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EFFECT OF TAMRA BHASMA IN HEPATOTOXICITY INDUCED BY MONOSODIUM GLUTAMATE – AN ANIMAL STUDY

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ABSTRACT

Most of the disorders caused in present scenario are due to unconventional etiology such as indiscriminate use of pesticides fertilizers in the farming, food adulteration, food additives, preservatives, sweetener, indiscriminate use of various synthetic drugs etc. For this unconventional etiology, these substances are known to cause oxidative stress in human body.[2] Although Monosodium Glutamate has been used in food since many years, recent concerns have developed over the safety of consuming large amounts of this compound over a prolonged period of time as it can lead to many health related issues.[1,17] Many types of research on animals have led to the conclusion that Monosodium Glutamate may be the cause of adverse health effects affecting every organ in human body eg obesity, nephrotoxicity, hepatotoxicity.[3] The Monosodium effect on liver can be compared with the concept of Gara visha. Tamra bhasma is used in different conditions like skin, pleeha, netra rog. In Agadtantra there is reference in Charak Samhita that it having Gara vishaghna property as well as doing Rasayana karma on body. Oxidative stress is one of the cause that is responsible for Monosodium Glutamate toxic effect on liver. The current study was designed to find out

Ayurvedic formulation to treat the hepatotoxicity with its in vivo study to screen its effectiveness in albino wistar rats. The pathological obtained results conspicuous observation have been discuss with scientific reasoning.

KEYWORDS: Tamra bhasma, Monosodium Glutamate, Hepatotoxocity.

INTRODUCTION

Garavisha is prepared artificially by mixing various substances; it produces *gada* (disease). As it takes some *kala* (time) for this type of poison to reach *vipaka* (metabolized) and produce it's toxic effects, it does not cause prompt death of person.[8] Ayurveda has no direct reference of hepatotoxicity caused due to drugs but as per the definition of *Gara visha*, all *kritrim visha* can be considered under the concept of *Gara visha.*[9]

Gara visha may enter the body by various routs, out of these methods *Anna* (food) is very comman route of administration of *Gara visha.*[10] Now a days due to influence of fast life and changes in food habits, peoples are exposed to toxins from many angles. One of the additives like Monosodium Glutamate is commonly used in all packed foods, prolonged use of Monosodium Glutamate may cause hazardous effects on human body.[15] Monosodium Glutamate is used in to food as per the provisions declared by FSSAI guideline. A subject to Good Manufacturing Practices (GMP), level and proper label declaration as provided in regulations of Food Safety and Standars.it shall not be added to any food for use by infants below twelve months.[17]

Consumption of Monosodium Glutamate between 0.3 and 1 gram daily has been reported to be safe, healthy persons to avoid consuming Monosodium Glutamate frequently.[16] As toxicogenesis and etiological factors of *Gara visha* mentioned in Ayurveda is similar to additives, synthetic food colors and synthetic drugs.[4] *Vagbhat* explains symptoms of *Gara visha* in details, 'Mahodaryakrutpleehi is one of the symptoms which is similar to hepatotoxicity induced by MSG.[9,4]

MATERIAL AND METHOD

Study design

1. Type of study design – Experimental study (In vivo study)

This study was carried out in two phases

a) Standard collection of Tamra bhasma from authentic pharmacy.

b) Observation of preclinical hepatotoxicity of Monosodium Glutamate in animal model.

MATERIAL

1 Test Item

Name of test item	*Tamra bhasma*
Physical State	Solid
Quantity Received	30 gm
Batch No	P230200303
Manufacturing Date	02/2023
Expiry Date	01/2028
Date of Receipt	09/05/2023

2 Standard Drug

Name of standard Drug	Silybon - 140 Silymarin
Physical state	Solid
Manufactured Date	Sep-2021
Expiry Date	Mar-2024
Batch No	Si050020
Marketed by	Micro Lab LTD

3 Induction Drug

Name of Induction Drug	Monosodium Glutamate
Physical state	Solid
Manufactured Date	Jan-2023
Expiry Date	Jan-2025
Batch No	SF009
Marketed by	Market Product

DOSE PREPARATION

Dose calculated by Paget and Barners Formula (Human to Animal dose conversion)

1) Tamra bhasma

1. 12 mg/ kg of Tamra bhasma i n Honey.

2) Si l ymarin

25 mg/ kg of Si l ymarin i n carboxymethylcellulose.

3) Monosodium Glutamate

30 mg/ kg of Monosodium Glutamate i n carboxymethylcellulose.

