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Research Article

Evaluation of acute and subacute toxicity of *Abhrasindoora*: As a mercury-based traditional herbo-bio-mineral metallic formulation

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ABSTRACT

Herbo-bio-mineral metallic preparations such as *Abhrasindoora* offer advantages over plant drugs under their stability over a period, lower dosage, and contain minerals and metals as an integral part of the formulations. The use of metals in medicine is often associated with toxicity. Present study was conducted to explore the acute and sub-acute toxicity study of *Abhrasindoora* in Wistar albino rats. Information related to possible health hazards, the estimate of no-observe-adverse-effect-level (NOAEL), hematology, and biochemical and histopathology parameters were studied. For the acute toxicity study rats were divided into two groups: Group I (Test group: 300 mg/kg) and Group II (Confirmatory group: 2,000 mg/kg) as per OECD 423 guidelines and for subacute toxicity study, rats were divided into four groups as Group I (water as the vehicle), Group II (600 mg/kg), Group III (800 mg/kg) and Group IV (1,000 mg/kg) as per OECD 407 guidelines. Clinical signs (daily); body weight and feed consumption (weekly) were taken. Blood samples were collected from the retro-orbital sinus before the necropsy (29^{th} day). For statistical analysis, one-way ANOVA and Dunnett's test were used. In comparison to the control group, no mortality or adverse effects were observed. No significant variations were observed in hematological and biochemical parameters. In target organs, no significant changes were observed in both acute and sub-acute toxicity studies. No deaths or any signs of toxicity were observed after oral administration in the acute toxicity study up to the dose of 2,000 mg/kg and in the sub-acute toxicity study up to the dose of 1,000 mg/kg.

Keywords:
Abhrasindoora, Herbo-Bio-Mineral Metallic formulation, Acute toxicity, Sub-acute toxicity

1. INTRODUCTION

Rasashastra is a pharmacotherapeutics of *Rasoushadhis* that deals with the processing of Mercurial preparations, the direction of its therapeutic use along with dietary regimen, and, the management of adverse effects of Herbo-Bio-Mineral Metallic compound[1]. The mercurial preparations are mainly categorized into four categories i. e. *Khalvi Rasayana*, *Parpati Rasayana*, *Kupipakwa rasayana* and *Pottali Rasayana*. This article emphasized toxicity studies of *Kupipakwa Rasayana* with special reference to *Abhrasindoora (ABS)* as a Sublimated mercurial Herbo-Bio-Mineral Metallic compound. There are four different pharmaceutical processes are explained for the preparation of *ABS* in *Rasashastra*[2]. Ayurvedic medications and their formulations have been thought to be safe and effective for ages due to their low risk of side effects. This notion may have influenced the rural population's indiscriminate usage of these formulations to a great extent. These formulations are typically given over a long period without sufficient dosage monitoring by professionals or understanding of the potentially harmful effects of such long-term use. As a result, scientific knowledge of oral toxicity is critical, not only for identifying

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doses that can be utilized later but also for revealing the potential clinical indications provoked by medicines under investigation. Regardless of the pharmacological benefits of the innovative Mercury-based Traditional Herbo-Bio-Mineral Metallic Formulation, little is known regarding the medicinal inorganic compounds' acute and subacute toxicity. As a result, the current research was carried out in an animal model to assess and focus on the acute and subacute toxicity of a Mercury-Based Traditional Herbo-Bio-Mineral Metallic Formulation.

Following *Rasendra Sambhava, Abhrasindoora* was prepared in a stepwise manner and the steps followed were a) bio-purification of Mercury, Sulphur, and Black mica followed by b) Preparation of jet-black colored (*Kajjali*) powder by trituration (*Mardana*) in the mortar and pestle c) Impregnation (*Bhavana*) process with latex of *Arka* and Juice of *Vatashunga* d) Cooking process in *Kachakupi* e) Collection of the final product as *Abhrasindoora* f) Trituration of the finished product in mortar and pestle to get bright red color fine powder of *ABS* which was further administered for toxicity study. It is indicated in *Shwasa* (Bronchial Asthma), *Tridoshaja Vikara* (All types of diseases) along with different adjuvants[3]. Purification of metals and minerals using organic *Shodhana* media is primary and important for *Samskara* to remove physical and chemical impurities. It makes *Rasadravya* brittle and facilitates it into small particles. *Shodhana* and *Bhavana's* process helps to make it in Herbo-Bio-Metal Mineral Compound form and remove physicochemical impurities[4]. The extensive use of *Rasoushadhis* for more than a millennium without any reports of any untoward events can be considered evidence of their safety, but no objective-verifiable data exists to support such claims. Pre-clinical studies of Ayurvedic drugs provide the scientific basis for their traditional use and prove that they are safe and efficacious[5]. Ayurvedic formulations consist of natural substances which are usually having a wide therapeutic range and effectiveness in a large number of diseases[6]. *Abhrasindoora* is a Herbo-Bio-Metal Mineral compound, so its safety is of high concern which needs to produce evidence-based data. Organic mercury compounds such as methyl mercury are extremely toxic to the biological system, and a small amount of them could lead to severe adverse effects in patients. Furthermore, the presence of free mercury is also a concern. To generate safety data on *Abhrasindoora* Acute toxicity and Subacute toxicity were conducted by following OECD guidelines along with Institutional Animal Ethical clearance. Acute systemic toxicity assesses the negative effects that occur after organisms are exposed to a single or repeated dose of a test material via a known route within 24 hours (oral, dermal, or inhalation). Subacute toxicity (repeat dose toxicity) is concerned with adverse effects that occur after a single or repeated dose of a test sample is given each day for a period of 14 to 28 days. Therefore, we have used *Abhrasindoora* and examined the alteration of various parameters such as Histopathology, Biochemical, and Haematology in Wistar Albino rats.

