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TOXICITY STUDY OF TAMRA BHASMA PREPARED WITH VARIOUS METHODS

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ABSTRACT
To assess the toxicity of Tamra Bhasma prepared with various methods, different samples of Tamra Bhasma were prepared by following different procedures, all the four prepared samples were subjected for acute & sub acute toxicity study by following Staircase method on Albino rats.
The results significantly suggest that Somanathi Tamra Bhasma was less toxic and few samples of Tamra Bhasma shown mild to moderate toxic signs & symptoms which are reversible.
Keywords: Tamra Bhasma, Somanathi Tamra Bhasma, Toxicity study.

INTRODUCTION

Toxicity means the poisonous effect of a substance and the Right dose differentiates a poison and a remedy, in Rasashastra dose of particular drug is of prime importance, for the expression of both efficacy and toxicity. As a raw material Tamra (copper) is considered to be highly poisonous and several times more poisonous than poison itself, because poison possesses only one dosha (toxic effect) while unpurified and unreduced copper may have eight doshas (toxic effects). And in Rasaratna samuchachaya in the context of Loha (metals), the concept of Marana was explained as, If Loha Marana is done by using Mercury and its compounds as media, the prepared bhasma is considered as first best category, Herbal drugs as media the prepared bhasma is considered as Second best category, Sulphur, Arsenic (Haratala) etc as media then the bhasma is considered as third categoryand if Arilohas (Anti-metals) used as media then the prepared bhasma is considered as inferior category and a special method of preparation of Tamra Bhasma was mentioned in Rasa Granthas (Texts) which is prepared by using Parada, Gandhaka, Haritala & Manashila media and named as Somanathi Tamra Bhasma which is claimed as less toxic & more effective even in excess dosage forms. Amritikarana process is recommended for Tamra bhasma to reduce its toxic effects. By considering all these concepts, to assess the toxicity of Tamra Bhasma prepared with different methods, Tamra Bhasma was prepared by following different procedures and subjected for acute and sub acute toxicity study on Albino Rats.

MATERIALS & METHODS

Materials

1) 4 Samples of Tamra Bhasma- were prepared with different methods. For toxicity Study at A.V.S. Ayurveda Mahavidyalaya College, Pharmacy & required materials were collected from same pharmacy & Herbal garden.
2) Albino rats - were collected from A.V.S. Ayurveda Mahavidyalaya Central Animal house
3) Di methyl car boxy cellulose Solution - For suspension preparation.

Methods

1) For Tamra Bhasma Preparation

a) Ist Sample (T.B1) of Tamra Bhasma (Tamra marana with parada media) was prepared by following reference from Rasa Ratna Samachchaya - 5 / 53.
b) IInd sample (T.B2) of Tamra Bhasma (Tamra marana with Gandhaka media) was prepared by the following reference from Ayurveda prakash - 3 / 139.
c) IIIrd sample (T.B3) of Tamra Bhasma (Tamra Bhasma after Amritikaran) was prepared by the following reference from Rasamritam – 2/ 45, 46
d) IVth sample (T.B4) of Tamra Bhasma that is Somanathi Tamra bhasma (Tamra marana with Parada,Gandhaka,Haritala and Manashila media) was prepared by the following reference from Rasa Ratna Samachchaya - 5 / 65, 66, 67

2) For 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.

Acute Toxicity

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 T.B 1, 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 next dose the staircase method was followed (decreasing the dose by factor 0.7 that is 750mg × 0.7 = 525 mg is the next dose).
IInd 525mg/200 gms of albino rat body wt was given to two albino rats following the same procedure, both rats were died after 6 minutes, this dose was also lethal, again following 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 T.B1 sample, 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 other samples were calculated.

Observation & Results of Acute Toxicity Study of Tamra Bhasma prepared with various methods

Table 1: Showing the Effect of Tamra bhasma prepared with Parada Media – T.B 1 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 2: Showing the Effect of Tamra bhasma prepared with Gandhak Media – T.B 2 in Acute Toxicity study on Albino Rats

Dose No	**Rats**		**Dose**	**Tremors**	**Convulsions**	**Jumping**	**Exopthalmus**	**Deep breathing**	**Death**
	No.	**Weight**							
Ist	1 2	200gms 199gms	250mg 250mg	- -	- -	- -	- -	- -	No death No death
IInd	1 2	199gms 199gms	270mg 270mg	- -	- -	- -	- -	- -	No death No death
IIIrd	1 2	200gms 210gms	280mg 280mg	- -	- -	- -	- -	- -	No death No death
IVth	1 2	200gms 201gms	290mg 290mg	+(2hr40min) + (2hr54min)	+ (4hr 10 min) +(4hr40min)	- -	+ _	+(5hr20min) +(5hr36min)	Died(1hr41min) No death