TEST SYSTEM AND MANAGEMENT

Species	Rat
Strain	Wistar
Source	
Sex	Male & Female (Female were non-pregnant & nulliparous)
Body weight range	180 g - 200 g
Identification	Identification mark to animals and cages
No. of animals	24 (12M & 12F)
Acclimatization	The rats were Acclimatized at test environment for 7 days.
Environmental conditions	Room temperature maintained between 22 + 30oC, relative humidity 55 + 5 % and 12-hours light and 12 hours dark cycle was maintained.
Accommodation	Three rats in each cage with clean paddy husk.
Diet	Pelleted feed supplied by Nutrivet Pvt. Ltd. ad libitum during the study.
Water	RO filtered water was provided ad libitum

STUDY DESIGN

Six rats in each group were used for this study, Test sample were applied orally as per standard protocol and animals were observed for signs and symptoms along with weekly Body weight.

Procedure

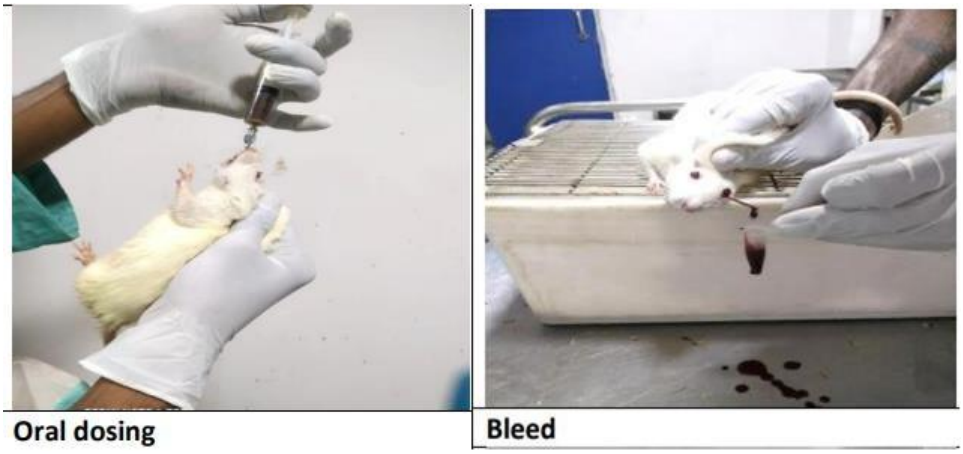
This hepatotoxicity study was conducted in 12 male wistar rat & 12 non-pregnant and nulliparous female wistarratsweighing 180-200 gm havingage between 6-10 weeks. Animals werekept foracclimatization under standard condition for 7 days. The rats were identified by color marking.

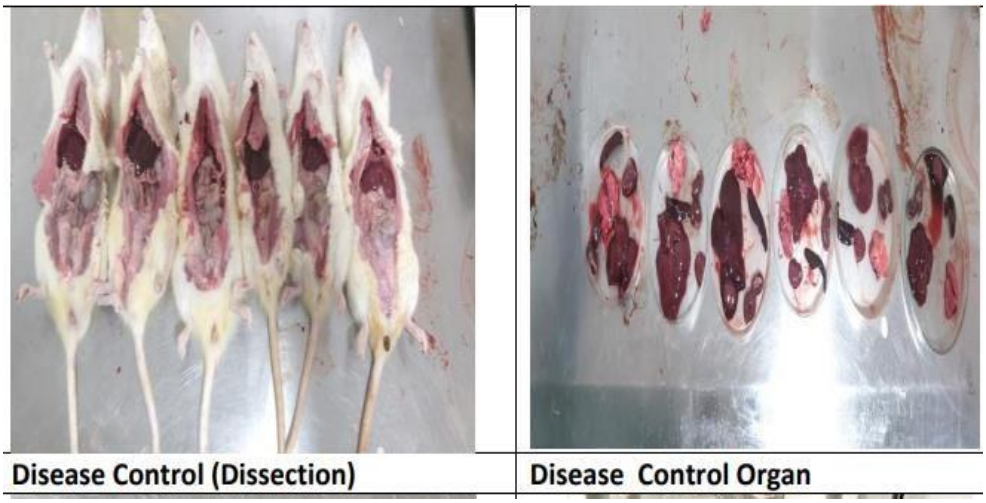
- Monosodium Glutamate (30 mg/kg body wt.) was administrated to each rat except control group
- (Group 1) for 15 days by oral route taking the help of oral gavage.
- All the animals were weighed before (day 1), and weekly thereafter till day 21.
- Monosodium Glutamate (30 mg/kg body wt.) was given to Disease control group (Group 2) till 21 days.
- Standard drug (Silymarin) was given to Standard group (Group3) for 7 days.
- *Tamra bhasma* was given orally to Test group (Group4) for 7 days.
- Anesthesia was provided to animals after completion of treatment and Collection of blood was done.

