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CONCEPT OF AMRITIKARANA AND ITS IMPORTANCE WITH SPECIAL REFERENCE TO TAMRA (COPPER)

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ABSTRACT
"Amritikarana" is a special procedure followed to remove the shista doshas (remained toxins) from the mrutha loha (Bhasma) even after shodhana and Maranadi procedures. To assess the importance of Amritikarana process, Tamra Bhasma was prepared and subjected for Amritikarana process. Both samples i.e. before and after Amritikarana process were subjected for Physico- chemical analysis and toxicity study. The results obtained strongly suggest that Amritikarana process significantly reduces toxicity and improves the quality of Bhasma.
Keywords: Amritikarana, Tamra Bhasma, Physico-chemical Analysis, Toxicity Study.

INTRODUCTION

Amritikarana literally means 'changing in to Nector' In most of the Rasagranthas the definition of Amritikarana is explained as "Amritikarana" is a special procedure followed to remove the shista doshas (remained toxins) from the mrutha loha (Bhasma) even after shodhana and Maranadi procedures. In Rasashastra raw metals and minerals are processed by following shodhana etc procedures to convert them in to body absorbable form and to remove their toxicity. Amongst these procedures Amritikarana play very important role in removing toxins from the Bhasma, which results in reduction of toxicity of metals and minerals. Amritikarana procedure is followed mainly for Abhraka, Swarna makshika, Loha and Tamra. In this article the scientific study of Amritikarana process of Tamra is included. Tamra is considered as a toxic material and possess eight toxic properties which are known as Asta doshas of Tamra, but that same Tamra if processed properly by following shodanadi procedures considered as very useful and used to treat may more disorders. To convert Tamra from toxic metal to non toxic Bhasma form, series of different procedures were followed, among these Amritikarana play a very important role, that's why to assess the importance of Amritikarana process, Tamra Bhasma was prepared and subjected for Amritikarana process and both samples i.e., before and after Amritikarana process were subjected for Physico- chemical analysis, elemental analysis, particle size analysis, structural cell shape study and Toxicity studies.

MATERIALS AND METHODS

Materials

For Tamra Bhasma preparation and Amritikarana

a. Two samples of Tamra Bhasma were prepared (sample-I before Amritikarana and Sample-II after Amritikarana) for Physico- chemical analysis and Toxicity study at A.V.S. Ayurveda Mahavidyalaya College, Pharmacy and required materials were collected from same pharmacy and Herbal garden.

a. Albino rats - were procured from A.V.S. Ayurveda Mahavidyalaya Central Animal house

b. Dimethyl carboxy cellulose Solution - For suspension preparation.

Methods

For Tamra Bhasma preparation and Amritikarana

a. Tamra Samanya Shodhana was made by following the reference of- R.R.S. 5/13

b. Tamra Visesha Shodhana was made by following the reference of- R.R.S. 5/30

c. Tamra Bhasma (Sample I) was prepared by following the reference of- R.R.S. 5/52

d. Tamra Bhasma Amritikarana (Sample II) was made by the following the reference of Rasamritam 2/45, 46

Toxicity Study

Staircase Method or Up and Down method was followed

Inclusive and Exclusive criteria

Inclusive criteria

- Adult healthy albino rats.
- Rat weighing 180-200gms
- Albino rats between 90-120 days were included.

Exclusive criteria

- Unhealthy albino rats
- Weight below 150gms and above 200gms
- Albino rats of below 90 days and above 120 days were excluded.

OBSERVATION AND RESULTS

Tamra Bhasma Preparation and Amritikarana

Pharmaceutical study

It is explained in classical reference that 3 gaja putas are required to prepare tamra bhasma. After 3rd gajaputa, prepared tamra bhasma was subjected for bhasma siddhi pareeksha. It was found negative. Then again 2 gaja putas were given following same procedure, after 5th gaja puta the bhasma siddhi tests were positive. (Table 1, 2)

Visesha shodhana of Tamra

Wt of Vishesha Shodita Tamra churna - 1980 gms
Loss in weight - 20 gms
Color - Blackish

