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Clinical trial

Effect of *Rasamanikya* (an Arsenical Formulation) on Ponderal and Blood Parameters

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ABSTRACT

***Introduction*:** Presence of heavy metals (like Mercury, Arsenic, Lead etc.) in Ayurvedic formulations became a major concern in current scenario. Therapeutic use of these formulations since ages without any noticeable side effects is ultimate proof for their safety. Considering the growing concerns, it becomes necessity to prove their safety and efficacy to generate scientific evidences. This can also be possible through animal experiments. The present study is carried out to evaluate the effect of *Rasamanikya* (RM), an arsenical preparation, in different dose levels on ponderal, hematological and biochemical parameters in Charles foster strain albino rats. ***Methods*:** RM was prepared as per classical guidelines and administered to Charles foster albino rats at 12mg/kg and 24mg/kg for 90 consecutive days followed by 30 days recovery. Blood was collected and rats were sacrificed on 91th day. Ponderal, hematological and biochemical parameters were studied. ***Results*:** Results showed significant increase in eosinophil counts, decrease in HDL and albumin at both dose levels. Comparatively, all the differences in between the groups are insignificant and no pathological changes at ponderal, hematological and bio-chemical levels were observed. ***Conclusion*:** *Rasamanikya* has potential to generate hematological and biochemical alternations on long standing usage. Hence, mandatorily be used under strict supervision of an Ayurvedic physician.

KEYWORDS

Arsenic, Blood parameters, Heavy metal content, *Rasamanikya*

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Ayurveda uses different formulations containing metals or minerals as an integral component since centuries with claimed efficacy without noticeable untoward toxic effects. But these formulations are repeatedly being targeted about presence of heavy metals and their toxicity such as mercury, lead, arsenic etc.[1-4] There is lot of discussions and debates going on globally on this aspect. Seers are fully aware about the toxicity of heavy metals when not following code of conduct described for their pharmaceutical and treatment procedures as per classics. Even if some untoward effects are noticed, the treatment modalities for such conditions have also been prescribed. *Haratala* (arsenic tri sulphide) is used as essential component in many Ayurvedic formulations. *Rasamanika* is one among such formulations attracted controversies due to presence of arsenic in it. It is beneficial in syphilis (*Firanga*), fistula (*Nadi vrana*), gout (*Vatarakta*), skin diseases (*Twak Roga*) and diseases due to aggravated *Vata* and *Kapha* etc.[5] Though this preparation is safely practiced since ages, there is a need to provide safety profile to generate scientific evidence. Though, acute, sub-chronic toxicity study of *Rasamanikya* is reported,[6] effect on hematological and biochemical parameters is not yet reported. Considering this, the present study is aimed at screening the ponderal, hematological and biochemical changes in Charles Foster albino rats after administration of *Rasamanikya* at different dose levels.

MATERIALS AND METHODS

Test drugs

Trial drug was prepared in the laboratory of *Rasashastra* & *Bhaishajya Kalpana*, Institute for Post Graduate Teaching & Research in Ayurveda (IPGT & RA), Gujarat Ayurved University (GAU), Jamnagar by following standard guidelines as prescribed in classical Ayurvedic literature.

Raw Patra *Haratala* was collected from Pharmacy, GAU, Jamnagar and *Shodhana* (a specific treatment procedure that removes undesirable substances from raw material) was done by *Swedana* (boiling) in equal quantity of *Kanji* and *Churnodaka*[7]. The *Shodhita* material filled in *Kachkupi* was processed in EMF at 450^{0}C for 33 mins and *Rasamanikya* was prepared.

Animals

Charles foster strain albino rats of either sex weighing 200 ± 20g were obtained from the animal house attached to the pharmacology laboratory, IPGT & RA, Gujarat Ayurved University, Jamnagar and were exposed to natural day and night cycles with ideal laboratory conditions in terms of ambient temperature and humidity. Animals were fed *ad libitum* with Amrut

brand rat pellet feed supplied by Pranav Agro Industries and tap water. The experiment was carried out after obtaining permission from Institutional Animal Ethics Committee (IAEC-07/2010/05/MD) and care of animals was taken as per the CPCSEA guidelines.

