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# Acute and Subacute Toxicity Study of *Lehana Yogas* in Wistar Albino Rats

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## ABSTRACT

*Lehana Yogas* is an Ayurvedic preparation which are known to enhance growth and development by providing sufficient nutrition and promoting health with improving intellect and speech and administered frequently with ghee and honey to children's as immune boosters. In this study we assessed the safety parameters of the *Lehana Yogas* mentioned and its effect on acute and subacute administration in Wistar albino rats. The Wistar strain albino rats were divided into group of five, vehicle group of *Ghrita-Madhu*, *Ghrita-Madhu* with *Suvarna Bhasma*, *Ghrita-Madhu -Suvarna Bhasma* with *Vacha Churna*, *Ghrita-Madhu -Suvarna Bhasma* with *Amalaki Churna*, respectively. The duration of administration was for 28 days. During the course of study, no mortality and no significant behavioral changes were noticed in any group studied. No major alterations were observed in hematology and serum biochemistry reports. Histopathology of vital organs also shown no toxic effects. **Materials and Methods:** Thirty albino rats of either sex were selected and assigned to 5 groups of 6 each. Drugs will be administered for the duration of 28 days and will be assessed for toxicity profile at the end of the study. **Result:** Animals who were administered daily with *Lehana Yogas* for longer duration did not show any mortality. Hence, it indicates safer use of drugs in terms acute and subacute level of administration. **Discussion:** It is concluded that the administration of *Lehana Yogas* is safe and non-toxic at the tested dose levels**.**

**Key words:** *Lehana, Suvarna Bhasma, Ghrita, Madhu, Vacha Churna, Amalaki Churna.*

## INTRODUCTION

*Kashyapa Samhita* emphasizes the importance of *Lehana* by stating that happiness and sorrow of child are dependent on *Lehana*.[1] *Lehana* is a simple remedy for common diseases in curative aspect. The preparation also imparts sufficient potency for the prevention of different diseases. Moreover, the drugs are easily accessible as well as administrable.[2] It has been aimed to stimulate and strengthen body's immunity. Hence, multifactorial, multidimensional approach has been explained by *Kashyapa* to bring all round growth and development of the child with the help of *Lehana*. Besides providing high calorific diet supplementation to the growing child, *Lehana* also takes care of physical, mental and immunological facets of the child; so that, he can grow as healthy adult.[3] There are many *Yogas* explained under *Lehana* by different acharyas. *Acharya Charaka* in his explanation of *Jatakarma Samskara*, explains about feeding of *Madhu & Ghrita* to the neonate.[4] *Acharya Kashyapa* explained *Suvarnaprashana*, where gold is rubbed and administered along with *Madhu* and *Ghrita* to the baby for a period of one month to six months to impart Intelligence and disease-free state.[5] *Swarna Vacha* explained by *Vagbhata*, where Gold and *Vacha Choorna* mixed with *Madhu* and *Ghrita* administered to the neonate will impart *Medhya, Rakshoghna*

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ISSN: 2456-3110 ORIGINAL ARTICLE August 2022

(Protective) and *Vagvivardhaka* (improves speech).[6] *Astanga Hridaya*, while discussing about care of the newborn, emphasizes on administering different *Prashana Yogas*. One such *Yoga* which is easy to administer is *Swarna Amalaka Yoga*, where Gold and *Amalaki Choorna* is administered to neonate along with *Madhu* and *Ghrita*.[7] When these *Yogas* are used in infants, may lead to optimum level of growth and development owing to stimulation of immune system and development of nervous system. Even though we have many references on *Lehana Yogas* but there is no reference regarding its safety in classics. Therefore, in this study we evaluated safety of this popular *Ayurvedic* incinerated gold preparation in experimental animals.

## Aim and Objectives

To determine the acute and sub-acute toxicity of *Lehana Yogas*

## Materials and Methods

*Suvarna Bhasma* was procured from SDM Ayurveda Pharmacy Udupi, Karnataka and Healthy Wistar albino rats of either sex weighing about 80-150g from animal house attached to SDM Centre for Research in *Ayurveda* and Allied Sciences Udupi. All animals were housed under standard conditions at an ambient temperature 22±3°C, relative humidity 45-55% and 12/12 h light/dark cycle. All animals were acclimatized in the laboratory about a week before commencement of the study. They had free access to standard pellet diet (Amruta brand animal feed, supplied from Bangalore) and fresh water *ad libitum*. Floor bed was changed every day, to maintain hygienic condition. Different groups of rats were placed in polypropylene cages with appropriate space for clear behavioral observations without any interference. The experimental protocol has been approved by the Institutional Animal Ethical Committee (IAEC), SDM College of Ayurveda, Udupi.

### Grouping

Thirty rats were divided into 5 groups of six each. Gr I - on normal diet and water *ad libitum* was considered as control, and Gr II - was administered with plain honey and ghee; Gr III - *Suvarnaprashana*; Gr IV - *Suvarnaprashana* with *Vacha Churna*; Gr V - *Suvarnaprashana* with *Amalaki Churna*, all along with normal standard animal diet and water *ad libitum*.

