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Investigation of Hematological and Biochemical Responses to Kasisa Bhasma in Wistar Rats

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ABSTRACT

In a controlled laboratory setting, Wistar rats were given Kasisa Bhasma orally for 28 days in order to assess its subacute toxicity. Twenty-four female rats were split into four groups and given doses of 225, 112.5, and 22.5 mg/kg/day of either Kasisa Bhasma or vehicle control. Clinical symptoms, organ weights, haematological and biochemical profiles, and histology were among the parameters evaluated. Throughout the trial period, no clinical abnormalities related to therapy or death were noted. Haematological and biochemical measures showed slight fluctuations, but they were not dose-dependent and stayed within normal biological bounds. At the greatest dose, some organ weights and values significantly decreased, but no corresponding histological alterations were found.

Keywords: *Toxicity, Rats, Hematology, Biochemistry, Histopathology.*

I. INTRODUCTION

In order to assess the safety and therapeutic potential of this age-old Ayurvedic formulation, it is essential to examine the haematological and biochemical reactions to Kasisa Bhasma in Wistar rats. In Ayurveda, an iron-based herbo-mineral mixture called Kasisa Bhasma is frequently used to treat anaemia and other conditions. Even if it has been widely used in the past, scientific confirmation through experimental investigations is necessary to guarantee its safety, especially in regards to systemic toxicity and physiological changes.

Wistar rats were used as the experimental model for this investigation because of their proven appropriateness in pharmacological and toxicological studies. To evaluate subacute toxicity, the animals were given Kasisa Bhasma orally at various dosage levels during a 28-day period. To identify any positive or negative physiological effects connected to the test drug's administration, haematological and biochemical parameters were thoroughly examined.

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Haemoglobin (Hb), total erythrocyte count (RBC), total leukocyte count (WBC), differential leukocyte count (DLC), and prothrombin time were among the haematological markers that were assessed. These metrics are crucial markers of the blood system's general health and functionality. Comparing the various dose groups to the control group, the results showed that most haematological parameters did not significantly change. Even though several metrics, such prothrombin time and eosinophil count, showed slight modifications at larger dosages, these changes were neither dose-dependent nor consistent and were within normal physiological bounds. This implies that when given at therapeutic doses, Kasisa Bhasma has no negative effects on the haematopoietic system.

The functioning state of important organs, including the liver and kidneys, was also evaluated using biochemical measures. Blood glucose, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum creatinine, and total protein levels were among the important variables examined. These indicators are frequently used to identify organ damage and metabolic disorders. The results showed that throughout all treatment groups, the majority of biochemical markers stayed constant. In the low-dose female group, there was a decrease in SGPT levels and a modest decrease in total protein levels at the maximum dose. However, because these alterations fell within typical biological ranges and showed no dose-dependent patterns, they were not suggestive of harm.

Under the circumstances of this investigation, Kasisa Bhasma does not appear to cause systemic toxicity based on the lack of notable changes in haematological and biochemical parameters. Its safety profile is further strengthened by the absence of mortality, clinical abnormalities, and persistent pathological alterations. The biochemical results were supported by the histopathological examination of important organs, such as the liver, kidney, heart, lungs, spleen, and brain, which did not show any abnormalities due to the medication.

II. REVIEW OF LITERATURE

Gautam, Dev Nath et al., (2016) An essential iron-containing mineral medication used in Ayurvedic Rasa Shastra, kasisa is used to treat a variety of conditions, including anaemia, skin, eye, and hair growth issues. In this investigation, calcination was carried out using a conventional heating setup using the Kanji (sour gruel) method, and shodhana was performed by triturating in lemon juice. To assess the safety of this iron-based ayurvedic remedy, histological and toxicological analyses were performed on both the calcined and purified Kasisa product. For the duration of the trial, thirty-two adult Charles Foster albino rats of either sex were used, sixteen for each drug (Kasisa Bhasma and Shodhita Kasisa). The brain, liver, kidney, and spleen were examined toxicologically. In contrast to Shodhita Kasisa, which had negative effects at doses of 25–50 mg/kg, Kasisa Bhasma at a greater dose of 100 mg/kg showed some negative effects in isolated organs of experimental animals, but the degree of damage was small. Compared to Shodhita Kasisa, Kasisa Bhasma is safer and nontoxic. To influence therapeutic efficacy, it might be given at a regulated dosage.

