Vol. 7/1 (2007) 58 - 65

JOURNAL OF NATURAL REMEDIES

Effect of *Tamrabhasma*, (an Indian Ayurvedic preparation of copper) on some general physiological, reproductive and laboratory parameters in rats

R. K. Goel[1]*, R. N. Maiti[1], T. Prabha[1], M. Mohan Kumar[1], Prakash[2] , G. Singh[2]

Departments of Pharmacology[1] and Anatomy[2], Institute of Medical Sciences, Banaras Hindu University, Varanasi - 221 005.

Abstract

Objective: The aim of the present study was to evaluate safety profile of *Tamrabhasma* (TMB), an Indian Ayurvedic preparation of copper found to possess significant ulcer protective activity both experimentally and clinically using some general, physiological, reproductive and laboratory parameters in rats and mice. Methods: Acute toxicity study was carried out in mice and rats of either sex treated orally with TMB (5, 20, 100 and 1000 mg/kg single oral dose). Subacute toxicity was carried out with 5, 20 and 100 mg/kg administered orally daily for 7 days while, 5mg/kg dose daily was used for 80 days for chronic toxicity study. In addition, teratological studies including both prenatal and postnatal studies were done in rats treated with TMB (5mg/kg single dose orally for 60 days plus during mating plus either upto 18 days of gestation or till delivery respectively to assess the safety profile of the drug. Results: TMB did not show any acute, subacute or chronic toxicity in terms of any change in general parameters (behaviour, mortality, weight gain, food and water intake). It did not produce any change in hematological profile, liver function tests and histopathological studies of major organs (liver, kidney, adrenals and testis) after 80 days study in rats. Teratological studies in rats showed no deleterious effect on organogenesis or intrauterine fetal development or any harmful effects on postnatal development of rat pups. Conclusion: The above observations are likely to strengthen the safe use of commonly used Ayruvedic medicine *Tamrabhasma* (TMB) in peptic ulcer diseases.

Key words: *Tamrabhasma*, toxicity, physiological, reproductive, teratological parameters

1. Introduction

Trace elements like copper, cobalt, manganese, gold etc. have got an important role in health and diseases [1]. *Tamrabhasma* (TMB) an Ayurvedic preparation of copper, is a potent antiulcerogenic drug as evidenced from various gastroduodenal ulcer studies [2-8]. The complex process of manufacture of TMB is said to reduce toxicity and improve the therapeutic quality of copper compound [9]. TMB has been indicated for the treatment of various diseases including “Amlapitta”, a clinical condition simulating peptic ulceration [10]. Antiulcerogenic effect of TMB was observed in various models of experimental

* Corresponding author
Email: rkgoel_bhu@yahoo.co.in

gastroduodenal ulcerations in rats and guinea pigs [2-4]. The mechanisms suggested for its antiulcerogenic effect are an inhibition of acid-pepsin secretion and an increase in mucin secretion and life span of mucosal cells without any effect on cell proliferation as observed in various studies [2, 4, 5]. Recently TMB has been reported to increase PGE and decrease LTC4 in human gastric and colonic mucosal incubates [6-8]. It was also found to inhibit lipid peroxidation which plays an important role in tissue damage [9].

The present work has been undertaken to elucidate the safety profile of TMB using 5, 20, 100 and 1000mg/kg by studying some of the general physiological, reproductive and laboratory toxicological parameters including the teratological study, if any, after acute, subacute or chronic administration in rats/mice for its safe use as an ulcer protective drug. The above doses of TMB were selected on the basis of our earlier reported work on experimental ulcers and gastric secretion including toxicity [2] and rat gastric mucosal resistance [8].

2. Materials and Methods

2.1 Drugs

Tamrabhasma (TMB, batch No. S6 150, MED 10/1985) was obtained from Dabur India Limited, New Delhi.

2.2 Animals

Charles -Foster strain of albino rats (120-160 g) and mice (25-30 g) of either sex (equal number of both the sexes), were used for acute and subacute toxicity studies while 25-30 g rats were used for chronic toxicity and teratological study. Animals were obtained from the Central Animal House of the Institute. They were kept in colony cages in the department temperature controlled room (26 ± 3°C) with 10:14 h light and dark cycles for at least 1 week before using them in the experiment. They were allowed free access to the diet and given water *ad libitum*. Prior permission was sought from the Institutional Animal Ethical Committee for conducting the study.