*Suvarna Bhasma* dose for this experimental study was selected by considering maximum recommended classical dose of *Swarna Bhasma* (15.62 to 31.25 mg).[8] Thus, *Swarna Bhasma* was calculated by extrapolating the human therapeutic dose to animal dose by using the standard dose calculation procedure based on the body surface area ratio.[9]

### Calculation formula

Rat dose = Human dose x Surface area factor 0.018 x 5, it gives per kg body weight dose

At the end of the study period, animals were kept overnight on fasting and blood was drawn through retro-orbital region. Blood samples were collected for hematological and serological examination. Biochemical and hematological parameters estimations was carried out at SDM Centre for Research in Ayurveda and Allied Sciences Udupi, India. Histological studies was carried out at Department of Pathology, Manipal. Biochemical parameter was estimated using Cobas c 111 analyzer using standard kit and hematological studies were estimated in an auto cell counter.

### Statistical analysis

All the values were expressed as Mean $\pm$ SEM and data were analysed by applying ANOVA with dunnet's multiple 't' test was used as post hoc test. Graph pad3 software was used for this purpose.

## Observations and Results

Overall, all the experimental animals were active throughout the experimental study. No evidence of toxicity or mortality was observed. Effect on food intake and water consumption and fecal and urine output remain unaffected during the study. No significant behavioral changes were observed in any of the groups.

### Effects on biochemical parameters

The biochemical parameters data related to the study are presented in Table 1

ISSN: 2456-3110 ORIGINAL ARTICLE August 2022

**Table 1: Showing the data related to biochemical parameters of experimental animals**

| Parameters | Group1 | Group2 | Group3 | Group4 | Group5 |
|---|---|---|---|---|---|
| Glucose mg/dL | 75.66±5.90 | 124.16±4.08** | 105±5.61** | 150.66±6.52** | 136.5±8.007** |
| Serum cholesterol mg/dL | 66.66±12.92 | 73.5±5.40 | 61.83±8.94 | 62.16±8.65 | 110.83±17.40* |
| Serum triglyceride mg/dL | 104.83±15.58 | 131.66±21.08 | 72.4±8.95 | 136.66±6.68 | 81.5±8.43 |
| SGOT IU/L | 142.66±7.06 | 94.66±13.72** | 119.83±7.51* | 111±3.08* | 106.16±5.82* |
| SGPT IU/L | 74.5±8.93 | 51.66±1.72* | 45.33±3.46** | 46.16±2.73** | 58.5±5.59 |
| Serum total protein g/dL | 8±0.57 | 5.8±0.48** | 5.75±0.25** | 4.45±0.19** | 5.76±0.38** |
| Serum albumin g/dL | 3.58±0.26 | 3.93±0.37 | 3.05±0.20 | 3.36±0.40 | 3.4±0.31 |
| Serum creatinine mg/dL | 0.66±0.03 | 0.58±0.02 | 0.50±0.03 | 0.6±0.07 | 0.70±0.07 |
| Total bilirubin mg/dL | 0.80±0.05 | 0.79±0.03 | 0.81±0.02 | 0.80±0.05 | 0.69±0.01 |
| Direct bilirubin mg/dL | 0.13±0.03 | 0.79±0.03** | 0.81±0.02** | 0.80±0.05** | 0.69±0.01** |
| Serum urea mg/dL | 43.83±2.75 | 38.66±2.02 | 111.4±74.93 | 37.5±1.72 | 35.33±3.49 |

### Effects on hematological parameters

The hematological parameters data related to the study are presented in Table 2

**Table 2: Showing the data related to hematological parameters of experimental animals**

| Parameters | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 |
|---|---|---|---|---|---|
| Hb g%/dL | 15.98±0.28 | 13.48±0.24** | 12.26±0.71** | 13.48±0.18** | 13.16±0.13** |
| Total count Cells/cumm | 11311.66±1163.6 | 10112±1520.9 | 10560±1410.2 | 9503.33±1023.1 | 10790±1212.7 |
| Neutrophil count Cells/cumm | 27.85±2.59 | 23.4±3.52 | 21.36±5.26 | 18.62±3.06 | 20.38±2.67 |
| Lymphocyte count Cells/cumm) | 66.41±3.61 | 72.86±4.26 | 72.38±3.41 | 78.1±3.33 | 76.58±3.05 |
| Monocyte count Cells/cumm | 4.98±1.33 | 3.1±0.79 | 3.9±0.59 | 2.8±0.70 | 2.66±0.70 |
| Eosinophils Cells/cumm | 0.43±0.17 | 0.21±0.09 | 0.08±0.03 | 0.10±0.04 | 0.24±0.09 |
| Basophils Cells/cumm | 0.25±0.03 | 0.24±0.02 | 0.24±0.06 | 0.22±0.05 | 0.25±0.03 |
| RBC Millions/cumm | 7.66±0.10 | 6.66±0.14 | 6.08±0.41 | 117.78±111.04 | 5.88±0.98 |
| PCV %cumm | 44.08±0.81 | 39.2±0.52 | 34.51±2.34** | 36.56±1.73** | 37.51±0.33* |
| MCV | 57.56±0.49 | 59.06±1.32 | 56.78±0.36 | 56.78±0.34 | 54.53±0.63* |

ISSN: 2456-3110 ORIGINAL ARTICLE August 2022

| | | | | | |
|---|---|---|---|---|---|
| MCH | 20.83±0.13 | 20.24±0.24 | 20.31±0.34 | 20.01±0.07* | 19.13±0.19** |
| MCHC | 36.26±0.13 | 34.28±0.42** | 35.78±0.54 | 35.25±0.11 | 35.06±0.14* |
| RDWCV | 23.03±0.42 | 21.36±0.34 | 21.3±1.28 | 20.86±0.12 | 23.28±0.69 |
| RDWSD | 53.66±1.39 | 51.02±1.58 | 49.08±3.11 | 47.63±0.58 | 51.33±1.21 |
| Platelet count lakhs/cumm | 6.01±0.33 | 5.52±1.13 | 7.92±1.47 | 6.42±0.32 | 8.19±0.35 |

### Histopathology of organs

All slides show liver tissue with lobular arrangement. Each lobule consists of a central vein and portal triads along the periphery of lobules. Numerous sinusoids pass radially from central vein and the spaces between the sinusoids contain liver cells. Histological changes like degeneration, necrosis is not seen in any slides. Compared with G1, chronic inflammatory infiltration seen in G4 and G5 groups. (Fig. 1,2,3,4,5,6 exhibit the histopathological sections of brain, heart, kidney, liver, lungs and spleen).

