PREVENTION OF LIVER CIRRHOSIS BY BETA-CAROTENE

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ABSTRACT

Purpose: This study was designed to evaluate the effects of Beta carotene supplementation on different biochemical parameters in thioacetamide induced cirrhotic rats. Methods: For this purpose 24 male Albino wistar rats were divided into four groups (n=6). Group I, remained healthy control rats, Group II, received thioacetamide (at a dose of 200mg/kg b.w, i.p, for 12 weeks, twice a week) in first phase and saline in second phase, Group III, received thioacetamide (200mg/kg b.w, i.p for 12 weeks, twice a week) in first phase and silymarin (orally at a dosage of 200mg/kg b.w, twice a week, for 8 weeks) in second phase and Group IV, received silymarin (orally at a dosage of 200mg/kg b.w, twice a week, for 8 weeks) in first phase and saline in second phase. Biochemical analysis was evaluated by total and direct bilirubin (Sherlock, 1951), liver specific enzymes (Retiman and Franhel, 1957), antioxidant enzymes[SOD (Kono et al., 1978), Catalase (Sinha et al., 1979), Glutathione reductase (Calberg and Mannervik, 1985), and MDA (Okhawa et al., 1979)] and plasma and intraerythrocyte sodium and potassium (Tabssum et al., 1996). Results: Marked increase in total and direct bilirubin and ALT activity was the indicative markers of liver cirrhosis while reduced antioxidant activity (SOD and GSH) and increased MDA and Catalase levels and disturbed electrolyte homeostasis were observed in cirrhotic group. Silymarin supplementation markedly reduced total bilirubin and ALT activity and restored the antioxidant enzymes (SOD and GSH), MDA and catalase activity and electrolyte homeostasis. Conclusion: These results indicate that silymarin successively attenuates the...
thioacetamide induced liver cirrhosis.

**Keywords**: Liver cirrhosis, Silymarin, Thioacetamide, Liver enzymes, SOD, GSH, Catalase, MDA, Plasma sodium and potassium, Intraerythrocyte sodium and potassium.

**INTRODUCTION**

Carotenoids are naturally present in abundance in vegetables. Bet-carotene serves as a precursor of vitamin A (Olson, 1987). Antioxidant nature of beta-carotene makes it a naturally occurring protective agent (Krinski and Deneke, 1982). Ziegler reported prevention of epithelial cancer by beta-carotene (Ziegler et al., 1992). Low concentration of beta-carotene is found in alcoholics than normal individuals. This can be related with malabsorption and malnutrition (Antonio et al., 1986; Majumdar et al; 1983). Burton and Ingold reported that beta-carotene acts as a good radical-scavenger and works at the partial pressures of oxygen less than 150 torr, oxygen pressure in normal air. Antioxidant activity of beta-carotene is lost at high oxygen pressures and an auto-catalytic pro-oxidant effect is shown by beta-carotene, especially at relatively high concentrations (Burton and Ingold, 1984).

Presence of conjugated carbon-carbon bonds makes the beta-carotene an effective quencher of singlet oxygen. By quenching excited triplet sensitizers it inhibits generation of singlet oxygen (Demmig and Admas, 2002). Ability of beta-carotene to scavenge lipid oxide and lipid peroxide radicals makes it an efficient chain breaking antioxidant (Krinsky and Johnson, 2005). Because of its unique structure and cleavage ability, beta-carotene appears as the most prominent provitamin A carotenoids (Tilman et al., 2010). Free radical quenching mechanism of beta-carotene includes free radical addition to carotenoids, removal of hydrogen, and transfer of electron. Antioxidant form of vitamin E can be regenerated by beta-carotene. In lipo-proteins, oxidation of vitamin E is restricted by beta-carotene which is itself oxidized before vitamin E. Beta-carotene also shows synergistic action in protecting lipids of membranes (Adrianne, 2004).

Thus, the aim of our present study is to evaluate the hepatoprotective role of beta-carotene in thioacetamide induced liver cirrhosis in rats.
MATERIALS AND METHODS
24 male Albino Wistar rats weighing 200-250gm were purchased from the animal house of ICCCBS (International center for chemical and biological sciences, Karachi, Pakistan) for the study. Animals were acclimatized to the laboratory conditions before the start of experiment and caged in a quiet temperature controlled animal room (23±4°C). Rats had free access to water and standard rat diet.

