RESEARCH PAPER

Subchronic oral toxicity study of *Rajat Bhasma* (RB) and green synthesized silver nanoparticles (AgNPs)

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ABSTRACT

Introduction: Several researchers have suggested using metal nanoparticles as an alternative to metal Bhasma—a traditional method of metal processing—to overcome the lengthy and time-consuming nature of Bhasmikaran. Therefore, it is crucial to compare the safety profiles of *Rajat Bhasma* (RB) and silver nanoparticles (AgNPs) synthesized through green methods.

Objective(s): This study compared the safety of *Rajat Bhasma* (RB) and silver nanoparticles (AgNPs) in a sub-chronic toxicity study.

Materials and Methods: In this study, 42 rats were divided into seven groups, each comprising six animals (three males and three females). Six groups were administered either *Rajat Bhasma* or silver nanoparticles orally for 28 days at therapeutically equivalent doses (TED) of 10.8 mg/kg, 10 × TED (108 mg/kg), and 20 × TED (216 mg/kg). Toxicity was evaluated based on general observations and hematological, biochemical, and histopathological assessments.

Results: The results demonstrated that *Rajat Bhasma* and silver nanoparticles administered at 10- and 20-fold TED doses adversely affected specific hematological parameters, including platelet count, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) levels. Additionally, AgNPs induced alterations in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, indicating hepatic toxicity, which was further corroborated by histopathological examination of the liver. Hydropic degeneration and congestion in the kidneys were observed in rats treated with RB at the 20 × TED dose and in all AgNP-treated groups.

Conclusion: RB and AgNPs were safe at therapeutically equivalent doses. However, AgNPs showed greater toxicity at higher doses compared to RB.

Keywords: *Nanoparticles, Rajat Bhasma, Silver nanoparticles, Subchronic toxicity*

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INTRODUCTION

Metals have been used in medicine since ancient times, with various civilizations employing them for therapeutic purposes. In Ayurveda, metals are utilized in the form of Bhasma, a medicinal preparation produced through specific processes known as Shodhana and *Marana*. Shodhana purifies red-hot metals by quenching them in selected liquid media, such as milk, cow urine, or herbal decoctions [1]. Marana refers to the repeated calcination cycles of the purified (Shodhita) metal combined with minerals and herbal substances [2]. *Rajat Bhasma* (RB) has traditionally been used to treat a variety of ailments, including Smritinasha (memory loss), Unmada (psychosis), Apasmara (epilepsy), Nidranasha (insomnia), Virya Kshaya (oligospermia), Vatarogas (joint disorders), Netrarogas (ocular diseases), Amlapitta (acidity), Aruchi (loss of taste), Jwara (fever), Sushka Kasa (dry cough), and Daurbalya (weakness), among others [3-4] The oral dosage of RB ranges from 30 to 120 mg, depending on the specific condition, and is typically administered with various anupanas (adjuvants).

Silver nanoparticles (*AgNPs*) have therapeutic and diagnostic applications in cancer, antibacterial activity, antifungal activity, antiviral activity, and the treatment of parasitic infections. *AgNPs* composites have been utilized in dentistry. [5] The green synthesis of *AgNPs* has gained significant attention in recent years due to its eco-friendly and sustainable approach. [6] Green synthesis advocates promote using herbal extracts to reduce metallic salts, resulting in nanoparticles with unique physical and chemical properties such as high specific surface area, strong surface reactivity, and nanoscale

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dimensions. [7] Generally, smaller nanoparticles exhibit better antimicrobial activity due to their increased surface area in contact with microbial cells. *AgNPs* of the same size range show antibacterial activity in the following order: triangular > pentagonal, hexagonal, cubic, nano-rod > spherical. [8]

Several review articles have compared nanoparticles with *Bhasma*. [9–11] Many researchers have proposed using nanoparticles as an alternative to metal-based *Bhasma* to simplify the extensive and time-consuming process of Bhasmikaran by leveraging recent advancements in nanotechnology. [12–13]

Green-synthesized nanoparticles have specific sizes and shapes depending on the synthesis technology employed, whereas *Bhasma* contains particles with a heterogeneous size distribution ranging from 10 to 300 nanometers. Green-synthesized nanoparticles consist of pure metals or their metallic compounds, while *Bhasma* comprises metallic oxides or sulfides along with various minerals and herbal constituents. Both silver nanoparticles and Ayurvedic *Bhasma* hold potential for medical applications; however, further research is necessary to fully elucidate their efficacy, safety, and long-term effects.