### Fig. 1: Effect on Brain.

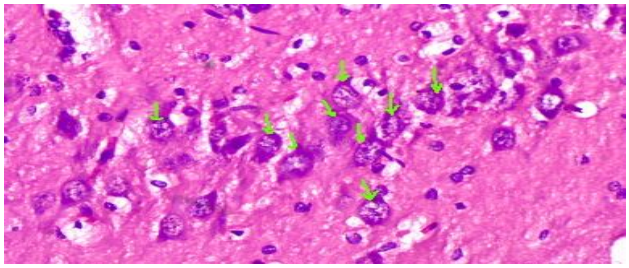

**Fig. 1a: Effect of Control on Brain.**

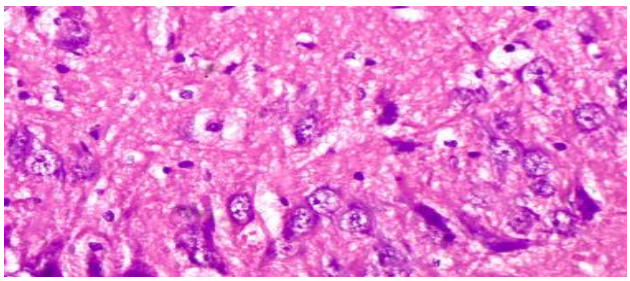

**Fig. 1b: Effect of honey and ghee on Brain.**

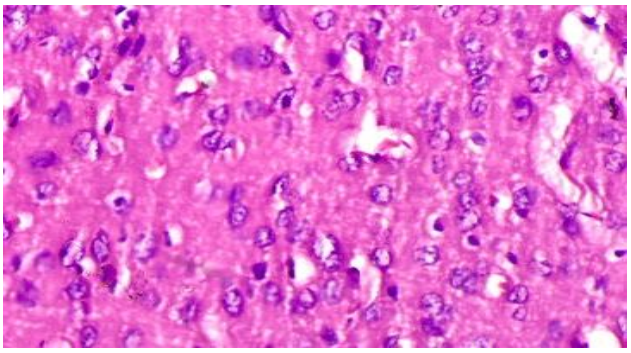

**Fig. 1c: Effect of Suvarna Bhasma on Brain.**

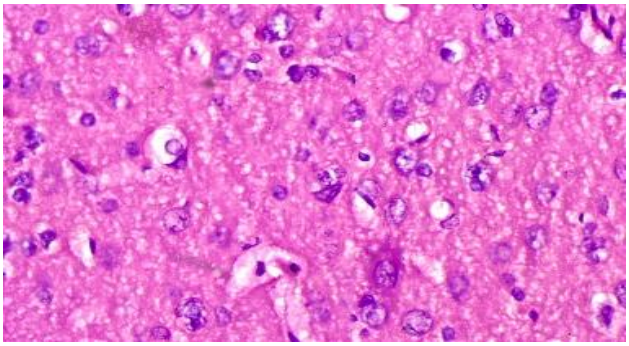

**Fig. 1d: Effect of Suvarna Bhasma with Vacha Churna on Brain.**

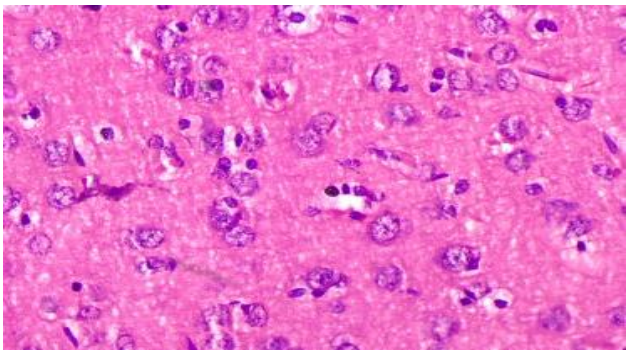

**Fig. 1e: Effect of Suvarna Bhasma with Amalaka Churna on Brain**

### Fig. 2: Effect on Heart.

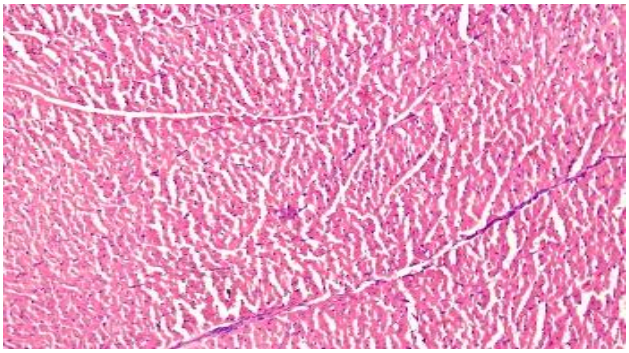

