Chapter VI

Other Parameters

Rifampicin administration usually with other antituberculin drugs showed increased lipid peroxidation liver of albino rats irrespective of their route of administration (Skakun and Suman'ko, 1985; Sodhi *at al*, 1997; 1998; Attari *et al* (2000). There is hardly any report on protection of rifampicin induced lipid peroxidation in liver and kidney of rat.

No reports are available on rifampicin induced formaldehyde formation in liver and kidney of rats.

Fifty mg per kg of rifampicin given for two weeks reduced glutathione and thiol content in liver and activities of glutathione peroxidase, glutathione-S-transferase and glutathione reductase in liver (Sodhi *et al*, 1998) and blood and liver (Sodhi *et al*, 1997_a). protein bound thiol content was increased without any change in non protein thiol content (Sodhi *et al*, 1997_b). While attari *et al* (2000) showed reduction in glutathione and thiol contents and antioxidant

systems after the administration of 50 and 100 mg/kg/day rifampicin + isoniazid for three weeks.

Oxidative stress related work has been done in liver of rifampicin and other antitubelculin drugs treated rat. Sodhi *et al* (1997a; b; 1998) showed drugs induced superoxide dismutase (an indicator of increase in free radicals) in rat liver. Malnutrition altered oxidative/antioxidative profile and showed decrease in superoxide dismutase and catalase. Protein and energy restriction also declined both these enzymes indicating relation with dietary proteins and energy (Sodhi *et al* (1997a; b; 1998).

MATERIAL AND METHODS

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. The livers and kidneys were for the assays of lipid peroxidation (Buege and Aust (1978), formaldehyde (Werringloer 1978), glutathione (Grunert and Phillips 1951) and protein oxidation (Levin *et al*, 1990).

RESULTS

Lipid peroxidation :

The data on the variations in lipid peroxidation are given in Table 1 Figure 1. Normal rat liver and kidney exhibited 116.08 ± 5.83 and 213.49 ± 11.72 nM of malondialdehyde. Malondialdehyde formation was enhanced in liver and kidney by 4.50 and 3.66 folds respectively after the administration of only rifampicin to group II rats. While lipid peroxidation was reduced by 11.27 % and 51.60 % in liver and kidney of mandur bhasma treated rats of group IV. Administration of mandur bhasma concurrent with rifampicin reversed the action of rifampicin by reducing lipid peroxidation in liver (78.49 %) and kidney (78.52%) when compared to the values observed

Table 1- influence of Mandur Bhasma on lipid peroxidation in liver and kidney during rifampicin induced liver necrosis

