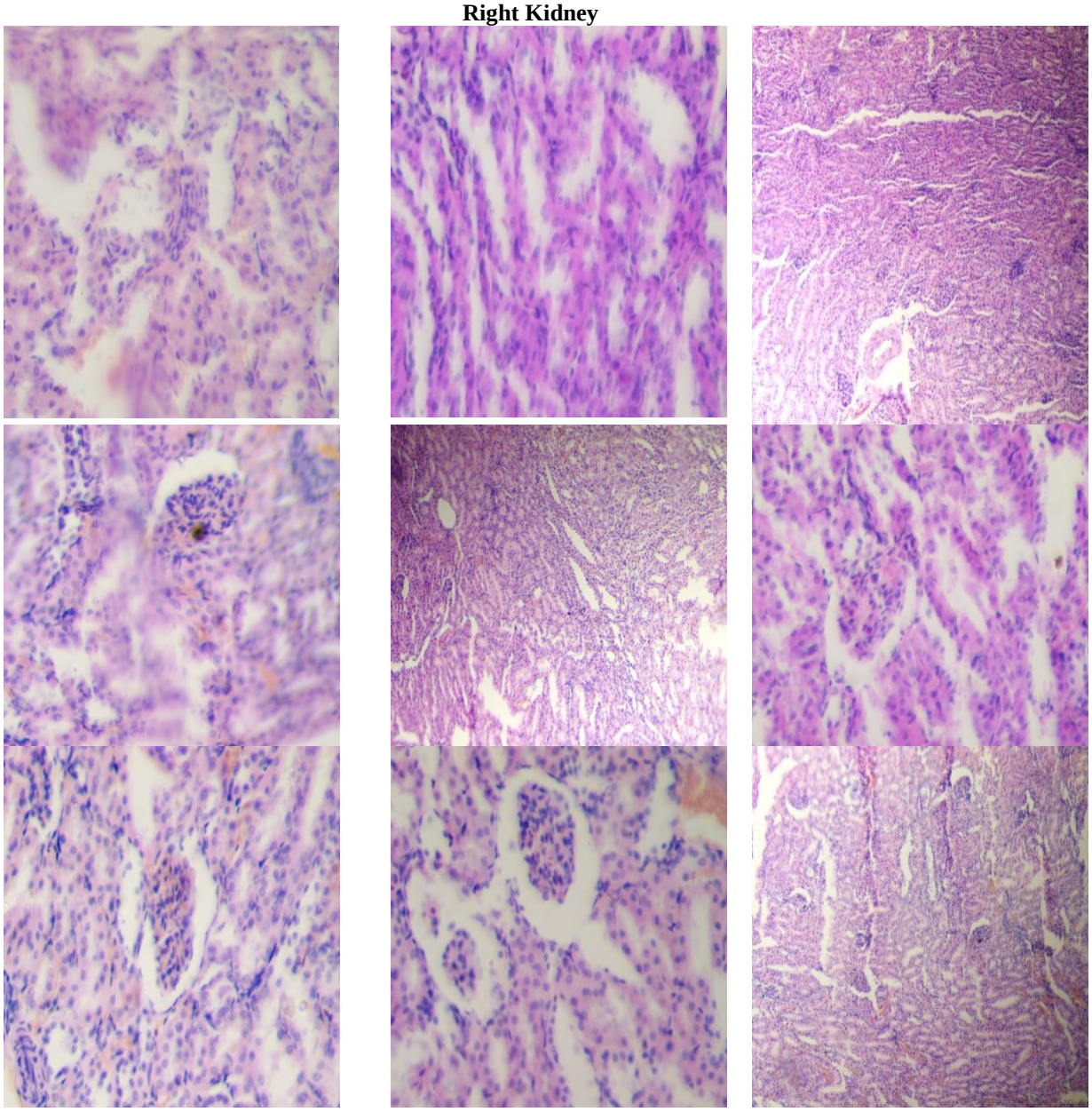
Control group 1- Observed moderate inflammation, necrosis, and Moderate degeneration of the renal cortex (glomerulus and Bowman's capsule) and nephron renal pyramids in histopathological examination.

