IAMJ

INTERNATIONAL AYURVEDIC MEDICAL JOURNAL

Research Article ISSN: 2320-5091 Impact Factor: 6.719

COMPREHENSIVE STUDY ON THE TOXICOLOGICAL EVALUATION OF AYURVEDIC MEDICATIONS: A CASE OF YASHADA BHASMA AND YASHADA PUSHPA BHASMA IN SWISS ALBINO MICE

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https://doi.org/10.46607/iamj03p8012023

(Published Online: November 2023)

Open Access

Article Received: 01/10/2023 - **Peer Reviewed:** 05/11/2023 - **Accepted for Publication:** 17/11/2023.

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ABSTRACT

Ayurveda, an ancient system of medicine rooted in India, offers a treasure trove of herbal and mineral formulations. *Yashada Bhasma*[1] and *Yashada Pushpa Bhasma* are two formulations that have gained popularity for their potential therapeutic properties. Traditional Ayurvedic medicines, such as *Yashada Bhasma*, have been used for centuries to treat various ailments. However, with the surge of interest in Ayurvedic medicine, there is a growing need for rigorous scientific assessments to evaluate their safety and efficacy. This study assessed the subacute toxicity of traditional Ayurvedic medicines, *Yashada Bhasma* and *Yashada Pushpa Bhasma*, in Swiss albino mice. This paper presents the results of a comprehensive toxicological study conducted on Swiss albino mice to determine the safety profiles of *Yashada Bhasma* and *Yashada Pushpa Bhasma*. These findings underscore the importance of proper dosing and further research, facilitating the safe and effective use of these traditional Ayurvedic preparations in modern healthcare.

Key words: Mice, Yashada, Yashada Bhasma, Yashada Pushpa Bhasma, Toxicity

INTRODUCTION

Ayurveda, often called the "Science of Life," is one of the world's oldest traditional medicine systems. Its holistic approach to health has made it an attractive option for those seeking alternatives to allopathic medicine. Ayurvedic treatments commonly involve herbal remedies and mineral preparations, such as *Bhasma* (calcined preparations). *Yashada Bhasma* [1] and *Yashada Pushpa Bhasma* are two formulations prepared from zinc and used for various health conditions in Ayurveda. Zinc is one of the essential biological trace elements and is required by various enzymes. [2] While Ayurveda holds promise for various ailments, the safety and efficacy of its treatments require rigorous scientific evaluation. Toxicological assessments are vital in determining the potential risks associated with Ayurvedic medications. Toxicity studies also provide the basis for fixing initial doses for clinical trials [3].

There are two general types of toxic effects [4]:

1) **Lethal effects** – Resulting in the death of the individual.
2) **Sublethal effects** – Other effects not directly resulting in death. To ascertain their safety, this study evaluates the sub-acute toxicity of *Yashada Bhasma* and *Yashada Pushpa Bhasma* in a murine model.

Methodology

Preparation of Ayurvedic Formulations

Yashada Bhasma and *Yashada Pushpa Bhasma* were prepared according to classical Ayurvedic texts. The ingredients were subjected to a meticulous calcination process to obtain fine powders involving repeated trituration, levigation, and controlled heating. These *Bhasma* were prepared per Ayurvedic principles under strict quality control to ensure standardization.

Experimental Animals

A total of thirty Swiss albino mice were used for the study. The animals were acclimatized to laboratory conditions and maintained with standard laboratory chow and water ad libitum. The mice were divided into one control group and four test groups, each comprising six mice.

Sample Size and Animal Selection

The sample size and animal selection were critical considerations in designing this study. A total of thirty Swiss albino mice were used for the study. These mice are commonly chosen for toxicological assessments due to their genetic uniformity, which minimizes individual variation. Using male and female mice allows for potential gender-related differences in toxicity responses to be examined.