Values are expressed in nM/mg protein

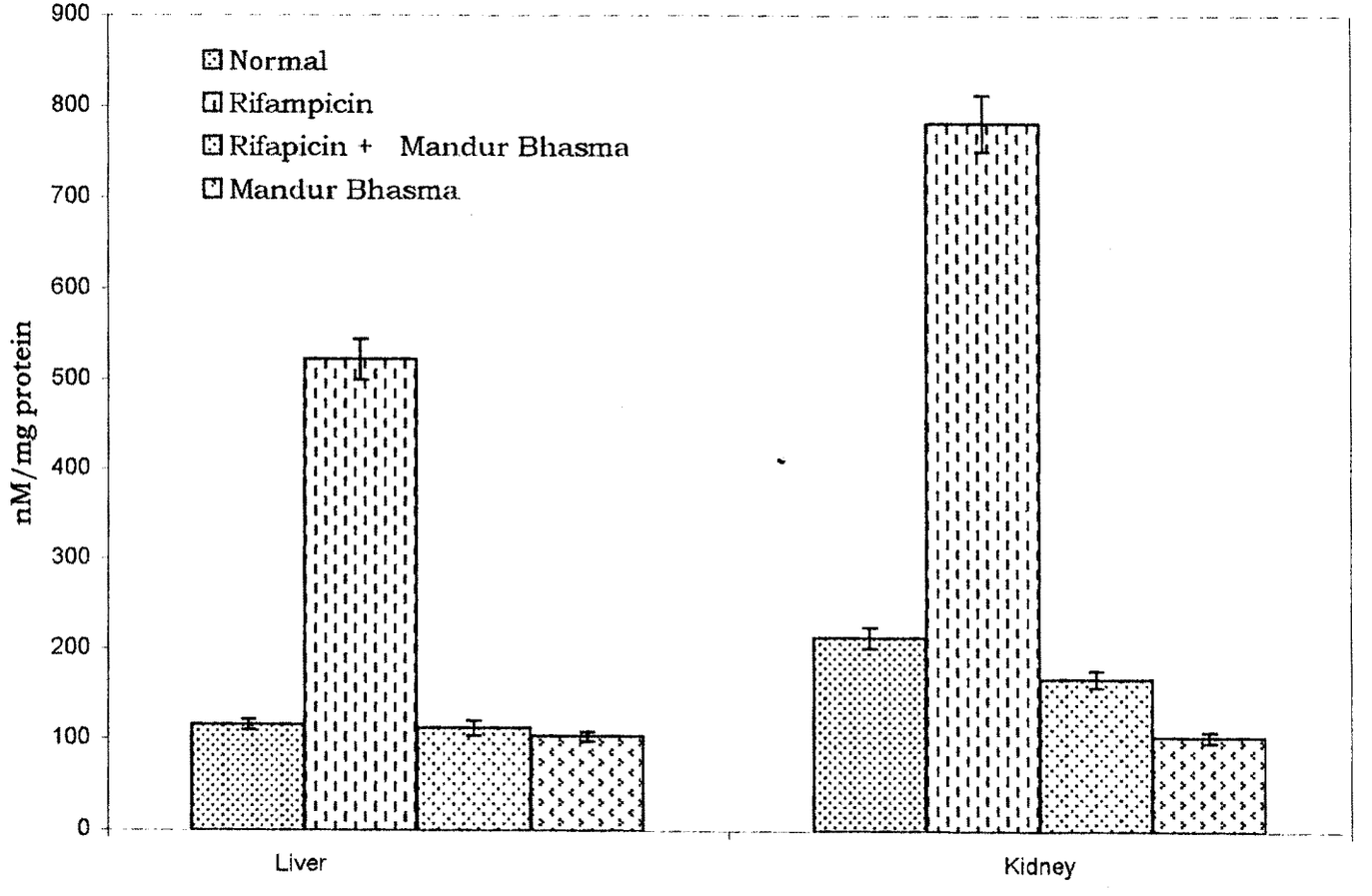
Group	Liver	Kidney
	30 Days	30 Days
Normal	116.08 ± 5.83	213.49 ± 11.72
Rifampicin	522.31 ± 22.58^{c}	781.38 ± 31.02^{c}
Rifapicin + Mandur Bhasma	112.36 ± 8.27^{a,g}	167.85 ± 9.14^{b,g}
Mandur Bhasma	103.43 ± 5.12^{d,g}	103.33 ± 6.13^{c,g}

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- influence of Mandur Bhasma on lipid peroxidation in liver and kidney during rifampicin induced liver necrosis



in rifampicin treated group II rats. On comparison with normal values lipid peroxidation in group III rats was decreased by 3.20 and 21.38 in liver and kidney.

Formaldeyde :

Rates of formaldehyde formation in rat liver and kidney are given in Table 2 Figure 2. Normal rat exhibited 9.00 ± 0.00 and 10.47 ± 0.02 μM/g mg protein formaldehyde in liver and kidney of rat normal. Administration of rifampicin to group II rats caused elevations of 12.11 and 8.77 folds in formaldehyde formation in liver and kidney. Mandur bhasma administration to group IV rats for 30 days did not alter formaldehyde formation in liver (2.11 %) and kidney (4.82 %). Simultaneous treatments of rifampicin + mandur bhasma resulted in the reductions of formaldehyde in rat liver and kidney by 96.10 and 88.97 % respectively, when compared the respective values noted in group II rats treated with rifampicin. Comparison of the glutathione values in the livers of group IV rats with normal values exhibited significant fall in liver (52.78 %) without change in kidney (2.56 % reduction).

