Ayurveda, the traditional system of Indian medicine, has enriched the historical background and is one of the great living traditions. Use of processed metals, minerals, and mercurial-processed herbs as a medicament has been an integral part of Ayurvedic practice. These metallic preparations have unique process of preparation, involving Shodhana (purification and/or detoxification) and Marana (incineration and/or calcination). Practitioners developed these methods to detoxify the raw material by chemical transformations and thus modify the properties of materials to enhance therapeutic potential[1,2]. Their extensive use of these medicaments since more than a millennium without any reports of any untoward events can be considered as a testimony to their safety; but no objective-verifiable data exists to support such claims. Pre-clinical studies of Ayurvedic drugs provide scientific basis for their traditional use and to prove that they are safe and efficacious[3].

Toxicological Studies of Rasasindura, an Ayurvedic Formulation

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Rasasindura is a unique, Ayurvedic mercurial preparation widely used by practitioners. This investigation is an attempt to perform acute and chronic oral toxicity evaluation of Rasasindura along with an adjuvant Guduchi Ghana (solidified aqueous extract of Tinospora cordifolia Will.) in rats. Oral acute toxicity study of test drug was carried at the limit dose of 2000 mg/kg orally in rats. For chronic toxicity, Rasasindura with adjuvant was administered at therapeutic equivalent dose (45 mg/kg, orally), therapeutic equivalent dose×5 (225 mg/kg, orally), therapeutic equivalent dose×10 (450 mg/kg, orally) for 90 days and an additional recovery group of therapeutic equivalent dose×10 for 30-day observation after the treatment period. Acute toxicity result showed that drug did not produce any signs and symptoms of toxicity or mortality up to an oral dose of 2000 mg/kg in rats. Chronic toxicity results showed that Rasasindura, even at a level as high as therapeutic equivalent dose×10 level, had no significant effect whatsoever on the ponderal and hematological parameters. Although the drug produced mild to moderate adverse changes (in kidney, liver, intestine, and stomach) at therapeutic equivalent dose×10 dose level, equivalent of which are unlikely to be ever employed in a clinical trial. The observed changes were not seen at the lower dose levels as well as in the recovery study. Hence, it is suggested that the Rasasindura, along with the adjuvant prepared as per the customary method, is safe for consumption at the therapeutic dose level.

Key words: Rasasindura, Guduchi ghana, Tinospora cordifolia, bhasma, toxicity

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be neutralized in the presence of purified sulphur\cite{6}. As per the classics, *Rasasindura* is administered with the adjuvant such as *Guduchi Ghana* (solidified aqueous extract of *Tinospora cordifolia* Will.)\cite{7}. *Rasasindura* has unique properties to pacify diseases such as diabetes, fistula, fever, lack of appetite, anaemia, oedema etc.; and it is considered equivalent to the elixir, which is known to overcome death\cite{4,7}. Since reports of toxicity evaluation of this classical preparation (along with the adjuvant) was not available during extensive literature review, it was thought worthwhile to undertake the detailed toxicity assessment in albino rats.

**MATERIALS AND METHODS**

*Aloe vera* and *T. cordifolia* were collected from the botanical garden of Gujarat Ayurved University, Jamnagar. The plant materials were authenticated and voucher specimens of each submitted to Pharmacognosy laboratory of Institute. *Rasasindura* and *Guduchi Ghana* (solidified aqueous extract of *T. cordifolia*) were prepared in Department of Rasashastra and Bhaishajya Kalpana, Gujarat Ayurved University, Jamnagar; and SOPs were prepared and documented\cite{4,8}. All chemicals used in the study were of analytical grade.

**Experimental animals:**

Wistar albino rats of either sex weighing 200±20 g body weight were used for the study. The animals were maintained under ideal husbandry conditions in terms of standard conditions of temperature (23±2\(^\circ\)), relative humidity (50 to 60\%), and exposed to 12 h light-and-dark cycles. All animals were exposed to the same environmental conditions and were maintained on standard diet and drinking water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC/10/2012/08, Ph.D.) as per the guideline of Committee for the Purpose of Control and Supervision on Experiments on Animals, India.