Tamra Bhasma Amritikarana (Tamra Bhasma Sample-II)
Initial weight – 520gms
Wt after puta – 350gms
Loss in weight – 170gms
Color – Brownish
Physico - Chemical Analysis
Table 3, 4 and 5
Estimation of copper volumetrically from the given Bhasma (Qualitative analysis)
The % of soluble copper in Tamra Bhasma Sample I = 26.16%
The % of soluble copper in Tamra Bhasma Sample II = 25.92%
Quantitative Analysis
The Elemental analysis and particle size assessment were done in IIT, Pawai, Using ICP/AES
Reports are given in Table 6.
Particle Size Assessment
The mean particle size value of Tamra Bhasma sample I was 2.32 µm.
The mean particle size value of Tamra Bhasma sample II was 2.03 µm.
The cell type in case of RT was Hexagonal with cell volume 390.984 A, in case of VST it was Monoclinic with cell volume 390.984 A, in case of Tamra Bhasma sample I it was Triclinic with cell volume 471.110 A, and in case of Tamra Bhasma sample II it was Triclinic with cell volume 445.638A. (Table 7)
The X-RD analysis
The X-RD analysis was done in Regional Research Laboratory Bhuaneshwara.
The final unit cell parameters for all the five samples have been given in Table 8
Acute Toxicity Study
Two healthy male albino rats were taken, weighing about 200gms, kept in separate cages, which were fasted over night. Both rats were administered with 1 ml of 1% DMC solution + 750 mg of Tamra Bhasma sample I (Tamra Bhasma before Amritikarana), through oral route, both rats were died after 6 minutes of feeding (one rat at 6th min. and another at 7th min.) so 750mg/200gms of albino rat body weight is a lethal dose. To assess the maximum tolerated dose the dose was reduced, to calculate the next dose the staircase method was followed (decreasing the dose by factor 0.7 i.e.,750mg × 0.7 = 525 mg is the next dose).
IInd dose 525mg/200 gms of albino rat body wt was given to another two albino rats following the same procedure, both rats were died after 6 minutes, this dose was also lethal, again following the same rule IIIrd dose of 367.5 mg was given to two albino rats, again both rats were died after 46 minutes, again dose was reduced to 257.25 mg following stair case rule, and administered to two albino rats, one rat was died after 1hour 41 minutes and one rat was survived, to know more accurate maximum tolerated dose of Tamra Bhasma sample I, again two rats were given 250mg dose and two were with 260 mg , here rats administered with 260mg dose were died and with 250mg were survived.
Following the same procedure the maximum tolerated and minimum lethal doses of Tamra Bhasma sample II was calculated.
Acute Toxicity Study (Table 9, 10)
Short-Term Chronic (Sub Acute) Toxicity
Test in which animals are dosed daily, the animals are maintained at the maximum tolerated dose for a period of 3 weeks to allow development of any pathological changes and then killed and subjected to full pathological and histological examinations. (Table 11)
Study Group
Group I – Tamra Bhasma sample I (Tamra Bhasma before Amritikarana), 250mg/200gms body weight of albino rat (MTD) with 1 ml of 1% D.M.C solution was administered.
Group III – Tamra Bhasma sample II (Tamra Bhasma after Amritikarana), 760mg/200gms body weight of albino rat (MTD) with 1 ml of 1% D.M.C solution was administered.
Group V – 1 ml of 1% D.M.C. Solution was administered. (Control)
(The animals were starved for 10 hours before the administration of medicine.)
Short-Term Chronic (Sub Acute) Toxicity Study of Two Samples of Tamra Bhasma (Table 12, 13)
Histopathological Study
Specimen; Rat Specimens (Table 14)
Measurements (mm): Brain: 22×18×15, Heart: 13×12×10, Rt Lung: 25×18×15
Lf Lung: 22×18×15, Liver: 45×40×40, Kidney: 17×11×10
Liver function Test
Before administration of drug blood samples were collected and after 21 days. Before killing again blood samples were collected. (Table 15)

Table 1: Variation in color and weight of Tamra during Samanya shodhana

	Weight	Wt loss	Color	Form
Raw tamra	3600 gms	-	Reddish shiny	Patra (flake)
In tila taila	3590 gms	10 gms	Blackish red	Patra
In takra	3580 gms	10 gms	Blackish red	Patra
In gomutra	3560 gms	20 gms	Reddish	Small pieces
In kanji	3555 gms	5 gms	Blackish red	Small pieces
In kulath kwath	3520 gms	35 gms	Reddish black	Coarse powder

Table 2: Preparation of Tamra Bhasma Sample-I

Putas	1st Gaja Puta	2nd Gaja puta	3rd Gaja puta	4th Gaja puta	5th Gaja puta
Initial weight	1600 gms	1480 gms	1434gms	1380gms	1320gms
Wt after puta	1280 gms	1234 gms	1180gms	1120gms	980gms
Loss in wt	320 gms	246 gms	254 gms	260gms	340gms
Color	Blackish	Blackish	Blackish	Blackish	Blackish