Table 2 –Variations in formaldehyde formation due to Mandur Bhasma during rifampicin induced liver damage

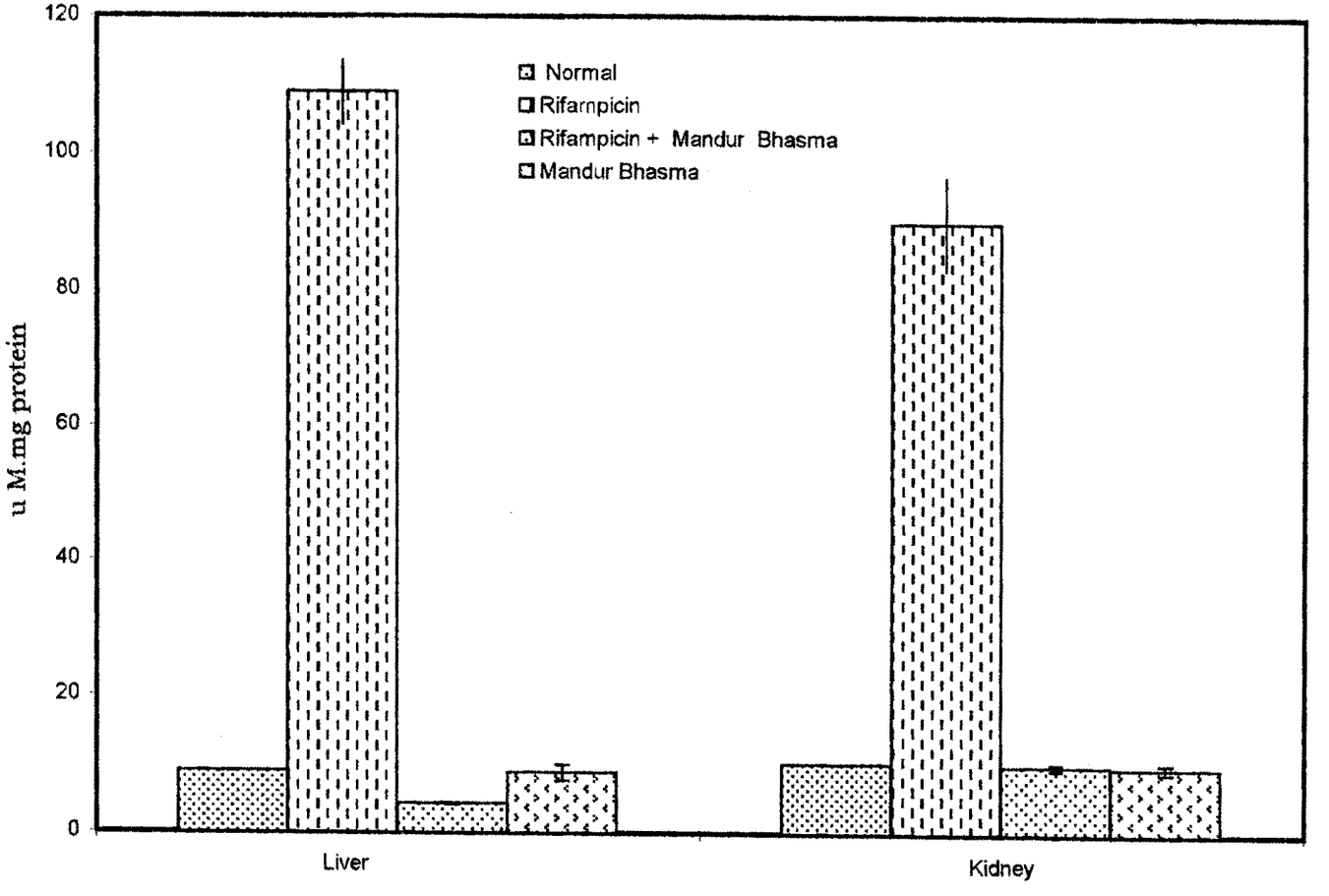
(Values are expressed as μ Moles/g mg protein)