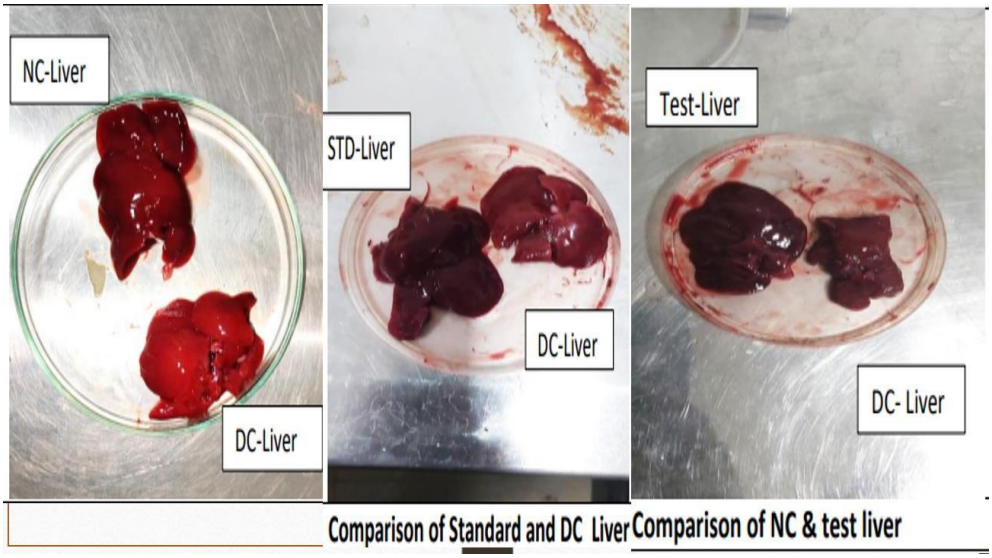
The 4 groups (n= 6, 3 male & 3 female per group) were as follows

Sr.No.	Animal Group	Group Name	Drug Specification	Dose in animal
1	Group 1	Normal Control (NC)	No treatment	-
2	Group 2	Disease Control (DC)	Only Monosodium Glutamate	30mg/kg
3	Group 3	Standard (STD)	Silymarin	25 mg/kg
4	Group 4	Test	*Tamra bhasma*	1.12 mg/kg

- Blood sample was collected on 0, 15th and 21 th days and serum biochemical was done.
- Biochemical estimations of various parameters such as serum glutamic- oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and alkaline phosphatase (ALP), bilirubin, and Albumin was assessed
- All animals were weighed, and visual observations fordeath, behavioralpatterns, and physical appearance changes are observed. Throughout the 21 day testing period, food and water intake were also assessed weakly.
- Animals from each group were sacrificed and Liver were removed, weighed and perfused in formalin solution for histopathology studies.







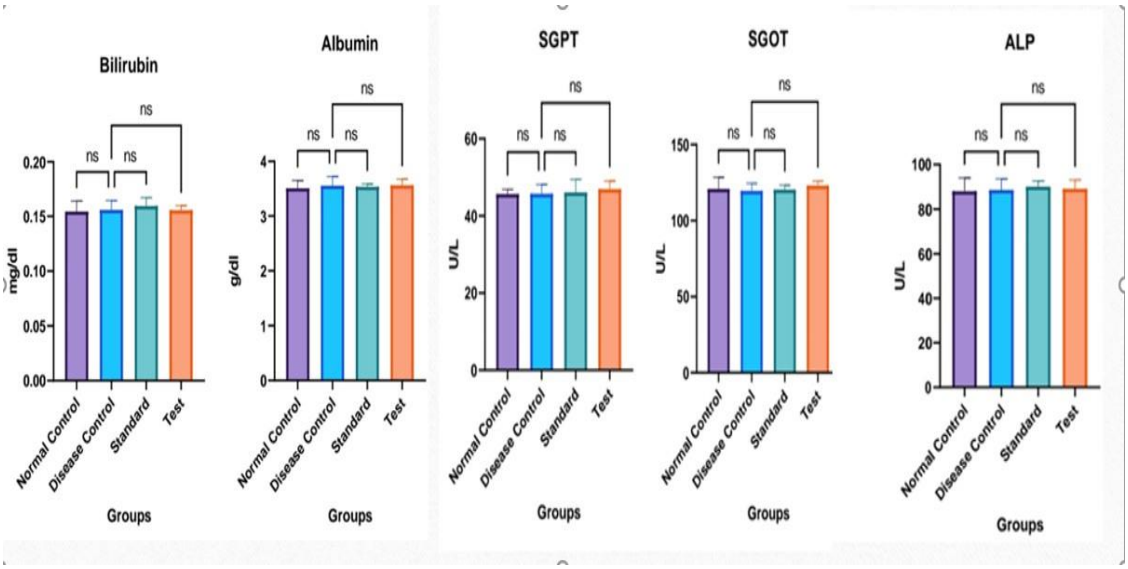
DATA ANALYSIS

Following are the value obtained from the blood report which is collected on 0, 15th and 21nd day of study.