Dose fixation and schedule

Clinical dose of *Rasamanikya* for humans is 125 mg per day[8]. The animal dose for rats was calculated by referring to table of Paget and Barnes[9]. On this basis, dose of the test drug for rats was found to be 12 mg/kg and considered as Therapeutically Equivalent Dose (TED). The test drug was administered in TED (12 mg/kg) and TED×2 (24 mg/kg) along with honey and ghee in equal quantity as vehicle. The drug was administered for 90 days, followed by 30 days of recovery period (Table 1).

Table 1. Test drug posology and methodology for toxicological study

Group	Description	Animals	Dose	Duration
I	Positive Control	6	-	90 days
II	RM TED	6	12 mg/kg	
III	RM TED×2	6	24 mg/kg	
IV	Recovery Control	6	-	
V	RM Recovery TED	6	12 mg/kg	
VI	RM Recovery TEDx2	6	24 mg/kg	

Experimental design

Rats were randomly assigned into six groups. Group I served as positive control (PC) receiving honey and Ghee. Group II, III received RM along with honey and Ghee at TED and TED×2 dose levels respectively. Group IV served as recovery control. Group V, VI served as recovery test group at TED and TED×2 dose levels. Body weight of all the animals was recorded at regular interval of fifteen days during the study. General behavioral pattern was observed on every week by exposing each animal to open arena. At the end of experimental period, all the animals were euthanized and gross pathological observations were performed.

Collection of Blood

At the end of experimental period, animals were anaesthetized with diethyl ether and blood was collected from supra orbital plexus in plain tube for hematological and serum biochemical investigations.

Hematological parameters

Levels of white blood cells (WBC), differential leucocytes count (DLC), red blood cells (RBC), hemoglobin (Hb), packed cell volume (PCV), platelet, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin content (MCHC) of different groups were carried out by following standard methods at pathology and biochemical lab, IPGT&RA, Jamnagar.

Biochemical parameters

Parameters were analyzed by auto analyzer (Fully automated Biochemical Random Access Analyzer, BS-200; Lilac Medicare Pvt. Ltd., Mumbai). Blood sugar level (BSL), Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvate transaminase (SGPT), Alkaline phosphatase (ALP), serum bilirubin (total), Serum cholesterol, serum triglycerides, HDL (high density lipoproteins), Blood urea, creatinine, uric acid, total protein, albumin, globulin and blood urea were studied.

Statistical analysis

The results were presented as Mean ± SEM in each group. Statistical comparisons were performed by both paired, unpaired student's t test by using Sigma stat software (version 3.1) for all the treated groups with the level of significance set at $P<0.05$.

RESULTS AND DISCUSSION

Body weight

Analysis of the data related to body weight changes in different groups reveal insignificant ($P>0.05$) increase in body weight in RM treated groups in comparison to normal control group (Table 2).

Table 2. Effect of test drugs on the body weight of albino rats recorded during toxicological study

Group	Initial (g)	Final (g)
I	190.00 ± 13.41	220.40 ± 11.75
II	193.00 ± 12.80	235.20 ± 23.77
III	181.00 ± 8.72	225.20 ± 18.95
IV	185.00 ± 5.40	207.50 ± 6.29
V	166.67 ± 16.66	190.67 ± 9.68
VI	161.25 ± 4.27	190.00 ± 18.71

Data: Mean ± SEM

Hematological parameters

Significant increase in eosinophil count was observed in group-II. Statistically significant decrease in RBC count was observed in group-V in comparison to group-IV. Platelet count was decreased insignificantly in all treated groups in both phases of the study. Other hematological parameters were not affected to significant extent in all treated groups in comparison to control group (Table 3).

