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# Acute and sub-acute toxicity studies of flower extract of *Tridex procumbens in albino rats*

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## ABSTRACT

**Aim-** The study was designed to evaluate the Acute & Sub-acute Toxicity Studies of Flower Extract of *Tridex procumbens*. **Material & Methods-** Dried powder of flower part was extracted by using chloroform: methanol solvent by using soxhlet apparatus for 72 hrs. Preliminary phytochemical screening was performed for the presence of various phytoconstituents. The chloroform: methanol extract was evaluated by using the acute and sub-acute toxicity studies. The acute toxicity study was also evaluated by administration of extract after oral and intraperitoneal extract. Various parameters i.e. body weight, clinical signs, hematological parameters and clinical chemistry parameters were evaluated. **Result-** In acute toxicity studies, there were no side effects at the dose of 2000 mg/kg. The sub-acute toxicity was performed at 100, 300 and 1000 mg/kg. There were some changes in body weight, organ weights, hematological parameters and clinical chemistry parameters. However, no toxic effects were seen at his dose. **Conclusion-** The results showed that no death of experimental rats at an oral dose of 2000 mg/kg body weight symptomatic of that the chloroform: methanol extract of *Tridex procumbens* flower is virtually non-toxic after oral dose exposure presuming that its components were engrossed. The extract can then be concluded to be safe for oral use as a traditional herbal remedy for the different ailments.

**Keywords:** Flower extract, acute toxicity, sub-acute study, chloroform: methanol extract, *Tridex procumbens*

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## INTRODUCTION

Toxicovigilance study of herbal medicines is debated issue in current scenario. Toxicity testing on herbal extracts is carried out on the same philosophy as the conventional medicine. Toxicity is the quantity to which a substance can damage humans or animals. It can be deliberate by its effects on the target organism, organ, tissue or cells. The toxic effects of a substance on animal physiology can variety from minor changes such as reduced weight gain, small physiological modification or change in the levels of circulating hormones, to severe effects in organ purposeful loss leading to death. Intermediate levels of toxicity may cause pain and suffering[1].

*In vitro* toxicity testing employs the use of models such as the brine shrimp lethality test (BST) whereas mice or rats are included for *in-vivo* methods[2]. Organization for Economic Cooperation and Development (OECD) guidelines are used during acute and sub-acute oral toxicity testing[3, 4, 5, 6]. It is significant to optimize the information obtained by using the smallest number of animals to comply with animal welfare regulations. Further, it is significant to avoid excessive pain or tissue damage in the animals, pharmaceuticals with aggravation or corrosive characteristics should not be administered in concentrations that produce severe toxicity after administration.

The main objective of this research work is to carry out acute toxicity and sub-acute toxicity of *Tridex procumbens* flower extract in rats. The acute toxicity study was evaluated by oral and intraperitoneal administration of flower extract in rats.

## MATERIAL & METHODS

### *Authentification of Plant Materials*

The flowers of *Tridex procumbens* were collected and materials were submitted to Department of Botany, Janata PG College, A.P.S. University, Rewa, M.P., and identified by Prof. Dr. S.N Dwivedi, Professor and Head of Department, Janata PG College, A.P.S. University, Rewa, M.P. as Voucher Specimen Number-/J/BOT/H-496.

### *Reagent & Chemicals*

For the estimation of various haematological and clinical chemistry parameters, diagnostic kits (Ranbaxy Pvt. Ltd.) were directly purchased form market. All chemicals for

extraction and for overall dissertation work were used of analytical grade (Merck Company Pvt. Ltd.).

## Methods

### *Preparation of Hydro alcoholic Extract*

Flowers of plant were dried under shadow and subjected to coarse powder for extraction process. Accurately weighed (200 gm) quantity of flowers of *Tridex procumbens* were extracted using chloroform: methanol solvent (50:50) by soxhlet apparatus for 72 hr. The chloroform: methanol extract was dehydrated under the reduced pressure to get crude extract and percent yield was calculated[7].

### *Preliminary Phytochemical Tests*

Qualitative chemical tests of chloroform: methanol extract were subjected to an assortment of chemical tests to detect various presences of various phytoconstituents [8, 9].

