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Chronic toxicity study of Somanathi Tamra Bhasma in albino- rats

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ABSTRACT

Somanathi Tamra Bhasma, a special method of Tamra bhasma preparation, was studied for its chronic toxicity in Wistar strain albino rats. In this study Somanathi Tamra Bhasma was administered orally daily to different groups of albino rats in TED (Therapeutically effective dose), TED×5 (5 times the therapeutically effective dose) and TED×10 (10 times the therapeutically effective dose) doses for 3 months by following Ayush guidelines for evaluation of ASU drugs. Behavioural, ponderal, hematological, biochemical and histopathological parameters were considered for the assessment of data. Somanathi Tamra Bhasma was found to be relatively safe at TED and TED×5 dose levels when behavioural, ponderal, hematological, bio-chemical and histopathological parameters were studied and mild to moderate toxic at TED×10 treated group. The overall chronic toxicity study data indicates that the test substance Somanathi Tamra Bhasma at TED and TED×5 dose levels is very well tolerated since no toxicity symptoms were observed in these groups in any of the parameters analyzed.

Keywords: Somanathi Tamra Bhasma, Chronic toxicity study, Histopathological.

INTRODUCTION

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[1] (toxic effects). But, if the same Tamra processed properly by following standard procedures, it can be converted into good and effective medicine. In most of the rasa granthas systematic methods of preparation of Tamra bhasma were well mentioned by using different medias. Somanathi tamra Bhasma[2] was a special method of tamra bhasma preparation, which was prepared by using shudha tamra, parada, gandhaka, haritala and manashila and considered to be more effective. In this present era, the medical practitioners have many legal bindings to prescribe safe and effective medicines. To provide essential scientific information and data related to safety profile of the drug, toxicity studies are essential. The chronic toxicity study will reveal the probable mode of action of the drug, its dose related response and toxicity profile in detail. Since there is no enough scientific references are available related to chronic toxicity of Somanathi Tamra Bhasma, which was regularly used by Ayurvedic practitioners, the present study was under taken to provide additional scientific proof for safety profile of Somanathi Tamra Bhasma.

MATERIALS & METHODS

All the materials required for the preparation of Somanathi Tamra Bhasma were procured from SDM Ayurveda Pharmacy, Udupi, after proper authentication of the ingredients the Somanathi Tamra Bhasma was prepared as per the reference of Rasaratna Samuchchaya[2] at Rasashastra & Bhaishajya kalpana practical hall of SDM college of Ayurveda Udupi. After the processing of Somanathi Tamra Bhasma, the bhasma sample was subjected for bhasma siddhi pareekshyas with Ayurvedic and Modern sophisticated tests, to confirm the quality of bhasma. After getting positive results the Somanathi Tamra Bhasma sample was utilized for experimental study.

Animals: In bread wistar strain albino rats of either sex of body wt. ranging from 170-200 g. were obtained from central animal house of the SDM Centre for Research in Ayurveda and Allied Sciences, Udupi. They were maintained at standard housing conditions and fed with standard animal pellet and provided with tap water ad libitum during the experiment. The institutional animal ethical committee (IAEC-SDMCAU/ACA-49/EC13/10-11) permitted the study.

Chronic Toxicity Study: The study was conducted as per Ayush guidelines for toxicity studies i.e., Guideline

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for toxicity studies i.e., Guidelines for evaluation of Ayurveda, Siddha and Unani drugs and other traditional medicine of India[3].

Wistar strain albino rats of either sex weighing 175- 200 g. in groups of 8 for each dose level of Somanathi Tamra Bhasma were used for experimental study. Chronic toxicity was assessed after single daily administration of Somanathi Tamra Bhasma at therapeutic dose 13.5(TED), 67.5(5 times TED) & 135 mg/200 g. (10 times TED), for a period of 90 days. Test drug was given as solution in 0.5% C.M.C., control group administered with the 0.5% C.M.C. solution. Toxicity was assessed in terms of body weight, behavior, food conversion ratio, histological study of vital organs and tissues (Brain, Heart, Lungs, Uterus, Testis, Kidney, Liver, Stomach and Bone marrow), bio- chemical assessment (S.G.O.T., S.G.P.T., Serum Alkaline Phosphate, Serum Total Protein, Serum Creatinine, Blood Urea & Blood Glucose) and hematological changes (Hemoglobin, W.B.C., R.B.C., P.C.V., M.C.V., M.C.H., R.D.W.C.V., R.D.W.S.D., M.C.H.C. & Platelet count).