Ethical guidelines
The experiments were conducted with ethical guidelines of institutional ERB (Ethical Review Board) and internationally accepted principles for laboratory use and care in animal research (Health research extension Act of 1985).

Study design
The rats were randomly divided into four groups, each of six rats. The duration of the study was 24 weeks, divided into two phases. Thioacetamide and beta-carotene were administered in either phase.

Group I: the control (remained untreated).
Group II: TAA-treated
Group III: TAA+ beta carotene treated
Group IV: Beta carotene treated

In Phase I, TAA-treated and TAA+beta-carotene groups received TAA, dissolved in 0.9% NaCl and were injected intraperitoneally at a dosage of 200mg/kg b.w, twice a week for 12 weeks. Beta-carotene group received beta carotene (orally at a dosage of 75mg/rat in a volume of 1.5ml on alternative days for 12 weeks). In phase II, TAA-treated group received TAA, TAA+beta-carotene group received beta carotene (orally at a dosage of 75mg/rat in a volume of 1.5ml on alternative days for 12 weeks) after TAA in first phase to study the protective role of beta carotene. Beta carotene group received saline in the second phase. At the end of experimental period, rats from all the groups were decapitated. The blood was collected from the neck wound in the lithium heparin coated tubes and centrifuged to collect plasma. Liver was excised, trimmed of connective tissues, rinsed with saline to eliminate blood contamination, dried by blotting with filter paper and weighed. The tissues then kept in freezer at −70°C until analysis.
Assessment of ALT and total and direct bilirubin
Plasma ALT (Retiman and Franhel, 1957) and total and direct bilirubin (Sherlock, 1951) were analyzed using commercially prepared reagent kits from Randox.

Preparation of post mitochondrial supernatant
Liver homogenate was prepared by taking 1g of liver tissue in 10ml of 5mM potassium phosphate buffer (pH 7.8) by using a homogenizer. The homogenates were centrifuged at 800g for five minutes at 4ºC to separate the nuclear debris. The supernatant so obtained was centrifuged at 10,500g for 20 minutes at 4ºC to get post mitochondrial supernatant which was used to assay SOD, Catalase, MDA, and glutathione reductase activity.

Estimation of thiobarbituric acid substances
The malonyldialdehyde (MDA) content, a measure of lipid peroxidation, was assayed in the form of thio- barbituric acid reacting substances (TBARS) by the lipid peroxidation method (Okhawa et al., 1979). Briefly, the reaction mixture consisted of 0.2 ml of 8.1% sodium dodecyle sulphate, 1.5 ml of 20% acetic acid solution adjusted to pH 3.5 with sodium hydroxide and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid was added to 0.2 ml of 10%(w/v) of PMS. The mixture was brought up to 4.0 ml with distilled water and heated at 95°C for 60 minutes. After cooling with tap water, 1.0 ml distilled water and 5.0 ml of the mixture of n-butanol and pyridine (15:1 v/v) was added and centrifuged. The organic layer was taken out and its absorbance was measured at 532 nm and compared with those obtained from MDA standards. The concentration values were calculated from absorption measurements as standard absorption.

Estimation of catalase
Catalase activity was assayed by the method of Sinha (Sinha et al., 1979). Briefly, the assay mixture was consisted of 1.96 ml phosphate buffer (0.01M,pH 7.0), 1.0 ml hydrogen peroxide (0.2 M) and 0.04 ml PMS (10%w/v) in a final volume of 3.0 ml. 2 ml dichromate acetic acid reagent was added in 1 ml of reaction mixture, boiled for 10 minutes, cooled. Changes in absorbance were recorded at 570 nm.

Estimation of SOD
Superoxide dismutase levels in the cell free supernatant were measured by the method of (Kono et al., 1978). Briefly 1.3ml of solution A (0.1 m EDTA containing 50 mM Na₂CO₃, pH 10.0), 0.5 ml of solution B (90µmNBTnitro blue tetra zolium dye) and 0.1 ml of solution C
(0.6% Triton X-100 in solution A), 0.1 ml of solution D (20 mM Hydroxyl amine hydrochloride, pH 6.0) were mixed and the rate of NBT reduction was recorded for one minute at 560 nm. 0.1 ml of the supernatant was added to the test cuvette as well as reference cuvette, which do not contain solution D. Finally, the percentage inhibition in the rate of reduction of NBT was recorded as described above. One enzyme unit was expressed as inverse of the amount of protein (mg) require in one minute.