In all the studies animals were divided into two groups. One group served as control and received distilled water orally while, the other group TMB orally through orogastric tube and served as test group.

2.3 Induction of toxicity

2.3.1 Acute toxicity study

TMB 5, 20, 100 and 1000 mg/kg as water suspension (1 ml/100 g body weight) was administered orally to the test groups (both rats and mice) while control groups received distilled water (DW) orally. All the groups were observed for 72 hours to note any changes in behavioural pattern including level of consciousness, gait, food and water intake and mortality.

2.3.2 Subacute toxicity study

TMB was administered orally in the dose of 5, 20 and 100 mg/kg (1ml/100 g body weight as water suspension) once a day for 7 days to the test group while the control group received DW for the same period. The animals were weighed on 8th day to note any change in body weight and blood was collected from the retro-orbital plexus after light ether anesthesia to study various blood parameters like hemoglobin percentage, total and differential leukocyte counts. The animals were sacrificed by decapitation and the adrenal glands were taken out for determination of their weights in both the groups.

2.3.3 Chronic toxicity study

TMB was administered orally to test group in the dose of 5mg/kg (1mg/ml water suspension) once a day for 80 days while the control group

received DW for same period. During treatment period, care was taken to separate male and female rats in each group as soon as the animal sex was differentiated and they were kept in separate cages till their full treatment i.e. up to 80 days. During the period of treatment behavioural pattern was observed daily, body weight was recorded at weekly interval or mentioned otherwise and daily food and water intake was noted in both the groups. At the end of 80 days treatment the blood was collected from the retro-orbital plexus of rats after light ether anesthesia to study hematological parameters like hemoglobin percentage, total and differential leukocyte counts, and liver function tests like bilirubin, alkaline phosphates, total protein and albumin. The animals were sacrificed by decapitation and the adrenal glands were taken out for determination of their weights in both the groups. Kidneys, testis, liver and spleen were also taken out from both the groups for histological study.

Table 1. Chronic toxicity studies with TMB (5 mg/kg) in rats (80 days treatment).

PARAMETERS	**MALE RATS**		**FEMALE RATS**	
	Control	**TMB**	**Control**	**TMB**
Organ weight (n=6)				
Liver (g)	2.78 ± 0.21	2.89 ± 0.23	2.95 ± 0.19	3.07 ± 0.23
Kidney (g)	0.79 ± 0.08	0.83 ± 0.13	0.64 ± 0.04	0.75 ± 0.03
Adrenals (mg)	26.0 ± 0.53	24.9 ± 0.76	23.7 ± 0.81	22.3 ± 0.52
Testes (g)	1.43 ± 0.07	1.66 ± 0.09	-	-
Haematological parameters (n=8)				
Hemoglobin (g %)	12.8 ± 0.49	13.1 ± 0.83	12.2 ± 0.58	11.8 ± 0.51
WBC (cells/mm^3)	6875 ± 930	7935 ± 507	7435 ± 675	8525 ± 976
Liver function tests (n=6)				
SGOT (IU/L)	0.61 ± 0.06	0.76 ± 0.12	0.76 ± 0.10	0.89 ± 0.13
SGPT (IU/L)	62.5 ± 3.35	70.3 ± 4.03	67.4 ± 2.6	67.9 ± 1.9
Total Protein (mg/dl)	5.29 ± 0.31	5.75 ± 0.16	6.56 ± 0.33	6.29 ± 0.41
Albumin (g/dl)	2.30 ± 0.74	2.41 ± 0.19	3.07 ± 0.21	2.98 ± 0.11
Renal function tests (n=6)				
Blood urea (mg/dL)	41.1 ± 6.7	38.9 ± 4.2	45.7 ± 2.6	41.1 ± 2.3
Creatitnine (mg%)	1.11 ± 0.09	1.21 ± 0.10	1.05 ± 0.13	1.09 ± 0.09
General paremeters (n=8)				
Body weight (g/rat)	248 ± 4.5	257 ± 3.5	233 ± 5.7	229 ± 6.1
Food intake (g/rat/day)	42.4 ± 3.71	41.8 ± 2.62	46.6 ± 2.73	48.7 ± 2.49
Water intake (ml/rat/day)	35.6 ± 2.01	34.4 ± 2.55	40.9 ± 2.56	42.7 ± 1.67

Values are mean ± SE

TMB: *Tamrabhasma*; WBC: White Blood Cells; SGOT: Serum Glutamate Oxaloacetate Transaminase; SGPT: Serum Glutamate Pyruvate Transaminase.