RESULTS

Table 1: Effect of Somanathi Tamra Bhasma on Food conversion ratio in Wister strain albino rats during chronic toxicity study

Groups	Food conversion ratio					
	15th day MEAN± SEM	30th day MEAN± SEM	45th day MEAN± SEM	60rd day MEAN± SEM	75th day MEAN± SEM	90th day MEAN± SEM
Control	2.99 ± 0.18	2.75 ± 0.13↓	2.66 ± 0.27↓	2.76 ± 0.25↑	2.38 ± 0.12↓	2.61 ± 0.31↑
TED	3.11 ± 0.14	2.72 ± 0.17↓	2.85 ± 0.28↑	2.61 ± 0.17↓	2.67 ± 0.29↑	2.42 ± 0.17↓
TEDx5	2.79 ± 0.18	2.82 ± 0.25↑	2.46 ± 0.09↓	2.50 ± 0.19↑	2.90 ± 0.14↑	2.92 ± 0.15↑
TEDx10	2.69 ± 0.14	2.87 ± 0.17↑	2.64 ± 0.23↓	2.11 ± 0.14↓	3.10 ± 0.25↑	3.09 ± 0.21↓

Data: MEAN±SEM ↑- Increase ↓- Decrease

Marginal decrease in food conversion ratio was noted in control and TED treated groups and marginal increase was noted in TED × 5 & TED × 10 treated groups, which were statistically non significant.

Table 2: Effect of Somanathi Tamra Bhasma on percentage changes in body weight of Wister strain albino rats during chronic toxicity study.

Groups	% changes in body weight					
	15th day MEAN± SEM	30th day MEAN± SEM	45th day MEAN± SEM	60rd day MEAN± SEM	75th day MEAN± SEM	90th day MEAN± SEM
Control	4.11 ± 1.30↑	17.33 ± 5.29↑	13.14 ± 2.77↑	16.16 ± 3.18↑	18.89 ± 3.49↑	22.22 ± 4.07↑
TED	5.49 ± 1.16↑	17.4 ± 5.70↑	17.95 ± 1.85↑	19.88 ± 1.90↑	23.92 ± 2.25↑	26.77 ± 2.59↑
TEDx5	15.34 ± 3.90↑	29.83 ± 9.10↑	33.31 ± 9.03↑	43.48 ± 11.55↑	40.72 ± 12.67↓	38.71 ± 11.07↓
TEDx10	18.51 ± 4.97*↑	31.29 ± 9.65↑	32.55 ± 9.61↑	37.21 ± 10.91↑	38.09 ± 11.65↑	40.96 ± 12.51↑

Data: MEAN±SEM, *P<0.05 ↑- Increase ↓- Decrease

A uniform increase in the body weight was observed in all the dose levels of test drugs, in comparison to their initial body weights, which are statistically non- significant.

Table 3: Effect of different dose levels of Somanathi Tamra Bhasma on organs weight (g.) of albino rats.

Name of the organ	Control (0.5% CMC Soln.)	TED (13.5 mg/200 g.)	TED×5 (67.5 mg/200 g.)	TED×10 (135 mg/200 g.)
Brain	1.90±0.05	1.98±0.05	1.47±0.08	1.32±0.03
Heart	0.81±0.08	0.85±0.10	0.76±0.03	0.74±0.02
Liver	7.11±0.44	8.01±0.40	7.79±0.42	8.31±0.20
Lungs	1.34±0.10	1.19±0.04	1.51±0.08	1.77±0.11
Kidney	1.43±0.08	1.57±0.08	1.94±0.19	1.84±0.06
Stomach	1.89±0.15	1.69±0.07	1.47±0.12	1.53±0.04
Testis	1.93±0.62	2.60±0.08	2.83±0.17	3.00±0.07
Uterus	0.56±0.09	0.58±0.07	0.70±0.06	0.70±0.04

Data: MEAN±SEM

Decrease in the weight of brain was observed in all the dose level groups in comparison to control group and TED × 5 & TED × 10 treated groups shown statistically significant decrease in absolute and relative weight of brain. Decrease in the weight of heart was observed in all the

dose level groups in comparison to control group and TED × 10 treated group shown statistically significant decrease in relative heart weight.