+: Present - : Absent

Table 3: Showing the Effect of Tamra bhasma after Amritikarana – T.B 3 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 4: Showing the Effect of Somanathi Tamra Bhasma (Tamra marana with Parada,Gandhaka,Haritala and Manashila media) – T.B 4 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	800mg 800mg	- -	- -	- -	- -	- -	No death No death
IInd	1 2	199gms 200gms	1200mg 1200mg	+ (4hr 6 min) + (4hr56min)	+ (5hr10 min) + (5hr16 min)	- -	+ -	+(5hr56min) +(6hr10 min)	Died (5hr56min) Died (6hr10min)
IIIrd	1 2	201gms 200gms	1100mg 1100mg	+ (5hr14 min) + (6hr2 min)	+ (6hr30 min) + (6hr45 min)	- -	- -	+(7hr56min) +(8hr16min)	Died(7hr56min) Died(8hr16min)
IVth	1 2	200gms 199gms	1050mg 1050mg	- -	- -	- -	- -	- -	No death No death
Vth	1 2	201gms 200gms	1060mg 1060mg	+(8hr56min) +(9hr48min)	+(12hr10min) +(11hr6min)	- -	- -	+(13hr14min) +(15hr10min)	Died(13hr14min) Died(15hr10min)

+: Present, - : Absent

SHORT-TERM CHRONIC (SUB ACUTE) TOXICITY OF TAMRA BHASMA PREPARED WITH VARIOUS METHODS

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.

Study Group

Group I – Sample T.B1, 250mg/200gms body weight of albino rat (MTD) with 1 ml of 1% D.M.C solution was administered.

Group II – Sample T.B.2, 280mg/200gms body weight of albino rat (MTD) with 1 ml of 1% D.M.C solution was administered.

Group III – Sample T.B3, 760mg/200gms body weight of albino rat (MTD) with 1 ml of 1% D.M.C solution was administered.

Group IV – Sample T.B4, 1050mg/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.)

Table 5: Showing the drug schedule for Toxicity Study (Maximum Tolerated Dose)

Group	No of animals	Drug	Dose / 200 gms	Duration
I	6	Sample TB1	250 mg with 1 ml 1%D.M.C.	21 days
II	6	Sample TB2	280 mg with 1 ml 1%D.M.C.	21 days
III	6	Sample TB3	760 mg with 1 ml 1%D.M.C.	21 days
IV	6	Sample TB4	1.050 mg with 1 ml 1%D.M.C.	21 days
V	6	D.M.C.	1 ml 1%D.M.C.	21 days

Observation & Results of Short-term chronic (sub acute) TOXICITY Study of Tamra Bhasma prepared with various methods.

Table 6: Showing the Changes observed in albino rats after administration of drugs for 21 days

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

Table 7: 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 1	202.17 ± 2.40	200.33 ± 1.86
Group 2	201.33 ± 1.51	196.67 ± 1.75
Group 3	199.67 ± 2.73	199.5 ± 2.95
Group 4	199.17 ± 2.04	198.67 ± 2.94
Group 5	197.25 ± 2.22	200.0 ± 2.71