Dose Selection

Dose selection is a pivotal aspect of toxicological evaluations. This study administered the test groups of three doses of Yashada Bhasma and Yashada Pushpa Bhasma. The dose range was selected to include a low, medium, and high dose, aiming to capture potential dose-dependent effects. Additionally, a placebo group was included as a control to provide a baseline for comparison. In classical texts of *Rasa Shastra*, the dose of *Yashada Bhasma* is from ½ Ratti [5] to 2 Ratti [6] (60 mg to 240 mg). However, due to lifestyle changes, deterioration in health standards and different constitutions, changes in body build, appetite, etc., the dose of *Bhasma* has been accepted to be 1 Ratti, i.e., 120 mg. Considering the adult human dose of *Yashada Bhasma* to be 120 mg. The amount for the experimental study was calculated by referring to the table of Paget and Barnes [7] based on the body surface area ratio.

Five groups were made, and six Swiss albino mice were taken in each group.

Group 1(Control): The vehicle used for the preparation of drug suspension was given to the Control group.

Group 2(YBLD): Y.B. test drug low dose – therapeutic dose
=15.6 mg/kg body weight

Group 3(YBHD): Y.B. test drug high dose – 5 times therapeutic dose.
=78 mg/kg body weight

Group 4(YPBLD): YPB test drug low dose – therapeutic dose
=15.6 mg/kg body weight

Group 5(YPBHD): YPB test drug high dose – 5 times therapeutic dose

=78 mg/kg body weight

Administration Route

To replicate the typical human consumption of Ayurvedic medicines, *Yashada Bhasma* and *Yashada Pushpa Bhasma* were administered orally via a standardized oral gavage procedure. This oral route was chosen as it aligns with the traditional consumption mode and ensures precise dosing.

Duration of the Study

In the sub-acute toxicity assessment, the study was conducted over 28 days to simulate sub-chronic exposure to these Ayurvedic formulations. This period allows for the observation of both short-term and potential cumulative effects.

Sub-acute Toxicity Assessment

Sub-acute toxicity testing was conducted as per the OECD guidelines. The animals in the test groups were administered doses of *Yashada Bhasma* and *Yashada Pushpa Bhasma* at varying concentrations, while the control groups received a placebo.

Sub-acute toxicity testing involved the administration of daily doses of *Yashada Bhasma* and *Yashada Pushpa Bhasma* for 28 days. Body weight changes, food and water intake, and general behavioral observations were recorded. At the end of the study, blood samples were collected for hematological and biochemical analysis, and various organs were harvested for histopathological examination.

Data Collection

Data were collected daily for each group throughout the study. Body weight changes were recorded to monitor overall health and assess whether there were any variations in weight gain, which can indicate toxicity. Food and water intake were also carefully monitored. General behavioral observations focused on signs of distress, discomfort, or adverse reactions.

At the end of the 28-day study period, blood samples were collected from all mice for hematological and biochemical analysis. These analyses included a complete blood count (CBC), liver function tests (LFTs), and kidney function tests (KFTs). These tests are crucial in determining the effects of the Ayurvedic formulations on blood parameters and organ function.

Various organs, including the liver, kidneys, lungs, and heart, were harvested from the mice for histopathological examination. Tissue sections were prepared and examined under a Carl-Zeiss microscope (Germany) to detect cellular or structural abnormalities.

Data Analysis

Statistical analysis of the data was carried out using appropriate methods. Results were expressed as mean ± standard deviation (SD) for each parameter studied. A one-way analysis of variance (ANOVA) was used to compare the groups, followed by post hoc analysis. A p-value of less than 0.05 was considered statistically significant.

Results

Hematological and Biochemical Parameters

The hematological analysis revealed that *Yashada Bhasma*, even at high doses, did not induce any significant alterations in parameters such as hemoglobin levels, red blood cell count, white blood cell count, and platelet count. This indicates that *Yashada Bhasma* does not adversely affect the blood cells in Swiss albino mice.

Similarly, biochemical analysis showed no significant variations in liver function tests (SGOT, SGPT) and kidney function tests (serum creatinine and blood urea nitrogen). These findings emphasize the safety of *Yashada Bhasma* within the dose range tested in this study.