Several studies have investigated the toxicity of nanoparticles synthesized via green technologies. [6] *AgNPs* can cross biological membranes and enter cells directly, accumulating in organs such as the brain, heart, liver, spleen, lungs, and kidneys, potentially impacting physiological functions. [14] The toxicity of *AgNPs* depends on the release of ionic silver and their size, shape, and surface characteristics. [15]

In this study, we aimed to assess the toxicity of *Rajat Bhasma* (RB) and *AgNPs* prepared using herbal media extracts, which are traditionally recommended for RB preparation. Although acute toxicity studies on RB and green-synthesized *AgNPs* have been conducted [16], no subchronic toxicity investigation directly comparing the two has been reported.

MATERIALS AND METHODS

Drugs and chemicals

Rajat Bhasma (RB) was prepared following the guidelines outlined in Ayurvedic classics. Silver nanoparticles (*AgNPs*) were synthesized using a reduction method involving herbal medicines. Silver plates with 99.89% purity were obtained from a local goldsmith in Nanded, Maharashtra. Following general [17] and specific purification [18] procedures, the plates were incinerated after mixing with purified sulfur, Kajjali (black sulfide of mercury), and the juice of *Aloe vera* Linn. [19] *AgNPs* were synthesized by reducing silver nitrate ($AgNO_3$) using an alcoholic extract of *Agasti Patra*. RB contains granular to massive incrustations of crystalline particles with monoclinic, slender to cubic shapes and an average size of 453.5 nm. [20] *AgNPs* consist of non-granular to less crystalline particles with a face-centered cubic shape and an average size of 197.26 nm. [20] All chemicals used in this study were of analytical grade.

Animals

Healthy Wistar albino rats of both sexes, aged 65 days and weighing 200 ± 20 g, were used under the Institutional Animal Ethics Committee (IAEC) guidelines. The animals were procured from the animal house of Jawaharlal Nehru Medical College, Sawangi (Meghe). Rats were housed in polypropylene cages within a well-ventilated animal facility and were weighed weekly throughout the study. A 12-hour light (130–400 Lux) and dark cycle was maintained, with a constant temperature of 22 ± 3°C and humidity ranging from 30% to 70%. Rice husks and corn husks were used as bedding materials. Before the experiment, the rats were acclimatized to laboratory conditions for seven days. All necessary procedures were followed to minimize stress, and animals were exposed to the same environmental conditions. They were maintained on a standard diet with ad libitum access to drinking water. The Institutional Animal Ethics Committee (IAEC) approved the experimental protocol of Jawaharlal Nehru Medical College, Sawangi (Meghe) under protocol number DMIMSU/IAEC/2021/02 on 19/06/2021. All animal handling and experimental procedures were performed under the guidelines of the Committee for Control and Supervision of Experiments on Animals in India (CPCSEA).

Dose Determination

In accordance with classical guidelines, the maximum therapeutic clinical dose of *Rajat Bhasma* (RB) is 120 mg/day. To compare the safety profiles of RB and silver nanoparticles (*AgNPs*), the dose of *AgNPs* was also set at 120 mg/day. The appropriate dose for rats was calculated using the Paget and Barnes table and determined to be 10.8 mg/kg body weight, considered the therapeutically equivalent dose (TED). The test substances were suspended in 1%

sodium carboxymethyl cellulose (CMC) and administered orally via an oral cannula.

Subchronic toxicity study (28-day model)

The subchronic 28-day toxicity study was conducted under OECD Test Guideline 407 [21] and the methodologies described by Kpemissi et al. [22], Liu et al. [23], and Agyigra et al. [24], with some modifications.