Singh, Rajendra et al., (2012) The goal of the current study was to evaluate the safety of standardised Panchakola Avaleha on albino rats (Wistar strain). For 28 days in a row, the animals received three oral doses of Panchakola Avaleha: a higher dose (500 mg/kg/day), a medium dose (250 mg/kg/day),

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and a therapeutic dose (50 mg/kg/day). The test drug's effects on haematological, biochemical, and histopathologic markers were assessed. Haematological, biochemical, and histological tests showed normal behaviour, no mortality, and no notable alterations.

Okediran, Babatunde et al., (2021) Animal research frequently uses overnight food and water restriction procedures, however data on the haematological and biochemical alterations brought on by prolonged food and water deprivation can reveal information about the stress these animals endure. Ten (n = 10) male albino rats kept in a metallic cage were used in this investigation. For six days, the rats were denied both food and drink. Serum was used for biochemical research, and blood samples were collected for haematological investigations on the third and sixth days. Red cell counts, packed cell volume, haemoglobin concentration, and mean corpuscular volume all significantly increased in comparison to the basal values, however mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration significantly decreased. When compared to the basal characteristics, there was a considerable increase in white blood cells, neutrophils, eosinophils, monocytes, and lymphocytes. Aspartate and alanine aminotransferase activities are higher than basal parameters after feeding and water restriction. Serum concentrations of total protein, albumin, creatinine, and blood urea were considerably higher than those of serum glucose, triglycerides, and cholesterol. We can deduce that rats that are denied food and water are more likely to experience stress, dehydration, and famine.

Gupta, Virupaksha et al., (2011) Lifestyle diseases (LSDs) are illnesses that are typically linked to significant changes in how people spend their lives. As such, they constitute a medical problem. An alternative is now urgently needed because using traditional medicine for these conditions on a regular basis may result in negative drug reactions. The medicinal application of Rasa Dravyas is being investigated, as evidenced by the growing number of papers confirming it, although Rasa Shastra has flourished since the mediaeval era. In terms of metallo-pharmaceutics, modern pharmaceutics also began to take advantage of these unique aspects. The usefulness of Rasaushadhis and their contribution to community improvement are the main topics of this article, with particular attention on LSDs.

III. MATERIALS AND METHODS

Test Animals and Housing

Before the program started, IAEC approval was obtained for the female Wister rats used in this investigation.

32 female Wistar rats weighing between 100 and 150 grams and aged between 6 and 7 weeks were chosen and divided into four groups, each including 8 females. During the seven days when the animals were acclimated, a health examination was conducted. For the duration of the trial, rats were kept separately in polypropylene cages, fed an animal pelleted meal, and given unlimited access to mineral water. The temperature was kept between 26 and 30 degrees Celsius, the relative humidity was between 60 and 70 percent, and the lighting was adjusted to provide roughly 12 hours of light and 12 hours of darkness.

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Test Drug

The dark red (Gairika) colour powder used in the test was made at the NIA Jaipur pharmacy.Kasisa Bhasma has a percentage of total iron (w/w) of 62.26, ferric iron (w/w) of 59.90, ferrous iron (w/w) of 2.36, and sulphate (w/w) of 4.02, according to the physico-chemical analysis.

The conventional dosage extrapolation approach was used to determine the study's doses. According to the Ayurvedic Pharmacopoeia, the therapeutic dose for humans in this derivation was 250 mg per day.

Experimental Design

A 28-day repeated dosage oral toxicity study was conducted on 32 females, who were split into 4 groups of 8 females each. For 28 days in a row, the rats were given vehicle control (honey 2:3 deionised water) and oral gavages of 225, 112.5, and 22.5 mg/kg/day of Kasisa Bhasma (dissolved in honey 2:3 deionised water). The drug or vehicle was given to both the test and control groups in the same volume according to body weight. Twice a day, at the start and finish of the workday, animals were examined for external appearance, general clinical observations, and mortality. On day 29, before euthanasia, a blood sample was taken.

After the animals were fasted for the entire night, diethylether-induced anaesthesia was used to take blood samples from the retro-orbital plexus. In order to measure haemoglobin (Hb%), total erythrocyte count (TEC), total leucocyte count (TLC), and prothrombine time, blood was drawn in a silica-coated vial with anticoagulant containing EDTA. In order to get serum for clinical chemistry parameters such as total protein, serum glucose, serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), and serum creatinine, blood was drawn in a centrifuge tube without anticoagulant. On the 29th day of the investigation, necropsy was carried out. Organs such as the liver, kidneys, heart, spleen, lungs, brain, testis/ovaries, and sciatic nerve were collected, weighed, and stored in 10% neutral buffered formalin. After that, tissues were cut and dehydrated using increasing alcohol grades. Blocks were created after the tissues were finally implanted in melted paraffin. The tissue sections were stained with haematoxylin and eosin after being cut at 3 to 5 microns using a microtome. The sections were then viewed under a microscope after being mounted with DPX. All obtained tissues from females in the control and 225 mg/kg/day groups underwent histopathological evaluation.