Histopathological Study

Table 8: Showing the Histo Pathological study reports

Specimen; Rat Specimens
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

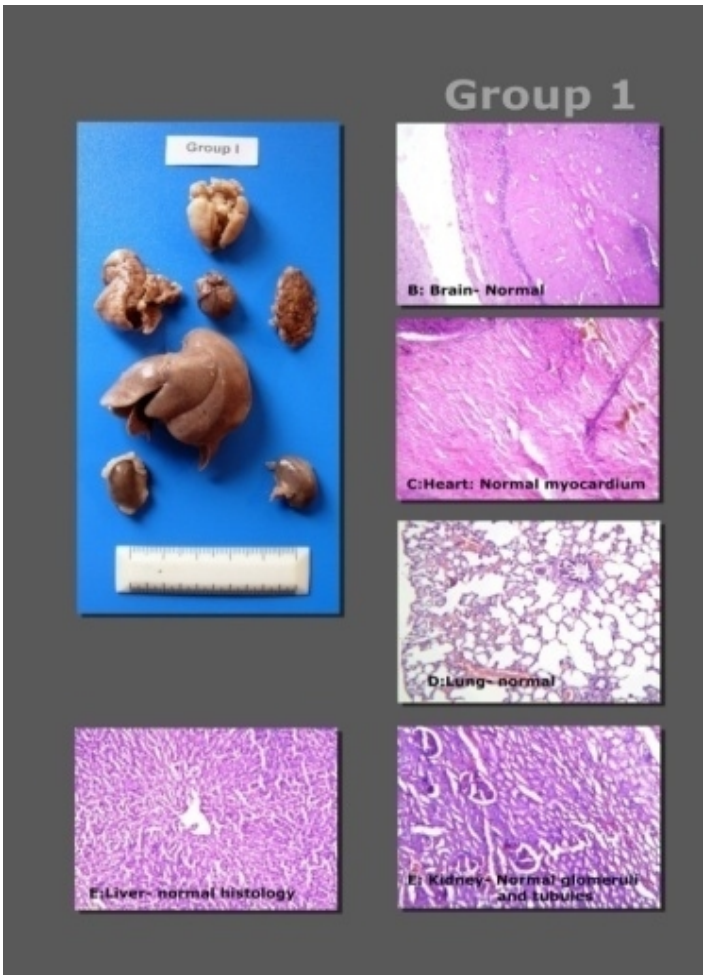
Microscopy Section from	Group I	Group II	Group III	Group IV	Group V
Brain	Normal tissue	Normal tissue	Normal tissue	Normal tissue	Normal tissue
Heart	Normal Mycardium & Endocardium	Normal Mycardium & Endocardium	Normal Mycardium & Endocardium	Normal Mycardium & Endocardium	Normal Mycardium & Endocardium
Lungs	Shows Congested Lung parenchyma with normal bronchioles	Shows Congested Lung parenchyma with normal bronchioles	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	Lobular architecture well maintained, hepatocytes shows microvesicular fatty droplet deposition indicating fatty liver (mild injury)	Normal Hepatic with normal Sinusoids	Normal Hepatic with normal Sinusoids	Normal Hepatic with normal Sinusoids
Kidney	Normal glomeruli	Normal glomeruli	Normal glomeruli	Normal glomeruli	Normal glomeruli
Impression	All organs within normal Study	Microvesicular Fatty Liver	All organs within normal Study	All organs within normal Study	All organs within normal Study