A total of 42 Wistar albino rats (both males and females) were used in the study, divided into seven groups of six animals each (3 males and three females per group). The body weights of the rats were recorded regularly. Each animal was handled individually and carefully observed for abnormal behavior or physical changes before treatment. The test substances, *Rajat Bhasma* (RB) and silver nanoparticles (*AgNPs*), were suspended in 1% sodium carboxymethyl cellulose (CMC) and administered orally via an oral cannula once daily for 28 consecutive days. The control group (Group 1) received distilled water. Group 2 was administered RB at the therapeutically effective dose (TED) of 10.8 mg/kg. Groups 3 and 4 received RB at higher doses of 108 mg/kg and 216 mg/kg, respectively. Group 5 was treated with *AgNPs* at the TED of 10.8 mg/kg, while Groups 6 and 7 received *AgNPs* at 108 mg/kg and 216 mg/kg, respectively. All test suspensions were administered orally once daily for 28 days. Details of the dosing regimen and group assignments are summarized in Table 1.

Table 1. Grouping and *Rajata Bhasma* Dose Schedule:

Groups	Details of Groups	Dose mg/kg body weight	Number of animals per group	
			Male	Female
Group-1	C-Control Group Distilled Water	10 ml/kg	3	3
Group-II	**RB 1-** TED	10.8	3	3
Group-III	**RB 2-***Rajata Bhasma* 10 X TED	108	3	3
Group-IV	**RB 3-***Rajata Bhasma* 20 X TED	216	3	3
Group-V	**AgNPs 1-**Silver Nanoparticles TED	10.8	3	3
Group-VI	**AgNPs 2-**Silver Nanoparticles 10 X TED	108	3	3
Group-VII	**AgNPs 3-** Silver Nanoparticles 20 X TED	216	3	3

The animals were meticulously monitored daily throughout the 28-day treatment period for signs of mortality, morbidity, behavioral changes, and food and water intake variations. At the end of the administration period, the animals were fasted overnight with free access to water. On day 29, they were anesthetized using ether, and blood samples were collected via retro-orbital puncture using capillary tubes prior to sacrifice.

Hematology samples were collected in tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA), while blood samples without anticoagulant were collected for clinical biochemistry analyses. The following hematological parameters were measured: total red blood cell count (TRBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC), percentages of neutrophils, lymphocytes, eosinophils, monocytes, packed cell volume (PCV), and platelet count (PLT).

Serum biochemical estimation

The serum separated from blood samples was used to estimate various biochemical parameters using diagnostic reagent kits on a Robonik biochemical analyzer. Serum total protein (g/dL) was measured by the biuret method, following the protocol described by Varley (2005). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels (IU/L) were determined using the UV kinetic method, and serum alkaline phosphatase (ALP) and creatinine (mg/dL) were measured using the alkaline picrate method. Serum lactate dehydrogenase (LDH) levels (U/L) were assessed by electrophoresis. Serum uric acid, urea, albumin, and glucose were estimated using the Athenese-Dx TRUEchemie Test Kit.

All organ tissues (kidney, liver, and spleen) from the experimental Wistar albino rats were collected and fixed in 10% buffered formalin. The tissues were processed using routine paraffin embedding and stained with hematoxylin and eosin (H&E) [25–26].

Statistical analysis

Data are expressed as mean ± standard deviation (SD) for six rats per experimental group. One-way analysis of variance (ANOVA) followed by Dunnett's multiple t-tests as a post hoc analysis was performed using GraphPad Prism software (version 8.0) to compare mean values of quantitative variables among groups. Additionally, paired and unpaired t-tests were conducted using GraphPad Prism (version 8.0) to assess significant