Table 3. Effect of RM on various hematological parameters

Parameters	Control	RM TED	RM TED×2	RC	RMR TED	RMR TED×2
WBC (/Cumm)	7460.00 ± 429.65	8000.00 ± 577.35	7000.00± 1064.42	5233.33 ± 491.03	4900.00 ± 1300.00	5700.00 ± 100.00
Neutrophil (%)	33.00 ± 5.15	25.50 ± 3.20	36.00 ± 2.91	29.67 ± 5.78	30.00 ± 2.00	28.50 ± 1.50
Lymphocyte (%)	60.20 ± 5.64	67.50 ± 2.72	58.20 ± 3.15	66.00 ± 6.11	66.00 ± 2.00	67.50 ± 2.50
Eosinophil (%)	3.00 ± 0.00	3.75 ± 0.25*	3.20 ± 0.37	2.33 ± 0.33	2.00 ± 0.00	2.50 ± 0.50
Monocyte (%)	3.80 ± 0.73	3.25 ± 0.48	2.60 ± 0.24	2.00 ± 0.00	2.00 ± 0.00	1.50 ± 0.50
Hb (gms%)	14.28 ± 0.22	14.22 ± 0.29	13.98 ± 0.52	14.87 ± 0.35	13.60 ± 0.30	13.60 ± 0.10
PCV (%)	43.94 ± 0.68	43.82 ± 0.90	42.96 ± 1.58	46.16 ± 1.07	43.15 ± 1.05	43.15 ± 1.15
RBC (mil/Cumm)	7.70 ± 0.07	7.71 ± 0.21	7.51 ± 0.20	8.04 ± 2.40	7.55± 14.5*	7.56 ± 29.50
Platelet (10^3/ul)	443.40 ± 46.12	418.25 ± 22.61	434.60 ± 23.61	903.00 ± 191.47	719.00 ± 357.00	884.50 ± 106.50
MCV	57.02 ± 0.97	45.85 ± 9.88	57.14 ± 1.41	57.37 ± 1.16	57.10 ± 0.30	57.10 ± 0.70
MCH	18.50 ± 0.32	18.45 ± 0.39	18.58 ± 0.47	18.50 ± 0.46	18.00 ± 0.10	18.00 ± 0.80
MCHC	32.44 ± 0.04	32.40 ± 0.041	32.50 ± 0.032	32.23 ± 0.84	31.50 ± 0.10	31.55 ± 1.05

Data: Mean ± SEM, * P<0.05

Biochemical Parameters

Significant decrease in HDL level was found in group-II. Highly significant decrease in albumin level was found in group-II and III. Other biochemical parameters related to liver function i.e. serum bilirubin (total), SGOT, SGPT, ALP, were found insignificantly decreased in different dose levels. Insignificant increase in blood sugar and triglyceride levels was found in different dose levels (Table 4) (Graph 3, 4, 5).