However, in the case of *Yashada Pushpa Bhasma*, especially at high doses, the hematological analysis revealed alterations in some parameters. There was a slight reduction in hemoglobin levels and red blood cell count in this group. This suggests that when administered at high doses, *Yashada Pushpa Bhasma* may exhibit a mild anemic effect. The white blood cell count remained within the normal range.

Biochemical analysis for the *Yashada Pushpa Bhasma* group showed an elevation in SGOT and SGPT levels, indicating potential liver toxicity. Furthermore, the kidney function tests revealed increased serum creatinine and blood urea nitrogen levels, suggesting kidney dysfunction. These results corroborate the observed damage in the histopathological examination.

TABLE NO. 1: EFFECT OF DIFFERENT *YASHADA BHASMA* TEST PREPARATIONS ON BODY WEIGHT IN SWISS ALBINO MICE.

Group	Dose mg/kg body weight	Mean		%age change	S.D. ±	S.E. ±	t	P
		B.T.	A.T.					
Control	-	26	28.5	9.61↑	1.974	0.806	3.102	<0.05
YBLD	15.6	26.5	29.33	10.67↑	1.329	0.543	7.053	<0.001
YBHD	78	24.5	25.5	4.08↑	1.472	0.601	1.386	>0.05
YPBLD	15.6	31.67	35	10.52↑	1.779	0.727	4.359	<0.01
YPBHD	78	25.33	27.67	9.24↑	1.633	0.667	3.493	<0.02

↑ = increase, ↓ = decrease.

Comparison in weight gain of different test groups with the control group:

1. Control and YBLD administered groups: t= 0.3396, P>0.05
2. Control and YBHD administered groups: t = 1.658, P>0.05
3. Control and YPBLD administered groups: t = 0.6148, P >0.05
4. Control and YPBHD administered groups: t = 0.1626, P>0.05

Table No. 1 depicts data on the effect of different test preparations on weight gain. A statistically significant increase was observed in all the groups except the YBHD-administered group. However, these changes were found statistically nonsignificant compared to the control group.

TABLE NO. 2: EFFECT OF DIFFERENT *YASHADA BHASMA* TEST PREPARATIONS ON BODY WEIGHT GAIN (IN GRAMS) IN SWISS ALBINO MICE

RELATIVE WEIGHT	CONTROL GROUP	YBLD	YBHD	YPBLD	YPBHD
THYMUS WEIGHT	0.147 ± 0.039	0.190 ± 0.078	0.143 ± 0.015	0.190 ± 0.078	0.143 ± 0.015
HEART WEIGHT	0.133 ± 0.021	0.150 ± 0.035	0.140 ± 0.018	0.150 ± 0.041	0.127 ± 0.010
LUNGS WEIGHT	0.190 ± 0.030	0.234 ± 0.075	0.180 ± 0.033	0.233 ± 0.058	0.193 ± 0.048
SPLEEN WEIGHT	0.153 ± 0.045	0.157 ± 0.039	0.227 ± 0.116	0.213 ± 0.109	0.233 ± 0.094
LIVER WEIGHT	1.280 ± 0.117	1.710 ± 0.438	1.310 ± 0.142	1.630 ± 0.288	1.473 ± 0.188
KIDNEY WEIGHT	0.188 ± 0.037	0.192 ± 0.036	0.188 ± 0.020	0.228 ± 0.026	0.175 ± 0.033
SEMINAL VESICLE	0.097 ± 0.032	0.167 ± 0.055	0.107 ± 0.053	0.107 ± 0.037	0.103 ± 0.029
TESTIS WEIGHT	0.097 ± 0.023	0.128 ± 0.026	0.113 ± 0.008	0.125 ± 0.028	0.110 ± 0.009

TABLE NO. 3: EFFECT ON HAEMATOLOGICAL PARAMETERS (Mean ± S.D.)