Table 3: Organoleptic characters of two samples of Tamra Bhasma

Sl. No.	Organoleptic Characters	Tamra Bhasma Sample I	Tamra Bhasma Sample II
1	Colour	Blackish	Brownish black
2	Odour	Odourless	Odourless
3	Taste	Tasteless	Tasteless
4	Touch	Soft	Soft
5	Appearance	Amorphous	Amorphous

Table 4: Physical constants of two samples of Tamra Bhasma

Sl. No.	Physical Constants	Tamra Bhasma Sample I	Tamra Bhasma Sample II
1	% Of Total ash	92%	96.2%
2	% Of Acid insoluble ash	90.4%	79.98%
3	% Of Water insoluble ash	88.8%	74.6%
4	P^H	5.52	5.38
5	Specific gravity	0.9949%	0.997%
6	Moisture content	0.4%	0.2%

Table 5: Solubility test of two samples of Tamra Bhasma

Samples	Solvents									
	D.W	Methanol	P.E	Acetone	Benzene	Tolune	Chloroform	E.A	Xylene	CCl_4
Tamra Bhasma Sample I	S.S	S.S	S.S	S.S	N.S	N.S	N.S	S.S	N.S	N.S
Tamra Bhasma Sample I	S.S	S.S	S.S	S.S	N.S	N.S	S.S	S.S	N.S	S.S

N.S – Not Soluble, S.S -Sparingly soluble

Table 6: Results in microgram/gm (PPM) or % as indicated

Elements	RT	SST	VST	Tamra Bhasma Sample I	Tamra Bhasma Sample II
CU	99.83%	79.56%	42.18%	29.94%	27.72%
Fe	0.057%	0.13%	0.41%	1.17%	1.11%
S	0.025%	0.096%	0.19%	6.64%	6.15%
Ni	0.021%	0.037%	0.019%	0.019%	0.019%
Ag	ND	54.65	32.91	42.36	45.64
Al	8.12	1.35%	2.60%	5.52%	5.12%
Si	94.27	0.021%	0.026%	0.029%	0.024%
Pb	0.043%	0.043%	0.020%	93.07	63.42
Na	-	-	12.60%	-	-
Hg	-	-	-	47.73	0.32%
As	-	-	-	-	-

ND-Not Detected

Table 7: Particle size Assessment

Samples	Count	Mean	Minimum	Maximum
Tamra Bhasma Sample I	1923	2.32 µm	0.96 µm	8.53 µm
Tamra Bhasma Sample II	1226	2.03 µm	0.96 µm	7.53 µm

Table 8: Final unit cell parameters

Sample	Volume(A**3)	Shape of the unit cell
R.T	637.852	Hexagonal
V.S.T	390.984	Monoclinic
Tamra Bhasma Sample I	471.110	Triclinic
Tamra Bhasma Sample II	445.638	Triclinic

Table 9: Showing the Effect of Tamra Bhasma sample I (Tamra Bhasma before Amritikarana) in Acute Toxicity study on Albino Rats

Dose No	Rats		Dose	Tremors	Convulsions	Jumping	Exopthalmus	Deep breathing	Death
	No.	Weight							
Ist	1 2	201gms 200gms	750mg 750mg	+(2 min) + (1.5 min)	+ (4 min) + (5 min)	+ -	+ +	+ (4min) +(5min)	Died(6min) Died(7min)
IInd	1 2	200gms 198gms	525mg 525mg	+ (3 min) + (5 min)	+ (7 min) + (8 min)	- -	+ -	+(7min) +8 min)	Died (8min) Died (9min)
IIIrd	1 2	200gms 200gms	367.5mg 367.5mg	+ (34 min) + (36 min)	+ (41 min) + (44 min)	+ (46 min) -	- -	+(46min) +(49min)	Died(46min) Died(49min)
IVth	1 2	200gms 201gms	257.25mg 257.25mg	+ (54 min) + (2 hrs)	+ (1hr 30 min) -	- -	- -	+(1hr40min) -	Died(1hr41min) No death
Vth	1 2	200gms 201gms	250mg 250mg	- -	- -	- -	- -	- -	No death No death
VIth	1 2	200gms 201gms	260mg 260mg	+ (56 min) + (1 hr)	+ (1hr 37 min) + (1hr 41min)	- -	+ -	+(1hr38min) +(1hr52min)	Died (1hr38min) Died (1hr52min)

+: Present, - : Absent

Table 10: Showing the Effect of Tamra Bhasma sample II (Tamra Bhasma after Amritikarana) in Acute Toxicity study on Albino Rats