2. MATERIALS AND METHODS

2.1. Institutional Animal Ethics committee (IAEC) Approval

As per the IAEC approval, the study was commenced in healthy, young, nulliparous, and non-pregnant Wistar Albino rats of both genders. All experiments and protocols described in the present study were approved by the Institutional Animal Ethics Committee (IAEC), Department of Pharmacology, Parul Institute of Pharmacy and Research and with the permission from Committee for Control and Supervision of Experiments on Animals (CPCSEA). The protocol number for the study is 984/2020-01.

Chemicals: Chemicals and reagents of analytical grade were procured from Siga Aldrich, Mumbai, and Durga scientific Pvt Ltd, Vadodara.

Drug: *Abhrasindoora* was prepared at Parul Ayurved Pharmacy, Parul Institute of Ayurved, Vadodara (Table 1).

Heavy metal analysis of *Abhrasindoora* was done from Vasu Research Centre, Vadodara (Table 2).

Table 1. Ingredients of *Abhrasindoora*.

S.N.	Name of ingredients	Scientific/English Name	Quantity
1	*Shodhita Parada*	Purified Mercury	1 part
2	*Shodhita Gandhaka*	Purified Sulphur	1 part
3	*Dhanyabhraka*	Powder of purified Mica	1 part
4	*Arkaksheera*	Latex of *Calotropis procera*	Q.S.
5	*Vatashunga swarasa*	Fresh Juice of *Ficus benghalensis*	Q.S.

Table 2. Heavy metal analysis of *Abhrasindoora*.

Heavy metals	Results
Mercury (Hg)	2.4%
Arsenic (As)	0.01%
Cadmium (Cd)	0%
Lead (Pb)	Not detected
Stibnite (Sb)	0%

2.2. Exposure of *Abhrasindoora* to Wistar Albino Rat for Acute toxicity study

OECD 423 guidelines were followed for acute toxicity study[7-8]. As the other medicinal herbs-mineral preparations were found to be safe in previous studies, the initial limit test at the dose of 2,000 mg/kg was done. Three animals were used in each step. The formulation was given at the dose of 2,000 mg/kg of body weight orally to each animal. Initially, the animals were observed every 30 minutes for 24 hours followed by once a day for the next 14 days.

Dosing schedule: The single dose of the drug was orally administered and then observed for 14 days. After completion of the treatment period of 14 days, all animals were sacrificed on day 15th.

2.3. Exposure of *Abhrasindoora* to Wistar Albino Rat for Subacute toxicity study

Sub-acute toxicity was carried out in Wistar albino rats (8-12 weeks). Animals were divided into four groups, each consisting of 5 male and 5 female rats, and division was made as Group I (water + 0.5/% CMC as the vehicle), Group II (600 mg/kg), Group III (800 mg/kg) and Group IV (1,000 mg/kg) as per OECD 407 guidelines[9]. Wistar albino rats were dosed according to body weight with a single oral daily dose for 28 days. Clinical signs such as body weight daily and food intake on weekly basis were documented. Blood samples were collected by retro-orbital sinus before the necropsy on the 29th day. Target organs were stored in 10% normal saline.

Dosing schedule: The test item was administered for 28 days. After the completion of the treatment period of 28 days, all animals were sacrificed on day 29.

Dose Preparation: *ABS* was triturated to get fine powder and mixed uniformly in the required proportion. The suspensions for low, medium, and high doses were prepared in 0.5% Carboxy Methyl Cellulose in distilled water and administered to animals orally with the help of a gastric catheter.

Drug Administration: The administration was done by oral gavage using an intubation needle (stainless steel, 20 gauge) fitted into a polypropylene disposable syringe. The concentration was adjusted accordingly to its weekly body weight. The doses for the study were selected based on a literature search and the result of an acute oral toxicity study in Rats.

Statistical analysis: Statistical analysis was carried out using one-way ANOVA followed by Dunnett's test. All the qualitative data were expressed as mean±Standard Error of Mean (SEM). Every statistical analysis was performed with a one-way analysis of variance (ANOVA). Statistically significant differences were accepted at $p \leq 0.05$.

2.4. Observations and results

Acute Toxicity study: An acute toxicity study was performed at the dose of 2,000 mg/kg level. No change in the skin, fur, eyes, or in the mucous membrane was observed. There was no occurrence of tremors, convulsions, increased salivation, diarrhoea, lethargy, sleep, coma, etc. There was no change in body weight and eating or drinking habits. Not a single death occurred during the study.

Body Weight: The weight of each animal was recorded one day before the initiation of the dosing (Day 0) and at weekly intervals throughout the study (Table 3).