differences between groups. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Daily oral administration of RB and *AgNPs* at doses of 10.8 mg/kg (RB1, *AgNPs*1), 108 mg/kg (RB2, *AgNPs*2), and 216 mg/kg (RB3, *AgNPs*3) for 28 consecutive days did not produce significant alterations in the general behavior of treated rats compared to controls. Both treated and control groups exhibited good health throughout the treatment period, and no mortality was observed. All animals in all groups, except those in the *AgNPs*3 group, remained normal and active. However, starting from day 15, rats in the *AgNPs*3 group displayed hyperactivity and aggression, accompanied by increased feed intake compared to other groups. Rats in the *AgNPs*1 group also exhibited mild hyperactivity and aggression. Feed intake was significantly increased across all groups during the experimental period, with the RB3 group showing particularly high intake. Weight gain was observed in the control and RB1 groups during the subchronic toxicity study. Rats in the RB1 group showed a highly significant increase in body weight, whereas no significant changes in body weight were observed in any of the *AgNPs* groups. There were no significant dose-related changes in body weight across the groups. Furthermore, the percentage change in body weight patterns among treated groups did not differ significantly from that of the control group [Table 2].

The effects of *Rajat Bhasma* (RB) on hematological parameters are summarized in [Table 3]. Among the eight parameters studied, a significant decrease in hematocrit and platelet count was observed at both the therapeutically equivalent dose (TED) and 20 × TED dose levels of RB; however, all values remained within normal physiological ranges. The significant reduction in platelet count at TED and 20 × TED doses suggests a potential toxic effect. A significant decrease in white blood cell (WBC) count was observed at TED and 10 × TED doses, although values were still within normal limits. Notably, administration of RB at the 20 × TED dose level caused a significant reduction in WBC count [Table 3]. All other hematological parameters remained within normal ranges and showed no significant alterations.

The effects of three different doses of silver nanoparticles (*AgNPs*) on hematological parameters are presented in [Table 3]. Among the eight parameters studied, a significant decrease in red blood cell (RBC) count and hematocrit was observed at the therapeutically equivalent dose (TED), 10 × TED, and 20 × TED dose levels compared to the control group. The 20 × TED dose of *AgNPs* also caused a significant reduction in white blood cell (WBC) and platelet counts; however, platelet counts remained within the normal range [Table 3]. All other hematological parameters showed no significant changes and remained within normal limits.

Table 2. Effect of RB and SNP on body weight (g) of albino rats in repeated dose 28-days oral sub-chronic toxicity study

Duration (week)	Treatments						
	Control	RB TED	RB TED X 10	RB TED X 20	AgNPs TED	AgNPs TED X 10	AgNPs TED X 20
0	321.66±32.11	179.33±7.33	239.66±40.16	251.66±13.82	262.33±53.43	263.16±66.00	251±44.62
1	329.66±26.99	193.66±10.83	243.16±33.51	274.83±11.90*	260±48.46	267.66±64.84	248.83±44.58
2	332.33±25.78	197±10.95	245.33±34.83	281±11.93**	259±50.28	261.5±60.28	248.5±44.13
3	334.66±24.08*	197.5±6.92*	244.16±39.50	286.66±14.62*	262±51.96	260.33±59.22	248.5±44.08
4	341±21.97*	213.66±19.40**	240.5±41.89	283.5±15.97**	261.33±44.89	261.66±44.08	251.66±44.00
% change	6.01↑	19.14↑	0.34↑	12.64↑	-0.38↓	-0.56↓	0.26↑

Data: Mean ± SD, RB: *Rajat Bhasma*, SNP: Silver Nanoparticles, TED: Therapeutically Equivalent Dose,↑: increase, ↓: decrease *P <0.05,** p < 0.01, *** P < 0.001, when compared to initial body weight (paired "t" test).