**Dose selection:**

As per the classical guideline, the therapeutic dose of *Rasasindura* is 125 mg/d\cite{9} and should be administered with the adjuvant as solidified aqueous extract of *T. cordifolia* (375 mg/d). Hence, total dose of drug with adjuvant is 500 mg/d. The suitable dose for rats was calculated by referring to table of Paget and Barnes\cite{10} and was found to be 45 mg/kg rat (considered as therapeutic equivalent dose, TED). The test drug along with adjuvant was administered orally with the help of cannula, in the form of suspension in honey and distilled water solution.

**Acute toxicity study:**

Young, healthy, nulliparous, and non-pregnant Wistar-strain albino, female rats were selected and acclimatized for seven days before the experiment. The *Rasasindura* along with adjuvant was orally administered at limit dose of 2000 mg/kg to overnight fasted female rats in sequential manner as per the OECD 425 guideline\cite{11}. The rats were observed closely for behavioural changes, signs and symptoms of toxicity, and mortality continuously for the first six hours; and thereafter, periodically up to 14 d. The body weight of each rat was noted on the last day and the rats were sacrificed. The abdomen was opened through mid-line incision to record the autopsy changes, followed by dissecting the important organs for histopathological changes.

**Chronic toxicity study:**

The chronic toxicity study was carried out followed by standard guideline with modification as per experimental need\cite{12,13}. Rats were randomized into six groups of six rats in each with three males and three females. Group (I) was kept as control group, received vehicle as honey solution in distilled water (5 ml/kg, orally). Group (II) to (IV) were administered with test drug *Rasasindura* along with adjuvant at TED (45 mg/kg, orally), TED×5 (225 mg/kg, orally), and TED×10 (450 mg/kg, orally), respectively. The suspensions of test drugs were administered orally once-a-day for 90 consecutive days in main study. Additional six animals were kept in satellite control group (V) and in the recovery TED×10 treated group (VI) for observation after the treatment period, for reversibility or persistence of any toxic effects. The duration of post-treatment period was fixed as 30 d (total of 120 d, including 90 d treatment period and 30 d recovery period). All the animals were dosed with constant dose volume of 5 ml/kg, orally.

The rats were observed daily, carefully for any overt and apparent signs and symptoms of toxicity. The body-weight change of an individual rat was noted initially and thereafter weekly during the study period. At the end of experimental periods, blood was withdrawn by the retro-orbital puncture under light-ether anaesthesia using capillary tube for estimation of serum biochemical and haematological parameters. The body weight of each rat was noted on last day and rats were sacrificed. The abdomen was opened through mid-line incision to...
record the autopsy changes followed by dissecting out
the important organs.

Haematological analysis was performed using an
automatic haematological analyser (Swelab, Sweden).
The parameters were total red blood cell (RBC),
haemoglobin (Hb), packed cell volume (PCV),
mean corpuscular volume (MCV), mean corpuscular
haemoglobin (MCH), mean corpuscular haemoglobin
concentration (MCHC), white blood cell (WBC),
neutrophils percentage (%N), lymphocyte percentage
(%,L), eosinophil’s percentage, monocyte percentage,
and platelet count (PC).

Serum bio-chemical parameters were carried out using
fully automated biochemical random access analyser
(BS-200, Lilac Medicare Pvt. Ltd., Mumbai). The
parameters were blood glucose[14], urea[15], creatinine[16],
total cholesterol[17], HDL-cholesterol[18], triglyceride[19],
VLDL-cholesterol, LDL-cholesterol, total protein[20],
albumin, globulin[21], alkaline phosphatase[22],
SGOT[23], SGPT[24], uric acid[25], direct bilirubin[24], total
bilirubin[26], and serum calcium[27].

Bone-marrow smear from the femur bone was prepared
using standard procedure. All the important internal
organs were carefully dissected namely brain, pituitary,
liver, heart, thymus, spleen, kidney, lung, stomach,
testis, prostate, seminal vesicle, uterus, ovary, adrenal gland, trachea, aorta, lymph node, and skin.

