Chapter III

Serological Studies

Increased enzyme activities (which play role in liver metabolisms) in plasma/serum is the sign of destruction of hepatocyte integrity. The altered cellular enzymes and bilirubin may indicate obstruction, proliferation, inflammation or neoplasia of the biliary system.

In clinical diagnosis serum AST (SGOT), ALT (SGPT) and alkaline phosphatase are commonly used as the parameters of liver function. Similarly alkaline phosphatase is widely used as a marker to detect hepatic damages especially cholestasis. It is also indicator of damaged condition of tissue mainly in rats.

Among the parameters other than enzymes are serum bile pigments (bilirubin) that are used for the diagnosis of liver disorders. Its level in serum is altered due to defect in hepatic clearance by microsomal conjugation, intrahepatic obstruction of bile canaliculi or extrahepatic obstruction of the bile duct. Conjugated and unconjugated bilirubin are increased in hepatic obstruction and cholestasis in rats (Stonard and Evans, 1995).

Since the levels of serum bilirubin, AST [SGOT], ALT [SGPT], alkaline phosphatase and bilirubin are utilized for the

diagnosis of hepatitis. During present study also these parameters are studied to evaluate the hepatoprotective action of abhrak bhasma in albino rats.

MATERIAL AND METHODS

Preparation of mandur bhasma :

Mandur bhasma was prepared in the laboratory by the method as described in Material and Methods.

Experimental protocol :

Ninety days old male albino rats [120 to 130 g] derived from Haffkine strain were used for the present study. The animals were maintained under standard laboratory conditions and were fed standard pellet feed and water *ad libitum*. The rats were divided into 4 groups each containing 6 animals.

Group I - The rats of this group were maintained as normal without any treatment.

Group II - The rats of IInd group were given rifampicin (50 mg/kg body wt; po) daily at 8-30 to 9-00 a. m. for 30 days.

Group III - Rifampicin (50 mg/kg body wt.) and mandur bhasma 10 mg/kg body wt. was given daily at 8-30 to 9-00 a. m. for 30 days to the rats of this group.

Group IV - Mandur bhasma (10 mg/kg body wt.) were given orally daily at 8-30 to 9-00 a. m.

The rats from all 4 groups were killed by giving ether anesthesia. Blood samples were aspirated from the left ventricles. Sera were prepared after clotting of the blood and were utilized for the assays of serum enzymes and bilirubin. AST and ALT were estimated using commercial kits according to the method of Reitman and Frankel [1957]. Determination of bilirubin was carried out by the method described by Jendlrassik and Grof (1938). Alkaline phosphatase was assayed according to Bergmeyer [1965]. Urea and creatinine were determined by the methods of Henry (1991) and Heinegard and Tiderstorm (1973).

RESULTS

Changes in serum enzymes of liver function tests :

Variations in serum AST, ALT and alkaline phosphatase are shown in Table 1 and Figure 1. The alterations in serum AST, ALT and alkaline phosphatase are given in Table 1. Normal rat exhibited 24.39 ± 1.08, 42.05 ± 2.36 and 35.21 ± 1.34 units of activities of AST, ALT and alkaline phosphatase in serum. Administration of rifampicin for 30 days elevated the activities in rat serum by 2.80, 1.53 and 2.51 folds. While administration of mandur bhasma concurrent with rifampicin for 30 days counteracted the action of rifampicin by reducing the levels of AST, ALT and alkaline phosphatase. The reductions of 59.67, 37.51 and 73.22 % were observed when compared with the levels of respective

Table 1 – Mandur Bhasma induced Changes in serum AST and ALT and alkaline phosphatase levels during rifampicin induced liver necrosis

(Values are expressed units/ml serum)