Table 3. Effect of test drug on hematological parameters of albino rats in repeated dose 28-days oral Sub- chronic toxicity study

Parameters	Control	RB TED	RB TED X10	RB TED X 20	AgNPs TED	AgNPs TED X10	AgNPs TED X20
WBC count (/mm³)	14.33±6.70	4.41± 2.44**	5.28±3.76*	3.15±2.80**	6.15±4.92	4.46±7.96	1.85±1.88**
RBC (mil/cum)	12.58±3.83	10.47±5.26	8.00±7.59	6.80±7.45	5.88±4.21*	6.31±5.66*	4.78±2.49**
Haemoglobin (g %)	13.32±2.98	11.28±3.77	14.66±7.24	16.18±6.09	13.93±2.06	10.7±5.32	13.83±1.89
MCV (fl)	53.03±24.02	60.83±12.35	51±12.56	37.66±23.50	49.5±17.81	49.83±24.76	56.83±0.98
MCH (pico gm)	17.98±8.99	19.9±5.58	19.51±4.89	17.81±9.28	143.6±268.36	11.06±9.12	18.7±19.56
MCHC (%)	26.7±11.00	31.31±5.26	33.86±8.58	39.83±32.84	23.23±18.12	91.61±156.60	18.93±30.43
Haematocrit	64.43±4.10	37.91±8.65****	39.83±32.84	29.66±36.13*	18.93±30.43**	7.65±1.05****	15.1±21.57***
Platelet count (10⁶ /mm³)	539±144.69	276.83±89.82**	493.5±506.84	204.56±273.06*	321.16±297.58	332.83±255.70	341.5±125.83*

Data: Mean ± SD, RB: *Rajat Bhasma*, AgNPs: Silver Nanoparticles, TED: Therapeutically Equivalent Dose, *P <0.05,** p < 0.01, *** P < 0.001, when compared to vehicle control group (paired "t" test). @P <0.05, @@p < 0.01, @@@P < 0.001, when compared to vehicle control group (One Way Annova followed by Dunnett's multiple "t" test)

Table 4. Effect of test drug on Bio-chemical parameters of albino rats in repeated dose 28-days oral Sub- chronic toxicity study

Parameters	Control	RB TED	RB TED X 10	RB TED X 20	AgNPs TED	AgNPs TED X 10	AgNPs TED X 20
Blood sugar (mg/dL)	78.77±6.63	63.28±7.88	51.78±11.46***	74.09±14.68	88.66±28.72	105.24±59.14	66.97±12.70
Total bilirubin (mg/dL)	0.44±0.34	0.42±0.30	0.30±0.36	0.49±0.29	0.43±0.23	0.30±0.23	0.37±0.22
AST (IU/L)	154.85±26.68	152.67±32.03	164.97±12.27	379.41±110.45***@@@	164.87±20.46	210.92±48.54*	162.40±28.35
ALP (IU/L)	75.42±3.07	61.25±4.77***	64.50±7.41**	221.79±61.01***@@@	65.69±10.39	80.53±10.35	61.97±8.56**
ALT (IU/L)	10.47±1.19	13.76±2.92*	16.38±4.44*	35.84±43.09	8.57±6.78	52.09±43.48*	68.90±42.86**@@
LDH (µ/l)	455.40±192.42	252.06±48.10*	518.00±216.90	868.38±374.60*@@	385.99±50.26	415.88±43.83	311.63±74.03
Blood urea (mg/dL)	62.64±7.67	57.62±10.41	53.81±16.40	56.73±24.27	63.13±12.49	64.67±15.48	54.96±11.66
Uric acid (mg/dL)	4.30±1.02	3.06±0.72*	4.52±1.02	6.13±1.86@	2.87±0.24**	4.52±0.65	3.48±0.99
Creatinine (mg/dL)	0.74±0.05	1.26±0.80	0.78±0.006	0.86±0.05**@	0.89±0.09**	0.81±0.04*	0.79±0.09
Total protein (g/dL)	8.31±2.70	9.29±1.61	8.14±0.64	7.94±1.18	7.94±0.61	8.09±0.69	8.68±1.20
Albumin (g/dL)	3.51±0.83	3.95±0.83	4.70±0.32**@@	4.45±0.58*@	4.47±0.41*@	4.80±0.34**@@	4.52±0.60*@

Data: Mean ± SD, RB: *Rajat Bhasma*, AgNPs: Silver Nanoparticles, TED: Therapeutically Equivalent Dose, *P <0.05,** p < 0.01, *** P < 0.001, when compared to vehicle control group (Unpaired "t" test). @P <0.05, @@p < 0.01, @@@P < 0.001, when compared to vehicle control group (One Way Annova followed by Dunnett's multiple "t" test)