Test group 2 Mild inflammation, necrosis, and Mild degeneration of the renal cortex (glomerulus and Bowman's capsule) and nephron renal pyramids was found in histopathological examination.

Standard group 3 Mild inflammation, necrosis, and Mild degeneration of the renal cortex (glomerulus and Bowman's capsule) and nephron renal pyramids was observed in histopathological examination.

Group 1 **Group 2** **Group 3**

Right Kidney



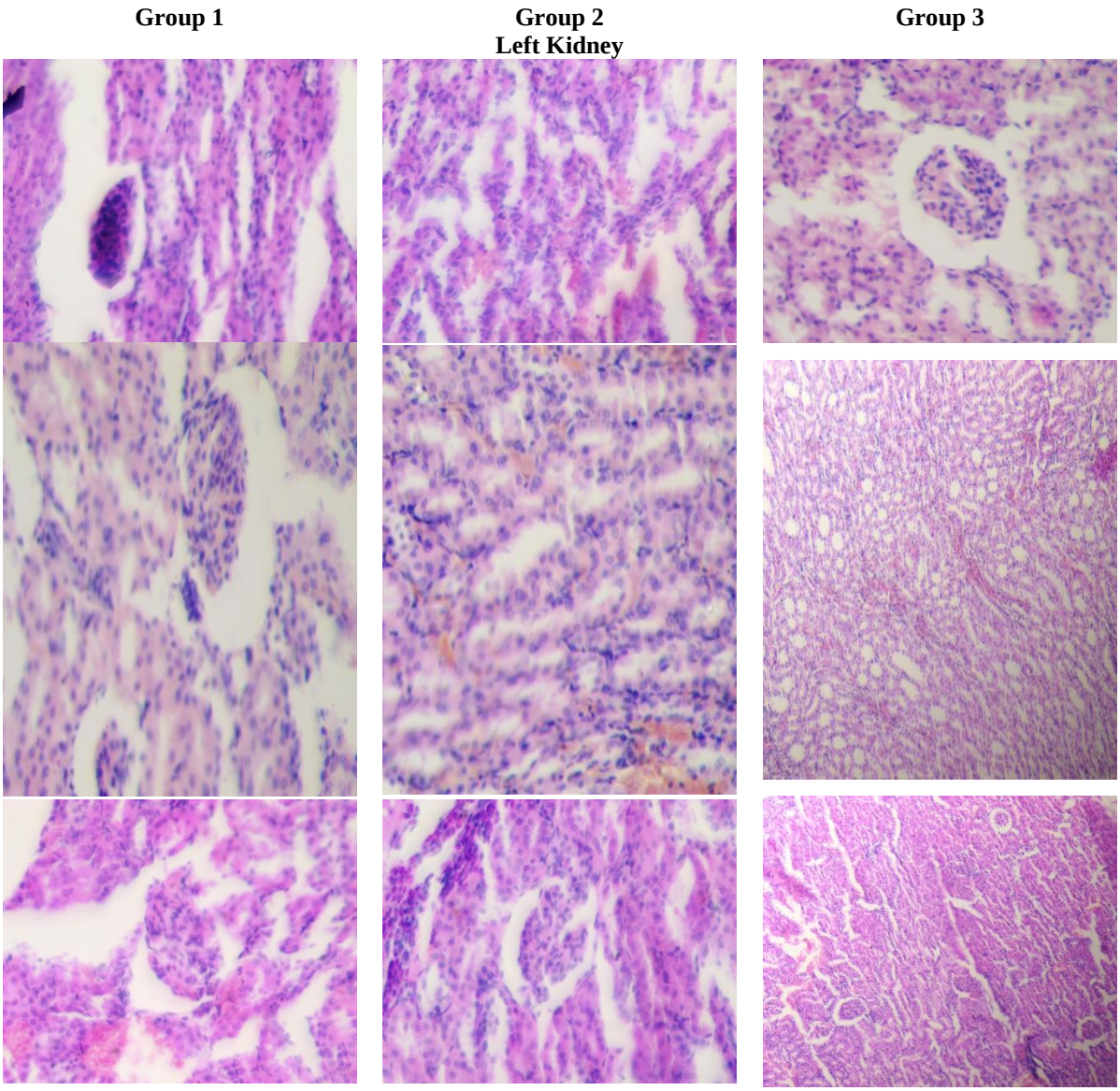


Figure 1: Histopathology in Kidney.

DISCUSSION

Nephrotoxicity remains a clinically significant concern, particularly with the overuse of widely accessible medications such as paracetamol. Although paracetamol is generally safe at therapeutic doses, its metabolism at higher concentrations leads to the formation of the toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI). This compound is responsible for inducing oxidative stress, glutathione depletion, and mitochondrial dysfunction, thereby causing structural damage to renal tubular epithelium and impairing renal function.

The constituents of *Shleshmatakadhya Agad* undergo *Avasthapaka* (primary digestion) facilitated by *Jatharagni* (digestive fire). The presence of *Tikta* (bitter) and *Kashaya* (astringent) *Rasa* enhances liver and kidney detoxification processes, while *Ushna Virya* (hot potency) of herbs like *Guduchi* promotes the bioavailability of active constituents through rapid absorption in the *Anna Vaha Srotas* (gastrointestinal tract). After absorption, the formulation is distributed via *Rakta Vaha Srotas* (blood channels), reaching the *Yakrit* (liver) and *Vrikka* (kidneys). Herbs with *Sukshma* (subtle) and *Vyavayi* (penetrating) properties facilitate deep tissue penetration and fast action. The *Madhura Vipaka* (sweet post-digestive effect) of several constituents supports the regeneration of *Rakta* and *Mamsa Dhatu* (blood and muscular tissue), aiding the repair of nephronal structures. Elimination of the formulation's