### *Selection and Procurement of animals*

Wistar albino rats of either sex between two to three months of age weighing 150-200 g were used which were procured from the central animal house of Oriental College of Pharmacy and Research, Oriental University, Indore (MP), India. All animals were housed in an animal room under normal condition, 12 hrs light and dark cycle. The animals were allowed free to access commercial rat pellet diet (Lipton India Ltd, Mumbai, India) and water *ad libitum.* The bedding materials of the cages were changed every day. All the experimental trial was carried out in agreement with the CPCSEA guidelines. The study designs were permitted by the Institutional Animal Ethical Committee of Oriental College of Pharmacy and Research, Oriental University, Indore (MP), India.

## Toxicity Studies

### Acute toxicity studies after oral administration

The extract was suspended in 2.5 % Tween 80 in normal saline. The test dose was administered in a single dose by gavages using appropriate intubation canula. Food was withheld for an additional 3-4 hours after dosing.

The Globally Harmonized Classification System (GHS) in acute toxicity category (ATC) method of the OECD was used to conclude the LD50 range [4]as per appendix 2. Since in attendance was no prior information on toxicity of *Tridex procumbens* flower extract, for animal wellbeing reasons, the starting dose was selected to be 300 mg/kg body weight (Appendix 3). Since no death occurred at this dosage level, then the next higher dose, 2000 mg/kg was used. In 2000 mg/kg category[5], all the three rats were treated with 2000 mg of extract per kg body weight of the rat. The volume of the extract given was designed according to the weight of each rat, ensuring that the volume fed to the rat did not exceed 2 mL.

The rats were observed independently after dosing at least once within the first 30 minutes, then periodically during the first 24 hour. The parameters of interest were changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems for signs of toxicity that may include; tremors, convulsions, salivation, diarrhea, lethargy, somnolence, or coma or death. The mortality rates for each dose group were recorded for the first 24 hours. Standard pathology events were followed to examine the dead or sacrificed animals and the pathological changes recorded.

### Acute toxicity after intraperitoneal administration

The procedure used conformed to the OECD guideline that is used in acute toxicity testing [4] and as per Appendices 2 to 4. The extract was dissolved in phosphate buffered saline with 3 % DMSO. The extract was first filtered through filter paper, and then all the way through 0.2 µm Millipore filters to ensure sterility of the solution for intraperitoneal administration. The concentration of the solution was adjusted to ensure that the volume delivered per animal based on individual body weight was between 1 ml and 2 ml, the volume recommended for rats. The solution was then injected via intraperitoneal route starting with the 2000 mg/kg body weight dosage level.

The GHS/ATC method was then used to estimate the LD50 range. For example, for a starting dose of 2000 mg/kg body weight (Appendix 2), three animals were injected with 2000 mg/kg each. If 0-1 death occurred within 24 hours, the experiment was frequent with 3 more animals. If during the repeat exercise 0-1 death occurred, it was accomplished that the LD50 range for the extract falls between 2000 and 5000 mg/kg body weight. On the other hand, if 2-3 deaths occurred during the first or repeat exercise it was assumed that the LD50 range was below 2000 mg/kg and therefore the experiment was shifted to test procedure with a starting dose of 300 mg/kg body weight.

### Sub-acute toxicity

Feed and water were provided *ad libitum* and the Wistar rats were allowed 7 days for becoming accustomed. The rats aged 6-8 weeks, were randomly owed and housed in cages, each containing 5 rats; males and females separately with wood shaving bedding changed twice a week to uphold hygiene. The animals were assigned at unsystematic to three treatment groups of 5 animals per sex and a control group. A total of 40 animals were used. Each treatment group received a different concentration of the plant extract by gavages as described in the acute toxicity study. The dosage levels were logarithmically spaced as follows; 100 mg/kg, 300 mg/kg and 1000 mg/kg body weight daily. Controls were administered with untreated vehicle comprising 2.5 % Tween 80 in normal saline. Animals were dosed daily for 28 days with the test material on the basis of weekly mean group weight in accordance with OECD guideline[5].Body weight of all animals was taken on weekly basis.

