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AN EXPERIMENTAL STUDY TO EVALUATE ACUTE AND SUB-ACUTE ORAL TOXICITY OF KSHARAGADA ON WISTAR ALBINO RATS

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ABSTRACT

Introduction: Drug-induced toxicity represents the adverse effects arising from pharmaceutical substances, potentially leading to severe complications. This study investigates *Ksharagada* (KA), a traditional Ayurvedic herbo-mineral formulation, for its safety profile through acute and sub-acute oral toxicity studies in female Wistar albino rats. **Materials and Methods**: *Ksharagada* was prepared using traditional methods, and its physicochemical properties were analyzed. Acute toxicity was assessed using five rats at doses of 2000 mg/kg and 5000 mg/kg, while sub-acute toxicity involved 24 rats divided into control and three dosage groups (540 mg/kg, 810 mg/kg, and 1080 mg/kg) over 28 days, followed by a 14-day observation period. Various biochemical parameters and histological examinations were conducted following OECD guidelines. **Results**: In the acute toxicity study, all rats survived without observable toxicity at doses up to 5000 mg/kg. The sub-acute study showed significant alterations in biochemical parameters, such as increased total bilirubin and decreased SGPT, SGOT, ALP, total protein, creatinine, urea, and uric acid in higher dose groups, suggesting potential hepatic and renal impacts. However, histopathological examinations of vital organs revealed no toxic effects. **Conclusion**: *Ksharagada* demonstrated a high safety margin in both acute and sub-acute toxicity studies, with an LD50 above 5000 mg/kg. Despite some biochemical alterations, the absence of

significant histopathological damage supports its safe use in traditional medicine. Further studies are recommended to validate these findings in clinical settings.

Keywords: *Ksharagada*, Physicochemical Analysis, Anticancer, Herbo-mineral Formulation, Drug-induced Toxicity

INTRODUCTION:

Drug-induced toxicity in humans refers to the adverse effects or harmful reactions caused by the administration of drugs or pharmaceutical substances. These toxic effects can range from mild discomfort to severe complications, including organ damage or failure, and even death. Some drugs may exert toxic effects due to their intended pharmacological actions. For example, chemotherapeutic agents used to treat cancer often cause toxicity to rapidly dividing cells, including normal tissues. Exceeding the recommended dosage of a drug can lead to toxicity. This can occur due to accidental overdose, misuse, or improper administration **[1]**. People may vary in their response to drugs due to factors such as genetic makeup, age, gender, underlying health conditions, or concurrent use of other medications. What may be a safe dose for one individual could be toxic for another **[2]**. Preventing drug-induced toxicity requires careful prescribing practices, patient education, monitoring for adverse effects, and prompt recognition and management of toxicity when it occurs. Ayurveda relies heavily on natural remedies, including herbs, minerals, and dietary supplements, which are believed to support the body's innate healing mechanisms. These natural remedies are often used to address underlying health conditions and promote overall well-being, potentially reducing the need for pharmaceutical drugs and their associated risks. *Agadas* or Anti-toxic formulation plays an important role in treating drug induced toxicity **[3]**. *Ksharagada* (KA) is a traditional Ayurvedic formulation renowned for its therapeutic efficacy **[4, 5]**. Ensuring its safety profile through rigorous toxicity studies is crucial for its continued use in traditional medicine. This study evaluates both the acute and sub-acute oral toxicity of KA in female Wistar albino rats, adhering to the OECD guidelines, specifically OECD 425 for acute toxicity and OECD 407 for sub-acute toxicity **[6, 7]**.

MATERIALS AND METHODS:

Pharmaceutical study: All the ingredients[4,5]for Ksharagada were collected from the Parul Institute of Ayurved Pharmacy and surrounding locations in Vadodara, Gujarat. The pharmaceutical experiment was carried out at the *Rasashastra* and *Bhaishajya Kalpana* Practical Halls, Parul Institute of Ayurved Pharmacy, Vadodara. *Taruna Palasha*

Kshara (Ash of Butea monosperma) was collected in, added six times to water in a 1:6 ratio, and stirred well. This mixture is kept for 2 hours and allowed to settle at the bottom of the glass jar. Filtration of the mixture is done with a double-folded cloth and transferred to a measuring jar. This filtering process was performed 21 times, and finally, *Ksharodka* (after the 21st filtration) was collected and measured. *Ksharodaka* was boiled in a steel vessel. After boiling for 30 minutes, *Sookshma churna* (fine powder) of all drugs was added and stirred well. Stirring continued until the mixture reached a thick consistency. After self-cooling, the *Ksharagada* was collected and stored in an airtight container and organoleptic features were observed.