Statistical Analysis

The Student's "t" test and ANOVA were used to analyse all the data.

IV. RESULTS AND DISCUSSION

Table 1: Impact of Kasisa Bhasma on Hematological Parameters in Rats After 28 Days of Treatment

Group and Dose (mg/kg)	Hb (g/dl)	RBC (10^6/mm^3)	WBC (10^3/mm^3)	Prothrombin Time (sec)
Control (vehicle)	14.07±1.37	4.20±0.06	6.20±0.10	7.00±0.00
Kasisa Bhasma 225	15.50±0.31	4.50±0.15	6.37±0.07	5.67±0.33^b
Kasisa Bhasma 112.5	14.03±0.91	4.27±0.03	6.37±0.03	7.00±0.58
Kasisa Bhasma 22.5	17.40±1.23	4.37±0.15	6.40±0.15	6.67±0.88

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The eosinophil count in the 112.5 mg/kg/day group and the prothrombine time in the 225 mg/kg/day group showed statistically significant decreases in the above table. (Table 1)

Table 2: Impact of Kasisa Bhasma on Rat Biochemical Parameters Following 28-Day Lingual Treatment

Group and Dose (mg/kg)	Blood Glucose (mg/dl)	SGOT (IU/L)	SGPT (IU/L)	Serum Creatinine (mg/dl)	Total Protein (g/dl)
Control (vehicle)	136.00±2.89	113.33±11.86	58.33±2.85	0.60±0.00	5.37±0.83
Kasisa Bhasma 225	119.33±7.54	122.00±6.35	50.67±2.40	0.60±0.00	6.10±0.00
Kasisa Bhasma 112.5	136.33±1.33	90.67±4.63	49.67±5.04	0.60±0.00	5.60±0.10
Kasisa Bhasma 22.5	138.33±4.70	96.00±4.93	37.67±4.37[b]	0.70±0.00	6.33±0.33

The females in the 22.5 mg/kg/day group had lower serum glutamic pyruvic transaminase (SGPT) levels, whereas the 225 mg/kg/day group had considerably lower total protein levels. Clinical chemistry values did not alter as a result of treatment. (Table 2)

Table 3: Impact of Kasisa Bhasma on Vital Organ Weights in Rats Following 28-Day Oral Administration

Group and Dose (mg/kg)	Liver	Heart	Brain	Lungs	Kidney	Spleen	Sciatic Nerve	Ovaries
Control (vehicle)	7.15±0.27	0.64±0.03	1.56±0.01	1.45±0.07	1.29±0.07	0.67±0.02	0.02±0.01	0.12±0.02
Kasisa Bhasma 225	4.70±0.84[a]	0.56±0.01[a]	1.54±0.06	1.11±0.20	1.13±0.08[a]	0.57±0.07	0.03±0.01	0.10±0.01
Kasisa Bhasma 112.5	6.12±0.48	0.55±0.03	1.52±0.01	1.07±0.09	1.21±0.03	0.54±0.01[b]	0.03±0.00	0.08±0.02
Kasisa Bhasma 22.5	6.61±0.56	0.64±0.04	1.45±0.19	1.20±0.15	1.39±0.06	0.61±0.04	0.02±0.00	0.10±0.02

No treatment-related lesions were found during the gross necropsy investigation. At 225 mg/kg/day, there was no discernible change in the weight of the liver, heart, or kidneys in females (Table 3).

Nevertheless, no treatment-related or dose-dependent alterations were found in any organ's histopathological assessment. (Figures 1 - 6)

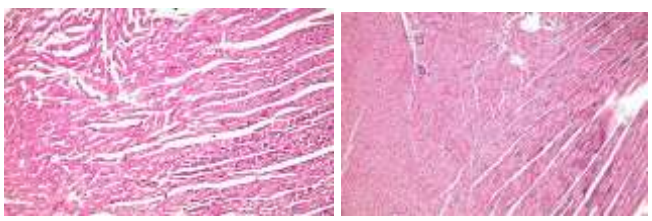

Figure 1: Heart of Control Rat and Heart of Rat Treated with 225 mg/kg Body Weight (H&E ×100)