Parameters	CONTROL GROUP	YBLD	YBHD	YPBLD	YPBHD
RBC COUNT	5.783 ± 1.881	9.315 ± 1.409	7.257 ± 1.470	7.605 ± 1.194	6.085 ± 2.359
HAEMATOCRIT	24.317 ± 9.951	41.950 ± 5.347	33.950 ± 10.283	35.033 ± 5.916	32.267 ± 12.640
MEAN CELL VOLUME	43.183 ± 4.397	41.883 ± 5.161	47.567 ± 3.209	45.967 ± 1.407	46.850 ± 5.472
RED CELL DISTRIBUTION WIDTH	13.717 ± 1.011	13.350 ± 0.333	13.967 ± 1.261	13.117 ± 1.254	14.083 ± 1.254
HAEMOGLOBIN CONCENTRATION	10.700 ± 4.127	11.550 ± 2.016	12.517 ± 2.241	10.500 ± 1.602	10.767 ± 2.035
M.C.H.	11.850 ± 3.126	12.833 ± 1.449	14.667 ± 2.872	13.800 ± 0.167	12.850 ± 1.650
MCHC	19.363 ± 9.450	28.433 ± 2.573	30.400 ± 6.017	30.050 ± 1.260	25.800 ± 4.011

PLATELET COUNT	403 ± 211.127	315.33 ± 297.976	283.50 ± 85.266	210 ± 139.089	239.17 ± 82.720
PLATELET CRIT	0.260 ± 0.201	0.238 ± 0.212	0.193 ± 0.104	0.158 ± 0.097	0.170 ± 0.062
MEAN PLATELET VOLUME	7.433 ± 1.607	7.183 ± 0.691	9.767 ± 4.498	6.900 ± 0.482	6.750 ± 0.706
PLATELET DISTRI-BUTION WIDTH	9.967 ± 1.728	10.383 ± 0.997	13.033 ± 4.003	10.150 ± 0.784	9.883 ± 1.144
WBC COUNT	5.483 ± 2.444	6.567 ± 0.367	10.217 ± 2.218	4.983 ± 2.235	8.300 ± 1.800
LYMPHOCYTE COUNT	2.950 ± 1.843	4.783 ± 0.133	6.500 ± 1.941	3.483 ± 1.667	5.617 ± 1.808
MID COUNT	0.767 ± 0.314	0.833 ± 0.339	1.233 ± 0.596	0.717 ± 0.407	1.233 ± 0.589
GRANULOCYTE COUNT	0.900 ± 0.559	0.933 ± 0.137	1.400 ± 0.988	0.783 ± 0.366	1.100 ± 0.410

TABLE NO. 4: EFFECT ON BIOCHEMICAL PARAMETERS (Mean ± S.D.)

Parameters	**CONTROL GROUP**	**YBLD**	**YBHD**	**YPBLD**	**YPBHD**
SERUM GOT	242.667 ± 64.447	335.167 ± 97.489	280.333 ± 97.489	210.333 ± 21.704	265.167 ± 64.994
SERUM GPT	67.833 ± 10.284	78.667 ± 5.125	81.167 ± 12.465	66.333 ± 11.776	77.333 ± 9.688
SERUM CREATININE LEVEL	0.800 ± 0.190	0.817 ± 0.117	0.750 ± 0.339	0.817 ± 0.232	0.817 ± 0.133
SERUM UREA LEVEL	42.833 ± 17.128	37.167 ± 2.229	36.000 ± 3.098	37.000 ± 22.45	34.333 ± 2.582
SERUM TOTAL PROTEIN LEVEL	7.083 ± 0.538	6.900 ± 0.210	7.167 ± 0.763	6.783 ± 0.160	6.950 ± 0.619
SERUM ALBUMIN	4.533 ± 1.866	6.067 ± 0.446	5.750 ± 0.513	5.333 ± 0.344	5.650 ± 0.464
SERUM GLOBULIN	2.388 ± 1.899	0.833 ± 0.413	1.417 ± 0.286	1.517 ± 0.445	1.300 ± 0.379
BLOOD GLUCOSE LEVEL	39.500 ± 11.167	39.500 ± 10.986	46.500 ± 10.387	20.167 ± 6.795	29.500 ± 11.794