Table 4: Effect of different dose levels of Somanathi Tamra Bhasma on hematological parameters in albino rats

Parameter studied	Control (0.5% CMC Soln.)	TED (13.5 mg/200 g.)	TED×5 (67.5 mg/200 g.)	TED×10 (135 mg/200 g.)
Haemoglobin (g)	15.35± 0.14	15.78±0.29	15.98±0.57	16.08±0.31
W.B.C. (10^3µL)	7166.66±997.22	7300±990.29	9033.33±1194.1	9950±889.85
R.B.C. (10^6µL)	7.90± 0.20	8.05±0.16	7.96±0.30	8.33±0.16
P.C.V. (%)	42.2± 0.71	43±0.45	44.68±1.46	45.06±1.10
M.C.V. (fL)	53.48± 0.77	53.58±0.60	55.13±0.82	54.31±0.39
M.C.H. (Pg)	19.78± 0.28	19.43±0.20	19.78±0.24	19.3±0.19
R.D.W.C.V. (%)	12.7± 0.24	13.23±0.14	12.94±0.20	13.5±0.31
R.D.W.S.D. (fL)	24.93±0.50	25.5±0.26	25.21±0.30	26.3±0.91
M.C.H.C. (g/dL)	37.31±0.30	36.95±0.33	35.75±0.17	35.85±0.25
Platelet (10^3/)µL	7.33±0.42	6.91±0.18	6.95±0.19	5.93±0.20

Data: MEAN±SEM

Increase in hemoglobin was observed in all dose level groups in comparison to control and which was statistically non significant. Marginally high increase was noted in W.B.C. count in TED×5 & TED×10 treated groups, non significant increase in red blood corpuscles count was observed in all dose level groups in comparison to control, Decrease in the Mean corpuscular hemoglobin concentration was observed in all the dose level groups in comparison to control group and TED × 5 & TED × 10 treated groups shown statistically significant decrease. Decrease in the platelet count was observed in all the dose level groups in comparison to control group and TED × 10 treated group shown statistically significant decrease.

Table 5: Effect of different dose levels of Somanathi Tamra Bhasma on bio-chemical parameters in albino rats.

Parameter studied	Control (0.5% CMC Soln.)	TED (13.5 mg/200g.)	TED×5 (67.5 mg/200g.)	TED×10 (135 mg/200 g.)
S.G.O.T. (IU/L)	67.83±6.75	56.5±4.58	78.31±5.26	111.66±1.94
S.G.P.T. (IU/L)	34.83±1.70	30.83±1.44	32.96±0.33	39.16±2.62
Serum Alkaline Phosphate (IU/L)	125.83±8.30	128.5±4.42	130.83±11.97	224.33±32.28
Serum Total Protein (g/dl)	5.65±0.09	5.76±0.08	6.5±0.05	6.98±0.08
Serum Creatinine (mg/dl)	0.4±0.04	0.43±0.03	0.58±0.01	0.66±0.02
Blood Urea (mg/dl)	29.83±5.57	30.33±6.36	66.33±6.24	79.83±3.04
Blood Glucose (mg/dl)	131±16.00	139.83±15.92	138.16±3.83	162.16±5.99

Data: MEAN±SEM

SGOT level was marginally reduced in TED treated group and it was increased in TED × 5 & TED × 10 treated groups, and TED × 10 treated group shown statistically significant increase. TED × 10 treated group shown statistically significant increase in serum alkaline phosphate level. TED × 5 & TED × 10 treated groups shown statistically significant increase in serum total protein level, Serum urea & Serum cretinine level.

Histopathological examinations of brain, heart, kidney, liver, lungs, stomach, testis, uterus and bone marrow shown (figure 1-4) normal cytoarchitecture in TED administered group. In TED × 5 treated group moderate stimulation in uterus and testis was observed and in TED × 10 treated group cell effusion and congestion in lungs, mild fatty changes in liver and kidney (Figure 4 c & d) and decreased spermatogenesis was observed.

