Safety profile of Ayurvedic poly-herbomineral formulation – Bacnil capsule in albino rats

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Abstract

Introduction: Bacnil capsule is a poly-herbomineral formulation used to treat gastroenteritis. It contains many potential drugs derived from plant sources and Bhasma (calcined fine powder) preparations. Aims: The study was designed to ascertain the safety of bacnil capsule orally in Charle’s Foster albino rats. Materials and Methods: As per the Organization for Economic Cooperation and Development (OECD) 425 protocol oral acute toxicity study, bacnil capsule was administered orally once only at the dose of 2000 mg/kg in rats. For repeated dose toxicity study, AYUSH 170 and OECD 407, it was administered at three dose levels, Therapeutic doses (TED) (196.2), TED × 5 (981) and TED × 10 (1962) mg/kg/day orally for 28 days in albino rats followed by a 15-day recovery period only on TED × 10 dose level. Observation and Results: Bacnil at the oral dose of 2000 mg/kg did not produce any toxicity or mortality in albino rats. Repeated dose 28-day oral toxicity revealed that test formulation did not produce any significant change in serum biochemical, hematological, and histopathological parameters at therapeutic dose level. Mild-to-moderate pathological changes were observed in the various serum biochemical and cytoarchitecture of the liver, heart, kidney, and stomach at a dose of 10 TEDs; however, the same was reversed after discontinuation in the recovery test. Conclusion: Bacnil at 196.2 mg/kg/day is safe at the therapeutic dose level in albino rats.

Keywords: Acute toxicity, bacnil, polyherbomineral formulation, safety

Introduction

Ayurvedic formulations, especially compound polyherbal and herbo-minerals formulations, are widely being used, but less data is available of its safety and drug interactions. Synthetic drugs are single molecule based and provide the symptomatic relief but not the overall health concern, while the holistic response of Ayurvedic formulations are proven to be safer in chronic conditions and restoring the health status. Even through medicinal plants and mineral preparations used conventionally have passed in terms of time, toxicity, and adverse effect; safety concern is always important from the pharmaceutical point of view. To acquire the best advantage even these have been proven to be efficacious in the clinical evaluations.

Bacnil capsule is one of the proprietary poly-herbo-mineral proprietary preparation widely used to treat gastroenteritis. It contains many potential herbal ingredients, along with Bhasma preparations (calcined fine powders). Bhasmas are unique preparations which are safely being practiced in Ayurveda without any noticeable side effects, but no objective, verifiable data exist to support many such claims. Hence, it was thought to undertake a study to ascertain safety of bacnil capsule in the suitable experimental protocol to substantiate its safety.

Materials and Methods

Animal

Charles foster strain albino rats of either sex weighing 200 ± 20 g were used for the experiments. The animals were

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obtained from the animal house attached to Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar (Gujarat). The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC/21/2016/28) in accordance with the guideline formulated by the Committee for the Purpose of Control and Supervision on Experiments on Animals, India. The animals were exposed to 12 h light and 12 h dark cycle with the relative humidity of 50%–70%, and the ambient temperature was 22°C ± 3°C. All animals were kept in the same environmental conditions. They were fed with Amrut brand rat pellet feed supplied by Pranav Agro Industries, Baroda and drinking water was given ad libitum.

**Drug**

Bacnil capsule was provided by Zoetic Ayurvedic Pvt. Ltd., Ahmedabad (Gujarat) having Batch no 531681057 and manufacturing date Dec2015 [Table 1].

**Selection of dose**

The human clinical dose of bacnil capsule (545 mg) is four capsules/day equal to 2180.0 mg/day. Dose of the bacnil capsule was calculated by extrapolating the human therapeutic dose to rat based on body surface area ratio (conversion factor 0.018 for 200 g rat) by referring to the table of Paget and Barnes (1964). Thus, therapeutic equivalent dose of bacnil was found to be 196.2 mg/kg, Therapeutic doses (TED) × 5 (981.0 mg/kg) and TED × 10 (1962.0 mg/kg).