### Clinical observations

Animals were pragmatic individually for clinical signs twice daily after dosing. Clinical explanations were recorded daily. The parameters of interest were changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, and autonomic, central nervous systems for signs of toxicity. Signs of toxicity included tremors, convulsions, salivation, diarrhea, lethargy, somnolence, or coma or death. The mortality rates for each dose group were recorded for the first 24 hours. Animals found declining or showing clinical signs of pain or distress was euthanized using diethyl ether.

### Haematological tests

For haematological studies, 2-3 ml of blood was collected using a capillary tube from the orbital sinus of the lightly ether-anaesthetized Wister rat into a test tube containing ethylenediamine tetra acetic acid (EDTA). Haematological parameters of interest included; haemoglobin concentration (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), total erythrocyte count (RBC), haematocrit (PCV). Also collected was total white blood cell

(WBC) and differential leukocyte count. Blood was collected before treatment and then thereafter fortnightly for 28th days.

**Clinical chemistry tests**

About 2-3 ml of blood samples per animal were collected in heparinized tubes. Plasma was obtained by centrifuging heparinized blood at 12,000 rpm for 5 min. Plasma was separated and stored at -20 degree centigrade until use. Clinical chemistry parameters built-in concentration of total proteins, albumin and the activities of Aspartate aminotransferase (AST). These parameters were calculated using the liquid-chemistry photometric methods. The plasma protein concentration was strong-minded calorimetrically at 540 nm using the Biuret method while total plasma albumin was determined by the bromocresol green method at 630 nm. The enzyme activity was determined as per the guidelines from the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)[10]. The plasma AST activity was determined by a photometric method with absorbance read at 340 nm.

**Statistical Analysis**

Data were expressed as the mean standard error of mean (S.E.M.) of the means and statistical analysis was carried out employing one-way ANOVA. Differences between the data were considered significant at $P < 0.05$.

## RESULTS

**EXTRACTIVE VALUE DETERMINATION**

Dried flowers of *Tridex procumbens* was extracted using chloroform: methanol solvent by soxhlet apparatus. The percent yields were determined by using the following formula.

$$\text{Percent yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

**Table 1:** Different extracts with their appearance and % yield (in gm)

| S. No. | Extracts | Color of dried extracts | Consistency of dried extracts | % Yield (W/W) |
|---|---|---|---|---|
| **1** | chloroform: methanol extract of *Tridex procumbens* | Dark brown | Sticky | 10.78 % |

## Preliminary Phytochemical Screening

The preliminary phytochemical analysis revealed that different active constituent present in different extracts such as carbohydrates, proteins, amino acids, fat, oils, steroids, terpenoids, glycosides, alkaloids, tannins and other phenolics compounds.

## Toxicity Studies

**Acute oral toxicity of *Tridex procumbens* flower extract**

**Medical Possessions**

There was no death throughout acute oral toxicity test of *Tridex procumbens* flower extract at 2000 mg/kg. All the animals showed clinical signs such as piroerectile, rubbing of nose and mouth and avoided feeds for the first 10 min post dosing. All the animals rubbed their mouth and nose with their front pawns and alongside the walls of the cage soon after dosing. All these symptoms moved out completely after 30 min of post dosing. The extract did not cause diarrhoea but the droppings in all test animals were wet and not well formed like pellets.

**Unpleasant Pathology**

Body weight changes during the 5 treatment days for the three animals dosed at 2000 mg/kg were not significantly different from the control group. All extracts treated animals showed a stable augment in body weights after 5th days. There was no confirmation of exudates in the peritoneal cavity during autopsy. The results are summarized in Table No. 2.