Analytical study

KA was subjected to organoleptic parameters like loss on drying, total ash, acid insoluble ash, extractive values, and pH. Physico chemical analysis, HPTLC and GCMS were carried out as per the WHO guidelines and Ayurvedic pharmacopeia and Indian pharmacopeia.[8,9,10] Physicochemical analysis and HPTLC were performed at the VASU laboratory in Vadodara, while GCMS was analyzed at the Sophisticated Instrumentation Centre for Applied Research and Testing - SICART in Anand, Gujarat.

Acute toxicity Study [6]

In an acute toxicity study, *Ksharagada* (KA) powder, which is light maroon, thick, and soft with a pH of 7.82 and 69.62% water solubility, was tested on five female Wistar albino rats. The rats were kept at 23°C and acclimatized for five days with food and water before dosing. They were then fasted, but still given water, before being administered KA. The initial dose was 2000 mg/kg body weight, with a limit test dose of 5000 mg/kg body weight. The stock solution was prepared at a concentration of 1 ml per 100 g of body weight. Distilled water was used as the vehicle for dosing.

Sub-Acute Oral Toxicity Study [7]

In Sub acute oral toxicity study on *Ksharagada* (KA) powder, which is light maroon, thick, and soft with a pH of 7.82 and 69.62% water solubility, 24 female Wistar albino rats were used, divided into four groups of six: Normal Control (NC), *Ksharagada* Low Dose (KALD), Medium Dose (KAMD), and High Dose (KAHD). The rats were kept at 23°C, acclimatized for five days, and given pellets and water throughout the study. They received KA doses of 540 mg/kg (KALD), 810 mg/kg (KAMD), and 1080 mg/kg (KAHD), with the stock solution prepared at 1 ml per 100 g of body weight. The dosing lasted for 28 days, followed by a 14-day observation period.

OBSERVATIONS AND RESULTS:

Observations and Results of AOT study

Throughout the study on *Ksharagada* (KA) powder, various parameters were monitored in the rats. Food and water consumption were normal, and body weight remained stable at approximately 250 grams. There was no mucus secretion, and faecal colour was normal. The animals did not exhibit diarrhoea, sedation, convulsions, or drowsiness. Eye colour, skin condition, and urination were all normal, and no signs of coma were observed. Importantly, all animals remained alive throughout the study. The observations for acute toxicity at both 2000 mg/kg and 5000 mg/kg doses showed that all five animals survived without any observable signs of toxicity. Histopathological examination of the liver and kidneys revealed normal structures. The kidneys showed normal histological structure of glomeruli and tubules, while the liver displayed a normal central vein with hepatic lobules and hepatocytes. Ksharagada did not induce mortality or any observable signs of toxicity in the tested rats at doses up to 5000 mg/kg, indicating an LD50 above this dose.

Observations and Results of Sub Acute Study

Table 1: Showing Effect of *Kshragada* on serum biochemical parameters in wistr albino rats

	NC	KALD	KAMD	KAHD
Total Bilurubin	**0.345±0.004**	**0.341±0.03**	**0.411±0.002**	**0.411±0.003**
SGPT	**26.71±0.438**	**27.25± 0.405**	**26±0.109**	**22.83±0.089**
SGOT	**42.75±0.112**	**42±0.224**	**30.33±0.211**	**30.33±0.211**
ALP	**124.83±0.516**	**124.7±0.143**	**111.8±0.075**	**111.7±0.167**
Total Protien	**6.718±0.005**	**6.717±0.003**	**6.557±0.002**	**6.308±0.003**
Albumin	**4.155±0.0034**	**4.157±0.004**	**4.072±0.004**	**4.157±0.004**
Globulin	**1.74±0.006**	**1.74±0.004**	**1.742±0.003**	**1.832±0.003**
Creatinine	**0.825±0.0034**	**0.823±0.003**	**0.753±0.003**	**0.718±0.003**
Urea	**21.83±0.004**	**21.25±0.092**	**19.43±0.088**	**18.83±0.003**
Uric Acid	**2.12±0.079**	**1.985±0.004**	**1.673±0.005**	**1.622±0.022**