Group	Liver A	Kidney B
Normal	9.00 ± 0.00	10.17 ± 0.02
Rifampicin	108.98 ± 4.69[c]	89.82 ± 6.81[c]
Rifampicin + Mandur Bhasma	4.25 ± 0.13[c,g]	9.91 ± 0.45[c,g]
Mandur Bhasma	8.81 ± 1.15[d,c]	9.68 ± 0.67[d,c]

Values are mean ± SE of 6 animals

p values are as in Table 1

Figure 2 –Variations in formaldehyde formation due to Mandur Bhasma during rifampicin induced liver damage



Glutathione :

Glutathione content in liver and kidney of normal rats is given in Table 3 and Figure 3. Normal rat liver and kidney exhibited 30.28 ± 2.06 and 32.39 ± 2.31 μM of glutathione/mg protein respectively. Treatment of rifampicin for 30 days caused reduction in glutathione content by 43.53 and 37.14 % in rat liver and kidney of groupa II. Administration of mandur bhasma along with rifampicin elevated glutathione levels in liver and kidney of group III rat by 2.02 and 1.75 folds in liver and kidney on comparison with the respective values in group II rats treated with rifampicin. The values of glutathione in liver (1.14 fold) and kidney (1.10 fold) of group II rats were higher than the normal levels. When only mandur bhasma was given to the rats of group IV caused the rises in glutathione contents in liver and kidney. The elevations of 1.43 and 1.47 folds were noted in liver and kidney of group IV rat as compared to the normal values.

Protein oxidation :

Table 4 and Figure 4 demonstrate the protein oxidation in liver and kidney of rat during present study.

Normal rat showed 681.81 ± 33.60 and 467.91 ± 21.35 mM carbonyl content/mg protein. Administration of rifampicin to group II

Table 3 - Influence of mandur bhasma on glutathione content of Liver and Kidney during rifampicin induced toxicity in albino rats

Values are expressed in μM/mg protein

Group	Liver	Kidney
Normal	30.28 ± 2.06	32.39 ± 2.31
Rifampicin	17.10 ± 1.38[c]	20.36 ± 1.52[c]
Rifampicin + Mandur Bhasma	34.56 ± 1.70[a,g]	35.55 ± 2.08[d,g]
Mandur Bhasma	43.29 ± 2.19[c,g]	47.59 ± 2.62[c,g]

Values are mean ± SE of 6 animals

p values are as in Table 1

Figure 3 - Influence of mandur bhasma on glutathione content of Liver and Kidney during rifampicin induced toxicity in albino rats