	Rats		Dose	Tremors	Convulsions	Jumping	Exopthalmus	Deep breathing	Death
	No.	Weight							
Ist	1 2	200gms 201gms	250mg 250mg	- -	- -	- -	- -	- -	No death No death
IInd	1 2	199gms 201gms	375mg 375mg	- -	- -	- -	- -	- -	No death No death
IIIrd	1 2	200gms 201gms	562mg 562mg	- -	- -	- -	- -	- -	No death No death
IVth	1 2	201gms 201gms	843mg 843mg	+ (1hr47 min) + (1hr56min)	+ (2hr 10 min) +(2hr18min)	- -	+ -	+(2hr40min) +(2hr36min)	Died(2hr40min) Died(2hr36min)
Vth	1 2	200gms 201gms	700mg 700mg	- -	- -	- -	- -	- -	No death No death
VIth	1 2	200gms 200gms	780mg 780mg	+ (6hr2 min) + (6hr4min)	+ (7hr 15 min) + (7hr 5min)	- -	- -	+(8hr16min) +(7hr56min)	Died (8hr16min) Died (7hr56min)
VIIth	1 2	201gms 200gms	770mg 770mg	+(13hr6min) +(16hr51min)	+(14hr22min) +(20hr10min)	- -	- -	+(15hr10min) +(23hr10min)	Died(15hr10min) Died(23hr10min)
VIIIth	1 2	200gms 199gms	760mg 760mg	- -	- -	- -	- -	- -	No death No death

+: Present, - : Absent

Table 11: Showing the drug schedule for Toxicity Study (Maximum Tolerated Dose)

Group	No of animals	Drug	Dose / 200 gms	Duration
I	6	Tamra Bhasma sample I	250 mg with 1 ml 1%D.M.C.	21 days
III	6	Tamra Bhasma sample II	760 mg with 1 ml 1%D.M.C.	21 days
Control (Group V)	6	D.M.C.	1 ml 1%D.M.C.	21 days

Table 12: Showing the Changes observed in albino rats after administration of drugs for 21 days

Observation	Before Administration	After 21 days of Administration		
		Group I	Group III	Control (Group V)
Color of eyes	Deep red	Deep red	Deep red	Deep red
Edema of eyes	Absent	Absent	Absent	Absent
Activity	Normal	Normal	Normal	Normal
Water intake	Normal	Normal	Normal	Normal
Food intake	Normal	Reduced	Reduced	Normal
Paralysis	Not observed	Not observed	Not observed	Not observed
Stool-color	Black	Black	Black	Black
Stool nature	Sticky	Hard	Hard	Sticky

Table 13: Showing the weight variation in albino rats, after 21 days of administration of Tamra Bhasma

Groups	1st day of administration (Mean ± SD) n = 6	After 21 days of administration (Mean ± SD) n = 6
Group I	202.17 ± 2.40	200.33 ± 1.86
Group III	199.67 ± 2.73	199.5 ± 2.95
Control (Group V)	197.25 ± 2.22	200.0 ± 2.71