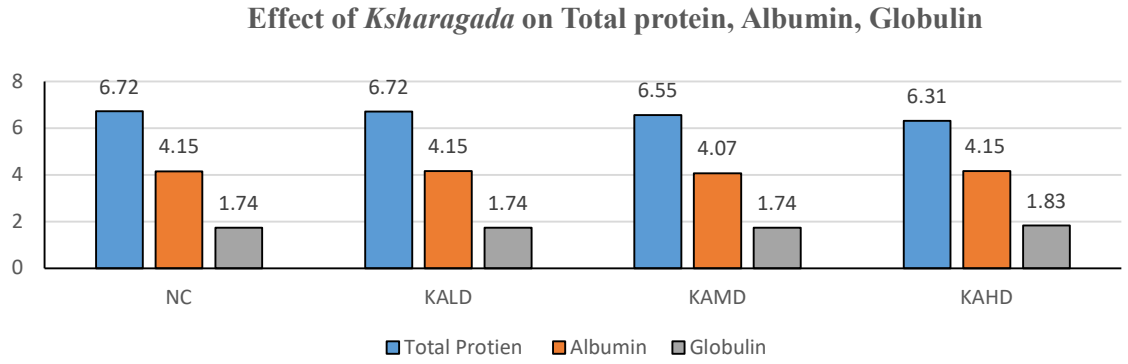


Figure 1: Showing Effect of *Ksharagada* on Total protein, Albumin, Globulin

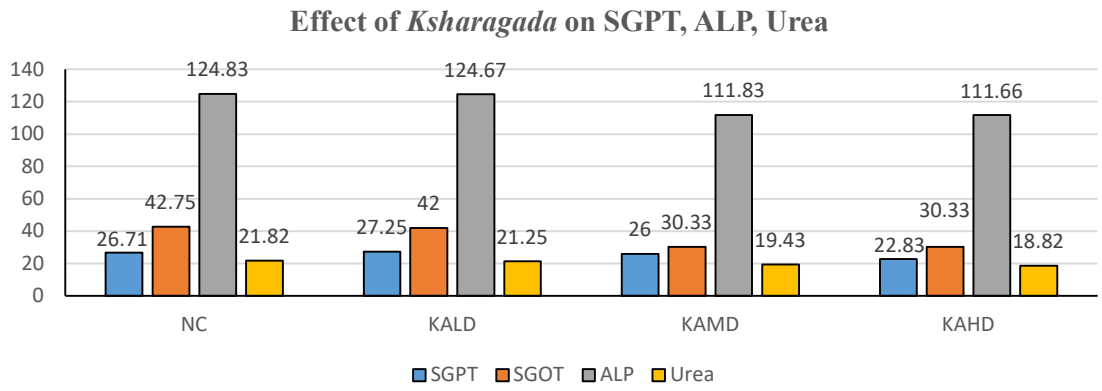


Figure 2: Showing Effect of *Ksharagada* on SGPT, ALP, Urea

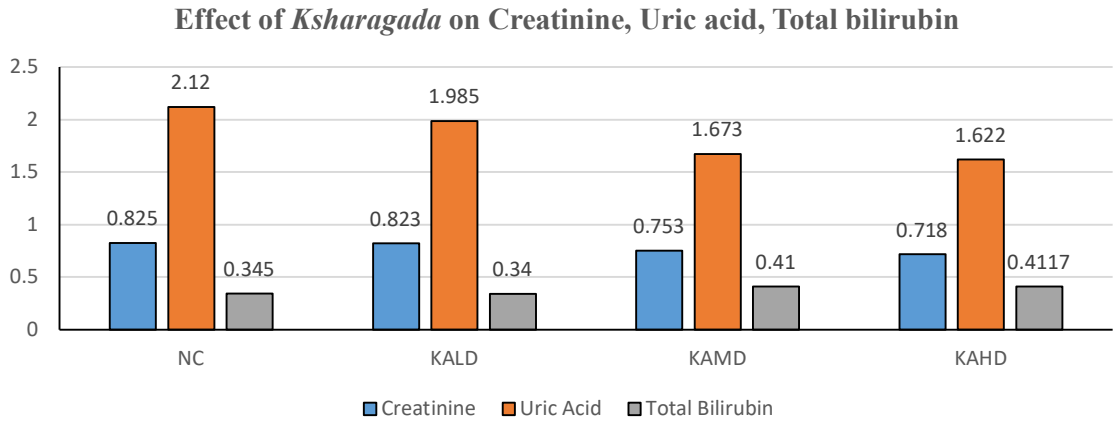


Figure 3: Showing Effect of *Ksharagada* on Creatinine, Uric acid, Total bilirubin