Food Intake: The quantity of the feed offered was based on the requirement of the animals in each cage. The intake of the feed was recorded on weekly basis. The feed consumed i. e. animal cage/week was calculated by subtraction of the left-over feed from the total quantity of the feed provided during that week (Table 4).

Skin and Fur Condition: After giving a single dose of the drug, there was no significant change in the skin, fur, eyes, and mucus membrane throughout the entire acute toxicity study period (Table 5).

Urine and Faeces observation: After giving a single dose of the drug, there was no significant change in urine and faeces throughout the entire acute toxicity study period (Table 6).

Histopathology of various organs: Organs from all the dead, moribund, or sacrificed or terminally sacrificed animals belonging to control and test groups were preserved in 10% formalin solution. The histopathological examination was performed initially for animals belonging to the control and test group (300 mg/kg). There were no significant changes observed in the microscopical examination, hence the study was further extended to 2,000 mg/kg dose.

Below mentioned organs of control and test group animals were collected and observed under a microscope for histopathological examination (Figure 1-5).

Sub-acute toxicity study: Sub-Acute toxicity study was performed in 3 different dose levels: low dose (600 mg/kg), medium dose (800 mg/kg), and high dose (1,000 mg/kg) (Table 7). No change in the skin, fur, eyes, or mucous membrane was observed. There were no occurrences of tremors, convulsions, increased salivation, diarrhea, lethargy, sleep, or coma. There was no change in body weight and or in eating and drinking habits. No deaths occurred.

Clinical signs: All the animals were observed daily for any abnormal clinical signs and behavioral changes. Appearance, changes, and disappearance of clinical signs were recorded for 28 days. The cage side changes observed included skin, fur, eye, and mucous membrane.

Autonomic changes like lacrimation, piloerection, pupil size and abnormal respiratory pattern, posture, gait, response to handling, presence of, tonic or clonic

Table 3. Observation table for body weight in Acute toxicity study of *ABS*.

Sl. No.	Group	0 days	7 days	14 days
1	Test group 300 mg	245 ± 8.62	251 ± 8.50	267 ± 13.57
2	Confirmatory group 300 mg	256 ± 10.14	263 ± 8.54	268 ± 13.01
4	Test group 2,000 mg	251 ± 9.03	258 ± 12.32	266 ± 10.65
5	Confirmatory group 2,000 mg	237 ± 9.53	241 ± 11.32	248 ± 11.46

Values are expressed in mean±SD

Table 4. Observation table for food intake in Acute toxicity study of *ABS*.

Sr No.	Group	0 days	7 days	14 days
1	Test group (300 mg/kg)	13 ± 1.63	17 ± 2.94	20 ± 2.44
2	Confirmatory group (300 mg/kg)	13 ± 1.69	12 ± 2.62	15 ± 2.86
3	Test group (2,000 mg/kg)	18 ± 2.86	21 ± 2.86	25 ± 2.16
4	Confirmatory group (2,000 mg/kg)	15 ± 2.24	15 ± 3.39	18 ± 1.24

Values are expressed in mean±SD

Table 5. Observation table of skin, fur, and eye condition in acute toxicity study of *ABS*.

Group	0 days			7 days			14 days		
	Skin	Fur	Eye	Skin	Fur	Eye	Skin	Fur	Eye
Test group (300 mg/kg)	N	N	N	N	N	N	N	N	N
Confirmatory group (300 mg/kg)	N	N	N	N	N	N	N	N	N
Test group (2,000 mg/kg)	N	N	N	N	N	N	N	N	N
Confirmatory group (2,000 mg/kg)	N	N	N	N	N	N	N	N	N

N-Normal

Table 6. Observation table of urine and feces condition in the acute toxicity study of *ABS*.

Group	0 days		7 days		14 days	
	Urine	Faeces	Urine	Faeces	Urine	Faeces
Test group (300 mg/kg)	N	N	N	N	N	N
Confirmatory group (300 mg/kg)	N	N	N	N	N	N
Test group (2,000 mg/kg)	N	N	N	N	N	N
Confirmatory group (2,000 mg/kg)	N	N	N	N	N	N

N-Normal

Table 7. Dosing details of *ABS* for subacute toxicity study.

Sr. No.	Group	Dose	Duration	Animals	Route
1	Control	Vehicle control	28 days	10	Oral
2	LD-ABS	600 mg/kg	28 days	10	Oral
3	MD-ABS	800 mg/kg	28 days	10	Oral
4	HD-ABS	1,000 mg/kg	28 days	10	Oral

LD-*ABS*= Low dose (600 mg/kg) *Abhrasindoora*, MD-*ABS*= Medium dose (800 mg/kg *Abhrasindoora*, HD-*ABS*= High dose (1000 mg/kg) *Abhrasindoora*.