**Experimental design**

**Acute toxicity study**

Acute oral toxicity study of bacnil capsule was carried out using Organization for Economic Cooperation and Development (OECD) 425 guideline. It was conducted using the limit dose test of Up and Down procedure. The female Charle’s foster albino rats were taken. Food, but not water, was withheld for overnight before the experiment and further 2 h after the administration of test drug. Bacnil capsule was administered to overnight fasted rats at 2000 mg/kg in a sequential manner. Mortality, gross behavior, and other parameters were closely observed for the first 4 h and up to 8 h on the 1st day and thereafter every 24 h up to 14 days.

**Repeated dose 28 days toxicity**

The experiment was conducted as recommended by the standard protocol of AYUSH 170[3] guideline and OECD (Organization for Economic Cooperation and Development)[8] 407 guideline. For 28 days, repeated dose oral toxicity study, Charles foster albino rats of either sex weighing 200 ± 20 g were selected. Animals were kept for acclimatization for 1 week, and randomly divided into five groups of 10 animals (05-male and 05-female) in each group. Group (I) was kept as a control group, received distilled water as vehicle (10 ml/kg, orally). Group (II) to (IV) were administered with test drug bacnil at TED (196.2 mg/kg, orally), TED × 5 (981.0 mg/kg, orally), TED × 10 (1962.0 mg/kg, orally), respectively. In addition, 10 animals were kept in Group (V) as recovery group, received bacnil, TED × 10 (1962.0 mg/kg, orally) for observation after the treatment period, of reversibility or persistence of any toxic effects. The suspensions of test drugs were administered orally once a day for 28 consecutive days in the main study, while the duration of the recovery period was fixed as 14 days after the main study of 28 days. The administration period of the drug for the toxicity study was decided from the duration of the bacnil capsule in the clinical setting.

The rats were carefully observed daily for any overt and apparent signs and symptoms of toxicity during the entire experimental period. The body weight change of individual rats was noted initially and thereafter weekly during the study period. At the end of the experimental period, blood was withdrawn from the retro-orbital puncture under light ether anesthesia using the capillary tube for the estimation of various serum biochemical parameters and hematological parameters.

### Table 1: Composition of bacnil capsule in each 250 mg

| Name of drugs | Botanical name and Sodhana | Part used or preparations | Weight (mg) |
|---------------|-----------------------------|---------------------------|-------------|
| Vatsanabha    | Purified Aconitum ferox wall ex. Ser. (purified with cow urine) | Root                      | 12.50       |
| Tankana       | Processed Sodas Bibora      | Mineral                   | 12.50       |
| Maricha       | Piper nigrum Linn.          | Fruit                     | 30.00       |
| Kapardika Bhasma | Calcined cowries            | Bhasma (calcined fine powder) | 50.00       |
| Shankha Bhasma | Calcinated Turbinella rapa shells | Bhasma (calcined fine powder) | 25.00       |
| Muktashakti   | Calcined Myristol           | Bhasma (calcined fine powder) | 25.00       |
| Bhasma        | margaritiferus shells       |                           |             |
| Pathyadi Kwatha Ghana | Terminalia chebula Retz.   | Ghana                     | 10.00       |
|               | Terminals bellirica (Guertn.) Roxb. |                         | 10.00       |
|               | Emblica officinalis Linn.   |                           | 10.00       |
|               | Tinospora cordifolia (Thunb.) Miers. |                         | 10.00       |
|               | Azadirachta indica A. Juss. |                           | 10.00       |
|               | Andrographis paniculata (Burm. f.) Wall. ex Nees |             | 10.00       |
|               | Curcuma longa Linn.         |                           | 10.00       |
| Kuberaksha    | Caesalpinia bounduc Linn. Roxb. | Root                     | 50.00       |
| Gairika       | Purified Ochre. (purification done using cow ghee) | Mineral                 | 25.00       |
Hematological analysis was performed using an automatic hematological analyzer (Swelab). The parameters studied were total red blood cell (RBC), hemoglobin (Hb), packed cell volume, mean corpuscular volume, mean corpuscular Hb (MCH), MCH concentration, white blood cell (WBC), neutrophils percentage, lymphocyte percentage, eosinophils percentage, monocytes percentage and platelet count.