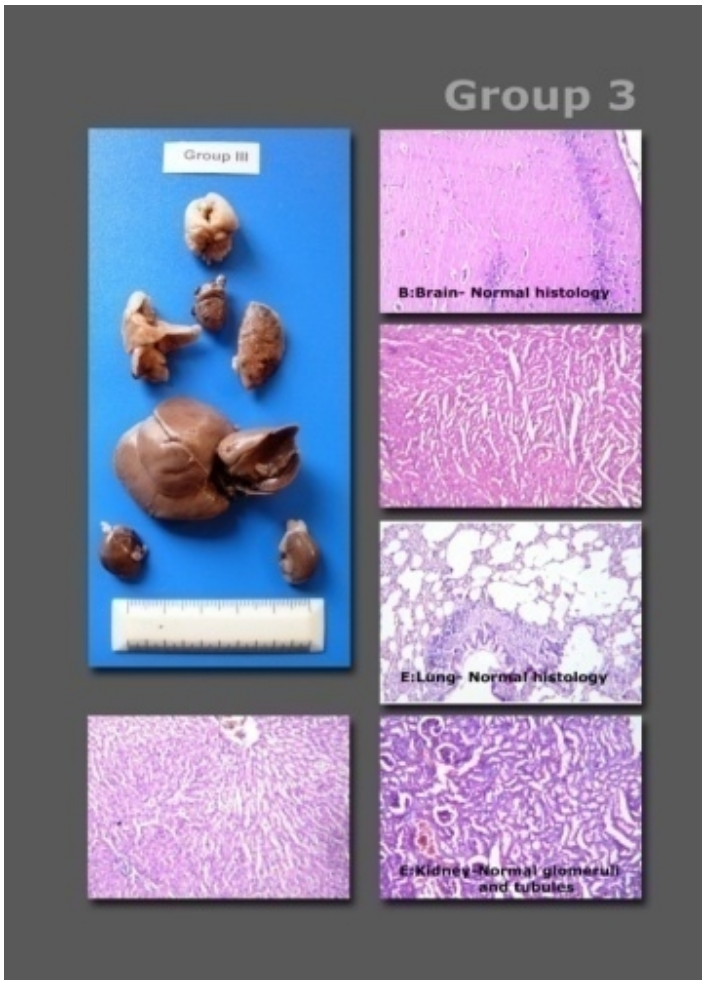
Liver function Test

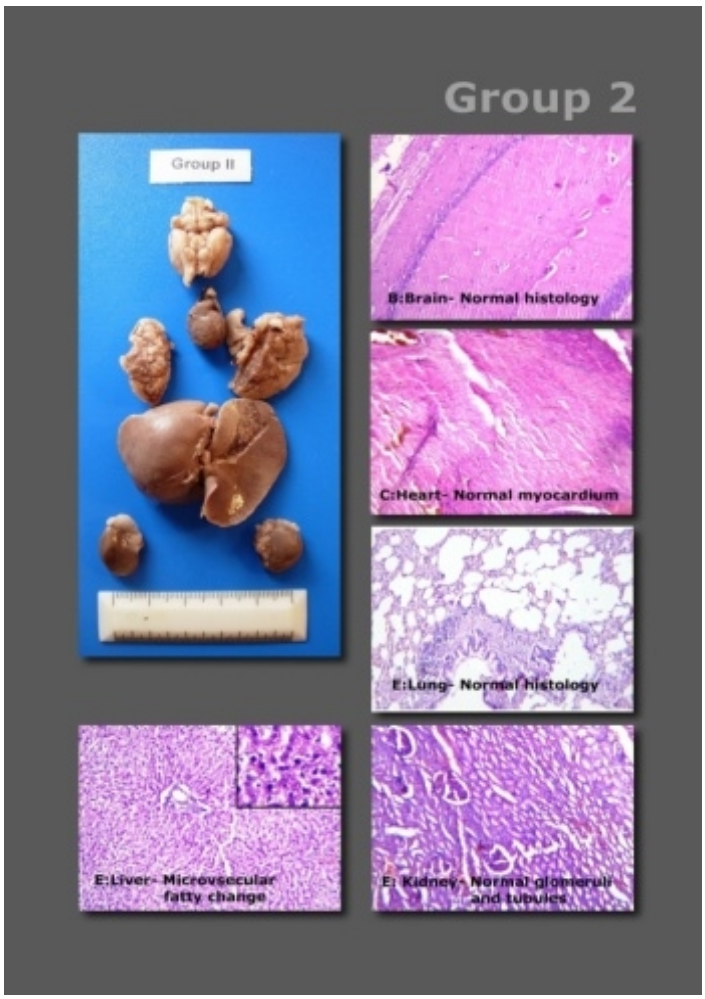
Before administration of drug blood samples were collected & after 21 days. Before killing again blood samples were collected

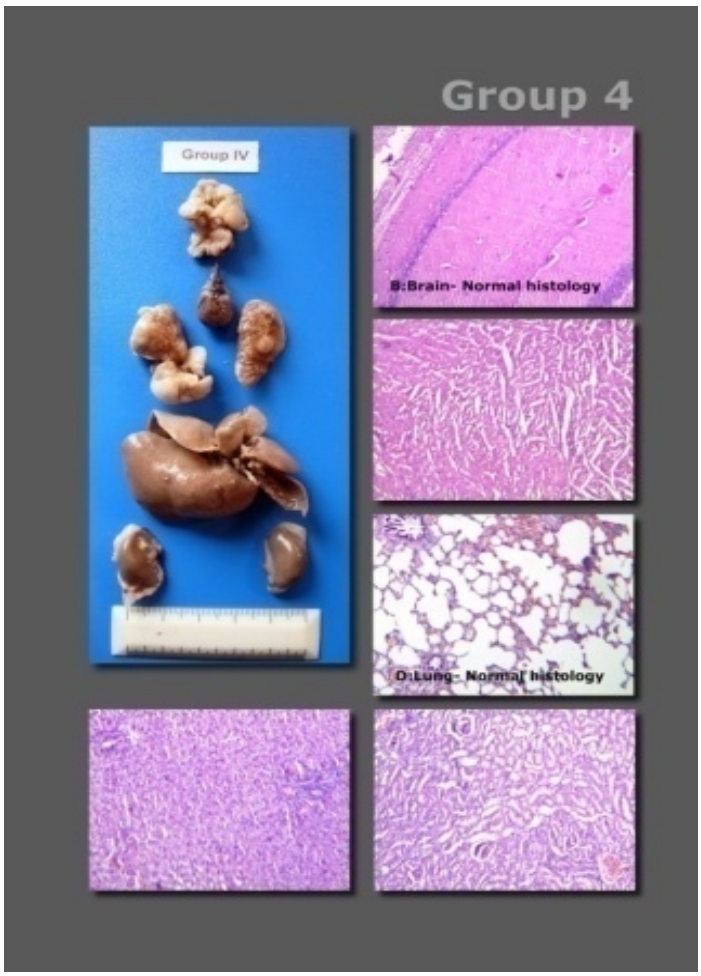
Table 9: Showing the Biochemistry Liver function Test of Different group Rats.