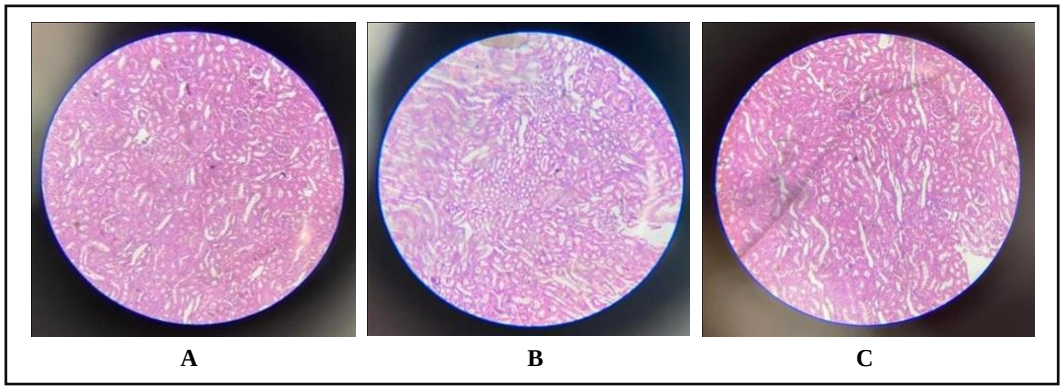

Figure 1. Histopathology of kidney tissue. Magnification (40x) a) Normal cytoarchitecture-control group, b) No significant changes in cytoarchitecture-treated with *ABS* (300 mg/kg), c) No significant changes in cytoarchitecture-treated with *ABS* (2,000 mg/kg).

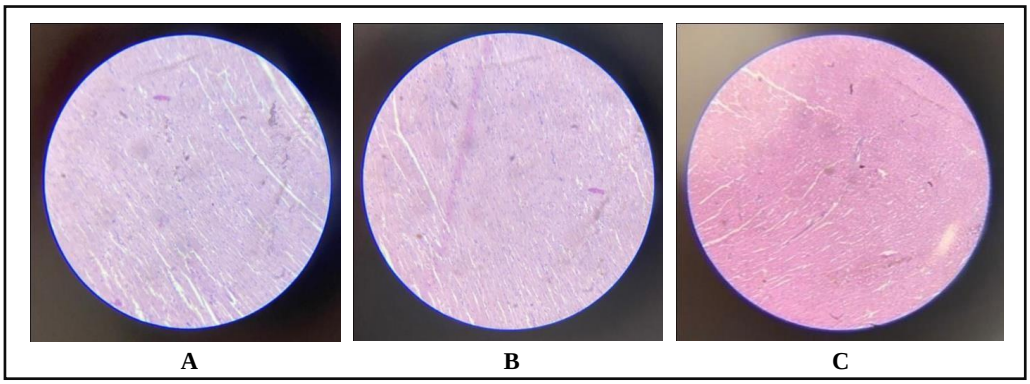

Figure 2. Histopathology of heart tissue. Magnification (40x) a) Normal cytoarchitecture-control group, b) No significant changes in cytoarchitecture-treated with *ABS* (300 mg/kg), c) No significant changes in cytoarchitecture-treated with ABS (2,000 mg/kg).

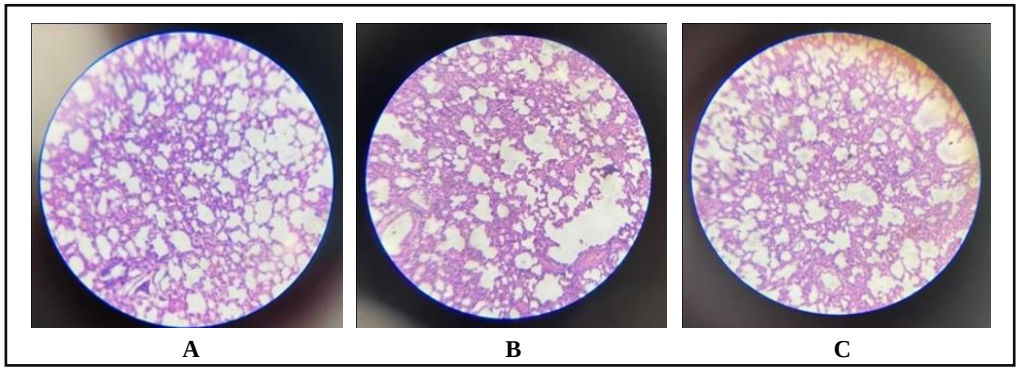

Figure 3. Histopathology of lung tissue. Magnification (40x) a) Normal cytoarchitecture-control group, b) No significant changes in cytoarchitecture-treated with *ABS* (300 mg/kg), c) No significant changes in cytoarchitecture- treated with *ABS* (2,000 mg/kg).

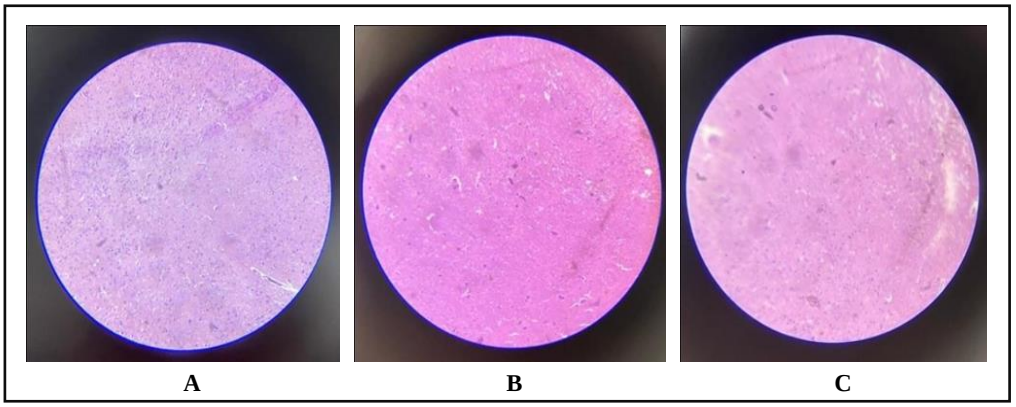

Figure 4. Histopathology of brain tissue. Magnification (40x) a) Normal cytoarchitecture-control group, b) No significant changes in cytoarchitecture-treated with *ABS* (300 mg/kg), c) No significant changes in cytoarchitecture-treated with *ABS* (2,000 mg/kg).