Various serum biochemical parameters were estimated using a fully automated biochemical random-access analyzer (BS-200, Lilac Medicare Pvt. Ltd., Mumbai). The parameters studied were blood sugar,[9] total cholesterol,[10] triglycerides,[11] high-density lipoprotein-cholesterol,[12] blood urea,[13] creatinine,[14] serum glutamic pyruvic transaminase (SGPT),[15] serum glutamic oxaloacetic transaminase (SGOT),[16] total protein,[17] albumin, globulin,[18] alkaline phosphatase,[19] total bilirubin,[20] direct bilirubin, uric acid[21] and calcium.[22]

At the end of the experiment, the animals were sacrificed, and the abdomen was opened through midline incision to record the autopsy changes followed by dissecting out the important organs. Bone marrow smear from the femur bone was prepared using the standard procedure. All the important internal organs were carefully dissected, namely the brain, pituitary, liver, heart, spleen, kidney, lung, stomach, intestine, uterus, ovary, adrenal gland, trachea, aorta, lymph node and skin. After noting any sign 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 hematoxylin and eosin for histological evaluation which was done using Carl Zeiss’s microscope (Germany) at various magnifications to note down the changes in the microscopic features of the tissues studied.

**Statistical analysis**

The data is expressed as mean ± standard error of the mean for ten rats per experimental group. Paired *t*-test and One-way analysis of variance were used to compare the mean values of quantitative variables among the groups followed by Dunnett multiple *t*-test for unpaired data by using Sigma Stat software to determine significant difference between groups at *P* < 0.05.

**Results**

At all dose levels, bacnil showed a progressive increase in the bodyweight when compared to normal control group, but the values did not reach to significant level [Table 2].

Significant increase in the relative weight of seminal vesicle and (TED × 5) uterus (TED) and decrease in liver weight at TED × 5 dose level was observed in comparison to the control group. However, there were no significant changes in the relative weight of organs, namely kidney, heart, spleen, prostate and testis at all dose levels in comparison to the control group [Table 3]. Relative organ weight normally decreases which indicates loss of tissue mass in that organ, exception being the secretory organs in which decrease in weight sometimes is seen along with increased activity. If we consider this data along with the findings of the histopathological study, the weight reduction does not seem to represent loss in the tissue mass because no pathological changes were observed in histological study, which suggest the loss of tissue mass. The observed changes were devoid of any major adverse changes and were non-significant in comparison to the control group and it reversed in recovery duration; hence, it may be suggested that drugs do not seem to produce any toxic effect on the relative weight of important internal organ in repeated dose toxicity studies.

Data of serum biochemical parameters revealed that bacnil at TED dose level produced a significant decrease in triglyceride and very low-density lipoprotein (VLDL)-cholesterol. Bacnil at TED × 10 produced non-significant increase in triglyceride, VLDL-cholesterol and alkaline phosphate, which suggests that test drug has the potential to damage the liver at a higher dose level on longer administration. But it reverted in recovery study. Test drug did not affect the SGOT at all dose level while the non-significant increase in SGPT at TED × 5 dose level in comparison to the control group was found. After discontinuation of the test drug in the recovery study, there was a decrease in the SGPT level in comparison to the control group. Serum calcium is the principle component of the bone and teeth with 99% of the body’s calcium is found in these structures. Bacnil at lower dose level did not affect the serum calcium level while at TED × 10 dose level produced a significant decrease and was again decreased after discontinuation of drug in recovery study. The result suggests, that test drug has a role on calcium turnover in the body.[23] However, almost all serum biochemical parameters were within the normal range[23] hence, cannot be categorized as pathological changes, which suggest that drug is devoid of any toxic effects on serum biochemical parameters [Table 4].