Table 2. Intrauterine development of rat fetuses at 18 day of gestation period in control (DW) and TMB treated females. (Naked-eye observation of rat uterus for fetal resorption and organs and touch reflex)

Observation	Control (DW)	TMB-Treated
No. of female rats	6	7
No. of fetuses	47	49
No. of resorbed fetus	2	Nil
Fetal Parameters:		
a) Any abnormality		
Eyes	Nil	Nil
Ears	Nil	Nil
Mouth	Nil	Nil
Limbs	Nil	Nil
b) Haemorrhage	Nil	1/49
c) Touch reflex present	47/47	49/49
d) Any other defect	Nil	Nil
e) C-R length (mm)	32.9 ± 0.81	32.1 ± 0.78
f) Cord length (mm)	24.3 ± 0.79	25.0 ± 0.67
g) Tail length (mm)	13.3 ± 0.31	13.2 ± 0.43
h) Total weight (mg)	3786.6 ± 151.7	3850.5 ± 238.1
i) Fetal weight (mg)	3101.4 ± 156.0	3145.6 ± 215.8
Placental parameters:		
a) Placental weight (mg)	685.3 ± 24.4	704.9 ± 32.7
b) Cord attachment (C/L)		
Central	46	47
Lateral	1	2

Values are mean ± SE. C-R: Crown and Rump.

Table 3. Postnatal development of rat pups up to 28 days of their life of control (DW) and TMB-treated parents

Observations	Control (DW)	TMB (5mg/kg)*
Pups per litter	8.0 ± 1.1	8.0 ± 0.8
No. of pups death	2	1
Haemorrhage	Nil	Nil
Congenital Abnormality:		
Eyes and ears, mouth, limbs	Nil	Nil
Appearance of hair	6-7 days	6-7 days
Opening of eyes	15 - 18 days	15 - 18 days
Opening of ears	15 - 16 days	15 - 16 days
Testes descent (in males)	19 - 21 days	19 - 21 days

* TMB orally for 60 days before mating and approximately for 30 days during mating and gestation period in both male and female rats (n=6)

2.3.4 Teratological study

The test group was treated with TMB in dose of 5mg/kg once a day for 60 days as followed above. On 61st day, the animals were then mixed in the ratio of 1 male and 3 female for mating in both the test and control group and treatment was continued as before. Vaginal smear of each female was examined daily for presence of sperms in the morning between 9 AM to 10 AM and day1 of pregnancy for each female rat was, thus, found. The respective treatment were then continued in the pregnant female rat either up to 18 days in case of prenatal study or continued till term for postnatal study i.e. for 21-22 days.

2.3.5 Prenatal study (intrauterine development of fetuses)

The female rats were anaesthetized with ether on day 18 of gestation in number of animals subjected to experiment. The fetuses were taken out of each litter and were studied for number of fetuses for dame, fetal resorption, and abnormality in touch reflex. Crown-rump (C-R), cord and tail lengths were measured for each fetus. Total weight (fetal + placental), fetal weight and placental weight were measured in each rat. Attachment of cord and its position on placenta was also noted.

2.3.6 Postnatal study

Treatment was continued till delivery for postnatal study. The number of rat pups per dame was noted. The development of rat pups was further noted up to 28 days of their postnatal

Table 4. Postnatal development of rat pups up to 28 days of their life of control (DW) and TMB-treated parents