After noting for any signs of gross lesion and ponderal
changes of major organs, all were transferred to
10% phosphate buffered formalin solution for fixation
and later on subjected to dehydrating, wax embedding,
sectioning, and staining with haematoxylin and eosin
(H and E) for histological evaluation by light microscopy.
The slides were viewed under trinocular research
Carl-Zeiss’s microscope at various magnifications to
note down the changes in the microscopic features of
the tissues.

Statistical analysis:
The data is expressed as mean±standard error of mean
for six rats per experimental group. One-way analysis
of variance (ANOVA) was used to compare the mean
values of quantitative variables among the groups,
followed by Dunnet’s multiple t-test for unpaired data
to determine significant difference between groups at
P<0.05.

RESULTS AND DISCUSSION

Acute toxicity study of test drug was carried out to
record immediate adverse signs and symptoms of drug
in female rats at dose levels that are several folds higher
than the therapeutic equivalent dose. Administration of
Rasasindura along with adjuvant did not affect any
behavioural changes and other parameters observed
during the acute toxicity test in female rats. No signs
and symptoms of toxicity and mortality were observed
up to oral dose of 2000 mg/kg of test drug in rats.
Further, drug did not affect the cytoarchitecture of
major organs like heart, kidney, liver, uterus, and ovary
which suggest that LD$_{50}$ value may be higher than
2000 mg/kg by oral route. As per UN classification,
any substance, which has oral LD$_{50}$ of more than
2000 mg/kg is considered as low hazard potential
and categorized as UN 6.1 PG III[28]. Thus as per the
above criterion Rasasindura along with adjuvant can
be categorized as substances with low health hazard
potential (Class 4 of GHS and UN 6.1 PG III).

There were no behavioural changes observed in
Rasasindura treated groups during the course of chronic
toxicity study. No symptoms of toxicity and mortality
was observed in treated groups at TED×10, TED×5,
and TED dose levels in the main study and TED×10
in the recovery study. Normal body weight gain was
observed in control rats during main study (90 d) as
well as recovery study (120 d). An increase in body
weight was found in Rasasindura treated groups at all
dose levels. Changes in body weight are an important
factor to monitor the health of an animal. Loss of body
weight is usually the first sign indicating the onset of
an adverse effect. The dose, at which body weight loss
is by 10% or more, is considered to be a toxic dose,
irrespective of whether or not it is accompanied by
any other changes[29]. The percentage change in body
weight pattern in test drug treated groups did not differ
significantly from the changes observed in the control
groups, which suggest the absence of serious toxic
effect of Rasasindura during chronic administration in
rats.

Out of the nine organs for which relative weights
were recorded (Table 1), Rasasindura treated group
showed significant decrease in relative weight of liver
in TED treated group in comparison to the normal
control, whereas in other dose levels the changes were
non-significant. A significant increase was observed
in relative weight of testis at TED, uterus weight at
TED×5, and kidney and testis at TED×10 dose levels;
however, the changes were almost reversed in the drug
treated recovery group at TED×10 dose level. Normally,
decrease in the weight of an organ is indicative of loss of
tissue mass in that organ, exception being the secretory organs in which the decrease in weight sometimes is seen along with the increased activity. In this case, the increase in the weight of reproductive organs may be indicative of stimulation of hormone secretion. In the present study, there were no remarkable changes observed in the relative weight of the organs at higher doses of test drugs. Hence, it may be, understood that the drugs do not tend to produce any serious toxic effect on the relative weight of the important internal organs in chronic toxicity studies.

Analysis of the effects of Rasasindura on haematological parameters (Table 2) revealed Non-significant increase in WBC count at each of the dose level studied in the main study; however, contrary effect was observed in the recovery phase in the drug treated group in comparison to the control group. However all the values were within the normal range[30]. The test drug at all dose level did not affect the RBC related parameters. This clearly indicates that the test drug did not affect the cellular and non-cellular elements of the blood to significant extent. Further, with the discontinuation of the drug, most of the values were similar to that observed in the recovery control group, which suggests that the drug is devoid of any serious haematological toxicity, even at higher dose on repeated administration.