Table 6: Biochemical Parameter – Day 0.

Group	Animal Marking	Billirubin	Albumin	SGPT	SGOT	ALP
Unit		mg/dl	g/dl	U/L	U/L	U/L
NC Male	H	0.142	3.31	45.61	125.65	84.95
	B	0.165	3.72	44.21	131.45	97.6
	T	0.166	3.59	47.13	115.65	88.56
NC Female	H	0.15	3.45	46.85	110.21	80.44
	B	0.148	3.56	44.37	118.54	91.36
	T	0.156	3.42	45.85	124.31	85.23
	Mean	**0.15**	**3.51**	**45.67**	**120.97**	**88.02**
	SD	**0.01**	**0.15**	**1.22**	**7.65**	**5.96**
		Disease Control (Mono Sodium Glutamate)				
DC Male	H	0.152	3.29	48.75	118.54	85.45
	B	0.169	3.55	41.85	122.85	96.46
	T	0.148	3.75	45.86	124.85	89.25
DC Female	H	0.146	3.46	47.47	110.56	82.45
	B	0.162	3.69	45.76	118.46	91.85
	T	0.158	3.59	44.78	121.85	86.46
	Mean	**0.16**	**3.55**	**45.75**	**119.52**	**88.65**
	SD	**0.01**	**0.17**	**2.37**	**5.05**	**5.00**
		Standard (Silymarin)				
STD Male	H	0.154	3.48	47.17	116.33	91.45
	B	0.149	3.48	44.81	118.89	90.69
	T	0.168	3.51	49.74	121.85	85.62
STD Female	H	0.159	3.58	43.12	119.78	92.96
	B	0.168	3.55	49.74	124.85	89.12
	T	0.159	3.61	41.85	120.75	90.86

	Mean	**0.16**	**3.54**	**46.07**	**120.41**	**90.12**
	SD	**0.01**	**0.05**	**3.35**	**2.87**	**2.53**
		Test (Tamra Bhasma)				
Test	H	0.159	3.57	47.23	125.11	88.96
Male	B	0.161	3.47	45.17	127.62	92.69
	T	0.155	3.59	49.85	121.45	81.65
Test	H	0.149	3.49	44.34	121.85	88.65
Male	B	0.155	3.49	46.25	119.85	90.63
	T	0.155	3.77	48.75	122.74	91.96
	Mean	**0.16**	**3.56**	**46.93**	**123.10**	**89.09**
	SD	**0.00**	**0.12**	**2.11**	**2.81**	**3.98**



Pair-Wise Comparison of Test vs other groups of Serum Biochemical Parameters on Day 0:

Serum Biochemical Parameters	Pairs	Mean Difference	p value	Inference
Sr. Bilirubin	Test vs NC	0.001167	> 0.05	Not Significant
	Test vs DC	-0.0001667	> 0.05	Not Significant
	Test vs SC	-0.003833	> 0.05	Not Significant
Sr. Albumin	Test vs NC	0.05500	> 0.05	Not Significant
	Test vs DC	0.008333	> 0.05	Not Significant
	Test vs SC	0.02833	> 0.05	Not Significant
SGPT	Test vs NC	1.262	> 0.05	Not Significant
	Test vs DC	1.187	> 0.05	Not Significant
	Test vs SC	0.8600	> 0.05	Not Significant
SGOT	Test vs NC	2.135	> 0.05	Not Significant
	Test vs DC	3.585	> 0.05	Not Significant
	Test vs SC	2.695	> 0.05	Not Significant
ALP	Test vs NC	1.067	> 0.05	Not Significant
	Test vs DC	0.4367	> 0.05	Not Significant
	Test vs SC	-1.027	> 0.05	Not Significant

Table 8: Mean biochemical parameter- Day 0

Groups	Bilirubin	Albumin	SGPT
Normal Control	0.15±0.01	3.51±0.15	45.67±1.22
Disease control	0.16±0.01	3.55±0.17	45.75±2.37
Standard	0.16±0.01	3.54±0.05	46.07±3.35
Test	0.16±0.00	3.56±0.12	46.93±2.11

Groups	SGOT	ALP
Normal Control	120.97±7.65	88.02±5.96
Disease control	119.52±5.05	88.65±5.00
Standard	120.41±2.87	90.12±2.53
Test	123.10±2.81	89.09±3.98

1) As compare to Disease Control group all the animals showed non-significant increase in all parameter. P value is 0.999 that is higher than 0.05. Results were found to be non-significant.