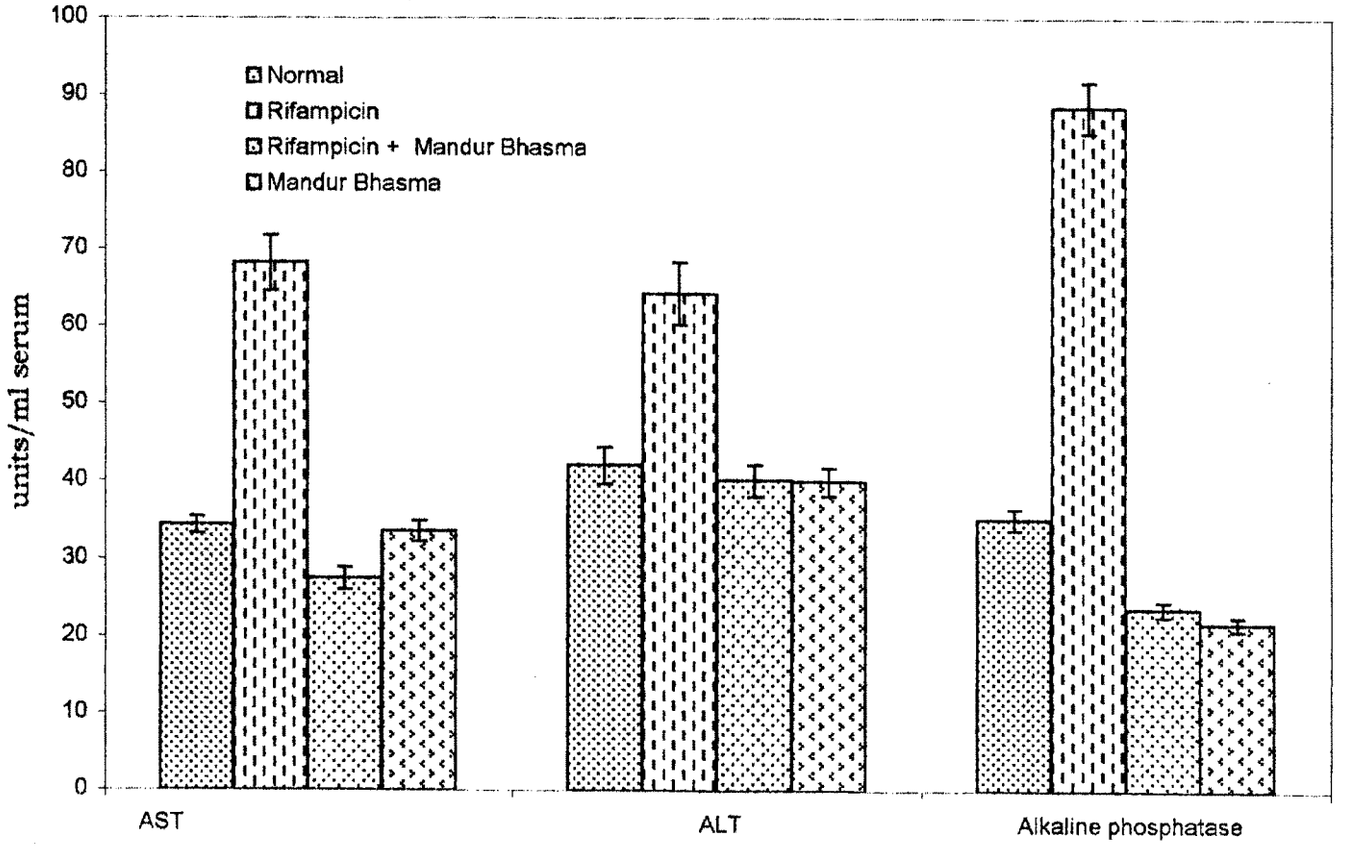
Group	AST	ALT	Alkaline phosphatase
Normal	34.39 ± 1.08	42.05 ± 2.36	35.21 ± 1.34
Rifampicin	68.22 ± 3.54^{c}	64.27 ± 4.05^{c}	88.45 ± 3.33^{c}
Rifampicin + Mandur Bhasma	27.51 ± 1.46^{a,g}	40.16 ± 2.04^{d,g}	23.69 ± 0.94^{c,g}
Mandur Bhasma	33.64 ± 1.32^{c,d}	39.97 ± 1.80^{d,h}	21.74 ± 0.87^{c,g}

Values are mean ± SE of 6 animals

P Values - a< 0.05, b< 0.01, c< 0.001 & d> 0.05

E < 0.05, f< 0.01, g< 0.001 & h< 0.05

Figure 1 – Mandur Bhasma induced Changes in serum AST and ALT and alkaline phosphatase levels during rifampicin induced liver necrosis



enzymes in normal rat serum. Comparison of enzyme activities of rifampicin + mandur bhasma treated rats with normal values exhibited the falls of 20.01, 4.49 and 32.72 % in serum AST, ALT and alkaline phosphatase levels. Marginal falls of 2.19 and 4.96 % were observed in AST and ALT of rat serum after the administration of only mandur bhasma for one month. While decrease of 38.26 % was noticed in rat serum alkaline phosphatase level after the administration only mandur bhasma.

