did not show any toxicity in animals throughout 28 d of exposure and therefore showed no morbidity and mortality. The body weight also did not show any significant variation. Similarly the organ:body weight ratio (Table 3.3) of treated animals did not show any significant variation. No gross significant changes were observed in the morphological characters and no pathological lesions were detected in any vital tissues like liver, kidney, testis, ovary and skin. Also, no significant change in hematological parameters was observed (Table 3.4).

The biochemical studies on the activity of GOT, GPT, ALP and proteins of the control and product-treated (1000 mg/kg/d) animals are shown in Table 3.5. No significance changes in the serum, protein and BUN between the control and experimental animals were observed. However, the activity of serum GAT and Alp were altered in both male and female rats. The serum GOT activity was significantly altered in male rats only.

The mucus membrane irritation test in female rabbits did not show any sign of erythema or oedema in the vaginal mucous membrane of rabbits. It was found to be non-irritant. The skin sensitization test in guinea pigs also indicated that the product was found to be unresponsive in guinea pigs.

3.7.5 Biochemical Studies in Blood Plasma of Leucoderma Patients

3.7.5.1 Screening of Tyrosinase Enzyme

The tyrosinase enzyme level was studied in the blood plasma of some of the leucoderma patients and was compared with healthy persons taken as a control. Standardization of the tyrosinase estimation methods was based on the conversion of dihydroxy-phenylalanine to red coloured oxidized product DOPA chrome. The absorbance of DOPA chrome was measured at 475 nm in spectrophotometer at two stages viz. before and after incubation. The change in absorbance at two levels is proportional to the enzyme concentration. The method standardization was done taking the blood plasma of white, variegated and black cows which showed respective variation, i.e. the highest levels in black and lowest in white cattle.

The comparative level of enzyme gave the following observation:

- Enzyme tyrosinase was found to be absent or beyond detectable limit in 15% of patients.
- In 60% of patients, the tyrosinase level was found to be lower as compared to healthy persons.
- In the rest of the the patients, the level of tyrosinase was found to be almost equal to that of healthy persons, but there was a severe deficiency of copper in their blood plasma.

3.7.5.2 Screening of Copper and Zinc in the Blood Plasma of Leucoderma Patients

With a view to standardize the anti-leucoderma ointment and its oral dose, screening of copper and zinc was carried out in the blood plasma of the patients. Copper acts as prosthetic group of enzyme tyrosinase, without which the enzyme becomes inactive. The estimation of both copper and zinc was done with the help of the Atomic Absorption Spectrophotometer. The following observation were recorded (Table 3.6):