**Table 2:** Effect of chloroform: methanol extract on weight of rats

| S No. | Rat Groups | Weight at Zero day (gm) | Weight at 5th day after treatment (gm) | Weight gain (gm) |
|---|---|---|---|---|
| **1** | 1 | 155 | 160 | +5 |
| **2** | 2 | 150 | 157 | +7 |
| **3** | 3 | 165 | 170 | +5 |
| **4** | Control | 155 | 162 | +7 |

**Acute toxicity study after intraperitoneal administration of flower extract**

**Clinical Special Effects**

Intraperitoneal administration of extract showed various serious complications and they lead to death of rats used in study. Initially, all the rats were treated by 2000 mg/kg, did not showed any toxic symptoms but all the rats died within two to three minutes. Immediately after dosing, the animals became restless, urbanized uncoordinated, jerky movements, then convulsed with their tails stretched and raised upward. In the 500 mg/kg body weight category, one rat died after 45 minutes, the second rat died after 4th hours and the third rat after 48th hours. However, symptoms were similar to 2000 mg/kg but milder. In third steps, 3 rats were dosed at 100 mg/kg survived without any observable symptoms for the next 24 hours and there was no mortality of rats. Dosing was continued for the next 5 days till the cumulative dose was equal to the next toxic dose of 500 mg/kg. The only observable transient signs that occurred within 30 minutes post extract administration included raised fur and mouth rubbing. From the above findings, it can be concluded that 100 mg/kg is safe dose for the pharmacological evaluation.

## Sub-Acute Toxicity

### Clinical Signs

All the animals in 100 mg/kg, 300 mg/kg and 1000 mg/kg dose categories did not exhibit any abnormality throughout the 28th day's oral management of *Tridex procumbens* flower extract. The only fleeting clinical signs that were most marked at 1000 mg/kg and lasted for about 30 minutes included raised fur, fast rates of respiration and rubbing at the oral cavity representative irritation. The animals looked dull and motionless immediately after dosing but this signs moved out after a few minutes. The motor functions were normal with no signs of gait irregularity. The mucous membranes were ordinary in all animals and there were no noticeable changes to the color of the eyes. The organ weight indices for various organs remained almost at a constant across the four treatment categories, that is, 1000, 300, 100 mg/kg body weight and in the control group implying that the oral doses tested had little or no impact on the weighed organs.

**Table 3:** Effects of the *Tridex procumbens* flower extract on mean body weight of rats

| S No | Dosage mg/kg | Mean Body Weight | | | | |
|---|---|---|---|---|---|---|
| | | Week 0 | Week 1 | Week 2 | Week 3 | Week 4 |
| 1 | Control | 175.3 ± 2.33 | 188.5 ± 3.22 | 195.3 ± 2.91 | 210.3 ± 2.24 | 220.7 ± 3.35 |
| 2 | 100 | 180.4 ± 2.45 | 185 ± 3.41 | 198.1 ± 3.94 | 210.9 ±3.22 | 222.3 ± 3.55 |
| 3 | 300 | 177.1 ± 3.91 | 180 ± 3.61 | 187.4 ± 4.41 | 195.7 ±6.22 | 212 ± 3.78 |
| 4 | 1000 | 185 ±4.33 | 190.6 ± 2.96 | 198.2 ± 3.55 | 210.5 ± 4.29 | 205.9 ± 4.71 |

**Table 4:** Effects of flower extract on actual weights & organ Weight indices of rats

| S No. | Dosage | Organ Weight | | | | |
|---|---|---|---|---|---|---|
| | | Liver AMW (mg) | Kidney AMW (mg) | Adrenal AMW (mg) | Heart AMW (mg) | Spleen AMW (mg) |
| 1 | Control | 7132 ±177 | 1.44 ±0.12 | 58 ±2.34 | 494±8.22 | 774±4.44 |
| 2 | 100 | 7135 ±288 | 1.25 ±0.22 | 61±3.22 | 498 ±7.34 | 780±8.22 |
| 3 | 300 | 7212 ±233 | 1.46 ±0.21 | 62 ±4.29 | 510 ±7.33 | 784±9.11 |
| 4 | 1000 | 7218 ±213 | 1.28 ±0.11 | 57± 2.45 | 522 ±4.55 | 785±6.66 |

Where- AMW-Average Mean Weight

### Haematological effects

The extract caused a modest increase in RBC count at dosages 100, 300 and 1000 mg/kg compared to the control. However, this augment was neither dose nor time related. There was a slight significant dissimilarity in RBC values ($p<0.0001$.) at dose 1000 mg/kg and the control in the fourth week at which summit RBC dropped significantly beneath the control values dropped. All the treatment groups and the control had a measured rise in haemoglobin, then a slight fall during week 2 to 4 but the levels remained higher than those taken before treatment. The variations between treatment groups were not significant in all time points. Red blood cell levels and haemoglobin levels showed an almost common trend. PCV levels did not show any meaning dissimilarity between all the treatment groups and the controls. The values for immature RBC in doses 100, 300 and 1000 mg/kg were below a digit while that for control was zero. There was dose unconnected fluctuations in the levels of MCH in all treatment groups with that of control experiencing a restrained fall from week 1 to week 4. In 1000 mg/kg category, the MCH level fell during week 1 to 2 then rose progressively during week 3 to 4.