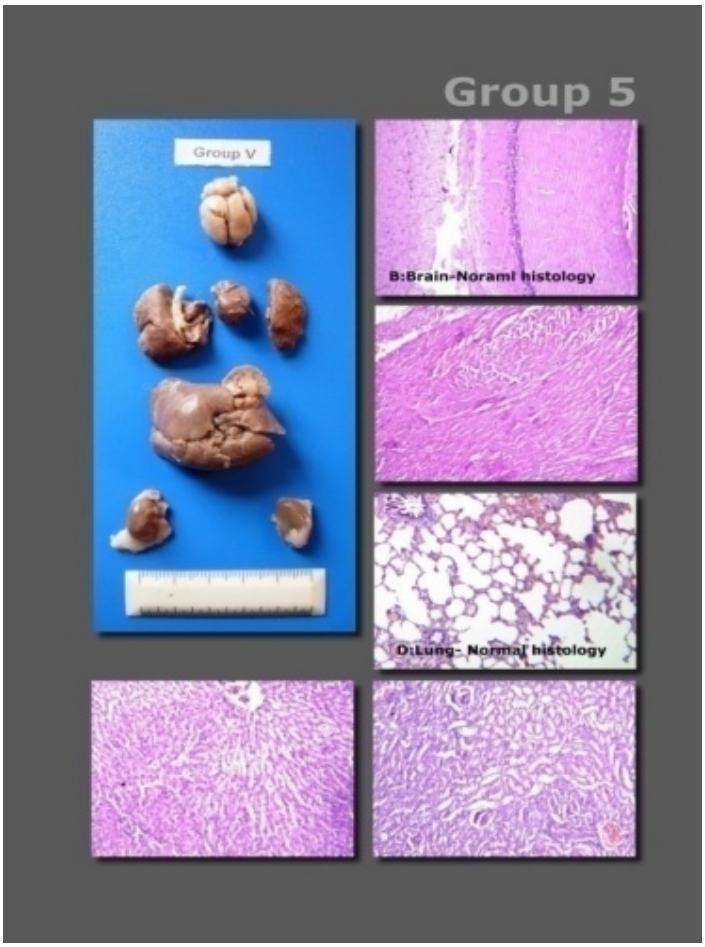
Name of test	Results						Units
	Gp1	Gp2	Gp3	Gp4	Gp5	Healthy Rat (Before Study)	
S. Bilirubin Direct	0.2	0.2	0.1	0.2	0.2	0.2	mg%
Indirect	0.2	0.3	0.3	0.3	0.4	0.4	mg%
Total	0.4	0.5	0.4	0.5	0.6	0.6	mg%
Serum Total Protein	6.9	6.7	7.1	6.3	6.8	6.1	gm%
Albumin	3.8	3.6	3.9	3.5	3.9	4.0	gm%
Globulin	3.1	3.1	3.2	2.8	2.9	2.1	gm%
A/G Ratio	1.3	1.2	1.2	1.1	1.3	1.9	
S.G.O.T	287	350	200	280	76	55	IU/L
S.G.P.T	102	164	66	92	63	60	IU/L
Serum Alkaline Phosphates	272	292	181	192	136	127	IU/L


Group 1
Group I
B: Brain- Normal
C:Heart: Normal myocardium
D:Lung- normal
E:Liver- normal histology
E: Kidney- Normal glomeruli and tubules


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


Group 2
Group II
B:Brain- Normal histology
C:Heart- Normal myocardium
E:Lung- Normal histology
E:Liver- Microvsecular fatty change
E: Kidney- Normal glomeruli and tubules


Group 4
Group IV
B:Brain- Normal histology
D:Lung- Normal histology



DISCUSSION

To evaluate toxicity of Tamra Bhasma prepared with various methods, different 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 prepared with Parada media (Mercury) 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 prepared with gandhaka media (without parada) was 280mg/200 gms body weight of albino rat, and minimal lethal dose was 290mg/200 gms body weight of albino rat.