**Fig. 2a: Effect of Control on Heart.**

ISSN: 2456-3110 ORIGINAL ARTICLE August 2022

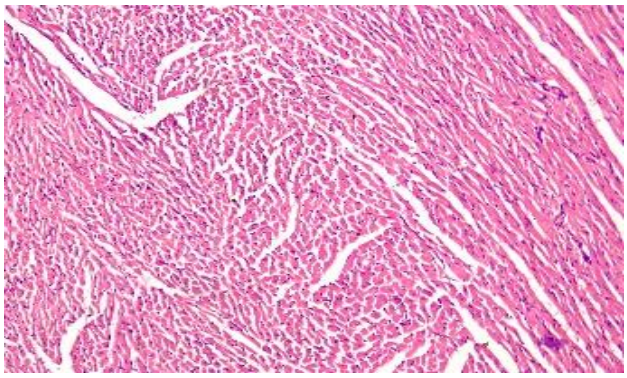

Fig. 2b: Effect of honey and ghee on Heart.

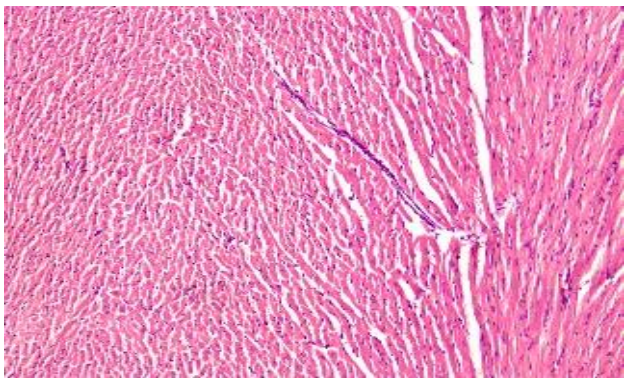

Fig. 2c: Effect of Suvarna Bhasma on Heart.

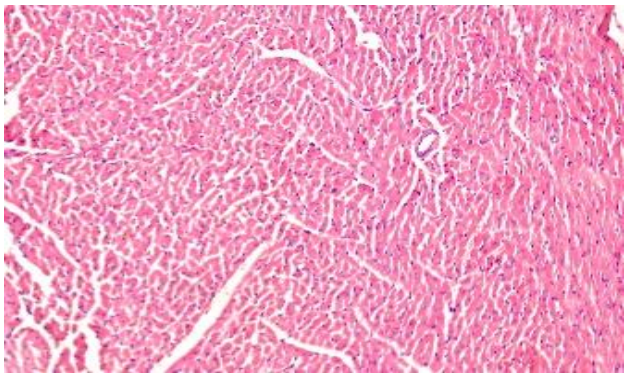

Fig. 2d: Effect of Suvarna Bhasma with Vacha Churna on Heart.

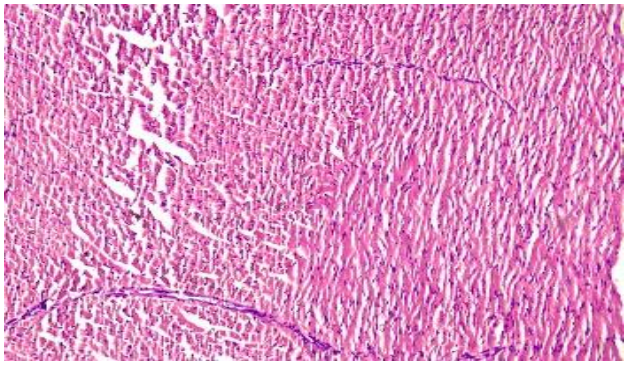

Fig. 2e: Effect of Suvarna Bhasma with Amalaka Churna on Heart.

Fig. 3: Effect on Kidney.

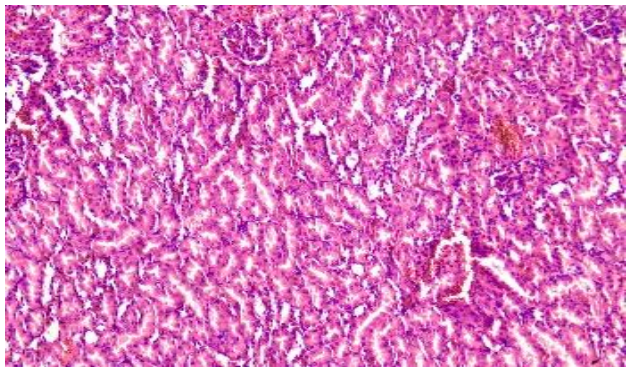

Fig. 3a: Effect of Control on Kidney.

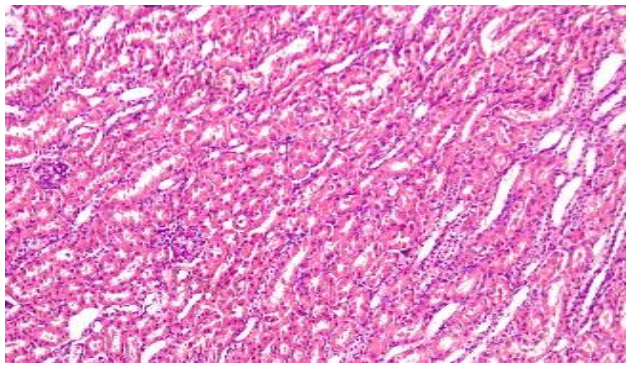

Fig. 3b: Effect of honey and ghee on Kidney.