The effects of Rasasindura on serum bio-chemical parameters are presented in Table 3. Out of eighteen parameters studied, significant decrease was observed in blood glucose level at TED and TED×5 treated groups, while significant increase in creatinine level at TED dose level and triglyceride level at TED×5 dose level in comparison to the control group in main study. However, similar significant changes were not observed in higher dose of Rasasindura. After discontinuation of test drug in the recovery group, the observed changes in glucose level, creatinine, and

### TABLE 1: EFFECT OF TEST DRUGS ON RELATIVE WEIGHT OF ORGANS OF RATS RECORDED DURING CHRONIC TOXICITY STUDY

| Relative weight | Control group | TED | TED×5 | TED×10 | R Control group | Recovery TED x 10 |
|-----------------|---------------|-----|-------|--------|-----------------|-------------------|
| Liver (g/100 g) | 3.57±0.17     | 3.12±0.10* | 3.27±0.06 | 3.26±0.09 | 2.72±0.07       | 2.99±0.11         |
| Heart (mg/100 g) | 280.41±8.88   | 266.04±5.36 | 289.12±9.69 | 281.65±6.78 | 240.44±8.42 | 269.80±12.81   |
| Kidney (mg/100 g) | 720.10±26.82  | 675.17±19.26 | 766.08±30.88 | 804.74±25.63* | 691.42±18.42 | 714.44±10.76   |
| Spleen (mg/100 g) | 194.50±9.32   | 173.56±8.14 | 206.28±18.26 | 189.69±10.14 | 158.85±7.44 | 179.45±8.87    |
| Thymus (mg/100 g) | 172.54±6.73   | 165.65±5.21 | 167.91±7.71 | 158.91±7.60 | 145.56±10.91 | 146.15±12.89   |
| Testis (g/100 g) | 648.26±28.37  | 818.50±41.74* | 684.25±123.10 | 868.99±45.83 | 792.01±61.68 | 661.95±39.86   |
| Prostate (mg/100 g) | 146.97±21.19  | 128.77±11.70 | 129.53±9.04 | 180.36±7.43 | 145.07±24.68 | 167.09±16.38   |
| S. vesicle (mg/100 g) | 504.08±95.14  | 504.55±55.60 | 481.70±32.76 | 527.32±47.38 | 421.09±62.60 | 477.01±58.91   |
| Uterus (mg/100 g) | 230.03±17.72  | 215.88±28.98 | 323.70±26.45* | 197.89±8.69 | 178.48±9.04 |

The results are expressed as mean±SEM, where n=6. SEM: Standard error of mean. *P<0.05, compared with control group

### TABLE 2: EFFECT OF TEST DRUGS ON HEMATOLOGICAL PARAMETERS IN RATS RECORDED DURING CHRONIC TOXICITY STUDY

| Hematological Parameters | Control group | TED | TED×5 | TED×10 | R control group | Recovery TED×10 |
|--------------------------|---------------|-----|-------|--------|-----------------|-----------------|
| RBC (10^5/ml)            | 7.55±0.08     | 7.69±0.17 | 7.41±0.10 | 7.86±0.22 | 8.67±0.26 | 8.74±0.26     |
| Hemoglobin (g/dl)        | 13.85±0.34    | 13.95±0.21 | 13.60±0.16 | 14.00±0.21 | 15.38±0.24 | 15.38±0.33   |
| PCV%                     | 42.18±0.58    | 42.48±0.95 | 41.40±0.43 | 44.41±1.01 | 48.76±1.22 | 49.60±1.30   |
| MCV (fl)                 | 55.86±0.41    | 55.25±0.47 | 55.84±0.48 | 56.54±0.75 | 56.32±1.18 | 56.75±0.80   |
| MCH (pg/red cell)        | 18.35±0.32    | 18.15±0.24 | 18.35±0.27 | 17.84±0.37 | 17.38±0.53 | 17.63±0.38   |
| MCHC (g/dl)              | 32.81±0.49    | 32.87±0.25 | 32.84±0.24 | 31.58±0.33 | 30.84±0.42 | 31.03±0.34   |
| WBC (10^3/ml)            | 6816.66±630.03 | 8114.28±1217.61 | 8114.28±1068.91 | 7585.71±570.47 | 7020.00±575.67 | 6233.33±276.48 |
| Neutrophil%              | 24.50±4.32    | 28.42±5.47 | 19.57±2.32 | 17.14±1.79 | 23.00±4.93 | 23.66±2.10   |
| Lymphocyte %             | 71.66±4.27    | 66.57±5.81 | 76.85±2.13 | 79.42±2.04 | 75.80±5.06 | 72.00±2.51   |
| Eosinophil%              | 2.33±0.21     | 2.71±0.28 | 2.00±0.21 | 2.00±0.21 | 2.60±0.24 | 2.33±0.21    |
| Monocyte%                | 1.50±0.22     | 2.28±0.28 | 1.57±0.20 | 1.42±0.20 | 2.60±0.24 | 2.00±0.36    |
| PLT (10^3/ml)            | 1152.83±54.10 | 1267.57±47.57 | 1154.00±62.82 | 1267.85±51.82 | 1115.80±45.11 | 1057.50±83.48 |