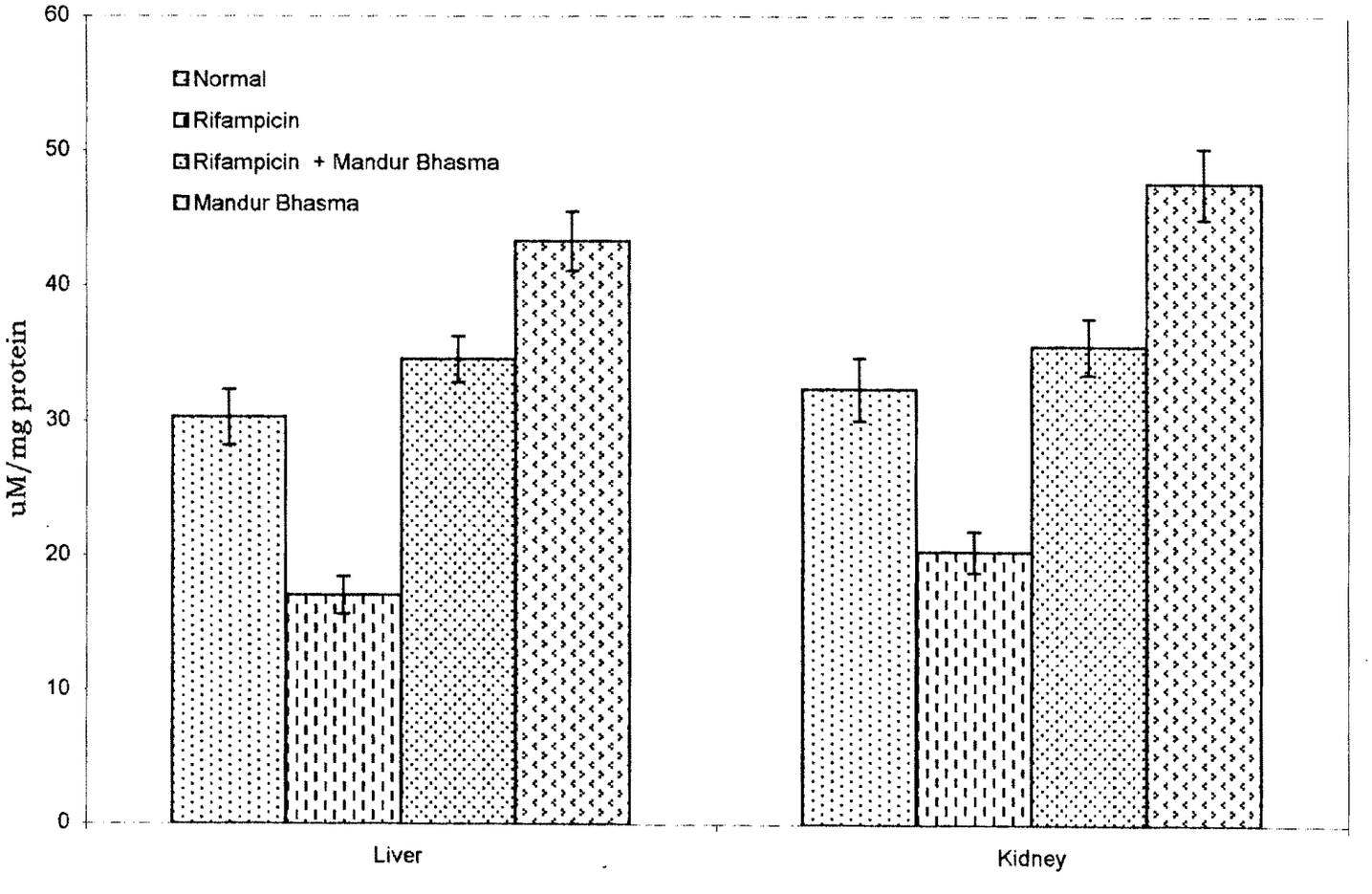


Table 4 - influence of Mandur Bhasma on Protein Oxidation in liver and kidney during rifampicin induced liver necrosis