Days	Body weight (mg)		Body length (mm)		Tail length (mm)		Leg length (mm)		Arm length(mm)	
	DW	TMB	DW	TMB	DW	TMB	DW	TMB	DW	TMB
1	5942	5467	48.8	48.8	20.2	19.3	12.4	11.9	9.9	9.4
	± 143	± 214	± 1.1	± 0.3	± 0.4	± 0.4	± 0.2	± 0.2	± 0.2	± 0.2
5	7792	7301	58.4	55.0	24.8	24.4	14.0	13.9	11.1	10.8
	± 307	± 421	± 2.3	± 0.7	± 1.2	± 0.8	± 0.1	± 0.4	± 0.1	± 0.4
9	11753	12503	67.3	67.1	33.0	33.1	16.7	17.1	12.5	12.6
	± 319	± 723	± 0.7	± 0.9	± 1.7	± 1.3	± 0.3	± 0.5	± 0.2	± 0.3
13	15207	15803	76.3	73.5	43.0	41.7	19.3	19.6	13.7	13.8
	± 930	± 903	± 1.9	± 2.1	± 2.3	± 2.5	± 0.4	± 0.2	± 0.3	± 0.3
17	19653	20583	83.9	80.5	53.7	54.0	21.5	22.4	14.9	15.2
	± 467	± 902	± 2.6	± 3.1	± 4.3	± 4.1	± 0.7	± 0.5	± 0.4	± 0.3
21	25801	26750	93.4	89.0	62.1	63.9	23.4	23.9	15.3	16.1
	± 133	± 221	± 2.4	± 1.5	± 6.0	± 4.8	± 0.6	± 0.5	± 0.4	± 0.3
28	40412	42216	101.9	102.1	83.2	83.9	27.7	28.4	16.5	16.9
	± 881	± 735	± 1.4	± 1.4	± 7.5	± 6.1	± 1.1	± 0.5	± 0.3	± 0.2

Values are Mean ± SE of litters of 6 mothers

life for various land marks. Number of rat pups per litter and any abnormalities in their eyes, ears, mouth, limbs or any other defect were noted at the time of delivery. They were also observed for various other developmental parameters like body weight, shape, body length, and arm, tail and leg lengths from day 1 to day 28 of their life. The days of eye opening, appearance of hair and descent of testes were also noted.

2.3.7 Statistical analysis

The results were expressed as mean ± SE of 6-8 rats in each group. The data were analysed following unpaired student's *t* test.

3. Results

The result of acute toxicity study with different doses indicated no remarkable changes in general behaviour, gait, food or water intake both rats and mice. There was no mortality in either group. The result of the subacute toxicity study when compared with control group did not show any change in body weight but tendency to increase in total leukocyte count (TLC) was observed in TMB-treated group at 100 mg/kg. Percent differential count (DLC) and adrenal gland weight did not show any difference from the control group while change in other parameters were either little or not at all at all the doses level.

The result of chronic toxicity study also showed a little or no change in various blood parameters, liver function tests and adrenal weights in TMB-treated group as in subacute study (Table 1). No change was also observed in general physiological parameters like body weight, food and water intake in TMB-treated group as compared to control when the animals

Fig. 1.
Post-natal development body length of rat pups up to 28 days of their life in control and TMB treated groups. Results are the mean ± SE of litters of 6 mothers.

Fig. 2.
Post-natal development body weight of rat pups up to 28 days of their life in control and TMB treated groups. Results are the mean ± SE of litters of 6 mothers.

Values for the Figure1

Post natal body length (cm) changes

Days	Control	TMB
1	4.88 ± 0.11	4.88 ± 0.03
5	5.84 ± 0.23	5.50 ± 0.07
9	6.73 ± 0.07	6.71 ± 0.09
13	7.63 ± 0.19	7.35 ± 0.21
17	8.39 ± 0.26	8.05 ± 0.31
21	9.34 ± 0.24	8.90 ± 0.15
28	10.19 ± 0.14	10.21 ± 0.14

Values for the Figure 2

Post natal body weight (g) changes

Days	Control	TMB
1	5.942 ± 0.143	5.467 ± 0.214
5	7.792 ± 0.307	7.301 ± 0.421
9	11.753 ± 0.319	12.503 ± 0.723
13	15.207 ± 0.930	15.803 ± 0.903
17	19.653 ± 1.467	20.583 ± 0.902
21	25.801 ± 2.133	26.750 ± 1.221
28	40.412 ± 2.881	42.216 ± 1.735

were followed at 80 days of treatment. Histological study done showed no gross change in structure, hemorrhage or necrosis in liver, kidney, adrenals and testis. The results of both pre and post natal studies have been summarized in tables 2-4. The prenatal study showed no obvious teratological defects in fetuses of both control and TMB treated dames. Gross observations of rat uterus revealed no fetal resorption (Table 2). Postnatal development study of rat pups up to 28 days of their life did not show any relevant teratogenic manifestation induced by TMB (Table 3,4). Similarly postnatal study showed little or no change in body length or weight (Figs. 1& 2).