One way anova was used to find out difference between DC, STD and Test group.

Table 10: Biochemical Parameter – Day 15.

Group	Animal Marking	Billirubin	Albumin	SGPT	SGOT	ALP
NC Male	H	0.149	3.34	47.25	128.69	87.65
	B	0.158	3.68	43.89	127.35	95.86
	T	0.166	3.62	48.95	118.46	85.65
NC Female	H	0.158	3.50	47.35	114.35	82.36
	B	0.147	3.54	45.69	116.75	88.36
	T	0.155	3.48	41.12	121.85	87.25
	Mean	**0.16**	**3.53**	**45.71**	**121.24**	**87.86**
	SD	**0.01**	**0.12**	**2.83**	**5.81**	**4.47**
		Disease Control (Mono Sodium Glutamate)				
DC Male	H	0.163	3.58	81.45	158.21	273.45
	B	0.181	3.57	78.987	163.85	294.85
	T	0.194	3.61	88.68	154.25	298.65
DC Female	H	0.156	3.55	79.68	157.95	277.21
	B	0.188	3.57	80.46	161.65	289.65
	T	0.183	3.59	77.85	159.85	283.25
	Mean	**0.18**	**3.58**	**81.18**	**159.29**	**286.18**
	SD	**0.01**	**0.02**	**3.87**	**3.32**	**9.94**
		Standard (Silymarin)				
STD Male	H	0.175	3.59	75.48	165.86	285.12
	B	0.186	3.62	83.57	159.69	265.45
	T	0.201	3.59	79.36	156.95	291.21
STD Female	H	0.21	3.55	84.63	160.25	274.28
	B	0.188	3.58	79.35	155.69	284.65
	T	0.195	3.6	78.65	152.12	289.12
	Mean	**0.19**	**3.59**	**80.17**	**158.43**	**281.64**

	SD	**0.01**	**0.02**	**3.38**	**4.68**	**9.85**
		Test (Tamra Bhasma)				
Test	H	0.175	3.69	81.65	164.32	295.45
Male	B	0.157	3.59	78.32	155.89	275.65
	T	0.159	3.62	80.25	159.14	289.65
Test	H	0.19	3.58	74.36	161.36	285.12
Male	B	0.182	3.59	81.18	157.25	265.12
	T	0.21	3.54	79.46	160.36	271.45
	Mean	**0.18**	**3.60**	**79.20**	**159.72**	**280.41**
	SD	**0.02**	**0.05**	**2.66**	**3.01**	**11.58**

Pair-Wise Comparison of Test vs other groups of Serum Biochemical Parameters on Day 15:

Serum Biochemical Parameters	Pairs	Mean Difference	p value	Inference
Sr. Bilirubin	Test vs NC	0.02333	< 0.05	**Significant**
	Test vs DC	0.001333	> 0.05	Not Significant
	Test vs SC	-0.01367	> 0.05	Not Significant
Sr. Albumin	Test vs NC	0.07500	> 0.05	Not Significant
	Test vs DC	0.02333	> 0.05	Not Significant
	Test vs SC	0.01333	> 0.05	Not Significant
SGPT	Test vs NC	33.495	< 0.01	**Very Significant**
	Test vs DC	-1.981	> 0.05	Not Significant
	Test vs SC	-0.9700	> 0.05	Not Significant
SGOT	Test vs NC	33.478	< 0.01	**Very Significant**
	Test vs DC	0.4267	> 0.05	Not Significant
	Test vs SC	1.293	> 0.05	Not Significant
ALP	Test vs NC	192.55	< 0.01	**Very Significant**
	Test vs DC	-5.770	> 0.05	Not Significant
	Test vs SC	-1.232	> 0.05	Not Significant