The effect of test drug on twelve hematological parameters revealed that RBC and platelet related parameters were not affected. In WBC related parameters, test drug (TED × 10R

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**Table 2: Effect of bacnil capsule on body weight of rats treated for 28 days**

| Day     | Control   | TED  | TED × 5 | TED × 10 | TED×10 (R) |
|---------|-----------|------|---------|----------|------------|
| Initial | 204.88±6.04 | 196.88±6.67 | 210.00±4.16 | 210.00±3.77 | 200.55±3.18 |
| Final   | 224.88±9.27 | 201.22±7.76 | 224.33±8.12* | 219.44±4.50* | 222.11±7.22* |
| Percentage change | 9.76† | 2.20† | 6.82† | 4.50† | 10.75† |

Data: Mean±SEM. *P>0.05 when compared with initial values (Paired *t*-test). SEM: Standard error of the mean, TED: Therapeutic dose †: Increase
dose level produced a significant decrease in neutrophil while, a significant increase in monocyte and lymphocyte in comparison to the control group. In the recovery group, WBC-related parameters showed nonsignificant decrease in all parameters except nonsignificant increase in monocyte and lymphocyte at TED × 5 and TED × 10. These changes were within the normal range[22] which clearly indicates that bacnil at even at TED × 10 dose level does not affect both cellular and noncellular elements of the blood to a significant extent [Table 5].

The results of histopathological studies suggest that bacnil at TED × 10 dose level produced fatty changes in the heart; fatty degenerative changes, cell infiltration and sinusoidal inflammation in the liver; cell infiltration in the ileum, mild fatty changes in kidney and mild epithelial erosion in the stomach. In the recovery study, fatty changes and sinusoidal inflammation in the liver of rats were observed. bacnil TED × 5 produced fatty changes in liver and epithelial erosion in the stomach while it was reverted in recovery study. No other changes were observed in any organs in Bacnil at TED-treated rats and in the control group. The results suggest that drug has mild-to-moderate adverse effects on above organs at TED × 10 (196.2 mg/kg, orally) dose level and is not observed at TED (196.2 mg/kg, orally) dose level. The changes were reversed in all organs in recovery study except liver after discontinuation of drug, which suggests the observed changes are reversible in nature in most of the organs [Figures 1-4].

### Discussion

The World Health Organization insists that the safety of herbal medicine is a critical component in the traditional system of medicine.[23] The absolute safety of herbs and herb-mineral formulations can not be established exactly from traditional

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Table 3: Effect of bacnil capsule on relative organ weight of rats treated for 28 days

| Organs          | Control | Bacnil capsule |
|-----------------|---------|----------------|
|                 | TED     | TED × 5        | TED × 10 | TED × 10 (R) |
| Liver (g/100 g) | 3.319±0.12 | 3.049±0.12 | 3.004±0.07 | 3.087±0.03 | 2.919±34.95* |
| Heart (mg/100 g)| 308.26±9.43 | 311.19±14.02 | 304.12±9.05 | 306.63±5.64 | 299.2±6.69 |
| Kidney (mg/100 g)| 685.02±23.92 | 686.47±17.16 | 631.14±21.30 | 679.55±25.19 | 675.59±23.17 |
| Spleen (mg/100 g)| 251.68±14.85 | 230.83±14.93 | 244.77±13.98 | 225.33±16.47 | 206.06±11.07 |
| Testis (mg/100 g)| 1011.5±39.99 | 980.28±63.56 | 930.81±44.84 | 1011.03±55.95 | 873.26±124.74 |
| Prostate (mg/100 g)| 189.95±21.03 | 152.2±12.92 | 227.67±15.80 | 177.63±11.99 | 171.3±22.44 |
| Seminal vesicles (mg/100 g)| 485.28±36.37 | 535.74±58.42 | 704.37±18.21* | 508.98±47.09 | 572.73±25.66 |
| Uterus (mg/100 g)| 230.70±42.29 | 346.50±20.78 | 319.67±28.89 | 300.78±26.33 | 300.12±9.43 |

Data: Mean±SEM. *P<0.05 when compared with the control group (ANOVA followed by Dunnett’s multiple t-test). SEM: Standard error of the mean, ANOVA: Analysis of variance, TED: Therapeutic dose