Serum bilirubin :

Variations in conjugated, unconjugated and total bilirubin are given in table 2 and Figure 2. The elevations of 82.16, 146.67 and 88.98 folds were noted in conjugated, unconjugated and total bilirubin contents of rat serum after the treatment of rifampicin for 30 days. Oral treatment of mandur bhasma concurrent with rifampicin lowered conspicuously the values of bilirubin contents. Reductions of 98.62, 99.31 and 98.74 % were observed in the contents of unconjugated, conjugated and total bilirubin after simultaneous administrations of rifampicin and mandur bhasma on comparison with the respective values observed in group II rats treated with only rifampicin. Comparison of the values of unconjugated and total bilirubin of rifampicin + mandur bhasma treated rats with normal values showed 13.73 and 12.28 % reductions respectively without any change in conjugated bilirubin

Table 2 –Variations in serum bilirubin content due to Mandur Bhasma during rifampicin induced liver damage

(Values are expressed as mg/dl serum)

Group	Direct	Indirect	Total
Normal	0.051 ± 0.003	0.006 ± 0.000	0.057 ± 0.003
Rifampicin	4.19 ± 0.180[c]	0.880 ± 0.000[c]	5.072 ± 0.230[c]
Rifampicin + Mandur	0.058 ± 0.004[a,g]	0.006 ± 0.000[d,g]	0.064 ± 0.004[a,g]
Mandur	0.049 ± 0.003[d,g]	0.006 ± 0.000[d,g]	0.055 ± 0.004[d,g]

Values are mean ± SE of 6 animals

p values are as in Table 1

Figure 2 –Variations in serum bilirubin content due to Mandur Bhasma during rifampicin induced liver damage