EFFECT OF *KSHRAGADA* ON SERUM BIOCHEMICAL PARAMETERS

In the study, several biochemical parameters were measured and compared across different groups, including NC (normal control), KAMD (group with a certain condition), and KAHD (another group with a different condition). The total protein levels were found to be decreased in both KAMD and KAHD groups compared to the NC group, indicating a potential impact of the conditions on protein metabolism or synthesis. Interestingly, albumin levels remained unchanged across all groups, suggesting that albumin synthesis or catabolism was not significantly affected by the conditions. Conversely, globulin levels showed an increase specifically in the KAHD group, which may point to an immune response or increased production of globulins in this group.

Enzyme activity assays revealed that SGPT (Serum Glutamate Pyruvate Transaminase) levels were decreased in the KAHD group, while SGOT (Serum Glutamate Oxaloacetate Transaminase) levels were reduced in both KAMD and KAHD groups, indicating potential hepatic

dysfunction or altered liver metabolism in these groups. Additionally, ALP (Alkaline Phosphatase) activity was decreased in both KAMD and KAHD groups.

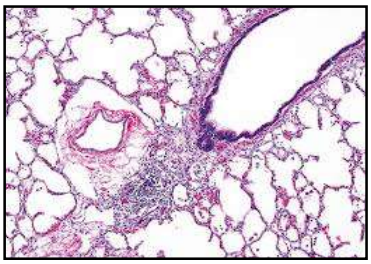
Kidney function markers also showed notable changes, with urea levels being decreased in KALD, KAMD, and KAHD groups, and creatinine levels were lower in KAMD and KAHD groups, suggesting alterations in renal function or protein catabolism. Uric acid levels were also reduced in KAMD and KAHD groups, which may be indicative of altered purine metabolism. Interestingly, total bilirubin levels were increased in KAMD and KAHD groups, which could point to altered bilirubin metabolism or excretion, potentially indicating liver dysfunction. Overall, these findings suggest significant biochemical alterations across various parameters in KAMD and KAHD groups, highlighting the impact of the respective conditions on liver and kidney function, as well as protein metabolism.

Table 2: Showing statistical analysis of *Ksharagada* on liver and kidney

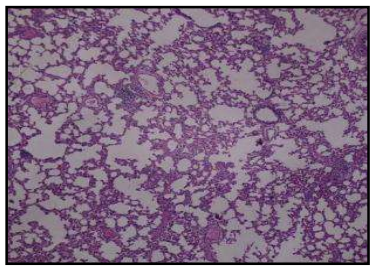
Parameters	NC vs KALD	NC vs KAMD	NC vs KAHD
Total Bilirubin	**p = 0.894**	**p < 0.0001*** **	**p < 0.0001*** **
	ns	**↑ in KAMD**	**↑ in KAHD**
SGPT	**p = 0.371**	**p = 0.130**	**p < 0.0001*** **
	ns	**ns**	**↓ in KAHD**
SGOT	**p = 0.015***	**p < 0.0001*** **	**p < 0.0001*** **
	ns	**↓ in KAMD**	**↓ in KAHD**
ALP	**p = 0.802**	**p < 0.0001*** **	**p < 0.0001*** **
	ns	**↓ in KAMD**	**↓ in KAHD**
Total Protein	**p = 0.878**	**p < 0.0001*** **	**p < 0.0001*** **
	ns	**↓ in KAMD**	**↓ in KAHD**
Albumin	**p = 0.722**	**p < 0.0001*** **	**p = 0.722**
	ns	**↓ in KAMD**	**ns**
Globulin	**p = 1.000**	**p = 0.790**	**p < 0.0001*** **
	ns	**ns**	**↑ in KAHD**
Creatinine	**p = 0.678**	**p < 0.0001*** **	**p < 0.0001*** **
	ns	**↓ in KAMD**	**↓ in KAHD**
Urea	**p < 0.0001*** **	**p < 0.0001*** **	**p < 0.0001*** **
	↓ in KALD	**↓ in KAMD**	**↓ in KAHD**
Uric Acid	**p = 0.141**	**p < 0.0001*** **	**p < 0.0001*** **
	ns	**↓ in KAMD**	**↓ in KAHD**