4. Discussion

Tamrabhasma (TMB) a traditional preparation of copper has been used by practioners of traditional medicine as a good remedy for *amlapitta*, a clinical entity resembling peptic ulceration [10]. It was also found to have ulcer protective effects against various acute gastro-duodenal ulcers in animals by affecting both offensive acid-pepsin secretion and mucosal lipid peroxidation and defensive mucin secretion and mucosal prostaglandins (PGs) synthesis and life span of mucosal cells [2-9].

TMB showed better potency on PGs release by human gastric and colonic mucosal incubates compared to $CuCl_2$ suggesting that other ingredients of TMB add on its effect [6]. It has an overall solubility in water of approximately 1% and 12 ng/ml of CuO forms a saturated solution at 50°C [11]. This may lead to low systemic absorption and toxicity with oral administered copper/TMB.

Single oral dose of TMB up to 1g/kg or 100mg/kg for 7 days or 5mg/kg for 80 days (effective ulcer protective dose 1-5mg/kg for 3 days) were not found to produce any mortality and change in general parameters like weight, food or water intake etc. No significant change was observed in any hematological parameters or on liver and kidney function test including histological studies of various tissues like liver, kidney, adrenals and testis between TMB and control groups.

There are four accepted criteria for a drug to be called as teratogen, namely 1) fetal resorption, 2) stunting in size or growth retardation, 3) malformation (gross or histological) and 4) functional disorders or behaviourial changes [12]. Teratological study with TMB using the dose of 5mg/kg for 60 days in both male and female mating rats and then continued in female pregnant rats for another 21 days or till delivery indicated no defects in the fetuses of both control or TMB-treated animals in prenatal and postnatal studies.

5. Conclusion

The data of present toxicity studies tend to agree that oral copper/TMB does not give rise to cumulative toxicity and will be safe for the treatment of peptic ulceration, which is however, a long drawn out affair. However, a detailed and elaborate toxicity studies on tissue enzyme activities, cell lines etc and tissue deposition of copper after long term treatment is needed to exclude any major toxicity of TMB.

6. Acknowledgements

The authors are grateful to Dabur India Ltd, New Delhi for providing the *Tamrabhasma* for the study.

References

1. WHO. Geneva. (1996) Trace elements in human nutrition and health; 124.

2. Sanyal AK, Pandey BL, Goel RK. (1982) *J. Ethnopharmacol.* 82(5): 79-89.

3. Das PK, Singh PP, Pandey BL, Goel RK. (1983) The scientific basis of the use of copper preparation in indigenous system of medicine. In: First Int. Conf. on Elements in Health and diseases. WHO, New Delhi, IHMMR: 178-183.

4. Pandey BL, Goel RK, Das PK. (1983) *Indian J. Exp. Biol.* 21: 258-264.

5. Tavares IA, Goel RK, Bennett A. (1990) *Adv. Prost. Thromb. & Leuk. Res*. 21: 785-788.

6. Goel RK, Tavares IA, Bennett A. (1992) *J. Pharm. Pharmacol.* 44: 862-864.

7. Goel RK, Maiti RN. (1992) In: F. Capasso, N. Mascolo (Eds.) *First International symposium on natural Drugs and the Digestive Tract*, Naples, Italy,EMSI, Roma: 73-76.

8. Goel RK, Maiti RN, Mukhopadhyaya K. (1994) *Indian J. Exp. Biol.* 32: 559-561.

9. Pattanaik N, Singh AV, Pandey RS, Singh BK, Mohan Kumar, Dixit SK, Tripathi YB. (2003) *Indian J. Clin. Biochem.* 18 (2): 181-189.

10. Bhavprakash Nighantu of Shri Bhava Misra (1500-1600 A.D.) (1969) In: Datuwade Varga, Misra BS, Vaisya RL. (Eds.) V Edn. Chaukhambha Sanskrit Santhan: Varanasi 605-606.

11. Hayward A, Hearn B, Hunt MR. (1967) *Nature* 215: 73-80.

12. Torchinsky A, Lishanski L, Wolstein O, Shepshelovich J, Orenstein H, Savion, Zaslavsky Z, Carp H, Brill A, Distein R, Toder V, Fein A. (2002) *BMC Dev. Biol.* 2 (1): 2.