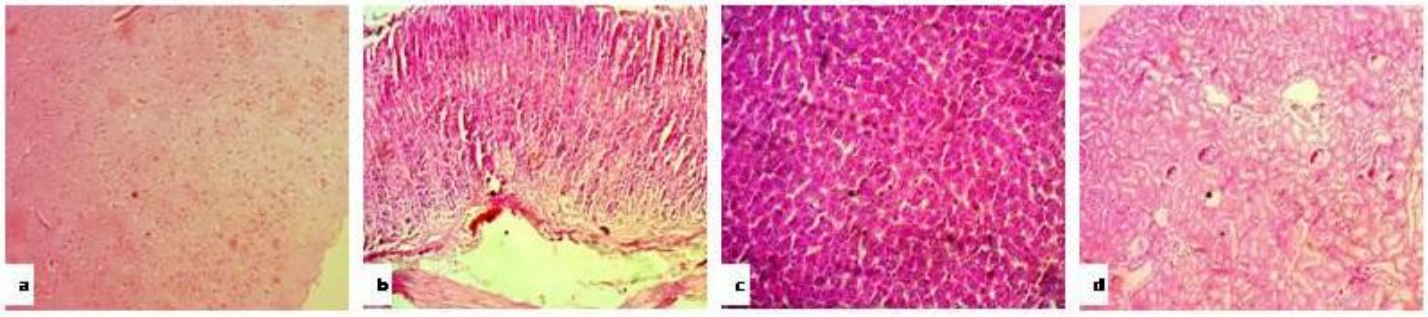

Figure 1: Photomicrograph of representative sections of Fore Brain (a), Stomach (b), Liver (c) & Kidney (d) of albino rats of control group (1×100 magnifications)

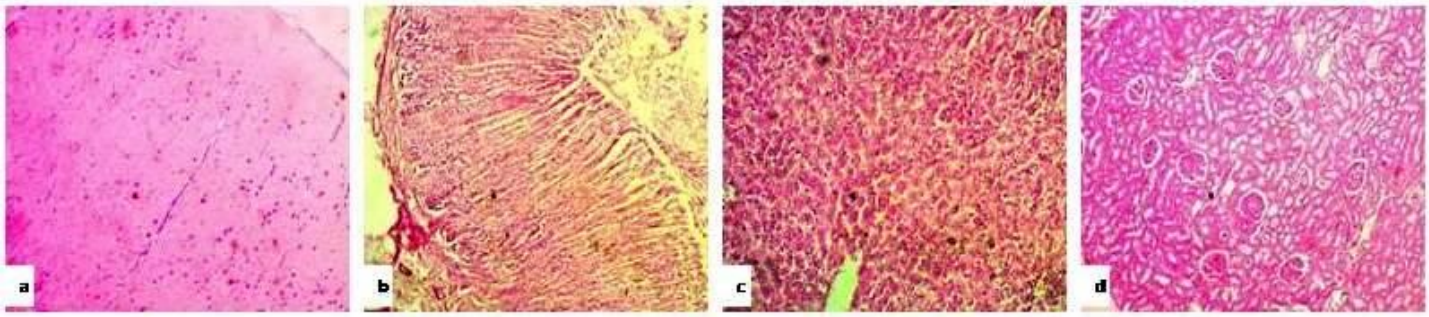

Figure 2: Photomicrograph of representative sections of Fore Brain (a), Stomach (b), Liver (c) & Kidney (d) of albino rats of TED group (1×100 magnifications)