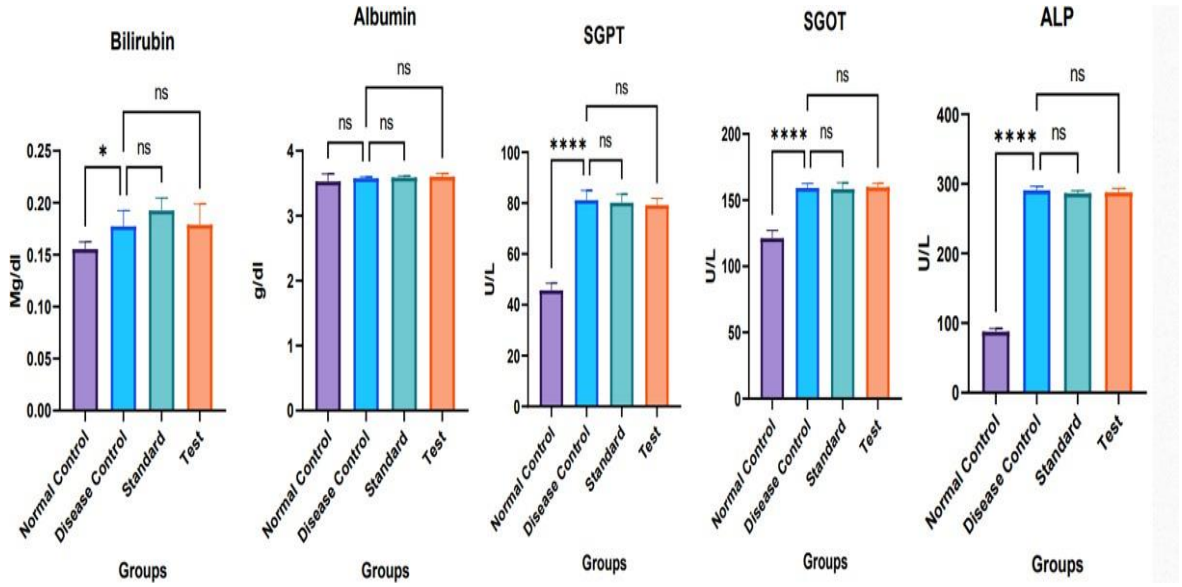


Table 12: Mean biochemical parameter- Day 15.

Groups	Bilirubin	Albumin	SGPT
Normal Control	0.16±0.01 *	3.53±0.12	45.71±2.83****
Disease control	0.18±0.01	3.58±0.02	81.18±3.87
Standard	0.19±0.01	3.59±0.02	80.17±3.58
Test	0.18±0.02	3.60±0.05	79.20±2.66

Groups	SGOT	ALP
Normal Control	121.24±5.81 ****	87.86±4.47****
Disease control	159.29±3.32	290.67±5.73
Standard	158.43±4.68	286.61±3.53
Test	159.72±3.01	287.94±5.60

1) Control animals were showed significant lower values of SGPT, SGOT, ALP and bilirubin. No significant changes were obtained in Disease control, Standard and Test group animals.

2) Disease control, Standard and Test group animals showed significantly increased SGPT, SGOT, ALP, and ALBUMIN after Monosodium Glutamate treatment. ($p<0.0001$).

One way anova was used to find out difference between DC, STD and Test group.

Table 14: Biochemical Parameter – Day 21.

Group	Animal Marking	Billirubin	Albumin	SGPT	SGOT	ALP
NC Male	H	0.152	3.50	46.85	128.85	90.25
	B	0.16	3.55	44.65	128.32	91.65
	T	0.158	3.59	47.32	121.65	88.65
NC Female	H	0.162	3.55	47.68	118.23	83.12
	B	0.152	3.57	46.56	116.23	86.21
	T	0.149	3.51	42.68	122.85	85.65
	Mean	**0.16**	**3.55**	**45.96**	**122.69**	**87.59**
	SD	**0.01**	**0.03**	**1.92**	**5.15**	**3.17**
		Disease Control (Mono Sodium Glutamate)				
DC Male	H	0.175	3.88	79.35	188.45	380.12
	B	0.185	3.65	76.12	155.65	374.25
	T	0.205	3.68	81.36	165.43	381.21
DC Female	H	0.165	3.78	79.36	174.65	378.65
	B	0.201	4.11	80.46	168.12	370.12
	T	0.185	3.95	82.35	159.12	371.85
	Mean	**0.19**	**3.84**	**79.83**	**168.57**	**376.03**
	SD	**0.02**	**0.17**	**2.16**	**11.82**	**4.60**
		Standard (Silymarin)				
	H	0.175	3.56	52.65	132.85	140.12
STD Male	B	0.182	3.59	60.95	139.52	149.26
	T	0.188	3.55	52.14	141.12	145.25

	H	0.179	3.57	59.58	138.52	148.35
STD Female	B	0.175	3.56	59.31	135.86	145.65
	T	0.181	3.58	58.46	140.52	144.25
	Mean	**0.18**	**3.57**	**57.18**	**138.07**	**145.48**
	SD	**0.00**	**0.01**	**3.80**	**3.15**	**3.25**
		Test (Tamra Bhasma)				
	H	0.178	3.64	61.22	149.88	150.85
Test	B	0.159	3.55	59.53	151.61	149.68
Male	T	0.155	3.58	63.45	145.85	151.86
	H	0.191	3.52	62.45	145.38	148.36
Test	B	0.185	3.58	59.86	149.85	151.69
Male	T	0.205	3.51	61.21	151.23	152.85
	Mean	**0.18**	**3.56**	**61.29**	**148.97**	**150.88**
	SD	**0.02**	**0.05**	**1.49**	**2.69**	**1.63**

Pair-Wise Comparison of Test vs other groups of Serum Biochemical Parameters on Day 21.