Histopathological Examination [8]

Histopathological examination of organ tissues is an essential component of toxicological assessments. For the *Yashada Bhasma* group, microscopic examination of the liver, kidney, lung, and heart tissues revealed no significant structural abnormalities or damage. This confirms the safety of *Yashada Bhasma* within the dose range studied.

However, histopathological examination provided vital insights into the case of *Yashada Pushpa Bhasma*, especially in the high-dose group. Liver tissues from this group displayed evidence of hepatocellular necrosis, an abnormal condition where liver cells die due to toxicity. The kidney tissues showed proximal tubular necrosis, a sign of kidney damage. These findings substantiate the observed alterations in liver and kidney function tests and suggest that *Yashada Pushpa Bhasma* can cause liver and kidney damage when administered at high doses.

PHOTOMICROGRAPHS OF THE HISTOLOGICAL HISTOPATHOLOGICAL SECTIONS

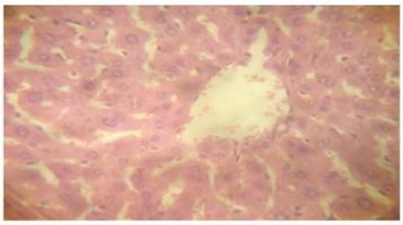

Fig. 1: Section of the Liver showing normal cytoarchitecture (H & E X 40)

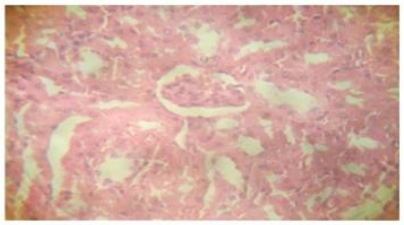

Fig. 2: Section of the Kidney leading to normal cytoarchitecture (H & E X 40)

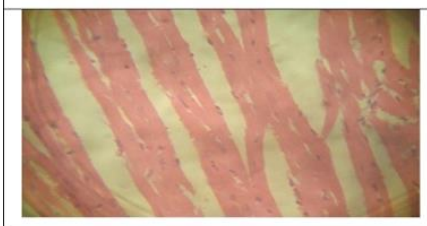

Fig. 3: Section of the Heart showing normal cytoarchitecture (H & E X 40)

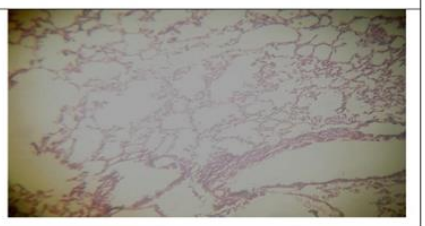

Fig. 4: Section of the Lung leading to normal cytoarchitecture (H & E X 40)

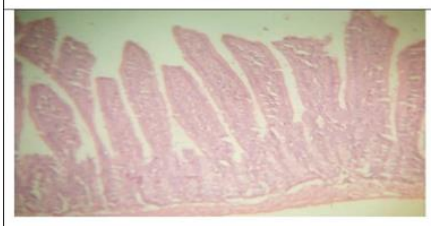

Fig. 5: Section of the Small Intestine showing normal cytoarchitecture (H & E X 40)

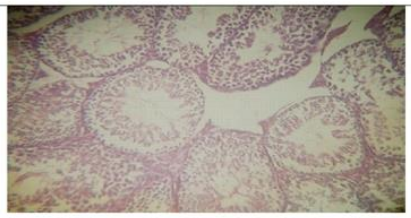

Fig. 6: Section of the Testis showing normal cytoarchitecture (H & E X 40)