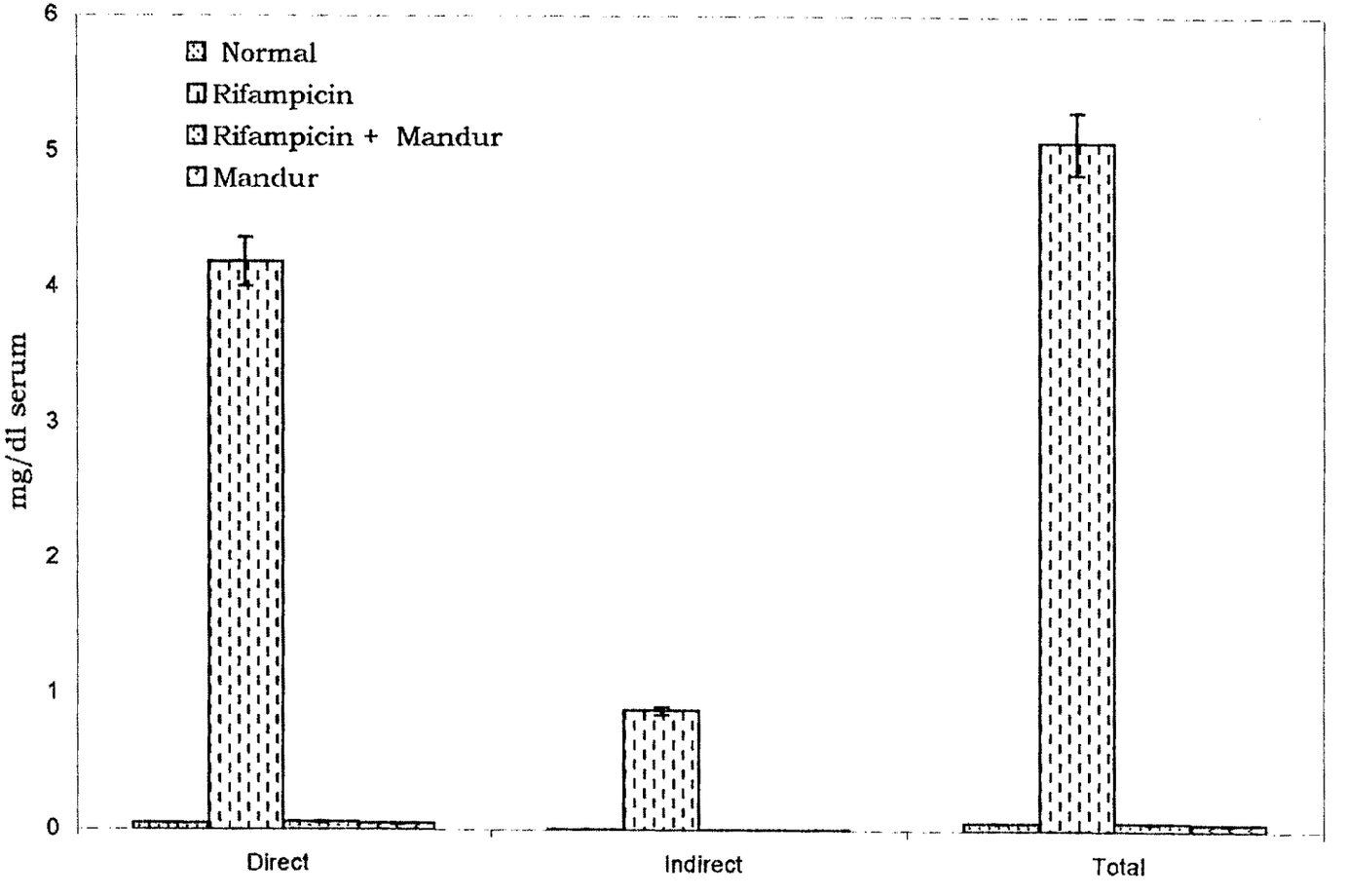


Table 3 – Action of Mandur Bhasma on serum urea and creatinine levels during rifampicin induced liver necrosis

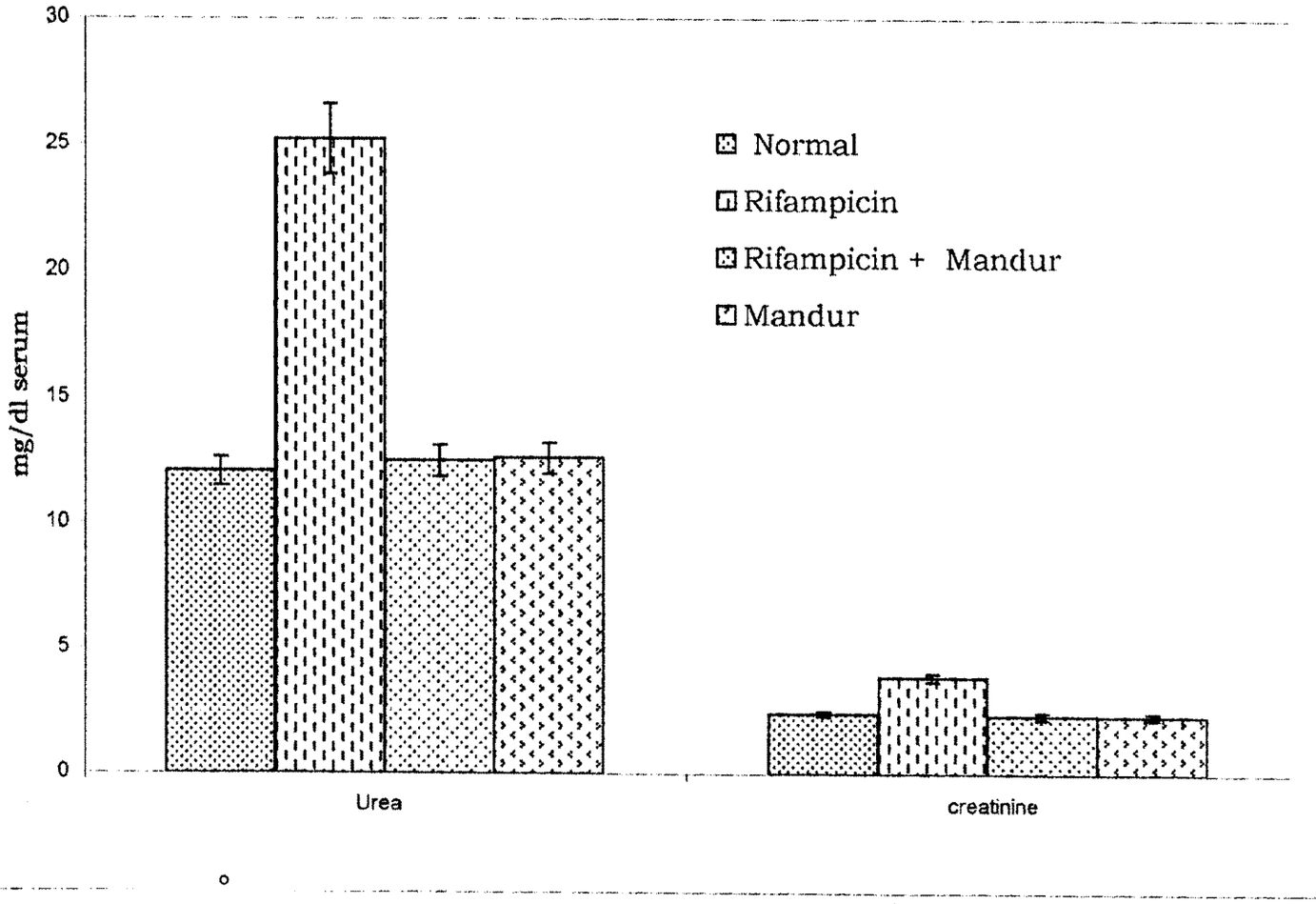
(Values are expressed as mg/dl serum)