The study's biochemical analysis revealed significant alterations in various parameters across different groups. Total bilirubin levels exhibited a highly significant increase in both KAMD and KAHD groups ($p < 0.0001$), suggesting considerable changes in bilirubin metabolism or excretion. SGPT levels significantly decreased in the KAHD group ($p < 0.0001$), while SGOT levels showed a significant reduction in both KAMD and KAHD groups ($p < 0.0001$), indicating potential hepatic dysfunction. ALP activity was also significantly decreased in both KAMD and KAHD groups ($p < 0.0001$), further supporting the notion of altered liver

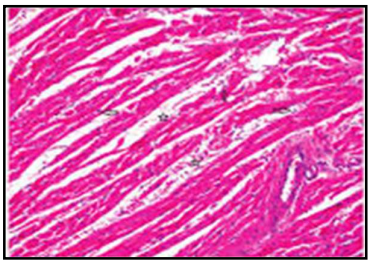
or bone metabolism. Total protein levels demonstrated a significant decrease in KAMD and KAHD groups ($p < 0.0001$), highlighting the impact of these conditions on protein metabolism or synthesis. Albumin levels significantly decreased in the KAMD group ($p < 0.0001$), while globulin levels significantly increased in the KAHD group ($p < 0.0001$), suggesting differential effects on these protein fractions. Kidney function markers also showed significant changes, with creatinine levels significantly reduced in KAMD and KAHD groups ($p < 0.0001$) and urea levels significantly decreased across all groups ($p < 0.0001$), indicating potential alterations in renal function. Uric acid levels were significantly decreased in KAMD and KAHD groups ($p < 0.0001$), reflecting changes in purine metabolism. These findings collectively underscore the substantial biochemical disruptions associated with the conditions in KAMD and KAHD groups.