The effects of oral administration of *Rajat Bhasma* (RB) on biochemical parameters are summarized in Table 4. A significant decrease in blood glucose levels was observed at the 10 × TED dose compared to the control group. Notably, alanine aminotransferase (ALT) levels increased significantly in the RB TED and RB 10 × TED groups, while higher doses resulted in nonsignificant increases; however, all ALT values remained within the normal reference range. A significant decrease in alkaline phosphatase (ALP) levels was noted in the RB TED and RB 10 × TED groups, with all values remaining within normal limits. In contrast, RB administered at 20 × TED significantly increased ALP, aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) levels, suggesting potential liver damage. Serum albumin levels increased significantly at RB 10 × TED and 20 × TED doses, although all values remained within the normal range. Significant uric acid and serum creatinine increases were observed at the 20 × TED dose, but these values were still within normal limits. No statistically significant changes were found in total bilirubin, serum urea, or total protein levels compared to the control group.

A significant increase in alanine aminotransferase (ALT) levels was observed in both the *AgNPs* 10 × TED and 20 × TED groups. In contrast, the lower dose of RB resulted in a nonsignificant increase in serum ALT levels. The *AgNPs* 10 × TED dose also caused a significant elevation in aspartate aminotransferase (AST) levels. A significant reduction in alkaline phosphatase (ALP) was noted at the *AgNPs* 20 × TED dose, although values remained within physiological limits. Increases in serum creatinine at the *AgNPs* TED and 10 × TED doses were also within normal physiological ranges. No statistically significant changes were observed in blood glucose, serum lactate dehydrogenase (LDH), total bilirubin, serum urea, or total protein levels compared to the control group.

Administration of *Rajat Bhasma* (RB) and silver nanoparticles (*AgNPs*) at all dose levels resulted in mild congestion in the spleen, except for the RB 20 × TED group, which exhibited extensive congestion. Congestion was also observed in the liver of both RB and *AgNPs* groups across all dose levels. Hydropic degeneration in the kidney was evident in all three *AgNPs* dose groups, whereas the RB groups exhibited only mild renal changes at all doses. Other examined organs displayed normal cytoarchitecture at TED, 10 × TED, and 20 × TED doses of both RB and *AgNPs* (Fig. 1).