**Table 5:** Overall cumulative effect of the extract on haematological values in rats

| Dose | WBC | RBC | PCV % | Haemoglobin (g/dL) | MCV (fL) | MCHC (g/dl) | Thrombocytes (×103 /_l) | Neutrophils (%) | Total Mature Neutrophils (%) | MCH (pictogram) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1000 mg/kg | 24022 ±3330 | 5.33 ±0.5 | 42.16 ±2.4 | 15.44 ±2.34 | 62.9 ±3.13 | 40.95 ±3.6 | 424.3 ±9.12 | 25.7 ±4.72 | 24.4 ±4.72 | 22.3 ±4.24 |
| 300 mg/kg | 2434 ±3545 | 5.9 ±0.6 | 41.2 ±3 | 15.67 ±3.12 | 61.5 ±3.83 | 39.1 ±3.92 | 430.5 ±11.52 | 26.8 ±2.54 | 25.3 ±4.32 | 24.4 ±4.73 |
| 100 mg/kg | 12135 ±5444 | 5.6 ±0.2 | 40.2 ±2.1 | 15.98 ±4.33 | 61.3 ±4.33 | 39.4 ±3.22 | 432.6 ±12.33.4 | 27.2 ±4.34 | 24.4 ±4.22 | 25.8 ±3.84 |
| Control | 28098 ±4023 | 6.2 ±0.8 | 43.3 ±1.22 | 16.22 ±3.12 | 62.6 ±3.99 | 40.4 ±3.91 | 433.8 ±13.69 | 26.7 ±4.23 | 26.4 ±4.24 | 26.7 ±3.39 |

There was a significant difference in the levels of thrombocytes at 1000 mg/kg treatment group and in the levels of WBC in the 300 and 100 mg/kg categories.

The WBC standards at dosage levels of 300 and 1000 mg/kg remained steady around 25,000 cell/µL between week 2 and 4. The high WBC levels for dosage 100 and 300 mg/kg at the establishment of the conduct experiment could have been due to laboratory errors or personage animal factors since they could not be connected to extract administration.

In general, the lymphocyte levels remained almost a constant in all treatment groups, variable between 65 and 75%. There was no noticeable transform in lymphocyte level in control group, but there was a slight boost in 300 and 1000 mg/kg categories. The levels in 100 mg/kg group fell somewhat from week 1 to 2 then rose from week 3 to 4.

There was a non-dose correlated slight MCV increase in all treatment groups between week 2 to 4 with control almost outstanding as a constant. However, the level in 100 mg/kg category knowledgeable a drop from week 1 to 2 followed by a rise during week 3 and 4.

Total neutrophils and mature neutrophils levels did not show signs of any appreciable differences amongst all the groups. The values were a steady during week 1 to 2 in 100 mg/kg category but there was a slight reduction during week 3 to 4. In 300 mg/kg group the principles fell steadily throughout the treatment period. In 100 mg/kg category, there was a small increase followed by diminish during week 1 to 2 and week 3 to 4 respectively.

**Clinical Parameters**

The effects of the flower extract on clinical parameters are summarized in Table Number 6. Throughout the treatment of experimental rats with the extract, there was a small dose un-related alter in protein levels during week 0 to week 2. The protein levels for dosage 1000 mg/kg and the control greater than before by less than half a unit whereas those for dosage 100 and 300 mg/kg abridged the same margin. From week 2 to week 4, the levels of protein in all behavior groups increase by a minute value.

All the dosage levels of the extract and the control showed similar trends in albumin concentration that was intimately similar to those in control group with values in all groups and the control changeable around 3.5 g/dL from weeks 2 to 4. There was therefore no important dissimilarity in albumin levels between all the treatment groups and the control.