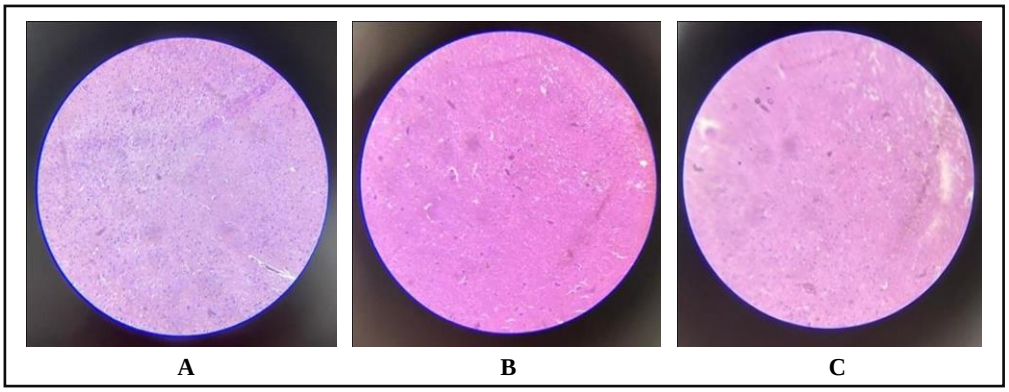

Figure 5. Histopathology of liver tissue. Magnification (40x) a) Normal cytoarchitecture-control group, b) No significant changes in cytoarchitecture-treated with *ABS* (300 mg/kg), c) No significant changes in cytoarchitecture-treated with *ABS* (2,000 mg/kg).

movement, stereotypes like excessive grooming and repetitive circling, or bizarre behavior like self-mutilation, walking backward, etc. were also recorded.

Body Weight: The weight of each animal was recorded one day before the initiation of the dosing (Day 0) and at weekly intervals throughout the study. The last body weight was recorded one day before blood collection (Table 8).

2.5. Histopathology of various organs

Organs from all the dead, moribund, or sacrificed or terminally sacrificed animals belonging to control, low, medium, and high dose groups were preserved in 10% formalin solution. The histopathological examination was performed initially for animals belonging to control, low, medium, and high dose groups. There were no significant changes observed in the microscopic examination.

Below mentioned organs of control, low, medium, and high dose group animals were collected and observed under a microscope for histopathological examination (Figure 6-10).

3. DISCUSSION

The examination of the poisonous properties of Metal and Mineral is frequently a preliminary step in screening natural items for pharmacological activity. The determination of LD_{50} is usually one of the first steps in such an examination. The acute toxicity study can provide preliminary information on an agent's mode of toxic action, serve as a foundation for classification and labeling, and aid in the dose determination of novel compounds in animal research. Furthermore, if a large dose (e.g., 2,000 mg/kg) is confirmed to be tolerable, no additional acute testing is required. The treated rats in this study had no adverse effects after 14 days of observation when given a novel herbo-bio-mineral metallic formulation at a dose of 2,000 mg/kg. (Table 2) Neither the weight nor the organs of the rats changed significantly. (Table 4). The ABS was shown to be non-toxic to the hemopoietic system when hematological parameters were compared between the control and treated groups. Furthermore, the majority of biochemical markers remained unchanged. In toxicity studies, hematology analyses also play a major role in evaluating the possible toxic effects induced by the oral treatment of the test material[10-11].

Further, changes in the hematological system of treated rats have a higher predictive value for toxicity in humans compared to animals when data is extrapolated from animal studies[12]. The effect of *ABS* on blood-related functions can be determined by assessing hematological parameters. In both humans and animals, the hemopoietic system is one of the most sensitive targets for hazardous substances and an important indicator of physiological and pathological conditions. The *ABS* showed no significant difference in red blood cell indices, implying that the formulation did not affect red blood cell erythropoiesis, shape, or osmotic fragility. White blood cells are the first line of defense against infectious pathogens, tissue damage, and inflammation. Furthermore, no significant alterations in neutrophils, lymphocytes, or monocytes were identified, implying that the *ABS* did

Table 8. Effect of *ABS* on the weight of rats in the subacute toxicity study.

Days	Control	600 mg/kg	800 mg/kg	1000 mg/kg
0 days	238.30 ± 15.30	264.22 ± 18.30	251.55 ± 17.60	237.74 ± 15.30
7 days	241.56 ± 15.45	265.58 ± 18.44	235.42 ± 17.76	239.44 ± 15.38
14 days	248.20 ± 17.37	266.78 ± 18.46	255.44 ± 16.89	240.25 ± 15.42
21 days	250.48 ± 17.56	268.23 ± 18.36	256.23 ± 17.12	242.48 ± 15.48
28 days	251.50 ± 17.60	268.20 ± 18.39	258.60 ± 17.35	246.50 ± 17.39

Values are expressed as a mean±SEM (n=10 and p<0.05).