The results are expressed as mean±SEM, where n=6. SEM: Standard error of mean
triglyceride were almost same as seen in the recovery control group. Cholesterol level and HDL-cholesterol level in rats were unaffected by the test drug at all dose level in comparison to the control group; hence, it can be inferred that the observed changes do not cause any serious toxic effect (Table 4).

Significant decrease in blood urea was observed in recovery TED \times 10 treated rats in comparison to the recovery control group however same was not observed in main study. Test drugs did not influence the level of serum transaminases, urea, and creatinine to a significant extent in main study; which suggests that the test drug may not affected the liver and kidney function in the treated rats. The observed values of above bio-chemical parameters are within the normal range[30].

The histopathological studies of twenty organs showed that Rasasindura along with adjuvant at highest dose level exhibited mild to moderate changes in kidney, liver, intestine, stomach, and lung in comparison to the control group. Rasasindura produced mild pigment deposition, fatty changes in epithelium, and oedematous changes in kidney tubule, whereas recovery group showed normal cytoarchitecture (fig. 1). Rasasindura TED\times 10 and TED\times 5 treated groups exhibited pigment deposition, mild necrosis, and fatty changes in the liver in comparison the control group, while the recovery group showed almost normal cytoarchitecture (fig. 2). Test drug at highest-dose level exhibited mild to moderate loss of villi and cell infiltration in intestine (fig. 3) and displayed mild to moderate sub-mucosal inflammation in the stomach (fig. 4). Mild effusion was seen in cytoarchitecture of lung of one of the rat at higher dose of TED\times 10 dose level in comparison to the control group; whereas, in other dose levels the changes were almost normal (fig. 5). Bone marrow smear and the other organs exhibited normal cytoarchitecture in the Rasasindura-preparation treated groups, both in the main study as well as in the recovery study, in comparison to the control groups.

The observed histopathological changes were not seen at therapeutic dose level and not in the recovery study. The result of bio-chemical parameters reveal that the drugs do not seem to produce any drastic changes in