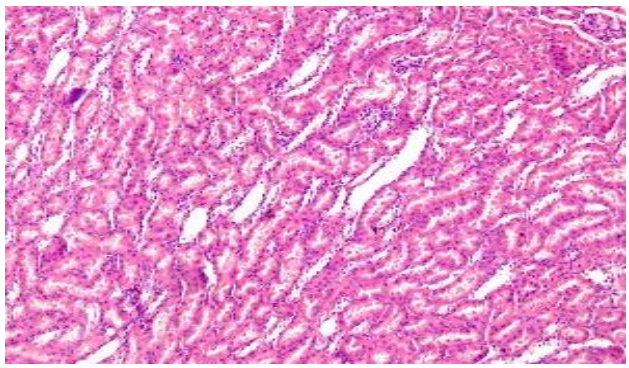

Fig. 3c: Effect of Suvarna Bhasma on Kidney.

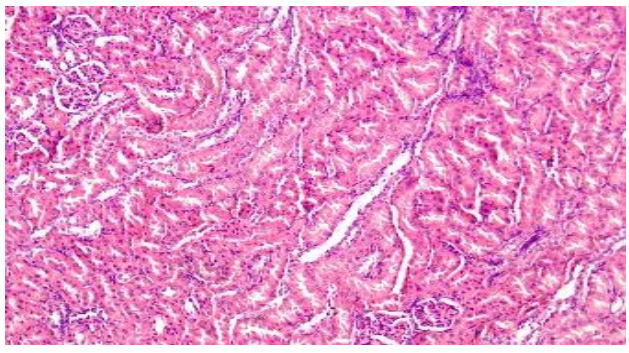

Fig. 3d: Effect of Suvarna Bhasma with Vacha Churna on Kidney.

ISSN: 2456-3110 ORIGINAL ARTICLE August 2022

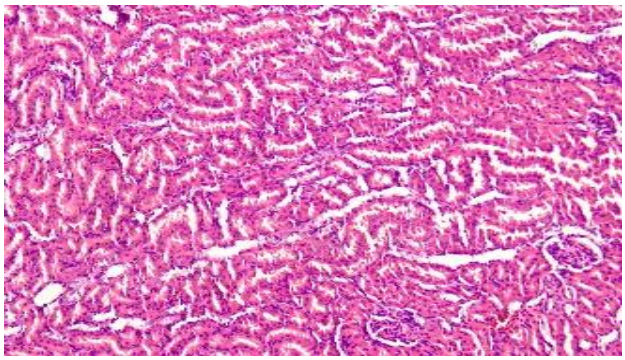

Fig. 3e: Effect of Suvarna Bhasma with Amalaka Churna on Kidney.

## Fig. 4: Effect on Liver.

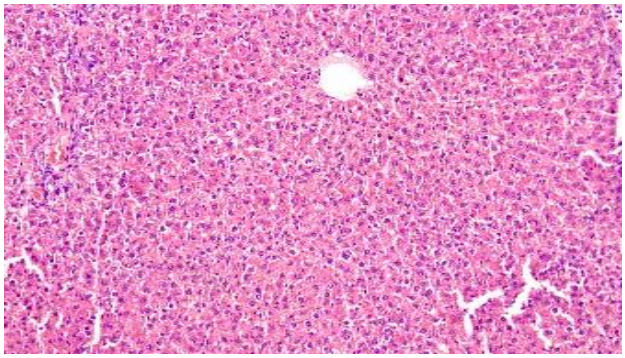

Fig. 4a: Effect of Control on Liver.

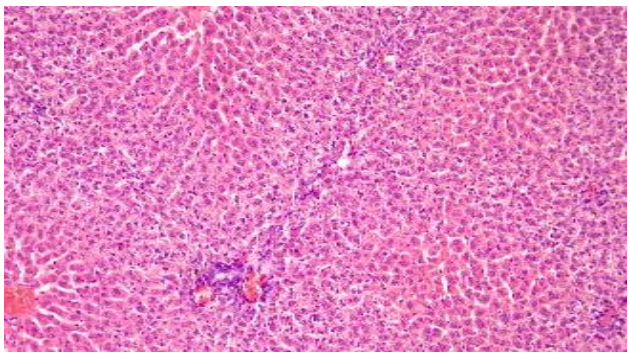

Fig. 4b: Effect of honey and ghee on Liver.

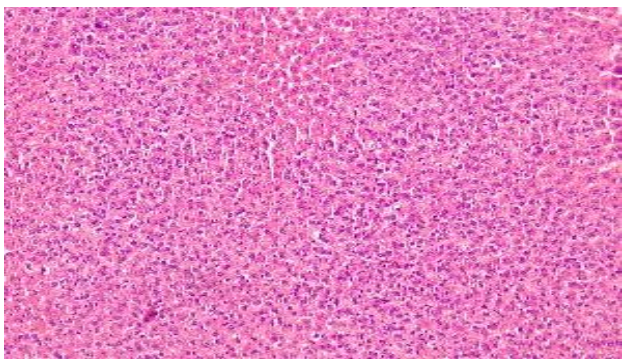

Fig. 4c: Effect of Suvarna Bhasma on Liver.