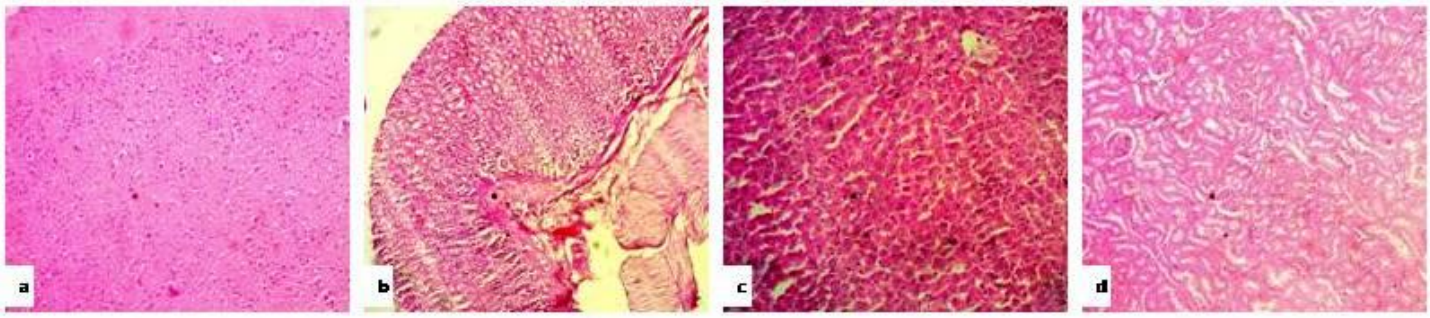

Figure 3: Photomicrograph of representative sections of Fore Brain (a), Stomach (b), Liver (c) & Kidney (d) of albino rats of TED×5 group (1×100 magnifications)

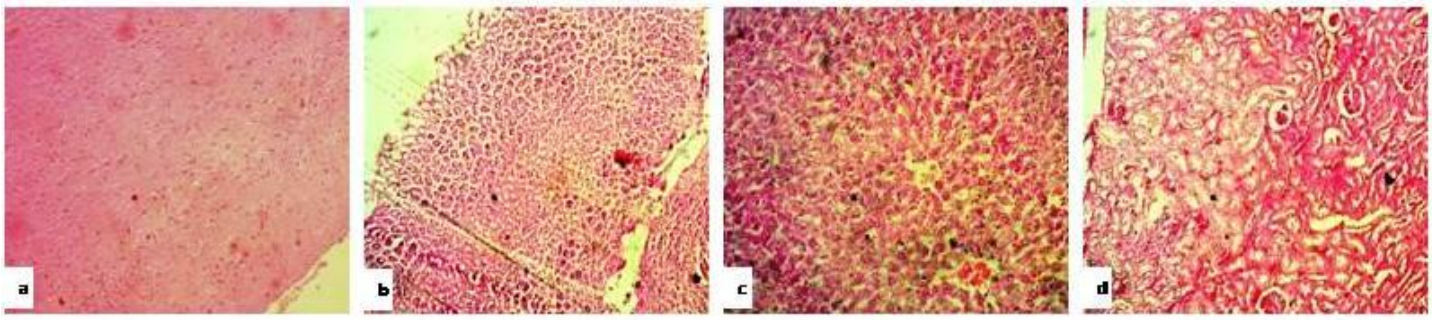

Figure 4: Photomicrograph of representative sections of Fore Brain (a), Stomach (b), Liver (c) & Kidney (d) of albino rats of TED×10 group (1×100 magnifications)

DISCUSSION

Chorionic toxicity of SomanathiTamraBhasma was studied as per the Ayush guidelines- "Guidelines for evaluation of Ayurveda, Siddha and Unani drugs and other traditional medicines of India. The study was conducted by using three dose levels, therapeutic dose, higher than therapeutic dose which produces toxicity and sub therapeutic dose (Inter mediate dose of previous doses). The higher therapeutic dose was decided as 10 times higher dose than the therapeutic dose, and sub therapeutic dose as 5 times higher the therapeutic dose.

After administration of drug in all the three dose levels for a period of 90 days, there was no any specific behavioral changes were noted, almost all group rats shown same behaviors.

The food conversion ratio shown marginal decrease in control & TED treated groups and marginal increase in TED×5 and TED ×10 treated groups, may be due to seasonal variation and test drug effect (Table1).

Uniform increase in the body weight was noted in control and also in test drug administered groups (Table 2).

Ponderal changes were recorded for 8 organs of Somanathi Tamra Bhasma treated all the three groups, most of the organs shown non significant mild variations in the weight, except brain and heart, where significant decrease in the weight was noted, in TED × 5 & TED × 10 treated groups, probably this may be due to the toxic effect of higher dose levels of Somanathi Tamra Bhasma administration for a period of 90 days (Table 3).