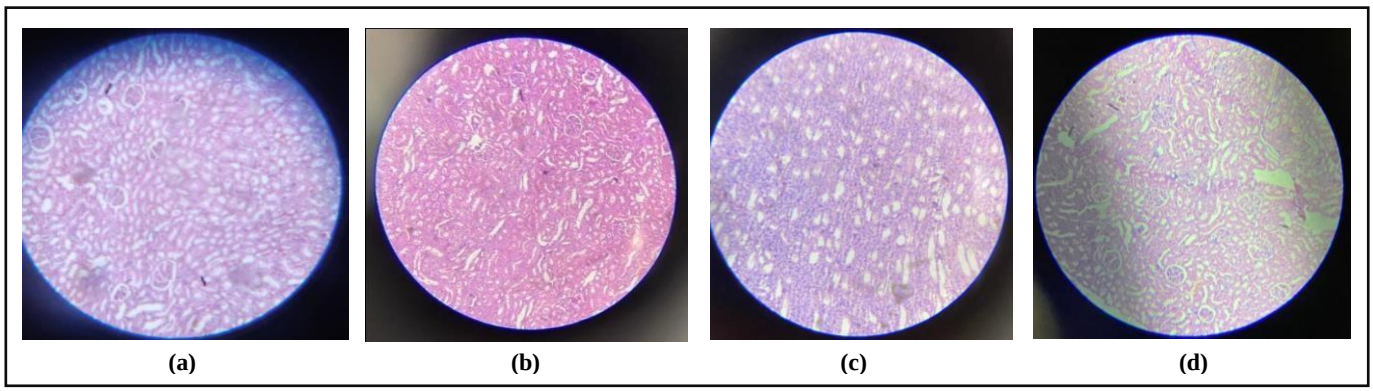

Figure 6. Histopathology of kidney tissue. Magnification (40x) a) Normal cytoarchitecture-control group, b) Not cause any adverse effect on the histoarchitecture-treated with LD *ABS* (600 mg/kg), c) Not cause any adverse effect on the histoarchitecture-MD treated with *ABS* (800 mg/kg). d) Not cause any adverse effect on the histoarchitecture-HD treated with *ABS* (1,000 mg/kg).

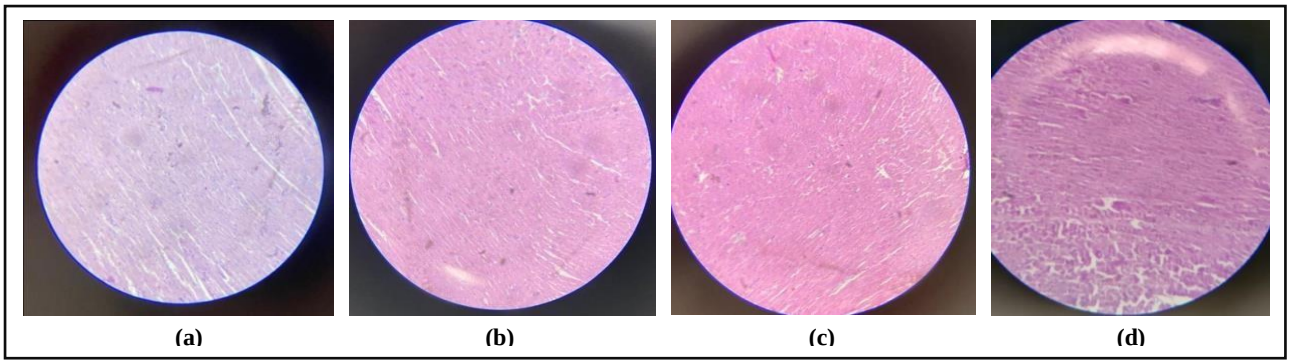

Figure 7. Histopathology of heart tissue. Magnification (40x) a) Normal cytoarchitecture-control group, b) Not cause any adverse effect on the histoarchitecture-treated with LD *ABS* (600 mg/kg), c) Not cause any adverse effect on the histoarchitecture-MD treated with *ABS* (800 mg/kg). d) Not cause any adverse effect on the histoarchitecture-HD treated with *ABS* (1,000 mg/kg).