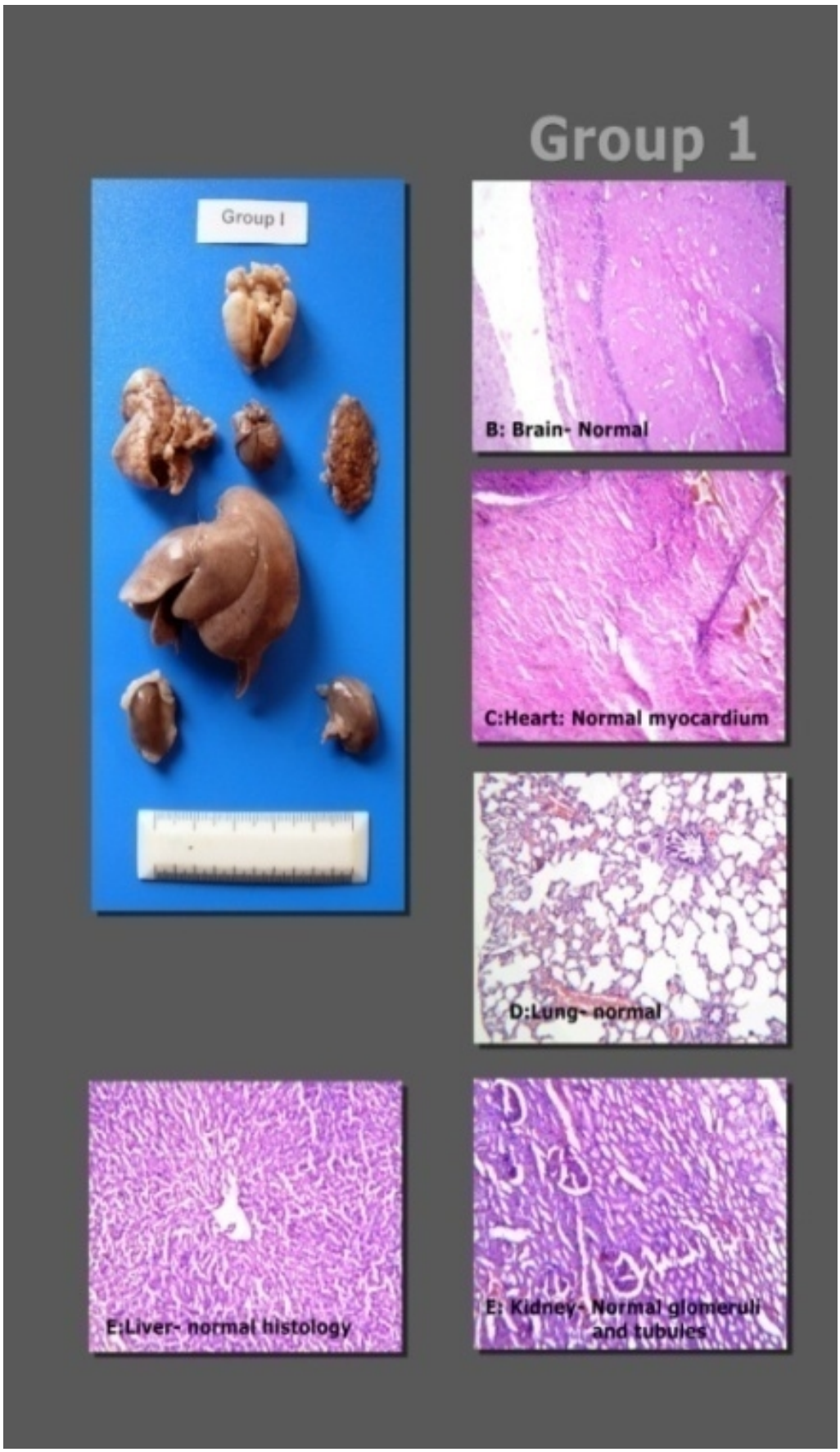
Table 14: Showing the Histo - Pathological reports

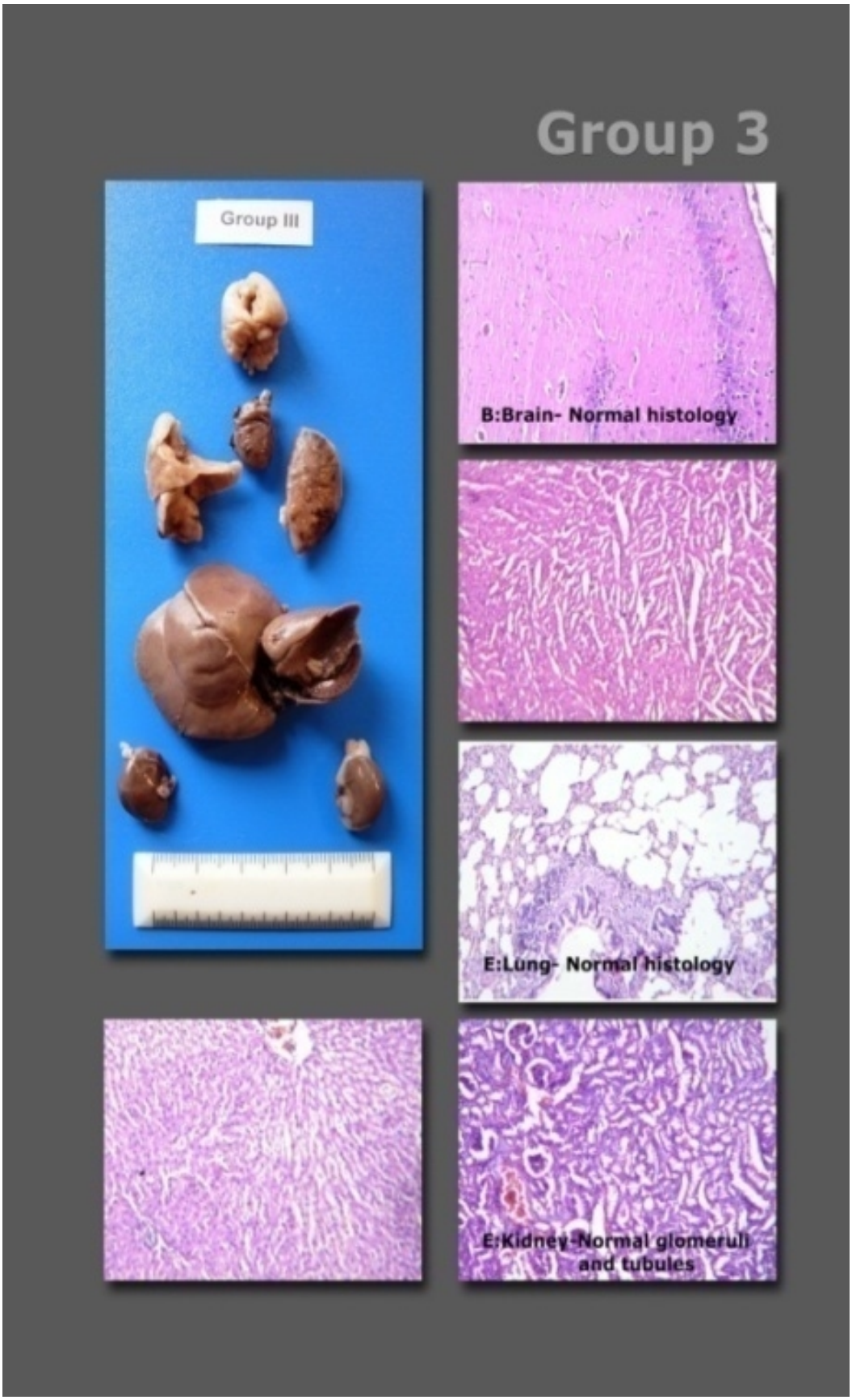
Microscopy Section from	Group I	Group III	Control (Group V)
Brain	Normal tissue	Normal tissue	Normal tissue
Heart	Normal Mycardium and Endocardium	Normal Mycardium and Endocardium	Normal Mycardium and Endocardium
Lungs	Shows Congested Lung parenchyma with normal bronchioles	Shows Congested Lung parenchyma with normal bronchioles	Shows Congested Lung parenchyma with normal bronchioles
Liver	Normal Hepatic with normal Sinusoids	Normal Hepatic with normal Sinusoids	Normal Hepatic with normal Sinusoids
Kidney	Normal glomeruli	Normal glomeruli	Normal glomeruli
Impression	All organs within normal Study	All organs within normal Study	All organs within normal Study