Maximum tolerated dose of Tamra Bhasma after Amritikarana, which is prepared with parada media (Mercury) 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 process administered to albino rats.

Maximum tolerated dose of Somanathi Tamra Bhasma was 1050mg/200 gms body weight of albino rat, and minimum lethal dose was 1060mg/200 gms body weight of albino rat. This may be the reason behind the high dose of Somanathi Tamra Bhasma mentioned in classical Rasashastra text in comparison to Tamra Bhasma samples prepared with other medias.

In short term chronic toxicity study

In group I, T.B1 was administered to 6 albino rats, In group II, T.B2 to 6 albino rats, In-group III, T.B3 to 6 albino rats, In group IV, T.B4 to 6 albino rats and In-group V, 1% D.M.C solution was administered to 6 albino rats.

After administration of drugs and during 21 days period, food intake was reduced, stool was black and hard in nature in group I, II, III and IV, may be due to Copper intake. There was reduction in body weight in group I, II, III & IV 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 sample TB1 shown no damage in brain, heart, lung, liver & kidney & all organs within normal study.

In group II (sample TB2) only liver shown, lobular architecture well maintained and hepatocytes shows micro vesicular fatty droplet deposition indicating fatty liver (mild injury) this may be due to preparation of Tamra bhasma without parada media, but with Gandhaka media.

In Group III, IV & V with sample TB3, TB4 & D.M.C solution respectively shown no any damage to heart, brain, liver kidney & lung & all organs were within normal Study.

In Liver function test in all groups except control the SGOT and Serum alkaline phosphate levels were increased but they were significantly increased in group II with sample T.B2, this may be due to copper metabolism.

CONCLUSION

Acute toxicity study

The maximum tolerated dose of Tamra Bhasma

a) Prepared with Parada media (Mercury) was – 250mg/200 gms body weight of albino rat
b) Prepared with Gandhaka media (without parada) was - 280 mg/200 gms body weight of albino rat
c) While Tamra Bhasma prepared with parada media after Amritikarana was - 760 mg/200 gms body weight of albino rat
d) Somanathi Tamra Bhasma was - 1050 mg/200 gms body weight of albino rat.

The minimum lethal dose of Tamra Bhasma

a) Prepared with Parada media (Mercury) was – 260mg/200 gms body weight of albino rat.
b) Prepared with Gandhaka media (without parada) was - 290 mg/200 gms body weight of albino rat
c) While Tamra Bhasma prepared with parada media after Amritikarana was - 770 mg/200 gms body weight of albino rat
d) Somanathi Tamra Bhasma was - 1060 mg/200 gms body weight of albino rat.

Tamra Bhasma prepared with Parada media has shown the Maximum tolerated dose 250mg/200 gms body weight of albino rat and minimal lethal dose 260mg. But the same sample after Amritikarana has shown maximum tolerated dose 760mg/200 gms body weight of albino rat and minimal lethal dose of 770mg. this clearly indicates that Amritikarana process reduces toxicity of Tamra Bhasma.

By observation it was noted that the Maximum tolerated dose and minimal lethal dose of Somanathi Tamra Bhasma were significantly high in comparison to other samples of Tamra Bhasma.

Short Term Chronic Toxicity Study

a) There was reduction in food intake and body weight in all the groups except Group V
b) In Group II (T.B 2), liver hepatocytes shown microvesicular fatty droplet deposition indicating fatty liver (mild injury), which is reversible.
c) SGOT and serum alkaline phosphate levels were increased in-group II.
d) Preparation time, cost, effectiveness, safety concerned somanathi tamra bhasma (sample T.B 4) may be considered as best when compared with sample T.B1, T.B2 and T. B3.

ABBREVATION

1) T.B 1 - Tamra Bhasma prepared with Parada media
2) T.B 2 - Tamra Bhasma prepared with Gandhaka media
3) T.B 3 - Tamra Bhasma after Amritikarana
4) T.B 4 - Somanathi Tamra Bhasma or Tamra Bhasma with Parad,Gandhka, Haratala & Manashila media
5) D.M.C. - Di methyl car boxy cellulose
6) M.T.D. – Maximum Tolerated dose

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