### TABLE 3: EFFECT OF TEST DRUGS ON BIOCHEMICAL PARAMETERS IN RATS RECORDED DURING CHRONIC TOXICITY STUDY

| Biochemical Parameters | Control group | Drug treated groups | R control group | Recovery TED \times 10 |
|------------------------|---------------|---------------------|----------------|-----------------------|
| Glucose (mg/dl)        | 92.83±8.63    | 68.57±2.61*         | 70.85±4.11     | 107.28±3.63          |
| Urea (mg/dl)           | 69.83±4.88    | 81.00±2.43          | 64.00±4.35     | 69.14±8.74           |
| Creatinine (mg/dl)     | 58.08±0.03    | 0.70±0.03*         | 0.62±0.03      | 0.55±0.02            |
| Total protein (g/dl)   | 7.08±0.17     | 6.94±0.19           | 6.95±0.24      | 7.12±0.10            |
| Albumin (g/dl)         | 3.58±0.16     | 3.61±0.15           | 3.67±0.23      | 3.72±0.08            |
| Globulin (g/dl)        | 3.50±0.17     | 3.12±0.10           | 3.28±0.17      | 3.40±0.09            |
| ALP (IU/L)             | 233.25±45.02  | 149.00±11.82        | 143.33±23.78   | 263.42±37.67         |
| SGOT (IU/L)            | 138.16±7.85   | 153.28±9.11         | 142.85±8.42    | 148.85±9.83          |
| SGPT (IU/L)            | 67.00±7.82    | 53.28±1.37          | 58.28±5.16     | 62.00±6.92           |
| Uric acid (mg/dl)      | 0.70±0.13     | 0.81±0.09           | 0.84±0.09      | 0.72±0.06            |
| D. Bilirubin (mg/dl)   | 0.13±0.03     | 0.11±0.01           | 0.10±0.00      | 0.12±0.02            |
| T. Bilirubin (mg/dl)   | 0.45±0.05     | 0.48±0.07           | 0.41±0.04      | 0.48±0.06            |
| Calcium (mg/dl)        | 9.58±0.30     | 8.98±0.17           | 9.81±0.25      | 9.22±0.22            |

The results are expressed as mean±SEM, where n=6. SEM: Standard error of mean. *P<0.05 compared with control group; @P<0.05 compared with recovery control group

### TABLE 4: EFFECT OF TEST DRUGS ON SERUM LIPID PROFILE IN RATS RECORDED DURING CHRONIC TOXICITY STUDY

| Serum Lipid profile      | Control group | Drug treated groups | R control group | Recovery TED \times 10 |
|--------------------------|---------------|---------------------|----------------|-----------------------|
| Total cholesterol (mg/dl)| 34.33±3.45    | 38.42±1.81          | 37.28±3.92     | 30.71±2.01            |
| HDL-cholesterol (mg/dl)  | 33.16±3.75    | 37.14±4.90          | 37.85±5.70     | 27.85±2.08            |
| Triglyceride (mg/dl)     | 61.33±3.67    | 76.14±6.55          | 78.42±6.07*    | 70.28±6.80            |
| VLDL-cholesterol (mg/dl) | 12.26±0.73    | 15.22±1.31          | 15.68±1.21     | 14.05±1.36            |
| LDL-cholesterol (mg/dl)  | 11.10±1.68    | 13.32±3.86          | 14.40±3.30     | 11.20±0.80            |

The results are expressed as mean±SEM, where n=6. SEM: Standard error of mean. *P<0.05 compared with control group; @P<0.05 compared with recovery control group
the liver and kidney function parameters in rats; which suggests that the organ damage as mentioned above in chronic toxicity is of mild intensity at higher dose level, however drug is relatively safe at therapeutic dose level. In previous 28-d toxicity study also demonstrated that Rasasindura (50-100 mg/kg) in Wistar-albino rats did not have any adverse effect on kidney[31]. Further, feeding on Rasasindura supplemented food did not elicit any evidence of heavy metal toxicity in larvae or flies, since there was neither any evidence of lethality, nor of any developmental defects in the emerging flies[32].

The results reiterates the fact that Bhasmas, despite their trace heavy metal content, are safe when appropriately manufactured and consumed as per directed instructions[33]. Toxic effects of mercury were said to be neutralized in the presence of sulphur[6]. In Ayurvedic system of medicine, Anupana (called vehicle, as a medium of administration) improves acceptability and palatability and helps in absorption of the main drug; additionally, it may also act as early antidote[32]. Guduchi used as adjuvant in current study is having antioxidant property acts as hepatoprotective drug and has potential against aflatoxins and heavy metal toxicity[34,35].

From the present study, it is concluded that Rasasindura along with adjuvant is not toxic on acute administration at a maximum oral dose level of 2000 mg/kg in female
rats. However, on chronic administration of test drug for 90 d produced mild to moderate adverse changes in the kidney, liver, intestine, and stomach of rats at TED×10 dose level, equivalent of which are not likely to be ever employed in clinical conditions, conversely Rasasindura at TED dose has no toxic potential. Rasasindura prepared as per customary method and administered with appropriate adjuvant is safe to consume at therapeutic dose level.

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