Group	Urea	creatinine
Normal	12.08 ± 0.57	2.42 ± 0.11
Rifampicin	25.21 ± 1.39^{c}	3.86 ± 0.17^{b}
Rifampicin + Mandur	12.50 ± 0.63^{d}	2.34 ± 0.15^{d}
Mandur	12.62 ± 0.62^{d}	2.33 ± 0.14^{a}

Values are mean ± SE of 6 animals

p values are as in Table 1

Figure 3 – Action of Mandur Bhasma on serum urea and creatinine levels during rifampicin induced liver necrosis



level (0.00 %). On the contrary administration of only mandur bhasma did not alter the contents of unconjugated (3.92 %) conjugated (0.00 %) and total bilirubin (3.51 %) in the serum of rat.

Kidney Function Tests :

Changes in serum urea and creatinine content of albino rat during present study are presented in Table 3 and Figure 3. Elevations of 2.09 and 1.60 folds were noted in urea and creatinine contents were observed in group II rats after the administration of only rifampicin. Administration of mandur bhasma concurrent with rifampicin counteracted the action of rifampicin by normalizing the serum levels of urea and creatinine. The contents of urea and creatinine were lowered by 50.42 % and 39.38 % respectively as compared to the respective values noted in group II rats treated with only rifampicin. However administration of only mandur bhasma to group III rats did not alter urea and creatinine contents significantly.

DISCUSSION

Serum levels of AST, ALT and alkaline phosphatase and bilirubin suggest the status of liver function (Talwar, 1980). During present study serum enzymes, AST, ALT and alkaline phosphatase were significantly elevated by rifampicin treatment indicating damage to the liver. The levels of bilirubin in serum also indicate

the damage to liver by rifampicin. Skakun and Shaman'ko (1981) showed rifampicin potentiated isoniazid toxicity. Skakun and Tabachuk (1992) showed increased AST, ALT and bilirubin alkaline phosphatase on rifampicin administration coupled with decreased bile secretion, excretion of bile acid and bilirubin content and cholesterol in liver. Biliriary excretion studied with help of dye decreased in rifampicin induced fatty liver (Khedun *et al,* 1992). Sodhi *et al* (1998) also showed increase in the level of AST, ALT in rifampicin induced fatty liver. Treatment of mandur bhasma concurrent with rifampicin normalized the levels of AST, ALT and alkaline phosphatase activities were lowered conspicuously by preventing/protecting liver and kidney from the toxic effects of rifampicin. Treatment of only mandur bhasma did not alter these parameters excepting alkaline phosphatase level, which was reduced significantly. Mandur bhasma also counteracted the action of rifampicin on bilirubin content of serum. The present observations suggest the hepatoprotective action of mandur bhasma, since increased serum enzymes are due to the leakage from liver during the damage induced by rifampicin and their values are lower in healthy state.

Creatinine and urea are increased in serum during renal failure Henry (1991) and Heinegard and Tiderstorm (1973). Therefore these parameters are used for the clinical evaluation

kidney function. Very little information is available on the toxic effect of rifampicin on serum urea and creatinine. Increased serum levels of urea and creatinine during present study by the administration of rifampicin indicate the damage to rat kidney. however administration of mandur bhasma concomitant with rifampicin reduced these serum parameters and maintained at normal level indicating mandur bhasma mediated protection to kidney against rifampicin. No alterations in serum urea and creatinine by mandur bhasma treatment suggests that mandur bhasma is not toxic.

From the foregoing observation it is clear that mandur bhasma protects both the liver and kidney against rifampicin toxicity. Therefore mandur bhamsa may be used to reduce the toxicity of rifampicin. Mandur bhasma may be helpful to cure tuberculosis, since it is recommended in ayurveda for the treatment of tuberculosis (Mishra, 1984).