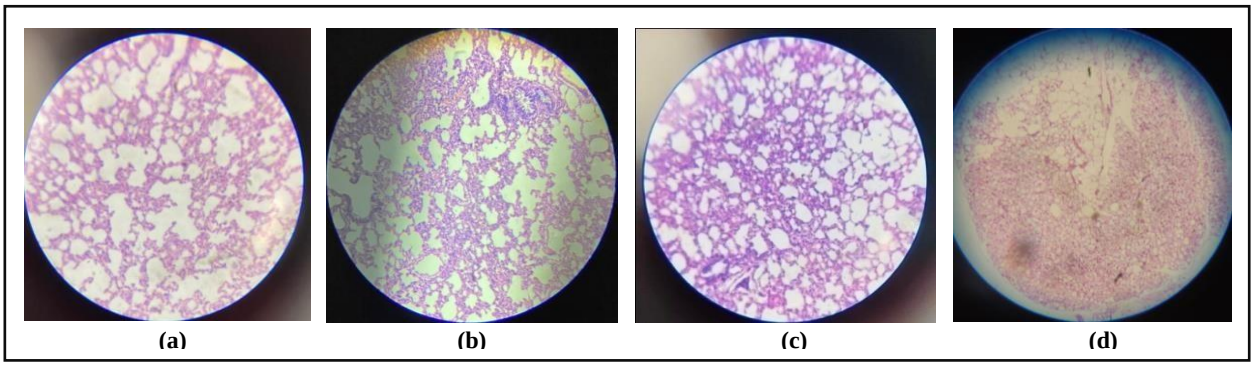

Figure 8. Histopathology of lung tissue. Magnification (40x) a) Normal cytoarchitecture-control group, b) Not cause any adverse effect on the histoarchitecture-treated with LD *ABS* (600 mg/kg), c) Not cause any adverse effect on the histoarchitecture-MD treated with *ABS* (800 mg/kg). d) Not cause any adverse effect on the histoarchitecture-HD treated with *ABS* (1,000 mg/kg).

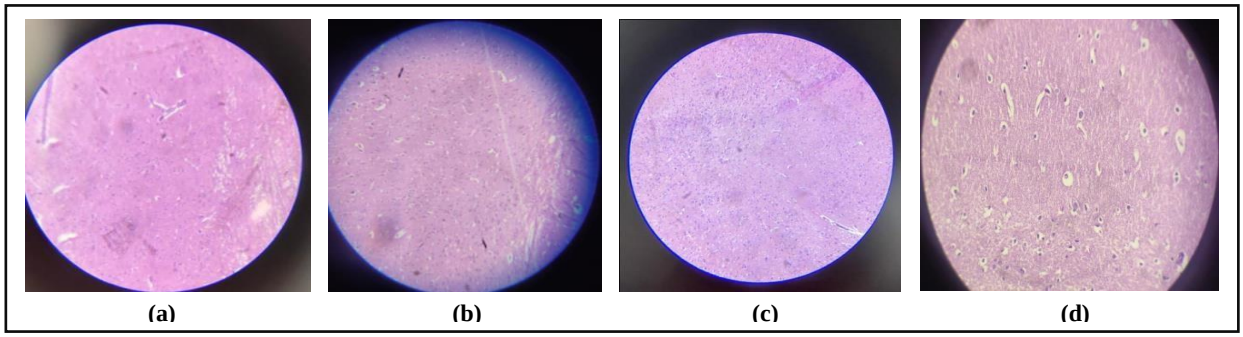

Figure 9. Histopathology of brain tissue. Magnification (40x) a) Normal cytoarchitecture-control group, b) Not cause any adverse effect on the histoarchitecture-treated with LD *ABS* (600 mg/kg), c) Not cause any adverse effect on the histoarchitecture-MD treated with *ABS* (800 mg/kg). d) Not cause any adverse effect on the histoarchitecture-HD treated with *ABS* (1,000 mg/kg).

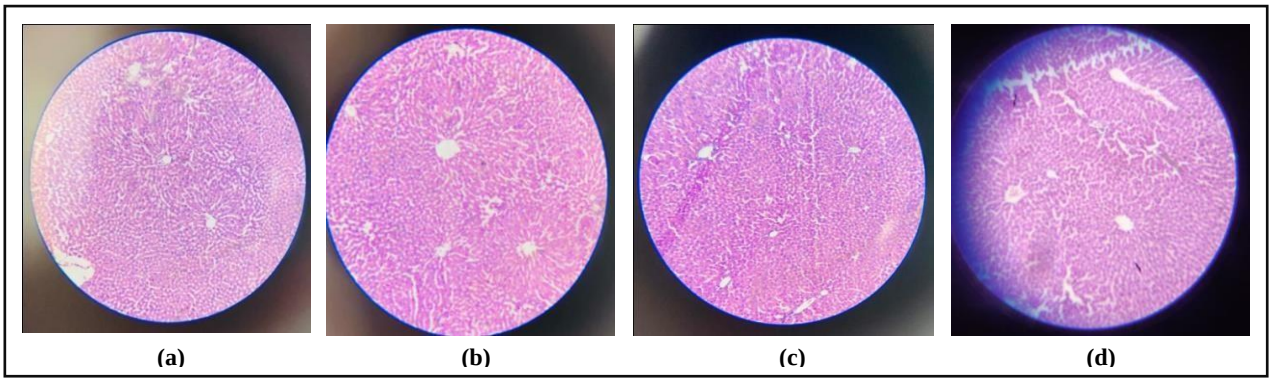

Figure 10. Histopathology of liver tissue. Magnification (40x) a) Normal cytoarchitecture-control group, b) Not cause any adverse effect on the histoarchitecture-LD treated with *ABS* (600 mg/kg), c) Not cause any adverse effect on the histoarchitecture-MD treated with *ABS* (800 mg/kg). d) Not cause any adverse effect on the histoarchitecture-HD treated with *ABS* (1,000 mg/kg).

Table 9. Effect of *ABS* on serum hematological parameters in the subacute toxicity study.