Serum Biochemical Parameters	Pairs	Mean Difference	p value	Inference
Sr. Bilirubin	Test vs NC	0.02333	< 0.05	**Significant**
	Test vs DC	-0.007167	> 0.05	Not Significant
	Test vs SC	-0.001167	> 0.05	Not Significant
Sr. Albumin	Test vs NC	0.01833	> 0.05	Not Significant
	Test vs DC	-0.2783	> 0.05	Not Significant
	Test vs SC	-0.0005	< 0.01	**Very Significant**
SGPT	Test vs NC	15.330	< 0.01	**Very Significant**
	Test vs DC	-18.547	< 0.01	**Very Significant**
	Test vs SC	4.105	< 0.05	**Significant**
SGOT	Test vs NC	26.278	< 0.01	**Very Significant**
	Test vs DC	-19.603	< 0.01	**Very Significant**
	Test vs SC	10.902	< 0.05	**Significant**
ALP	Test vs NC	63.293	< 0.01	**Very Significant**
	Test vs DC	-225.15	< 0.01	**Very Significant**
	Test vs SC	5.402	< 0.05	**Significant**

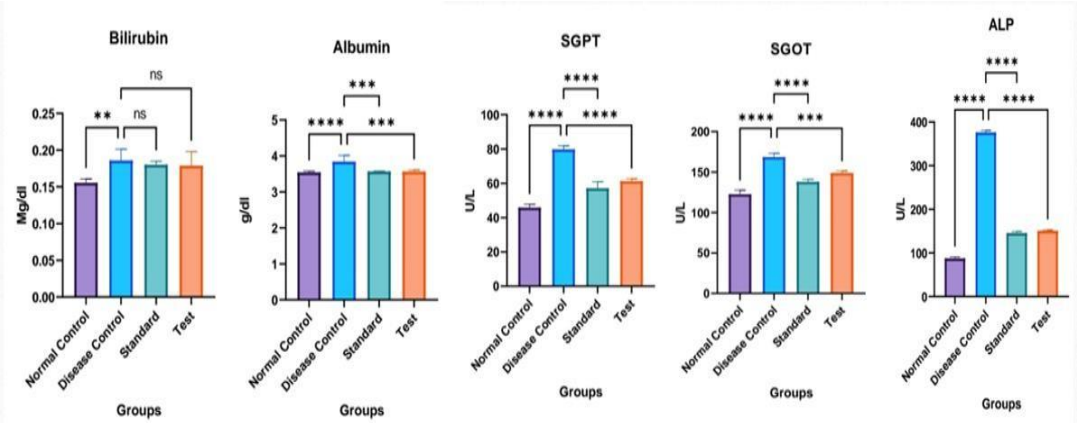


Table 16: Mean biochemical parameter- Day 21.

Groups	Bilirubin	Albumin	SGPT
Normal Control	0.16±0.01 ****	3.55±0.03 ****	45.96±1.92****
Disease control	0.19±0.02	3.84±0.17	79.83±2.16
Standard	0.18±0.00 ****	3.57±0.01 ****	57.18±3.80****
Test	0.18±0.02 ****	3.56±0.05 ****	61.29±1.49****

Groups	SGOT	ALP
Normal Control	122.69±5.15 ****	87.59±3.17 ****
Disease control	168.62±4.75	376.03±4.60
Standard	138.07±3.15 ****	145.48±3.25 ****
Test	148.97±269 ****	150.88±1.63 ****