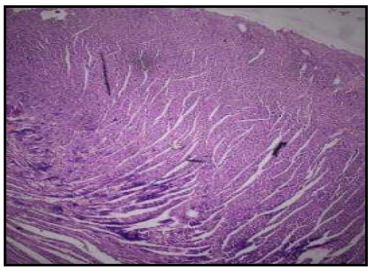
a) Normal Lung



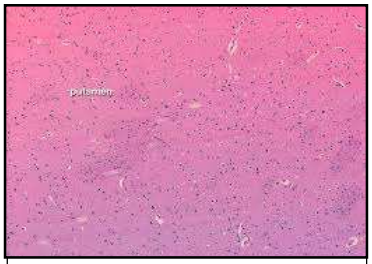
a) KAHD Lung



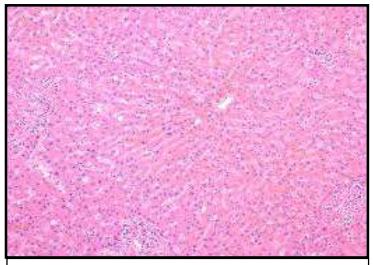
b) Normal Heart



b) KAHD heart



c) Normal brain



c) KAHD brain

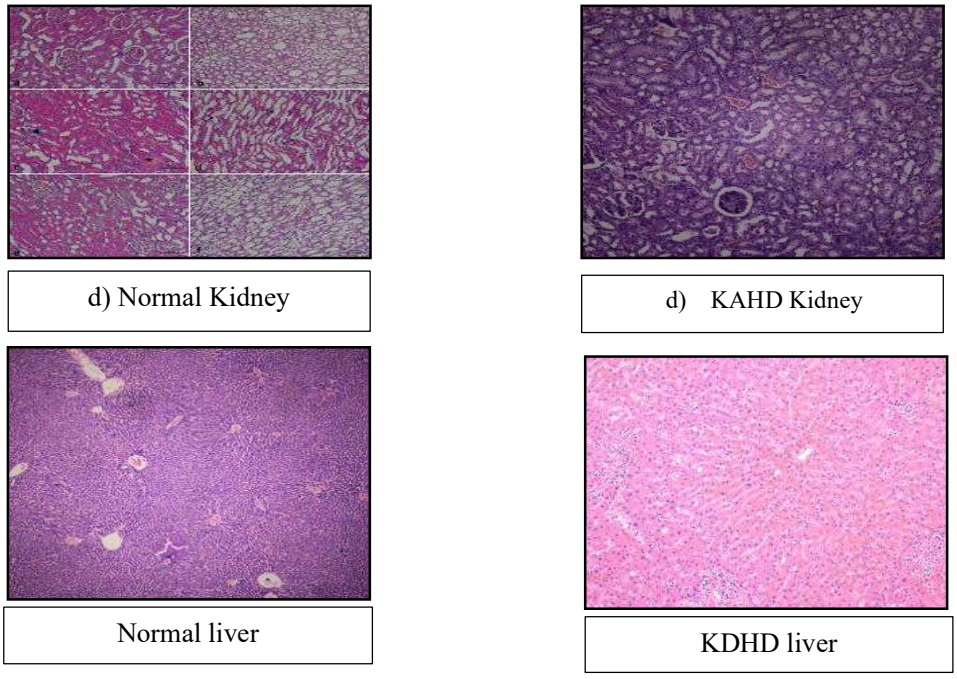


Figure 4: Showing histopathological examination of organs of Normal and KAHD - Lung, Heart, Brain, Kidney and Liver

Histopathological Examination (Figure 4): The liver, kidney, heart, lung, and brain tissues showed normal histological structures, indicating no toxic effect from *Ksharagada*.

DISCUSSION

The study aimed to evaluate the acute and sub-acute oral toxicity of *Ksharagada* (KA), a traditional Ayurvedic herbo-mineral formulation, in female Wistar albino rats. This evaluation is critical for establishing the safety profile of KA, which is essential for its therapeutic use. The study followed OECD guidelines, ensuring that the methodology and findings are robust and credible. In the acute toxicity study, *Ksharagada* was administered at doses of 2000 mg/kg and 5000 mg/kg. The results showed no mortality or observable signs of toxicity in the rats. The absence of adverse effects, even at high doses, suggests a high safety margin for KA. Histopathological examination of vital organs such as the liver and kidneys revealed normal structures, supporting the conclusion that KA does not induce acute toxicity at these doses. The sub-acute toxicity study involved administering KA at doses of 540 mg/kg, 810 mg/kg, and 1080 mg/kg over 28 days, followed by a 14-day observation period. Various biochemical parameters were measured to assess the potential toxic effects of KA on the rats' physiological functions.

The biochemical analysis indicated significant alterations in several parameters, particularly at higher doses. Notably, there were increases in total bilirubin and decreases in SGPT, SGOT, ALP, total protein, creatinine, urea, and uric acid levels. These changes suggest potential impacts on hepatic and renal functions **[8-11]**. Elevated total bilirubin levels might indicate altered bilirubin metabolism or excretion, while reduced SGPT and SGOT levels point towards hepatic dysfunction. Decreased ALP levels further support potential liver or bone metabolism alterations. Additionally, changes in kidney function markers, such as reduced creatinine and urea levels, suggest possible impacts on renal function or protein catabolism. The decrease in uric acid levels in the higher dose groups indicates alterations in purine metabolism. Despite these biochemical changes, histopathological examinations of the liver, kidneys, heart, lungs, and brain tissues showed normal histological structures. This finding is significant as it indicates that, although there are measurable biochemical alterations, there is no corresponding histological damage to the vital organs **[12]**.

CONCLUSION:

The conducted studies provide a comprehensive assessment of the safety profile of *Ksharagada*. Both acute and sub-acute toxicity studies confirm that *Ksharagada* is safe for oral administration in Wistar albino rats at doses significantly higher than traditional therapeutic doses. *Ksharagada* demonstrated a high safety margin with no mortality or observable toxicity at doses up to 5000 mg/kg. The LD50 is therefore above this dose, indicating low acute toxicity. While there were significant alterations in several biochemical parameters at higher doses, these changes were not associated with histopathological damage to vital organs. The biochemical alterations observed are within acceptable ranges and do not indicate severe toxicity. These findings support the continued use of *Ksharagada* in traditional medicine, given its high safety margin and lack of significant adverse effects in both acute and sub-acute settings.

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