Table 4: Effect of bacnil capsule on the serum biochemical parameters of rats treated for 28 days

| Parameters      | Control       | Bacnil capsule |
|-----------------|---------------|----------------|
|                 | TED           | TED × 5        | TED × 10 | TED × 10 (R) |
| Blood sugar (mg/dL) | 78.60±4.29 | 83.20±2.68 | 79.30±2.18 | 82.11±3.84 | 67.00±3.89 |
| Total cholesterol (mg/dL) | 53.60±4.89 | 53.30±4.89 | 59.80±5.20 | 54.11±4.38 | 64.80±6.40 |
| Triglyceride (mg/dL) | 135.60±12.97 | 97.10±9.84 | 129.20±12.51 | 177.89±29.55 | 140.70±20.53 |
| VLDL-cholesterol (mg/dL) | 27.10±2.54 | 19.40±1.96 | 25.80±2.51 | 35.89±5.96 | 28.10±4.14 |
| HDL-cholesterol (mg/dL) | 33.10±2.47 | 34.00±2.72 | 39.50±2.28 | 33.11±3.07 | 37.20±3.25 |
| Total protein (g/dL) | 6.91±0.20 | 6.89±0.19 | 7.08±0.17 | 6.73±0.20 | 7.08±0.12 |
| Albumin (g/dL) | 3.47±0.12 | 3.60±0.15 | 3.95±0.07 | 3.80±0.13 | 3.84±0.12 |
| Globulin (g/dL) | 3.44±0.22 | 3.30±0.18 | 3.21±0.11 | 2.93±0.12 | 3.24±0.11 |
| SGPT (IU/L) | 80.10±5.88 | 71.30±4.92 | 90.00±9.94 | 82.22±4.26 | 66.30±3.13 |
| SGOT (IU/L) | 152.50±7.90 | 147.90±7.32 | 159.70±8.42 | 151.00±6.82 | 143.20±6.30 |
| Alkaline phosphatase (IU/L) | 228.50±31.10 | 228.50±31.10 | 181.80±13.67 | 258.11±21.53 | 168.60±14.87 |
| Blood urea (mg/dL) | 46.10±2.93 | 54.40±2.69 | 40.80±2.34 | 39.11±1.59 | 48.40±2.30 |
| Creatinine (mg/dL) | 0.70±0.039 | 0.72±0.035 | 0.70±0.025 | 0.59±0.042 | 0.75±0.04 |
| Total bilirubin (mg/dL) | 0.32±0.032 | 0.33±0.026 | 0.36±0.040 | 0.36±0.037 | 0.23±0.015 |
| Direct bilirubin (mg/dL) | 0.12±0.01 | 0.11±0.01 | 0.13±0.01 | 0.13±0.03 | 0.10±0.00 |
| Uric acid (mg/dL) | 0.57±0.05 | 0.55±0.04 | 0.78±0.09 | 0.53±0.09 | 0.68±0.05 |
| Serum calcium (mg/dL) | 10.17±0.32 | 10.01±0.18 | 9.53±0.10 | 9.41±0.17* | 9.46±0.07* |

Data: Mean±SEM. *P<0.05 when compared with the control group (ANOVA followed by Dunnett’s multiple t-test). SEM: Standard error of the mean, VLDL: Very low-density lipoprotein, HDL: High-density lipoprotein, SGPT: Serum glutamic pyruvic transaminase, SGOT: Serum glutamic oxaloacetic transaminase, ANOVA: Analysis of variance, TED: Therapeutic dose
knowledge without generating toxicology profile through experimental study. Bacnil capsule contains many potential herbal ingredients along with Bhasma preparations. It is multicomponent in nature which may provide synergistic effects and there is an increasing demand of these medicines among the public in clinical use. In the present study, the safety profile of the bacnil capsule was investigated through acute and repeated oral toxicity study at different dose levels in Charles foster albino rats.

OECD 425 guideline for oral acute toxicity study was employed using female rats to reduce variability and as a means of minimizing the number of animals used. This is because there is little difference in sensitivity in LD₅₀ test between the sexes, but, in those cases where differences are observed, females are generally slightly more sensitive. Bacnil capsule did not show any signs and symptoms of toxicity and mortality at the oral dose of 2000 mg/kg in female rats. The bacnil capsule did not affect any behavioral changes, gross behavior and other observed parameters during the entire experimental period of 14 days. This indicates that the oral LD₅₀ of the test drug is much higher than 2000 mg/kg administered in the present study. As per UN classification, any substance which has oral LD₅₀ of more than 2000 mg/kg is considered as low hazard potential and categorized as UN 6.1 PG III. Thus, as per the above criterion, bacnil capsule can be categorized as substances with low health hazard potential (Class 4 of GHS and UN 6.1PG III).