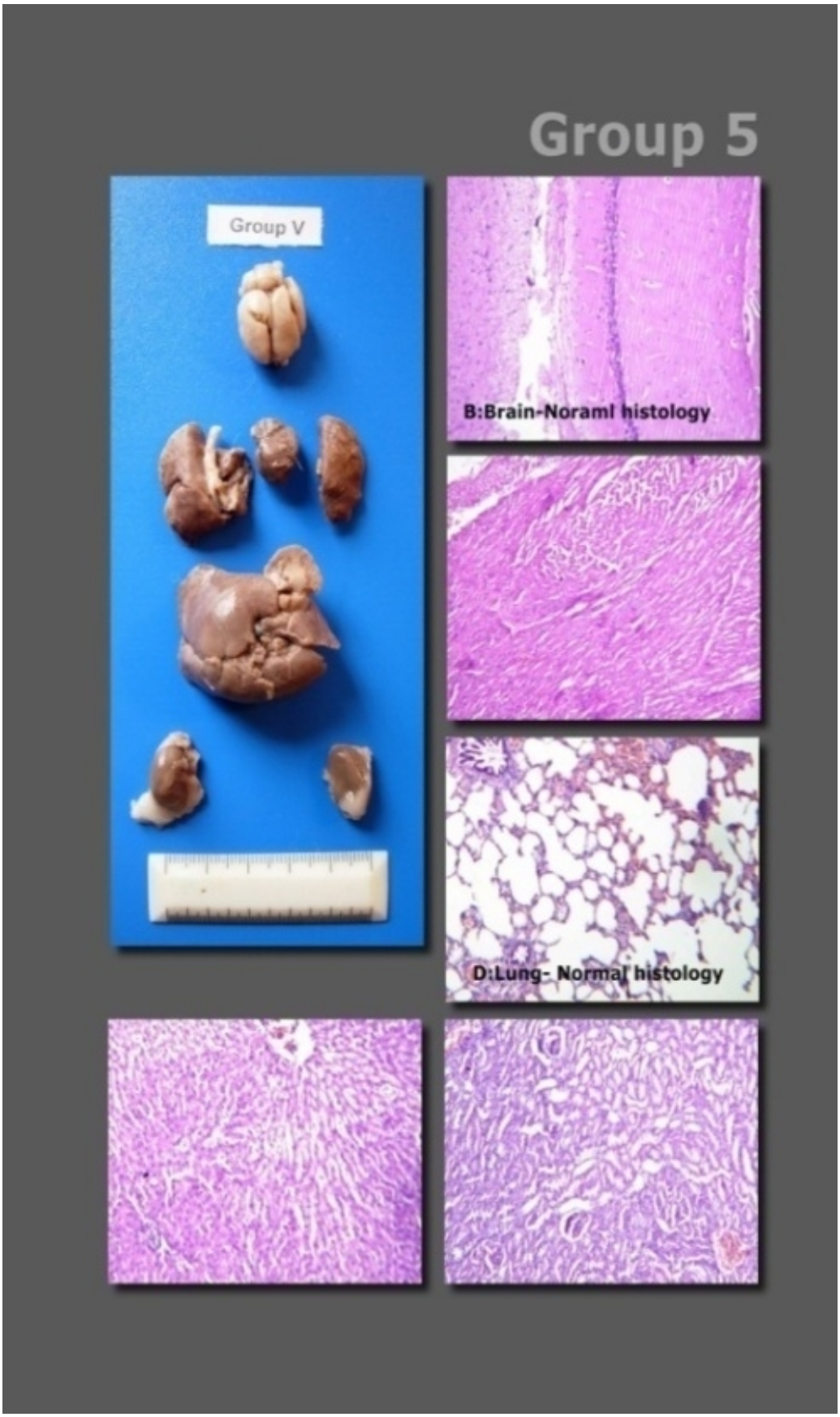
Table 15: Showing the Biochemistry Liver function Test of Different group Rats