Table 4. Effect of RM on various biochemical parameters

Parameters	Control	RM TED	RM TED×2	RC	RMR TED	RMR TED×2
Bl Sugar (mg/dl)	87.00 ± 6.27	98.80 ± 6.15	93.00 ± 5.40	90.33 ± 2.03	87.67 ± 3.28	84.00 ± 10.54
Cholesterol (mg/dl)	64.60 ± 2.15	56.40 ± 4.85	60.80 ± 6.54	60.00 ± 8.18	79.00 ± 1.53	61.33 ± 3.93
Triglyceride (mg/dl)	85.60 ± 12.19	110.40 ± 15.90	112.00 ± 33.60	155.67 ± 3.39	102.33 ± 20.50	107.33 ± 18.21
HDL (mg/dl)	38.20 ± 1.71	27.00 ± 4.10*	30.80 ± 5.71	32.67 ± 3.76	45.33 ± 3.28	35.00 ± 5.86
Bl urea (mg/dl)	51.20 ± 5.66	48.40 ± 5.14	61.00 ± 10.13	116.00 ± 15.31	108.67 ± 8.76	101.000 ± 8.96
Creatinine (mg/dl)	0.80 ± 0.00	0.72 ± 0.06	0.84 ± 0.07	0.78 ± 0.09	0.73 ± 0.03	0.67 ± 0.07
SGPT (IU/L)	54.20 ± 9.10	65.40 ± 11.48	64.80 ± 5.08	140.00 ± 38.76	116.00 ± 6.56	120.33 ± 12.87
SGOT (IU/L)	149.00 ± 21.56	166.20 ± 25.36	162.40 ± 15.96	339.00 ± 61.17	312.67 ± 52.59	430.67 ± 49.17
Total Protein (gm/dl)	7.86 ± 0.30	7.64 ± 0.29	7.86 ± 0.30	7.07 ± 0.23	7.03 ± 0.19	6.90 ± 0.38
Albumin (gm/dl)	7.86 ± 0.30	3.80 ± 0.26***	3.84 ± 0.31***	3.43 ± 0.03	3.83 ± 0.17	3.87 ± 0.20
Globulin (gm/dl)	3.94 ± 0.38	3.64 ± 0.08	3.76 ± 0.07	3.63 ± 0.20	3.20 ± 0.20	3.03 ± 0.19
Alkaline Phospatase (IU/L)	132.60 ± 19.68	139.20 ± 22.89	167.00 ± 22.03	181.33 ± 53.25	182.00 ± 29.94	182.67 ± 57.43
Billirubin total (mg/dl)	0.50 ± 0.089	0.62 ± 0.05	0.54 ± 0.05	0.76 ± 0.14	0.87 ± 0.32	0.77 ± 0.17
Uric acid (mg/dl)	1.48 ± 0.17	1.26 ± 0.13	1.36 ± 0.17	2.83 ± 0.98	1.73 ± 0.55	1.93 ± 0.28

Data: Mean ± SEM, * P<0.05, *** P<0.001

Ayurvedic drugs are widely popularized in Indian subcontinent due to their origin from natural resources. They are safely practiced since centuries without any noticeable adverse effects. But due to presence of heavy metals in their formulations, there is a need to provide safety profile of these drugs. Arsenic, one among such heavy metals is reported as toxic and carcinogenic. Ingestion of inorganic arsenic has been shown to cause cancer in humans, resulting in tumors of the skin, lung, liver, urinary bladder and other sites proven as human carcinogen by the International Agency for Research on Cancer (IARC)[10]. Though almost all the systems are being affected, liver and kidneys are most susceptible to arsenic toxicity[11]. During a metabolic process, arsenic gets methylated in liver by arsenic methyl transferase to form organoarsenics that are excreted by kidney through urine[12]. So, they are considered as primary organs of target in arsenic toxicity. Hence, the present study was designed to assess effect on ponderal, hematological and biochemical parameters due to administration of RM. Non significant increase in the body weight was observed in all the dose levels.

Eosinophil count was significantly increased in group-II at the end of the 90th day, which came back to normal level during the recovery period. Decreased RBC count in the recovery period is insignificant.

On chronic administration, RM insignificantly decreased platelet count in all treated groups. The haematopoietic system gets affected by arsenic exposure resulting in anemia, leucopenia, and thrombocytopenia. This has been reported as resulting from acute[13], intermediate[14] and chronic[15] oral exposures. These effects may be due to a direct haemolytic or cytotoxic effect on the blood cells[16] and a suppression of erythropoiesis[17]. RM administration at TED level significantly decreased HDL cholesterol. Two possibilities that can be proposed to explain the decrease are the test drugs may impair the transfer of cholesterol from both very low density lipoproteins and tissue to HDL fraction or they may be promoting the metabolism of this fraction by enhancing the activity of the key enzymes involved in HDL cholesterol metabolism. Significant decrease in albumin level was found group-II and III. It is most commonly associated with the decreased production of albumin in liver possibly due to destruction of hepatocytes. It can also be seen in nephritic syndrome due to excessive loss in urine. Observed decrease in serum albumin level may be attributed to impairment of liver function. Other biochemical parameters like triglyceride, SGPT, SGOT, serum alkaline phosphatase were insignificantly increased in all dose levels. The results in relation with above biochemical parameters revealed that drug has potentiality to cause hepatic impairment. Similar study done by administering RM for 28 days at TED dose level did not show any alteration in biochemical parameters (Table 5). This gives an inference that, *Rasmanikya*, being an arsenical preparation should be carefully used under a strict supervision.