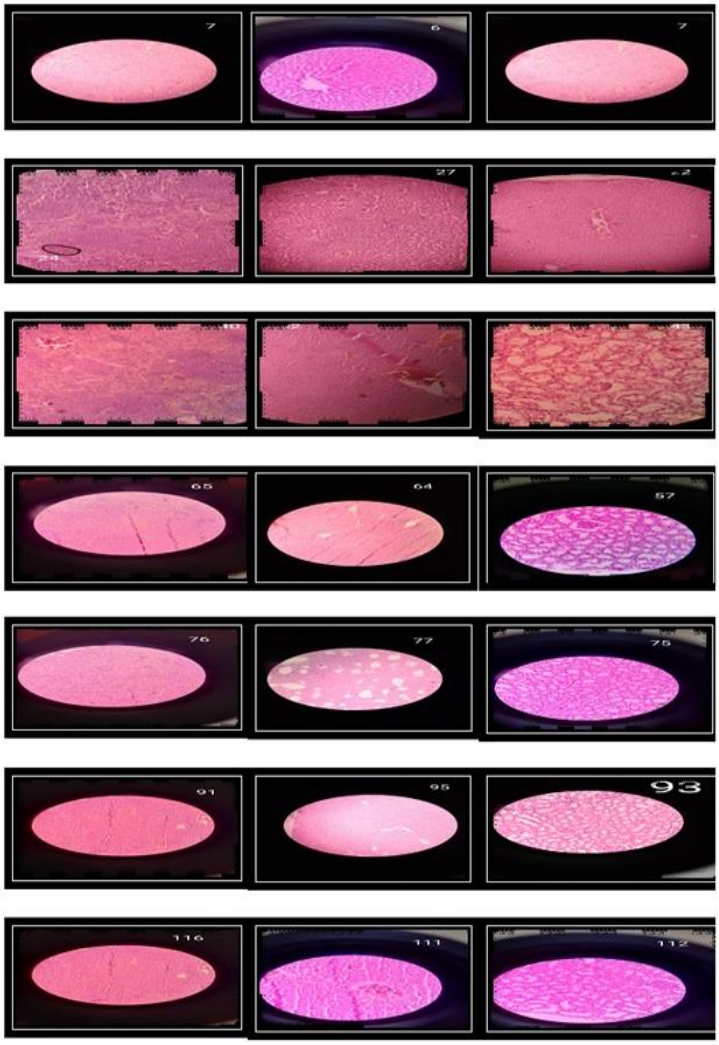

Fig. 1. First Column- Spleen Histopatholgy, Second Column- Liver Histopatholgy, Third Column- Kidney Histopatholgy. First row- Control Group, Second Row- RB TED x 10, Third row- RB TED x 20, Fourth row- AgNPs TED, Fifth row- AgNPs TED X 10, Sixth row- AgNPs TED X 20, Seventh row- AgNPs TED X 20.

Table 5. Histological findings from rats

Organ	Histopathological Findings	Control	RB TED	RB TED X 10	RB TED X 20	AgNPs TED	AgNPs TED X 10	AgNPs TED X 20
Spleen	Normal	03/06	04/06	03/06	03/06	03/06	03/06	03/06
	Congestion	01/06	02/06	02/06	02/06	03/06	03/06	03/06
	Extensive congestion	02/06	00/06	01/06	01/06	00/06	00/06	00/06
Liver	Normal	05/06	04/06	03/06	03/06	03/06	03/06	03/06
	Mild Congestion	01/06	00/06	00/06	00/06	00/06	00/06	00/06
	Congestion	00/00	02/06	03/06	03/06	03/06	03/06	03/06
Kidney	Normal	05/06	04/06	05/06	04/06	03/06	03/06	03/06
	Mild Congestion	01/06	01/06	00/06	00/06	00/06	00/06	00/06
	Cloudy changes in glomerulus	00/06	00/06	00/06	01/06	00/06	00/06	00/06
	Hydropic changes	00/06	00/06	01/06	01/06	00/06	00/06	00/06
	Mild hydropic degeneration	00/06	01/06	00/06	00/06	00/06	00/06	00/06
	Hydropic degeneration	00/06	00/06	00/06	00/06	03/06	03/06	03/06
Pancreas	Normal	06/06	06/06	06/06	06/06	06/06	06/06	06/06
Heart	Normal	06/06	06/06	06/06	06/06	06/06	06/06	06/06
Brain	Normal	06/06	06/06	06/06	06/06	06/06	06/06	06/06

The Effect of RB and AgNPs on Histopathology of Organs:

Histopathological findings of different organs are shown in Table 5.

DISCUSSION

Silver is not involved in any known biological processes and is neither a macro- nor micronutrient essential for living organisms. However, its antibacterial properties have been recognized for centuries. [27] Although silver exposure was historically considered rare, the increased use of silver in commercial and medicinal products has led to greater human exposure [28]. Systemic effects of silver exposure may include argyria of the eyes [29], leukopenia [30], and damage to the liver and kidneys [31]. Additionally, silver has been reported to cause brain damage [32], seizures [33], and mortality in animal studies [34].

Improper processing of RB can lead to various health complications, including Sharirsantap (a sensation of increased body heat), Vidabaddhata (constipation), Shukranash (oligospermia), Balanasha (loss of potency), and Ayuhanti (reduced lifespan) [35].