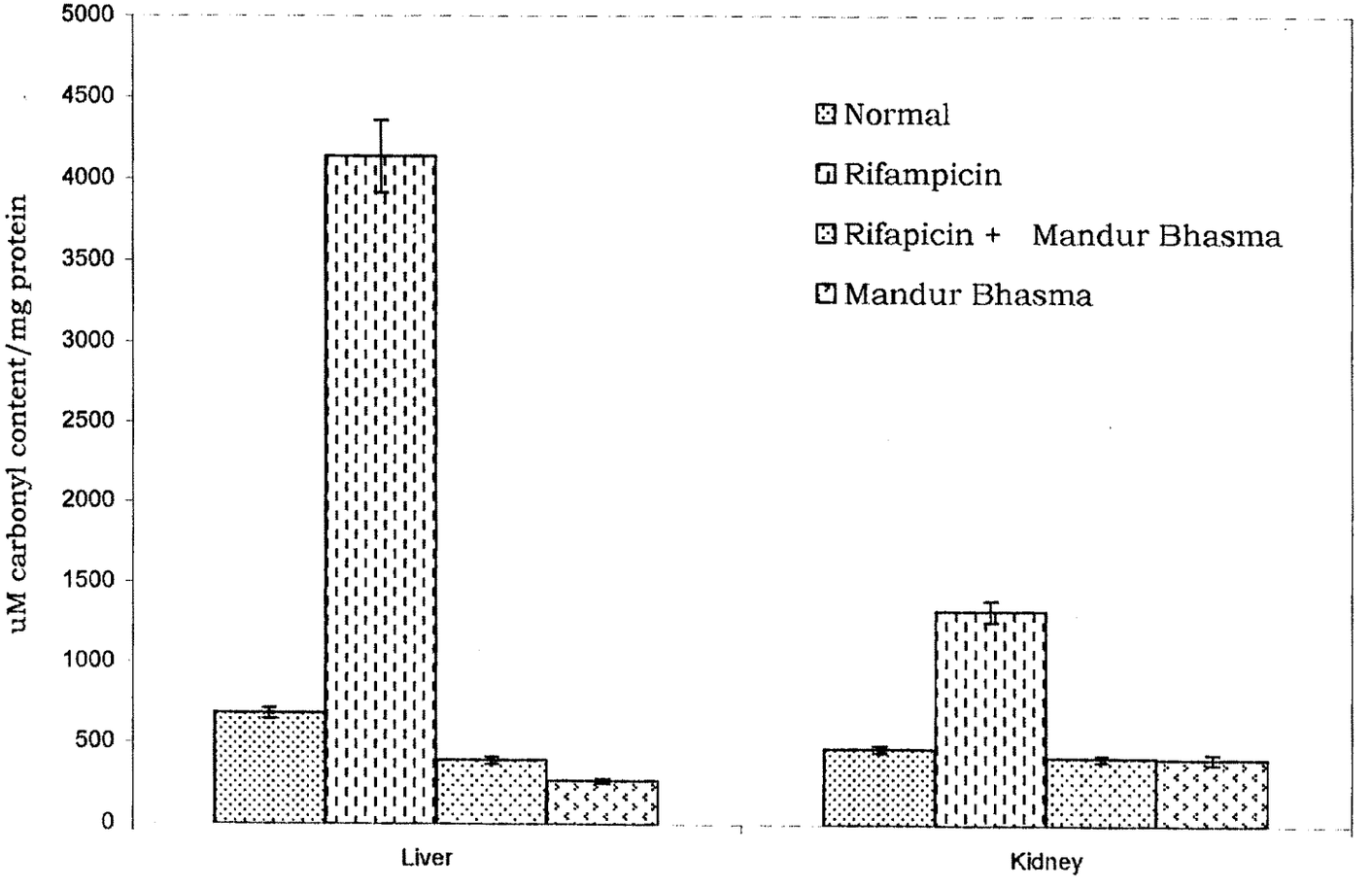
Values are expressed as mM carbonyl content/mg protein

Group	Liver	Kidney
	30 Days	30 Days
Normal	681.81 ± 33.60	467.91 ± 21.35
Rifampicin	4136.36 ± 223.08[c]	1325.74 ± 66.67[c]
Rifapicin + Mandur Bhasma	392.52 ± 23.06[c,g]	413.64 ± 19.68[d,g]
Mandur Bhasma	268.19 ± 15.26[c,g]	409.09 ± 30.26[d,a]