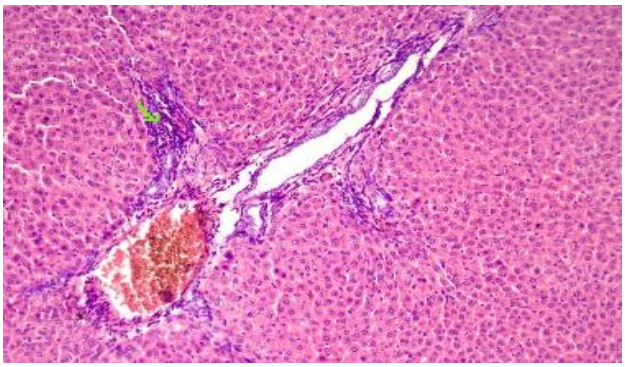

Fig. 4d: Effect of Suvarna Bhasma with Vacha Churna on Liver.

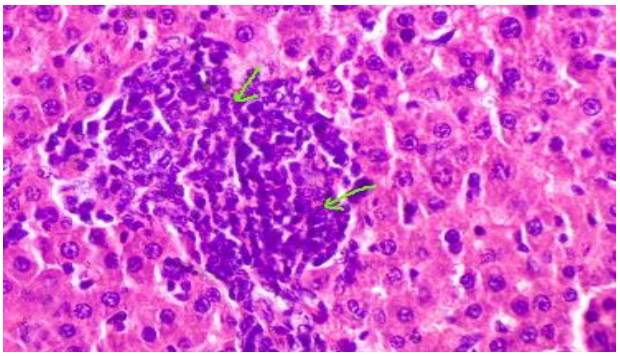

Fig. 4e: Effect of Suvarna Bhasma with Amalaka Churna on Liver.

## Fig. 5: Effect on Lungs.

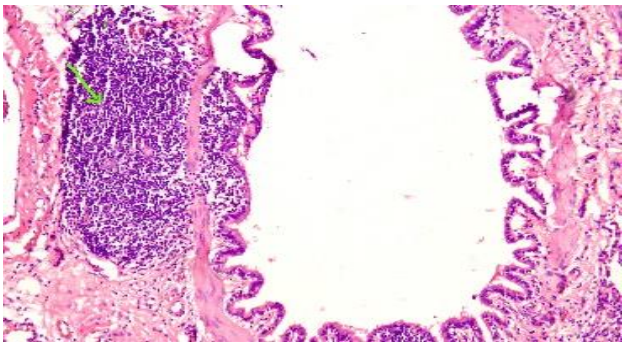

Fig. 5a: Effect of Control on Lungs.

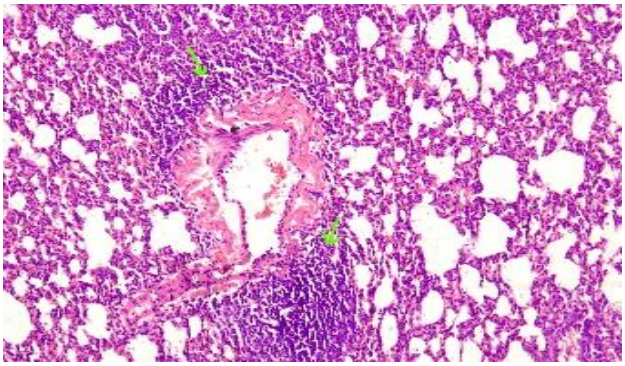

Fig. 5b: Effect of honey and ghee on Lungs.

ISSN: 2456-3110 ORIGINAL ARTICLE August 2022

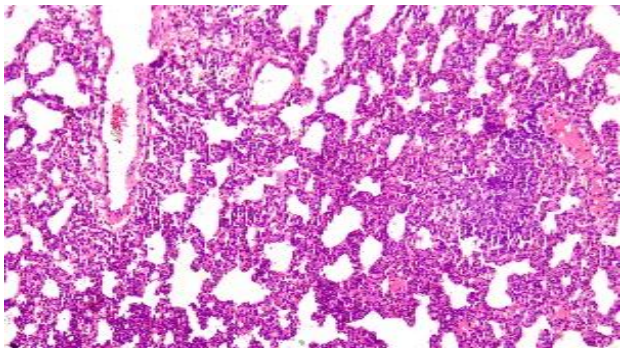

Fig. 5c: Effect of Suvarna Bhasma on Lungs.

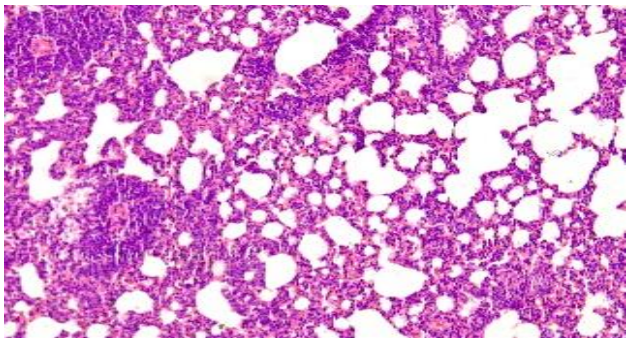

Fig. 5d: Effect of Suvarna Bhasma with Vacha Churna on Lungs.

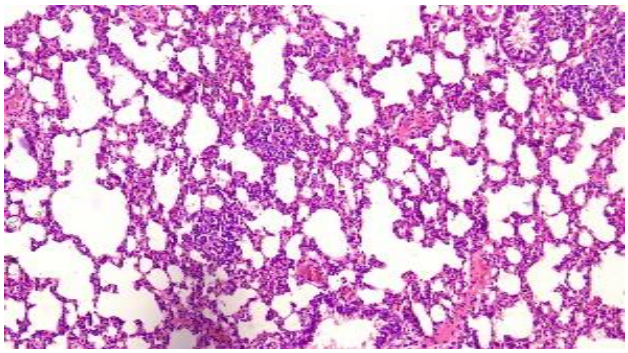

Fig. 5e: Effect of Suvarna Bhasma with Amalaka Churna on Lungs.