Aspartate aminotransferase (AST) levels dropped progressively from week 1 to week 4 but levels in control group rose again reasonably between weeks 2 and 4. The decreasing trend in AST levels predicted that any continuous dosing of the animals ahead of week 4 could cause further decline in the levels of AST.

**Table 6:** Effects of chloroform-methanol flower extract on the clinical chemistry in rats

| Dosage | Total Protein level (g/dL) | | | Mean Albumin level (mg/dl) | | | Mean AST level IU/L | | |
|---|---|---|---|---|---|---|---|---|---|
| | Week 0 | Week 2 | Week 4 | Week 0 | Week 2 | Week 4 | Week 0 | Week 2 | Week 4 |
| **1000 mg/kg** | 7.8± 1.4 | 7.7 ± 1.3 | 7.5± 1.9 | 4.6±0.44 | 3.5± 0.23 | 4.9± 0.25 | 20.4± 2.14 | 14.4± 2.16 | 12.5± 2.33 |
| **300 mg/kg** | 7.7± 1.4 | 7.3 ± 1.4 | 7.8± 1.8 | 4.5± 0.55 | 3.6± 0.16 | 4.1± 0.22 | 19.4± 2.35 | 13.8± 2.36 | 13.1± 3.24 |
| **100 mg/kg** | 6.3± 1.7 | 7.9± 2.8 | 7.4± 1.3 | 4.8± 0.26 | 3.7± 0.25 | 4.7± 0.14 | 19.6± 3.67 | 14.3± 1.55 | 12.3± 1.33 |
| **Control** | 6.2± 2.5 | 7.6± 1.3 | 7.9± 1.4 | 4.7± 0.19 | 3.7± 0.35 | 4.8 ± 0.27 | 18.8± 2.51 | 14.5± 2.74 | 12.9± 3.64 |

## DISCUSSION

The actuality that an oral dose of 2000 mg/kg body weight did not reason death to laboratory rat suggests that the chloroform: methanol extract of *Tridex procumbens* flower is practically non-toxic after oral dose disclosure presuming that its components were absorbed.

The increase in body weight pragmatic during treatment at the high oral dose confirms the non toxicity of the extract. Organ weight is one of the most sensitive drug toxicity indicators, and it changes often come first morphological changes. The data collected on organ weight was used to compute the organ weight index (OWI) for each isolated organ. As per Society of Toxicological Pathology (STN), the favored organs include the liver, heart, kidney, brain, adrenal glands and the testis. The organ weight indices for various organs remained almost a steady across the various dosage levels and in the control groups. This implies that the presence of the extract in the animal and the various oral concentrations used during the test had modest or no impact on the particular organs.

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) are the markers of liver function and are only unconfined from cytosol and sub-cellular organelles during cell grievance. Alanine aminotransferase is more hepatocellular specific whereas creatinine is a pointer of muscle wasting. Alanine aminotransferase is a critical restriction for recognition of possible drug-induced injury in both pre-clinical studies and human patients [11,12]. It is evident from the results that the ALT and AST values were actually moribund and no increases in these parameters were renowned.

The haematological parameters tested did not show any important variations associated with toxicity to haemopoeitic organs. The modest variations noted in any parameter were well within the normal biological range since none of the parameter greater than before or decreased significantly in relation with the control values at all dosage levels. There was a small non-dose related enlarging in the mean haemoglobin levels and thrombocytes count with time in all treatment levels and the control. In a situation whereby the haemopoeitic organs are affected by the toxic agents, the blood film shows an elevated level of undeveloped neutrophils and nucleated RBC.

The extract may therefore be well thought-out safe when used orally, but further investigations are required to be acquainted with its effects if used via additional routes.

## CONCLUSION

The results showed that no death of experimental rats at an oral dose of 2000 mg/kg body weight symptomatic of that the chloroform: methanol extract of *Tridex procumbens* flower is virtually non-toxic after oral dose exposure presuming that its components were engrossed. The extract can then be concluded to be safe for oral use as a traditional herbal remedy for the different ailments.

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