The hematological parameters studied in TED treated group shown no any significant changes, in TED × 5 treated group significant decrease in MCHC was noted and no any other parameters were significantly changed. In TED × 10 treated group also significant decrease in MCHC & Platelet counts were noted apart from other non significant minor variations in other parameters (Table 4). The Significant decrease in MCHC % in TED × 5 and TED × 10 tolerated groups may be due to microcytic anemia which may resulted due to the toxicity of the drug in

higher doses for long duration. The significant decrease in platelet count in TED × 10 treated group may be due to thrombocytopenia and may be caused because of trapping of platelets in the spleen, reduced production of platelets or due to increased break down of platelets due to toxic effect of the drug in higher doses for long term.

The Bio-chemical parameters studied shown increased values of SGOT & SGPT in TED × 5 and TED × 10 treated groups and specifically SGOT level was significantly increased in TED × 10 treated group, which was indicative mark of liver inflammation and injury and which was well established by histopathological examination of liver tissues from the same group, suggestive of potential toxicity of Somanathi Tamra Bhasma in higher dose levels in chronic administration for 90 days. Serum alkaline phosphate was significantly increased in TED × 10 treated group, which is suggestive of cholestasis in the biliary tract leading to liver injury and hence can be considered as one of the bio-marker for hepato- toxicity and which was well established by histopathological examinations too. Proteins are the building blocks of the body and are in state dynamic equilibrium and subject to constant chemical attack. Changes in protein level are the favored mechanism long term adaptic changes. In present study serum protein level was significantly increased in TED × 5 and TED × 10 treated groups.

Serum creatinine was increased in the all the groups studied but significantly increased in TED × 5 and TED × 10 treated groups. The serum creatinine level is considered as a marker of kidney functions, the elevation may be due to increase in muscle mass or impairment in lipid metabolism on kidney functions in liver disorders, revels the impact of impaired lipid metabolism on kidney function leading to elevation of Serum creatinine levels, which is again suggestive of toxic effect of Somanathi Tamra Bhasma in higher dose levels in chronic use.

Blood urea level was significantly increased in TED × 5 & TED × 10 treated groups (Table 5); which is indicative of renal dysfunction, again suggestive of toxicity of higher dose levels of Somanathi Tamra Bhasma. Serum blood sugar level was found to be non- significantly increased in all treated groups, this may be due to alteration in the metabolism, while using test drugs or may the effect of test drug on glucose metabolism.

Besides this the test drug Somanathi Tamra Bhasma did not produce any noticeable significant changes in any organ cytoarchitecture of TED administered group. In TED×5 treated group uterus and testis shown moderate stimulation & moderate spermatogenesis respectively. In TED×10 treated group lungs, stomach, liver, kidney, uterus and testis shown moderate cell & fluid effusion & congestion, mild erosion in epithelial layer, mild fatty changes, mild fatty changes in tubular epithelium, increased in size & epithelial folding and decreased spermatogenisis respectively (Fig 4 a,b,c &d), suggestive of mild to moderate toxicity of Somanathi Tamra Bhasma in higher dose levels when administered for 90 days, which can also well co-related with hematological and bio-chemical parameters.

CONCLUSION

After considering the data obtained from behavioural, ponderal, hematological, biochemical and histopathological parameters, it may be concluded that the test drug Somanathi Tamra Bhasma at TED × 10 treated group shown mild to moderate toxicity in higher doses, when administered for 90 days and these changes can be considered as non-specific and reversible. TED×5 administered group shown well tolerance in most of the parameters, though few changes were noted which were not of serious effect. TED administered group shown absolutely well tolerance in all the parameters studied suggesting no toxicity.

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CONFLICTS OF INTEREST

None declared.

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LIST OFABBREVIATIONS

C.M.C. - Carboxy methyl cellulose
T.E.D. - Therapeutically effective dose
W.B.C. - White blood corpuscles
R.B.C. - Red blood corpuscles
P.C.V. - Packed cell volume
M.C.V. - Mean corpuscular volume
M.C.H. - Mean corpuscular hemoglobin
R.D.W.C.V. - Red cell distribution width coefficient variation
R.D.W.S.D. - Red cell distribution width standard deviation
M.C.H.C. - Mean Corpuscular Hemoglobin Concentration
S.G.O.T. - Serum glutamic oxaloacetic pyruvic transminase
S.G.P.T. - Serum glutamic pyruvic transminase

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