**Fig. 6: Effect on Spleen.**

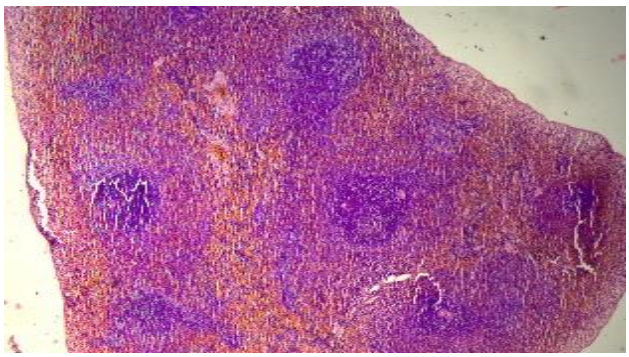

Fig. 6a: Effect of Control on Spleen.

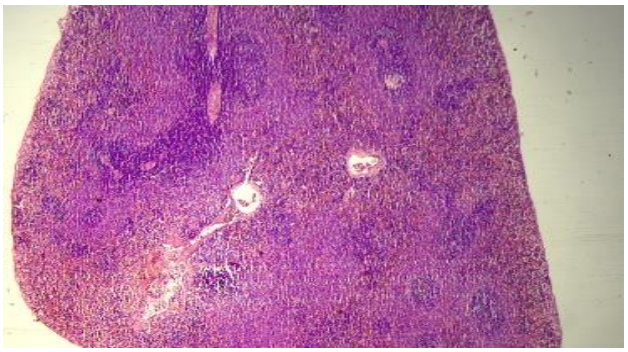

Fig. 6b: Effect of honey and ghee on Spleen.

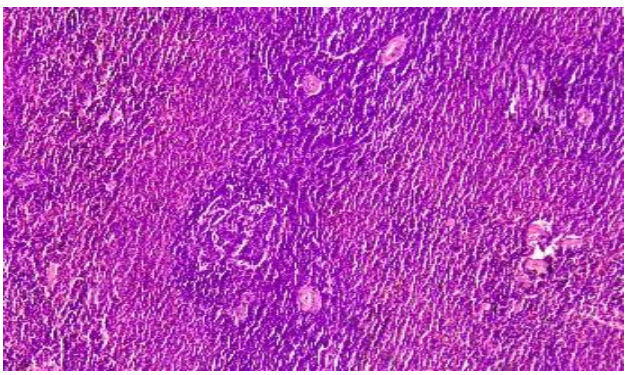

Fig. 6c: Effect of Suvarna Bhasma on Spleen.

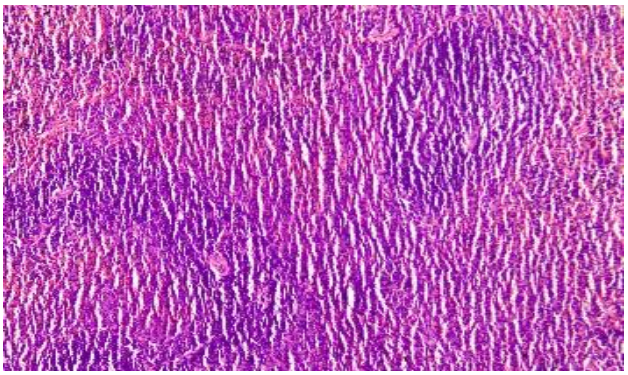

Fig. 6d: Effect of Suvarna Bhasma with Vacha Churna on Spleen.

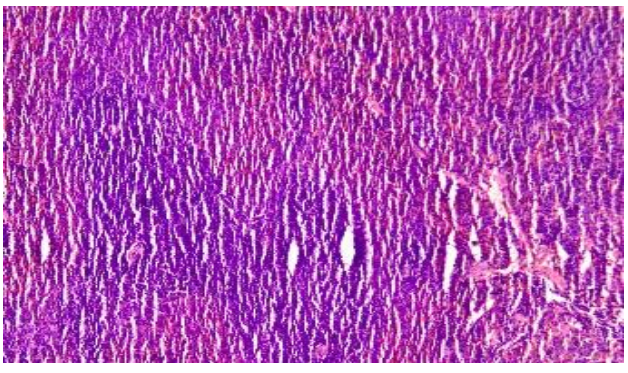

Fig. 6e: Effect of Suvarna Bhasma with Amalaka Churna on Spleen.

ISSN: 2456-3110 ORIGINAL ARTICLE August 2022

## DISCUSSION

In this study, from the point of toxicity, the maximum clinical *Swarna Bhasma* dose was converted to animal dose and was administered by considering the weight of individual rats. Impaired food and water intake is one of the important signs of toxicity.[10] However, in this study it was observed that food intake and water intake were unaffected. The passage of fecal material was normal. Weight gain was observed in all the animals. This is suggestive of the test drug is not causing any serious organ damage or any serious changes in physiological function.