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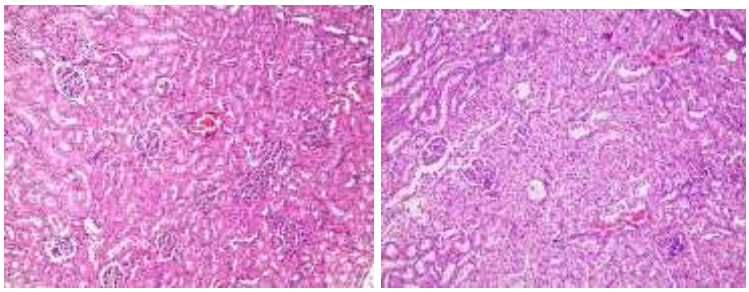

Figure 2: Kidney of Control Rat and Kidney of Rat Treated with 225 mg/kg Body Weight Dose Group (H&E ×100)

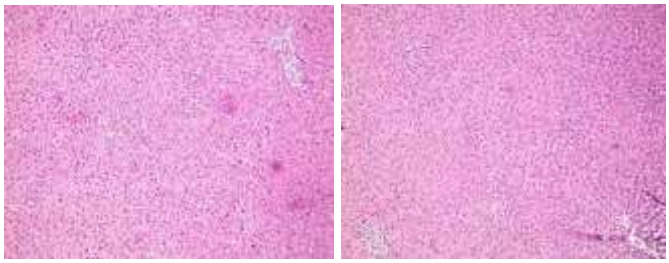

Figure 3: Liver of Control Rat and Liver of Rat Treated with 225 mg/kg Body Weight Dose Group (H&E ×100)

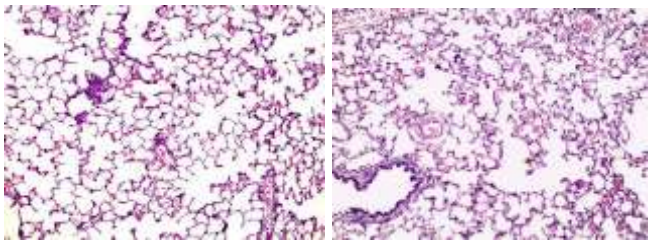

Figure 4: Lung of Control Rat and Lung of Rat Treated with 225 mg/kg Body Weight Dose Group (H&E ×100)

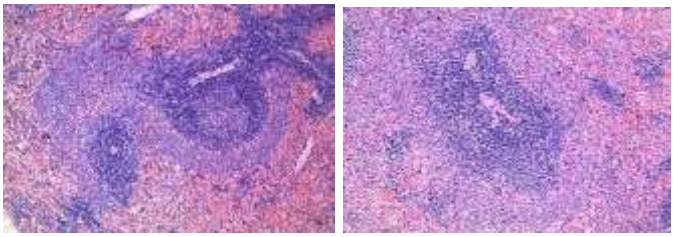

Figure 5: Spleen of Control Rat and Spleen of Rat Treated with 225 mg/kg Body Weight Dose Group (H&E ×100)

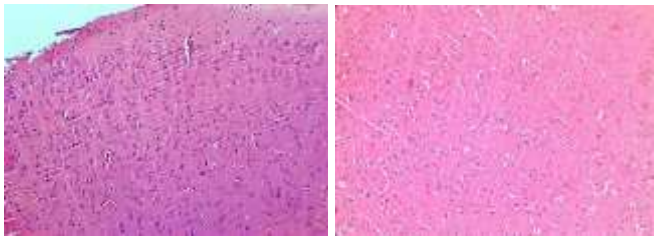

Figure 6: Brain of Control Rat and Brain of Rat Treated with 225 mg/kg Body Weight Dose Group (H&E ×100)

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V. CONCLUSION

The current study shows that when given to Wistar rats for 28 days in a row, Kasisa Bhasma has no appreciable harmful effects on haematological and biochemical measures. The drug's favourable safety profile under experimental conditions is indicated by the lack of mortality, behavioural abnormalities, and significant clinical symptoms. The majority of haematological indices, such as haemoglobin, RBC, WBC, and differential counts, stayed within physiologically normal ranges, indicating that the blood system was not negatively impacted. Similarly, there were no clinically significant changes in biochemical markers associated with liver and renal functioning, including SGOT, SGPT, serum creatinine, blood glucose, and total protein. Higher doses caused slight changes, although they were not dose-dependent and stayed within safe biological parameters. Additionally, the formulation's non-toxic nature was confirmed by the histological analysis of key organs, which revealed no structural abnormalities. Thus, under subacute exposure conditions, Kasisa Bhasma is safe up to a level of 112.5 mg/kg/day in Wistar rats. To determine its safety and effectiveness in humans, more long-term and clinical research is advised.

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