Parameters	Unit	Control	600 mg/kg	800 mg/kg	1000 mg/kg
RBC	10^9/L	6512.67 ± 11.37	6521.68 ± 7.02	6524.66 ± 5.03	6526.67 ± 4.04
WBC	10^{12}/L	6994.67 ± 0.58	6987.67 ± 4.04	6987.33 ± 2.52	6985.21 ± 4.01
Hb %	g/dl	12.41 ± 0.06	12.47 ± 0.02	12.51 ± 0.05	12.50 ± 0.05

Values are expressed as a mean±SEM (n=10, p<0.05). RBC: Total red blood cell count; WBC: Total white blood cell count, Hb %: Haemoglobin.

Table 10. Effect of *ABS* on serum hematological parameters in the subacute toxicity study.

Parameters	Unit	Control	600 mg/kg	800 mg/kg	1000 mg/kg
ALT	**(U/L)**	67.77 ± 0.06	67.82 ± 0.03	67.85 ± 0.06	67.89 ± 0.05
ALP	**(U/L)**	101.48 ± 0.04	101.48 ± 0.07	101.54 ± 0.07	101.56 ± 0.09
Creatinine	**(mg/dL)**	0.26 ± 0.00	0.26 ± 0.00	0.26 ± 0.00	0.26 ± 0.00
Urea	**(mg/dL)**	13.57 ± 0.01	13.57 ± 0.02	13.61 ± 0.02	13.62 ± 0.02

Values are expressed as a mean±SEM (n=10 and p<0.05). (ALP: serum alkaline phosphatase; ALT: serum alanine aminotransferase).

not pose a challenge to the animal's immune systems (Table 9). Levels of ALT, AST, urea, and creatinine, which are reliable predictors of liver and renal function, showed no significant changes (Table 10). The liver, kidney, spleen, heart, and uterus are primary organs, affected by metabolic reactions caused by toxic compounds[13]. In both acute and subacute toxicity studies, macroscopic examination of internal organs in rats of all test groups does not show any changes in color and texture compared to the control group rats during necropsy. By evaluating the results of the acute toxicity study, it is possible to suggest that *Abhrasindoora* has no acute adverse effects at the dose tested and with LD_{50} values of more than 2,000 mg/kg. Therefore, it is possible to suggest that LD_{50} of *ABS* is above 2,000 mg/kg body weight via the oral route. According to the Globally Harmonized System of Classification and Labelling of Chemicals under OECD guideline, 423, *ABS* can be classified into category 5 (LD_{50}>2,000 mg/kg), which was the lowest toxicity class in the classification. As no hazardous effects were observed during the acute toxicity study, a further study was carried out to assess the sub-acute toxicity of *ABS* for 28 days to compile comprehensive toxicological records. Sub-acute studies reveal the dosage regimens, target organ toxicity, and detect obvious adverse effects that may shorten the average lifespan of experimental animals. Hence, *ABS* was tested in rats for 28 days at doses of 600, 800, and 1,000 mg/kg. Body weight fluctuations are critical indicators of an animal's overall health. All the animals showed a normal increase in body weight after 28 days of *ABS* therapy. (Table 10) Loss of body weight is usually the first sign indicating the onset of an adverse effect[14]. The dose, at which body weight loss is by 10% or more, is considered to be a toxic dose, irrespective of whether or not it is accompanied by any other changes[15]. The significant increase in food and water intake is thought to be the cause of the increase in body weight. So, it can be concluded that *ABS* had no adverse effect on the animal's regular metabolism proving 600, 800, and 1,000 mg/kg as safe doses.

To assess any changes in renal and hepatic functioning due to the effect of *ABS*, serum biochemistry was examined. The hepatocellular and secretory activities of the liver are also affected by the change in the level of total protein, albumin, globulin, and total bilirubin. The absence of significant changes in the levels of aspartate aminotransferase (ALT), alanine transaminase (AST), Urea, and Creatinine are good indicators of liver and kidney function, suggests that sub-chronic administration of *ABS* did not affect rat hepatocytes and kidneys, nor on the animal's normal metabolism. The drug did not affect the cytoarchitecture of major organs like the heart, kidney, liver, lung, and brain i. e. histopathological examination of the organ corroborated these conclusions. (Figure 1-10) The results of the present study reiterate the fact that *Bhasmas*, despite their trace heavy metal content, are safe when appropriately manufactured and consumed as per directed instructions. The safety of *Rasoushadhis* was assessed with the help of micronucleus assay and comet assay[16]. The toxic effects of mercury were said to be neutralized in the presence of sulphur[17].

4. CONCLUSION

Present study provides valuable data on acute and subacute toxicity studies of *Abhrasindoora*. Since there were no deaths or signs of toxicity in treated rats during the acute toxicity study, it is possible to suggest that the LD_{50} of *ABS* is greater than 2,000 mg/kg body weight via the oral route. Observations made during the subacute toxicity study suggest that the long-term intake (28 days) of *ABS* at tested dose levels including the therapeutic dose does not induce any toxic effects in treated rats in comparison to control group rats. Thus, it can be concluded that the oral treatment of Mercury-based Traditional Herbo-Bio-Mineral Metallic Formulation (*ABS*) in rats has a wide margin of safety and potential for the development of a novel therapeutic agent for the treatment of Asthma. Before using this dose of *ABS* in human beings, a dose-finding study should be done. A similar method will be applicable.

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