Blood glucose levels was significantly increased in all the above four groups. It indicated that there is decrease of activity or damage to beta cells of Islets of Langerhans or it also implies that it will decrease the uptake of sugar in peripheral tissues. Also indicates that sweetness of honey has contributed to rising of glucose level in all administered groups, but cholesterol and triglycerides levels are not changed. There is significant decrease in SGOT count of group 2, 4 and 5. This indicates that there is loss of synthetic function of the liver in sub-acute administration of drugs. There is non-significant decrease in group 3. There is significant decrease in SGPT count of group 2, 3 and 4. This indicates that there is loss of synthetic function of the liver in sub-acute administration of drugs. There is non-significant decrease in group 5. There is significant decrease in Total protein count of all the above four groups, this decreased level of Total protein along with significant decrease in SGOT and SGPT clearly indicates that liver has lost its synthetic function. Liver is the site of albumin synthesis and also possibly of some of α- and β- globulins. There is non-significant decrease in Albumin count of group 3, 4 and 5 and non-significant increase in group 2. There is non-significant decrease in Total bilirubin count of group 2, 4 and 5 and non-significant increase in group 3. Elevated levels of direct bilirubin may indicate liver damage or liver disease. Higher than the normal levels of direct bilirubin may indicate that the liver isn't clearing bilirubin properly. There is significant increase in Direct bilirubin count of all the above four groups. It is due to significant decrease seen in SGOT, SGPT and total proteins. However histopathological report shows no changes like degeneration, necrosis is not seen in any slides but when compared to G1, chronic inflammatory infiltration seen in G4 and G5 groups. Urea helps to monitor problems that affect the kidney. In the present study, there is non-significant decrease in Urea count of group 2, 4 and 5 and non-significant increase in group 3. This shows that there is no association to impairment of kidney functions by administration of these drugs in sub-acute form. Creatinine is an important parameter for the assessment of functioning of kidney; increased creatinine level is a sign of abnormal kidney function or indication of nephrons damage in kidney. There is non-significant decrease in Creatinine count of group 2, 3 and 4 and non-significant increase in group 5. This shows that there is no association to impairment of kidney functions by administration of these drugs in sub-acute form.

Haemoglobin is the principal molecule responsible for the transport of both oxygen and carbon dioxide in blood. There is significant decrease in hemoglobin count of all the above four groups. This can be due to increased destruction of cells in circulation or by suppressing the bone marrow activity. Significant decrease in hemoglobin group can also indicate anemia. Maybe due to long term usage. There is non-significant decrease in RBC count of group 2, 3 and 5 and non-significant increase in group 4. Here it suggests that in sub-acute administration of these drugs, it will destruct the red blood cells. The platelet count estimated by the impedance method of analyzer counts fraction of each cell leading to over estimation. Also, the sub-acute administration of drugs might have led to destruction of islets of Langerhans in pancreas or due to increased uptake. In the present study, there is non-significant decrease in platelet count of group 2 and non-significant increase in group 3, 4 and 5. There is non-significant decrease in total count of all the above four groups. This also can be understood as a result of increased destruction of cells in circulation or due to suppression the bone marrow activity. There is non-significant decrease in neutrophil count of all the above four groups. There is non-significant increase in

ISSN: 2456-3110 ORIGINAL ARTICLE August 2022

lymphocyte count of all the above four groups. This suggests that it is not the absolute increase but it's the relative increase. There is non-significant decrease in monocyte count of all the above four groups. There is non-significant decrease in eosinophils count of all the above four groups. There is non-significant decrease in basophils count of all the above four groups. There is non-significant decrease in PCV count of group 2 and significant decrease in group 3, 4 and 5. There is non-significant increase in MCV count in group 2 and non-significant decrease in group 3, 4 and significant decrease in group 5. There is non-significant decrease in MCH count of group 2, 3. In group 4 and 5 significant decrease in observed. There is significant decrease in MCHC count of group 2. In group 3 and 4 non-significant decrease is observed and significant decrease in group 5 is observed. There is non-significant decrease in RDW-CV count of group 2,3,4. In group 5 non-significant increase in observed. There is non-significant decrease in RDW-SD count of all the above four groups.

No remarkable observations were seen in histopathological report of brain, lungs, heart, liver, kidney and spleen in the experimental animals of *Suvarna Prashana* group. In brain, very mild histological changes seen in brain of G2, G3, G4 and G5 group. Compared with G1 group, G4-2 shows very mild chronic inflammatory infiltration in cerebrum. In lungs, all the slides show severe infiltration of acute and chronic inflammatory cells in interstitial space, around the blood vessels and bronchioles. Widening of interstitial space due to inflammatory infiltration seen. In spleen, Compared with G1, apoptotic changes in lymphocytes (tingible body macrophages) and extramedullary hematopoiesis seen in G3, G4 and G5 groups. Few slides of G4 and G5 also shows expanded red pulp with mild reduction in white pulp. In heart, none of the slides show necrosis. Compared with G1, mild chronic inflammatory infiltration seen in 1 slide of G4 and G5 group. In kidney, no histological changes like inflammatory infiltrate, necrosis, and degeneration seen in any of the groups. In Liver, Histological changes like degeneration, necrosis is not seen in any slides. Compared with G1, chronic inflammatory infiltration seen in G4 and G5 groups.

## Conclusion

Animals that were administered daily with *Suvarna Prashana* for longer duration did not show any mortality. Hence, it indicates safer use of drugs in terms acute and subacute level of administration. Increase in body weight was also noted. This implies the drug is well tolerated and is not having any effect on the vital organs and its physiological processes. Histopathology report showed mild to moderate degenerative changes in all the groups in terms of toxicity report. All the groups showed more protective action against organs of kidney and heart.

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**ISSN: 2456-3110** **ORIGINAL ARTICLE** August 2022

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