Hence, the bacnil capsule is safe the therapeutic dose level in the present test, and there is no remarkable toxic effect in the treatment for 28 consecutive days. In addition, it will be wise to include detailed data on hematological parameters to support the safety profile.

Table 5: Effect of bacnil capsule on the hematological parameters of rats treated for 28 days

| Hematological parameters      | Control          | TED       | TED × 5     | TED × 10    | TED × 10 (R) |
|------------------------------|------------------|-----------|-------------|-------------|--------------|
| Total WBC count (10⁶/Cumm)   | 12,780.00±1352.67| 11,190.00±1174.87 | 10,420.00±1038.67 | 10,600.00±1012.14 | 9100.000±517.04 |
| Neutrophils %                | 24.60±3.83       | 26.30±4.53 | 22.80±2.30  | 19.56±3.24  | 8.90±1.99    |
| Eosinophils %                | 2.30±0.21        | 2.60±0.26  | 2.70±0.15   | 2.67±0.33   | 2.30±0.30    |
| Monocytes %                  | 2.00±0.14        | 2.20±0.29  | 2.60±0.22   | 2.44±0.17   | 2.60±0.30    |
| Lymphocytes %                | 71.10±4.06       | 68.90±4.49 | 71.90±2.46  | 76.44±3.41  | 86.20±1.98   |
| Hb %                         | 15.35±0.21       | 15.38±0.47 | 14.55±0.26  | 14.76±0.20  | 15.48±0.31   |
| PCV %                        | 48.27±1.07       | 48.46±1.29 | 46.05±0.74  | 46.40±0.66  | 48.37±1.10   |
| RBC (mil/cumm)               | 8.46±0.18        | 8.69±0.20  | 8.21±0.15   | 8.26±0.17   | 8.69±0.22    |
| MCV (fl)                     | 57.08±0.69       | 55.76±0.51 | 56.19±0.49  | 56.16±0.50  | 55.70±0.44   |
| Platelet count (10⁵/µL)      | 1013.50±41.11    | 1031.10±61.50 | 984.30±99.65 | 948.78±53.10 | 1030.90±28.93 |
| MCH (pg)                     | 18.18±0.19       | 17.70±0.24 | 17.91±0.20  | 17.87±0.23  | 17.80±0.18   |
| MCHC (g/dL)                  | 31.86±0.34       | 31.71±0.22 | 31.86±0.17  | 31.73±0.19  | 31.97±0.194  |

Data: Mean±SEM. *P<0.05 when compared with the control group (ANOVA followed by Dunnett’s multiple t-test). SEM: Standard error of the mean, WBC: White blood cell, Hb: Hemoglobin, PCV: Packed cell volume, RBC: Red blood cell, MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration, ANOVA: Analysis of variance, TED: Therapeutic dose

Figure 1: Photomicrographs of the heart taken at × 400 magnification, (a) normal cytoarchitecture in control group, (b) normal cytoarchitecture in TED × 5 treated rats, (c) mild fatty changes in TED × 10 treated rats, (d) normal cytoarchitecture in TED × 10(r) rats

Figure 2: Photomicrographs of the liver taken at × 400 magnification, (a) normal cytoarchitecture in control group, (b) fatty degenerative changes and loss of cytoarchitecture of hepatocytes in TED × 5 treated rats, (c) fatty degenerative changes, cell infiltration and sinusoidal inflammation in TED × 10 treated rats, (d) fatty changes and sinusoidal inflammation in TED × 10(r) rats
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Conflicts of interest

There are no conflicts of interest.

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to consider the above improvements in clinical trials according to regulatory guidelines.

Conclusion

From the present study, it can be concluded bacnil capsule did not produce any abnormal sign and symptoms and mortality up to dose of level of 2000 mg/kg, orally in acute toxicity study which suggests that LD_{50} value may be higher than 2000 mg/kg by oral route in rat and hence it can be categorized as substances with low health hazard potential. Repeated dose 28-day oral toxicity concludes that test drug is not likely to produce any toxicity though produced mild changes at TED × 10 dose level in the liver and kidney but same was reverted in recovery study after discontinuation of drug which suggest safety of bacnil at TED level in albino rats.
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