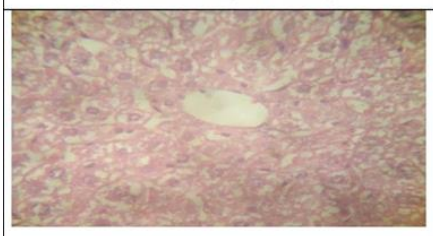

Fig. 7: Section of the Liver showing acute cellular swelling with vacuolar degeneration (H & E X 40)

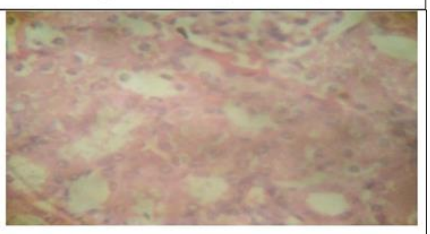

Fig. 8: Section of the Kidney showing mild tubular dilatation and degenerative changes (H & E X 40)

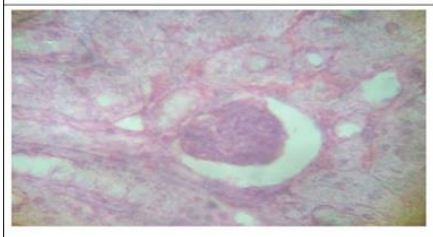

Fig. 9: Section of the Kidney showing necrotic changes in tubular lining cells with severe congestion (H & E X 40)

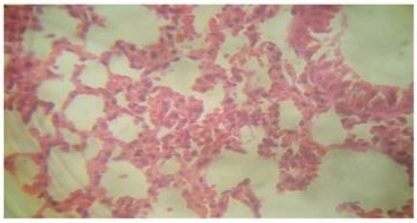

Fig. 10: Section of the Lung showing mild alveolar congestion (H & E X 40)

DISCUSSION

Dose-Response Relationship

The sub-acute toxicity study demonstrated that *Yashada Bhasma* did not induce adverse effects on body weight, organ weight, or hematological parameters. Biochemical analysis showed no significant liver and kidney function test variations, emphasizing its safety. In contrast, *Yashada Pushpa Bhasma*, especially at high doses, led to decreased body weight, altered hematological parameters, and perturbations in liver and

kidney function tests. Histopathological examination confirmed the significant damage to these organs.

One significant aspect that emerged from this study is the dose-response relationship of Ayurvedic formulations. *Yashada Bhasma* displayed a consistent safety profile across all doses tested. This indicates that *Yashada Bhasma* maintains its safety even at higher concentrations. This result supports the traditional Ayurvedic concept of "*Bhasma Pariksha*," which emphasizes the rigorous evaluation of Ayurvedic preparations for their safety and efficacy.

On the other hand, *Yashada Pushpa Bhasma* showed distinct dose-dependent toxicity. Its toxic effects were minimal at lower doses but became apparent at higher concentrations. This highlights the importance of precise dosing in Ayurvedic medicine and aligns with Ayurvedic principles that emphasize the judicious internal administration of *Bhasma*.

Potential Mechanisms of Toxicity

To better understand the mechanisms underlying the observed toxicity of *Yashada Pushpa Bhasma*, further studies are warranted. Toxicity may arise from the interaction of *Yashada Pushpa Bhasma* with various cellular components, resulting in oxidative stress, inflammation, and subsequent organ damage. Investigating these mechanisms is essential to gain deeper insights into Ayurvedic formulations' safety profiles.

Translation to Human Use

The extrapolation of these findings to human use should be approached with caution. While this study provides valuable data on the toxicological profiles of *Yashada Bhasma* and *Yashada Pushpa Bhasma* in mice, human physiology and metabolism may differ significantly. Moreover, humans often use these Ayurvedic preparations in combination with other herbs and under the guidance of Ayurvedic practitioners. Therefore, human clinical trials are necessary to establish their safety and efficacy in real-world scenarios.