Silver nanoparticles can enter biological systems through various routes. Factors such as particle size and nature, surface area, shape, aspect ratio, coating, crystallinity, dissolution, and agglomeration influence the toxicity of nanosilver [36]. The route of administration, concentration, duration, size, tissue distribution, penetration, and cellular uptake of nanosilver also play significant roles in determining its toxic effects [37–38]. Studies on the interaction of these nanoparticles with biological systems have revealed that, unlike pure silver nanoparticles, *Bhasma* is non-toxic to cells and facilitates the passage of both small and large molecules across the epithelial cell monolayer, possibly by relaxing tight junctions between cells [39]. To evaluate the safety of nanosilver, a sub-chronic toxicity study was conducted comparing nanoparticles prepared via modern green synthesis and ancient Ayurvedic methods in the form of *Bhasma*.

Body weight variations in animals are a critical indicator of their health. A decline in body weight is typically considered the first sign of an adverse outcome. A dose is deemed hazardous if it causes a 10% or greater reduction in body weight, regardless of any other changes that may occur [40]. The percentage change in body weight patterns in the RB-treated groups did not differ significantly from those observed in the control groups, suggesting the absence of serious toxic effects of RB during 28 days of oral administration in rats. No significant weight loss was observed in any of the three AgNPs groups, indicating the absence of serious toxic effects of AgNPs during 28 days of oral administration in rats. Previous studies have reported that oral administration of AgNPs (56 nm) at 125 and 500 mg/kg body weight does not significantly decrease body weight up to 10 and 4 weeks, respectively [41].

Significant decreases in platelet count were observed in the RB TED (10.8 mg/kg body weight) and RB TED × 20 groups (216 mg/kg body weight). The observed effect was not dose-dependent and remained within the normal range. Significant leucopenia was noted after oral administration of RB (216 mg/kg body weight) and AgNPs (216 mg/kg body weight). Significant decreases in RBC count and hematocrit were observed in all AgNPs groups, with dose-dependent effects.

Administered at higher doses (TED × 20), RB was observed to significantly increase ALP, AST, and LDH levels, indicative of liver damage. A significant increase in ALT levels was noted in the AgNPs TED × 10 and TED × 20 treated groups, further suggesting liver damage. However, it is worth noting that a lower dose of AgNPs did not cause liver damage, as indicated by the non-significant increase in ALT levels.

Histopathology reports indicate that RB and AgNPs accumulate in the liver, spleen, and kidney, causing histopathological changes. Previous investigations have revealed that approximately 10–15% of ingested silver is absorbed in the gastrointestinal tract and transported throughout the body via the circulatory system. The remaining silver is eliminated through urine and feces. [42]. In a study involving mice fed silver-doped water (500 ppm AgNO3), the highest quantity of silver was found in the kidneys (1.2×10^4 µg/kg wet weight), followed by the spleen (8.7×10^3 µg/kg wet weight) and liver (3.9×10^3 µg/kg wet weight). [43]. Other research has suggested that the liver is one of the primary locations for cuproenzyme expression in the body, and silver accumulation there may disrupt copper homeostasis. [44]. Many researchers have examined the subchronic toxicity of AgNPs, but none have investigated the toxicity of AgNPs synthesized using herbal medications employed in manufacturing RB. Additionally, compared to earlier studies, the particle size of AgNPs in this investigation differed. This study demonstrates that RB is safer than green-synthesized AgNPs at higher dosages. Further studies are needed to compare the safety of various metallic Bhasma and green-synthesized nanoparticles.

CONCLUSION

Rajat Bhasma and silver nanoparticles administered at 10- and 20-times TED have demonstrated adverse effects on specific hematological parameters, including platelet count, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) levels. Silver nanoparticles also induce changes in ALT and AST levels, indicating liver toxicity, which is further supported by histopathological findings in the liver. Rajat Bhasma at 20 times the TED dose and silver nanoparticles at all tested doses showed hydropic degeneration and congestion in the kidney. However, it is important to note that Rajat Bhasma and silver nanoparticles are safe at therapeutically equivalent doses. Silver nanoparticles exhibit greater toxicity at higher doses than Rajat Bhasma.

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CONFLICTS OF INTERESTS

The authors declared no conflicts of interest in this article.

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