metabolic by-products is achieved through the *Mutra Vaha Srotas* (urinary channels) and *Purisha Vaha Srotas* (fecal route), aligning with its mild *Shodhana* (cleansing) and *Ama-pachana* (detoxifying) properties. Notably, the Prabhava (specific action) of *Shleshmatakadhya Agad* is its ability to act as a *Vishaghna* (antitoxic agent), targeting *Dushi Visha*—a concept in Ayurveda that refers to latent toxins accumulated in the body due to chronic exposure to low-grade poisons such as drugs, environmental toxins, and improper lifestyle. In this context, paracetamol toxicity

closely parallels *Dushi Visha*, and the observed therapeutic effects of *Shleshmatakadhya Agad* correspond with this traditional concept.

CONCLUSION

The present experimental study demonstrated that *Shleshmatakadhya Agad* exerts significant nephroprotective effects in a paracetamol-induced nephrotoxicity model in Wistar rats. Administration of the formulation led to a substantial reduction in elevated blood urea and serum creatinine levels, indicating improved renal function. Although kidney weight changes were not statistically significant in the test group, histopathological evaluation confirmed reduced inflammation, necrosis, and degeneration in renal tissues compared to the control group.

The observed effects can be attributed to the synergistic action of ingredients possessing antioxidant, anti-inflammatory, and detoxifying properties. These findings are consistent with the Ayurvedic concept of *Dushi Visha*, under which chronic low-grade toxicity such as that from prolonged drug exposure is classified. The formulation's efficacy also supports its traditional use in *Visha Chikitsa* (toxicology) as described in *Yogaratnakara*.

Thus, *Shleshmatakadhya Agad* holds promise as a safe and effective herbal formulation for mitigating drug-induced nephrotoxicity and may serve as a potential complementary nephroprotective therapy.

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