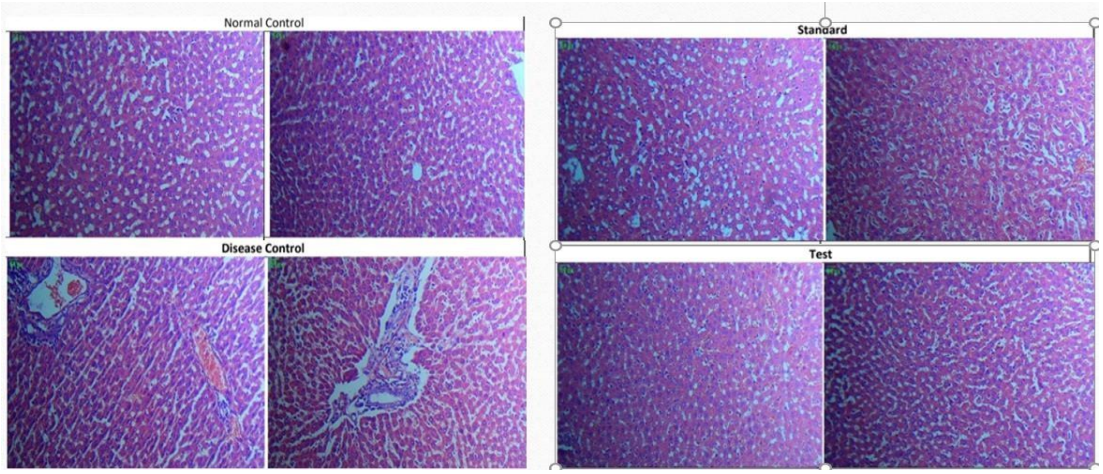
As compare to Disease Control group all the animals showed significant decrease in SGPT, SGOT, ALP and ALBUMIN.

3) Standard and Test group animals showed significantly decreased SGPT, SGOT, ALP, and ALBUMIN after Silymarin and *Tamra Bhasma* treatment. ($p<0.0001$).

One way anova was used to find out difference between DC, STD and Test group.

HISTOPATHOLOGY PARAMETER

Sections of 3-5 µm thickness of Liver were cut using a rotary microtome and stained with haematoxylin and eosin dye. The stained sections were observed under a light microscope, and photomicrographs were taken for both the control and experimental groups. Microscopic examination of liver organs from 4 groups was carried out.



DISCUSSION AND RESULTS

There were significant changes in biochemical markers of liver function test summarized in table 10 and 11. Alanine aminotransferase (ALT), aspartate aminotransferase (AST),

Bilirubin, and Alanine phosphatase (ALP) levels were raised in disease control group.

Statistical difference were observed in day 0, day 15 and day 21 biochemical parameter. Before induction (Day 0) of monosodium glutamate the biochemical parameters did not show any significant ($p>0.05$) change in their value when compared to the value of disease control.

After induction (Day 15) of monosodium glutamate the values of SGPT, SGOT and ALP were high in disease control, standard and test group.

The values of SGPT, SGOT and ALP were reduced in standard and test group after Treatment (Day 21) table 14 and 15. Normal control, standard Silymarin and test group animals showed significantly reduced levels of SGPT, SGOT, BILIRUBIN and ALP when compared with disease control group. So it concluded that no statistically changes were observed before induction, however SGPT, SGOT, BILIRUBIN and ALP were increased after induction of monosodium glutamate whereas SGPT, SGOT, BILIRUBIN and ALP decreased after treatment of Standard Silymarin and Test *Tamra bhasma.* Silymarin treated standard group showed significant decrease when compared to disease control group. The test *Tamra bhasma* group was found to be more effective similar to the Silymarin treated group.

The histological features of the normal liver as indicated a normal liver lobular architecture and cell structure. There were no pathological changes in healthy control livers. In Monosodium Glutamate-treated group, the reticular fibers have become thinner around the CV (Central Vain) compared to the normal liver section indication liver injury.

The histopathological changes induced by Monosodium Glutamate were significantly improved in animals treated with 1.12 mg/200g *Tamra bhasma*, showing lesser necrotic changes as in compared to the Monosodium Glutamate treated group. The liver section displayed the recovery of the hepatocytes into normal polyhedral shapes with some cell linings almost similar to the healthy liver.

CONCLUSION

Monosodium Glutamate is a potent hepatotoxic for rats. It is also known to produce marked liver damage in exposed animals. There was a highly significant changes observed in that group of animals which received Monosodium glutamate for 21 days (Disease control group).

Various Histopathological and biochemical changes were also recorded in the animals that received Monosodium glutamate. Hence, based on the results, it can be concluded that the deleterious effects of Monosodium glutamate were observed.

In the present study treatment with *Tamra bhasma* was found to significantly reverse the hepatotoxicity induced by Monosodium Glutamate. Biochemical parameters like SGPT, SGOT, Bilirubin, and ALP levels were controlled due to treatmentby standard as well as test drug.

During a 21 days observation period. In present study it is concluded that *Tamra bhasma* effectively protect against liver damage.

According to contemporary view Tamra bhasma showing Gara vishaghna property and preservative, additives, synthetic colouring agents which comes in Gara visha concept in Ayurved. Nature and effect of Monosodium Glutamate can be compared Garavisha concept in Agadtantra as it is artificial and does not cause immediate mortality, it is consumed with food and its bad effect can be seen over the time period. Present preclinical study shows hepatoprotective effect of Tamra bhasma against Monosodium glutamate.

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