Table 5. Effect of RM on various biochemical parameters after 28 days

Parameters	Control	RM TED
Bl Sugar (mg/dl)	113.60 ± 4.74	124.60 ± 4.13
Cholesterol (mg/dl)	77 ± 10.13	78 ± 11.83
Triglyceride (mg/dl)	162.20 ± 45.40	123.80 ± 26.01
HDL (mg/dl)	40.80 ± 4.77	36.80 ± 2.67
Bl urea (mg/dl)	30.60 ± 3.11	31.00 ± 01.30
Creatinine (mg/dl)	1.08 ± 0.06	1.05 ± 0.06
Total Protein (gm/dl)	7.16 ± 0.24	7.12±0.22
Albumin (gm/dl)	3.46 ± 0.31	3.62 ± 0.16
Globumin (gm/dl)	3.6 ± 0.30	3.4 ± 0.18
Alkaline Phospatase (IU/L)	65.6 ± 7.60	67.20 ± 8.47
Billirubin total (mg/dl)	0.42 ± 0.10	0.38 ± 0.66

CONCLUSION

Current research work concludes that *Rasamanikya* has potential to generate mild alterations in hematological and biochemical profiles when used for a long period of 90 days. On the other hand, short term use (28 days) is found to be safe. Hence, RM should be used judiciously by observing a gap at regular intervals, along with specified adjuvant. This gap may help in washing out of the metabolic by products and checks the accumulation in the body. Care should also be taken while using the drug in patients with hepatic and renal impairment. However to arrive at the final conclusions; further extensive studies on larger groups of animals at different doses and duration are needed.

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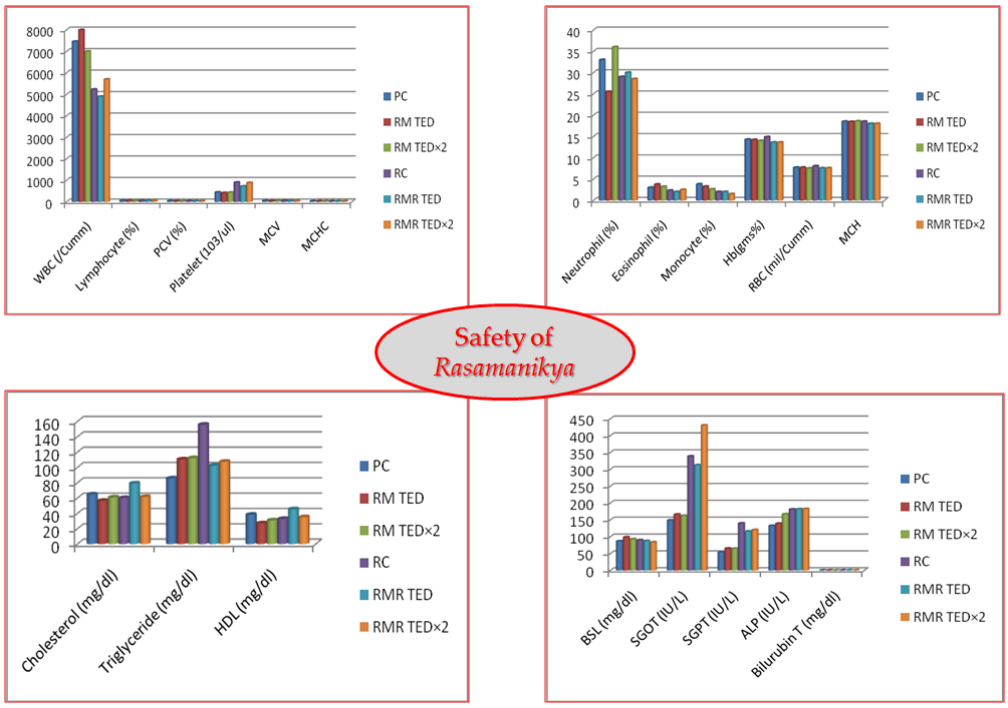
GRAPHICAL ABSTRACT



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