Name of test	Results				Units
	Group I	Group III	Control (Group V)	Healthy Rat (Before Study)	
S. Bilirubin Direct	0.2	0.1	0.2	0.2	mg%
Indirect	0.2	0.3	0.4	0.4	mg%
Total	0.4	0.4	0.6	0.6	mg%
Serum Total Protein	6.9	7.1	6.8	6.1	gm%
Albumin	3.8	3.9	3.9	4.0	gm%
Globulin	3.1	3.2	2.9	2.1	gm%
A/G Ratio	1.3	1.2	1.3	1.9	
S.G.O.T	287	200	76	55	IU/L
S.G.P.T	102	66	63	60	IU/L
Serum Alkaline Phosphates	272	181	136	127	IU/L

Histo- Pathological Images of different groups under study




Group 3
Group III
B:Brain- Normal histology
E:Lung- Normal histology
E:Kidney-Normal glomeruli and tubules


Group 5
Group V
B:Brain-Noraml histology
D:Lung- Normal histology

DISCUSSION

Physico- Chemical Analysis

Two Samples of Tamra Bhasma (before and after Amritikarana) were subjected to Organoleptic, Physical and Chemical analysis.

It was observed that both samples of Tamra Bhasma shown, odorless, amorphous to touch and tasteless property, appears as powder form, Tamra Bhasma sample I was Blackish and Tamra Bhasma sample II was Brownish Black in colour.

Samples of Tamra Bhasma were subjected to pH study, pH of Tamra Bhasma sample I was 5.52 and pH of Tamra Bhasma sample II was 5.38 respectively.

Test for Physical constants of 2 samples of Tamra Bhasma were carried out, Physical constants of Tamra Bhasma sample I were noted as, ash value 92%, acid insoluble ash 90.4%, water insoluble ash 88.8%, Specific gravity 0.9949% and Moisture content 0.4 %.

Physical constants of Tamra Bhasma sample II were noted as, ash value 96.2%, acid insoluble ash 79.98%, water insoluble ash 74.6%, Specific gravity 0.997% and Moisture content 0.2%. Tamra Bhasma sample I and II were sparingly soluble in Distilled water, Methanol, Petroleum ether and Ethyl alcohol, Tamra Bhasma sample II was also sparingly soluble in Acetone, Chloroform, and Carbon tetra chloride.

Both Samples of Tamra Bhasma were subjected to Volumetric analysis of copper, the mean % of copper – 30.92 was noted in both the samples.

Analytical reports of samples were obtained by using inductively coupled plasma atomic emission spectroscopy.

Cu, Fe, S, Ni, Al, Si and Pb were detected in sample RT, SST, VST and 2 samples of Tamra Bhasma. Ag was not detected in sample of RT, but it was present in sample S.S.T, V.S.T, Two samples of Tamra Bhasma. Hg was detected in Tamra Bhasma sample I and Tamra Bhasma sample II may be due to the use of Mercury media in the preparation of these two samples of Tamra Bhasma.