Values are mean ± SE of 6 animals

p values are as in Table 1

Figure 4 - Influence of Mandur Bhasma on Protein Oxidation in liver and kidney during rifampicin induced liver necrosis



rats caused conspicuous elevation in carbonyl content in liver and kidney. Carbonyl contents were enhanced by 6.07 and 2.83 folds in liver and kidney of group II rat as compared to normal values. Administration of only mandur bhasma resulted in 60.67 and 12.57 % reductions in carbonyl contents in liver and kidney respectively on comparison with normal values. Administration of mandur bhasma concurrent with rifampicin counteracted the action of rifampicin on protein oxidation by reducing carbonyl content in liver and kidney by 90.51 and 68.81 % as compared to the respective values noted in group II rats. Comparison of protein oxidation in the liver and kidney of group III with normal values exhibited fall of 42.43 and 11.60 % respectively.

DISCUSSION

Lipid peroxidation was conspicuously elevated in rat liver and kidney after the administration of rifampicin to the rats. This was reverted by the administration of mandur bhasma to the rats along with rifampicin. The values of lipoid peroxidation were significantly lower than the respective normal values. Administration of only mandur bhasma decreased lipid peroxidation in rat liver and kidney as compared to normal values. Malondialdehyde estimated in present study formed from the polyunsaturated fatty acids by microsomal

cytochrome P-450 mediation. The results indicate that the microsomal membrane system is protected in liver and kidney cells by mandur bhasma, which is also confirmed by the histological normal architecture of liver and kidney of rifampicin + mandur bhasma treated rats. The values of lipid peroxidation in the liver and kidney of only mandur bhasma treated rats indicated that mandur bhasma does not damage microsomes. Devarshi *et al*, (1986) showed inhibition of lipid peroxidation in mandur bhasma treated rat liver and kidney.

Formaldehyde formation was also enhanced conspicuously in rat liver and kidney after the administration of rifampicin. However administration of mandur bhasma concurrent with rifampicin counter acted the action of rifampicin by normalizing formaldehyde contents in rat liver and kidney. Treatment of only mandur bhasma reduced formaldehyde content significantly without significant change in kidney. Varieties of NADPH and oxygen dependent reactions catalyzed by the micirosomal reactions of many tissues have been recognized to yield formaldehyde as the product. The most common and frequently studied reaction is N-demethylation of secondary or tertiary amines is mediated by microsomal cytochrome P-450 bound mixed function

oxygenase system (Werringloer, 1978). Mandur bhamsa seem to protect liver and kidney by preventing the formation of formaldehyde.

Glutathione contents of liver and kidney were reduced significantly due to the treatments of rifampicin. While it was normalized by the administration of mandur bhasma concomitant with rifampicin. Administration of only mandur bhasma raised significantly glutathione contents in liver and kidney.

Carbonyl groups were increased conspicuously in the proteins of rat liver and kidney by the administration of rifampicin indication increased metal catalyzed oxidation of proteins. However treatment of mandur bhasma along with rifampicin reduced carbonyl groups conspicuously in the proteins rat liver and kidney. The carbonyl contents in the proteins of rifampicin + mandur bhasma treated rat liver and kidney were significantly lower than respective normal values. Similarly carbonyl contents in the proteins of rat liver and kidney of only mandur bhasma treated rat were significantly lower than respective normal values.

Thus the oxidative stress related lipid peroxidation, protein oxidation and formaldehyde contents were increased and glutathione was decreased in rifampicin induced toxicity in liver and also in

kidney. The simultaneous treatment of mandur bhasma with rifampicin reversed the trend and resulted in increased glutathione and decrease in other parameters. These results show that the increased glutathione clears the harmful products of oxidation that induce stress. Increased glutathione in mandur bhasma treated rats is the indicator that mandur bhasma stimulates the glutathione production. This also confirms that the Ayurvedic drug stimulates the natural path of clearance without bringing any new stress on the body.