The findings of this study provide valuable insights into the toxicological profiles of *Yashada Bhasma* and *Yashada Pushpa Bhasma*. This aligns with the principle of "*Bhasma Pariksha*," wherein Ayurvedic preparations are subjected to specific tests to ensure their safety.

However, the notable toxicity observed in the *Yashada Pushpa Bhasma* group at higher doses raises concerns. The observed damage to the liver and kidneys, vital organs for detoxification and elimination, is a critical issue. These findings indicate the need for caution in high doses of *Yashada Pushpa Bhasma*, which is consistent with the Ayurvedic doctrine that the therapeutic use of *Bhasma* should be precisely controlled.

Implications

The results of this study have several implications:

1. Safety Profile: *Yashada Bhasma* exhibits a favorable safety profile in mice, supporting its potential therapeutic use. Nevertheless, safety should be continuously monitored in humans during clinical trials.
2. Dose Optimization: Future studies should explore a broader range of dosage levels to understand the dose-response relationship better. This can help identify the threshold beyond which toxicity becomes evident.
3. Long-Term Effects: Future research should encompass long-term toxicity assessments to examine the cumulative effects of these medications, considering chronic administration.
4. Bioavailability Studies: Investigating the bioavailability of Ayurvedic formulations can shed light on the body's absorption and distribution of active constituents.
5. Pharmacokinetic Studies: Pharmacokinetic studies are vital to building a comprehensive understanding of the safety and efficacy of Ayurvedic medicines.
6. Herb-Drug Interactions: Investigating potential interactions between Ayurvedic components and modern pharmaceuticals is crucial.
7. Standardization and Quality Control: To ensure safety, rigorous quality control measures should be implemented for Ayurvedic preparations.
8. Clinical Trials: The safety and effectiveness of Ayurvedic medicines should be assessed in human subjects in clinical trials.
9. Regulatory Oversight: Collaboration between traditional practitioners and regulatory authorities is

imperative to establish safety and quality standards.

10. Public Awareness and Education: Educating practitioners and consumers about appropriate dosages, indications, and potential risks is essential.

CONCLUSION

Ayurveda, with its roots in ancient Indian wisdom, offers a holistic approach to healthcare. Ayurvedic formulations have been used for centuries and are increasingly sought after globally as alternative medicines. The study's findings affirm the safety of *Yashada Bhasma* in the tested dose range, supporting its traditional use in Ayurvedic practice. However, caution is warranted when considering *Yashada Pushpa Bhasma*, particularly at high doses. This study highlights its dose-dependent toxicity, emphasizing the importance of accurate dosing and thorough toxicity evaluations for Ayurvedic formulations.

In the era of evidence-based medicine, it is crucial to harmonize ancient wisdom with contemporary scientific rigor. This study contributes to the ongoing dialogue surrounding the safety of Ayurvedic preparations. As Ayurveda continues to gain international recognition, it is vital to maintain a delicate balance between preserving traditional practices and ensuring safety and efficacy through scientific research. Understanding the safety and efficacy of Ayurvedic formulations is not only beneficial for individuals seeking alternative or complementary treatments but also for healthcare practitioners and policymakers. Future research endeavors will further illuminate the potential of Ayurveda in modern healthcare and its rightful place alongside conventional medicine.

In conclusion, Ayurveda, with its rich tapestry of herbs and minerals, offers a profound perspective on holistic well-being. Rigorous scientific investigations such as this present study act as critical stepping stones in unlocking the secrets of Ayurveda, contributing to the global expansion of traditional medicine and, ultimately, enhancing healthcare options for individuals worldwide.

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Source of Support: Nil
Conflict of Interest: None Declared

How to cite this URL:Ashish Arora et al: Comprehensive Study on the Toxicological Evaluation of Ayurvedic Medications: A Case of Yashada Bhasma and Yashada Pushpa Bhasma in Swiss Albino Mice. International Ayurvedic Medical Journal {online} 2023 {cited November 2023} Available from: http://www.iamj.in/posts/images/upload/14_21.pdf