There was decrease in the % of copper from raw to shodita, shodita to visesha shodita and so on till Tamra Bhasma and Amritikarana. This may be due to different purification and incineration processes carried out during bhasmikaran process. (RT-99.83%, SST- 79.65%, VST-42.18%, Tamra Bhasma Sample I- 29.94% and Tamra Bhasma Sample II -27.72%).

There was a decrease in the percentage of Sulphur and Mercury from Tamra Bhasma sample I to Tamra Bhasma sample II, this may be the also effect of Amritikarana process.

There was decrease in the percentage of Lead from Tamra Bhasma sample I to Tamra Bhasma sample II (from 93.07 ppm to 64.42 ppm) this is also again the effect of Amritikarana process.

Particle size assessment was done at IIT Powai, Mumbai. The mean particle size value of Tamra Bhasma sample I was 2.32 µm where as mean particle size value of Tamra Bhasma sample II was 2.03 µm. Due to amritikarana process particle size of Tamra Bhasma was reduced again.

X-RD analysis was done at Regional Research Laboratory Bhoovaneshwar, it was observed that the cell type in case of RT was Hexagonal with cell volume 390.984 A. the cell type of VST was Monoclinic with cell volume 390.984 A, this indicates that when RT was converted in to VST there was change in the cell type, it changes from hexagonal to monoclinic.

VST has been converted in to Tamra Bhasma sample I by following Marana process during the course of conversion the cell type changes from Monoclinic to Triclinic with cell volume 445.638 A. this may be the effect of Marana process which is followed to prepare Bhasma.

Tamra Bhasma sample I has been converted in to Tamra Bhasma sample II by following Amritikarana process during the course of conversion the cell volume is reduced from 471.110A to 445.638A. This may be the effect of Amritikarana process.

Toxicity Study

Acute Toxicity Study

To evaluate toxicity of Tamra Bhasma before and after Amritikarana, both samples were subjected for acute and short term chronic (Sub acute) toxicity study on albino rats.

In acute toxicity study it was noted that, Maximum tolerated dose of Tamra Bhasma before Amritikarana was 250mg/200 gms body weight of albinorat, and minimum lethal dose was 260mg/200 gms body weight of albino rat.

Maximum tolerated dose of Tamra Bhasma after Amritikarana, was 760mg/200 gms body weight of albino rat, and minimum lethal dose was 770mg/200 gms body weight of albino rat. From this (dose 760 mg) it clearly indicates that Amritikarana process remarkably reduced the copper toxicity, when compared with Tamra Bhasma without Amritikarana (250mg) process when administered to albino rats.

In short term chronic toxicity study

In group I, Tamra. Bhasma Sample I was administered to 6 albino rats, In-group III, Tamra Bhasma Sample II was administered to 6 albino rats, In-group V, 1% D.M.C solution was administered to 6 albino rats (Control).

After administration of drugs and during 21 days period, food intake was reduced, stool was black and hard in nature in group I and III may be due to Copper intake. There was reduction in body weight in group Iand III may be due to reduced food intake.

After 21 days duration of drug schedule, from each group albino rats were anesthetized and scarified to obtain fresh Kidney, Liver, Heart, Brain and Lung tissues. The tissues were subjected for Histopathological study.

In-group I Tamra Bhasma sample I shown no damage in brain, heart, lung, liver and kidney and all organs within normal study.

In Group III and V with Tamra Bhasma sample II and D.M.C solution respectively shown no any damage to heart, brain, liver kidney and lung and all organs were within normal Study.

In Liver function test in group I Serum bilirubin direct, SGOT, SGPT and Serum alkaline phosphate were significantly increased when compared with group III, this clearly suggest that Amritikarana process reduces Copper Toxicity.

CONCLUSION

The acid insoluble ash & water insoluble ash percentage values were reduced after Amritikarana process of Tamra Bhasma.

Sulphur & Mercury percentage were decreased after Amritikarana process.

The mean particle size value & cell volume of Tamra Bhasma was also reduced after Amritikarana process.

The Maximum tolerated dose of Tamra bhasma was increased after Amritikarana process.

By considering the data obtained by this study, we can clearly say that Amritikarana process significantly reduces toxicity.

ABBREVIATIONS

1) RT - Raw Tamra
2) SST - Samanya Shodhita Tamra
3) VST - Vishesh Shodhita Tamra
4) Tamra Bhasma Sample I - Tamra Bhasma before Amritikarana
5) Tamra Bhasma Sample II - Tamra Bhasma after Amritikarana
6) D.M.C. - Dimethyl carboxy cellulose
7) M.T.D. - Maximum Tolerated dose

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