**Estimation of glutathione reductase**

GSH activity was determined by continuous spectrophotometric rate determination (Calberg and Mannervik, 1985). In a clean glass test tube, 0.3 mL of 10% BSA, 1.5mL of 50mM potassium phosphate buffer (pH 7.6), 0.35mL of 0.8mM β-NADPH and 0.1mL of 30mM oxidized glutathione was taken and finally added 0.1mL of homogenate, mixed well by inversion. Absorbance was recorded at 340nm at 25ºC for 5 minutes on kinetic spectrophotometer PRIM 500 (Germany) with automatic aspiration and thermostat. The activity was calculated using the molar coefficient for NADPH of 6.22 µmol⁻¹ × cm⁻¹ and expressed in unit/gram tissue.

**Estimation of plasma sodium and potassium**

Plasma was diluted 1:100 with 0.1N HCl and was used for simultaneous determination of sodium and potassium. The emission intensities of standards and samples were recorded against the respective blank solutions. The emission intensities of sodium, potassium were recorded at 589 and 768nm respectively.

**Erythrocyte membrane preparation**

The packed red cells extracted by centrifugation at 4ºC, 450g for 15 minutes were resuspended and diluted in 25 volumes of 0.011 mol/L Tris-HCl buffer at pH 7.4. The hemolyzed cells were then centrifuged for 30 min at 12,000 rpm at 4ºC and the membrane pellet was resuspended in 30 ml of 0.011 mol/L Tris-HCl buffer. This centrifugation step was repeated three times. The final concentration of the membrane suspension was ~4 mg protein/ml of Tris buffer. The membrane suspension was stored at -80ºC until the assay was performed.

**Estimation of intraerythrocyte sodium and potassium**

Heparinized blood was centrifuged and plasma was separated. Buffy coat was aspirated and discarded. Erythrocytes were washed three times at room temperature by suspension in the
magnesium chloride solution (112mmol/L), centrifugation at 450g at 4°C for 5 minutes and aspiration of the supernatant as described earlier (Fortes and Starkey, 1977). Final supernatant was retained for the estimation of intraerythrocyte sodium and potassium concentration. Neither electrolyte was detectable in the final wash. Washed erythrocytes were then used for the estimation of intraerythrocytes sodium and potassium (Tabssum et al., 1996).

**Histopathological examination**

Left lobe of liver was removed quickly and immersed in 10% formalin. Slices of liver then fixed in a solution containing ethanol (150ml), formaldehyde (60ml), acetic acid (15ml) and picric acid (1 g) for 2 hours. Then the samples were incubated in phosphate buffered formaldehyde until embedded in paraffin, sectioned at 5 µm and stained with hematoxylin and eosin (H/E), and analyzed by light microscopy.

**STATISTICAL ANALYSIS**

Results are presented as mean ± SD. Statistical Significance and difference from control and test values evaluated by Student’s t-test. Statistical probability of **P<0.05, *P<0.01** were considered to be significant.

**RESULTS**

**Effect of thioacetamide and beta-carotene treatment on body weight in control and treated rats.**

Decreased body weight was observed after chronic administration of TAA in TAA and TAA+beta-carotene groups in first phase of experimental period. Rats of TAA+beta carotene group regained their body weight after beta carotene supplementation in second phase. Rats of TAA group continuously lost their body weights. Rats of beta carotene group and control group gained their body weights throughout the treatment (fig 1).
Effect of thioacetamide and beta carotene treatment on liver weight and liver to body weight ratio in control and treated rats

Increased liver weight and liver-body weight ratio was observed in TAA group after 12 week administration of TAA as Compare to control (6.33±1.31 p<0.01) (0.028±0.003 p<0.01) (Table-1) whereas reduction in the liver weight and liver-body weight ratio was observed in TAA + beta-carotene group as compare to control (5.55±0.77 p<0.01) (0.03±0.005 p<0.01) respectively. Beta-carotene treated group showed reduced liver weight and liver -body weight ratio (5.18±0.72 p<0.01) (0.027±0.002 p<0.05).

Effect of thioacetamide and beta carotene treatment on liver weight, liver - body weight ratio in control and treated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver Weights</th>
<th>Liver body-weight ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.33±1.31*</td>
<td>0.028±0.003*</td>
</tr>
<tr>
<td>TAA-treated</td>
<td>6.82±0.71*</td>
<td>0.39±0.002*</td>
</tr>
<tr>
<td>TAA+beta-carotene treated</td>
<td>5.55±0.77*</td>
<td>0.030±0.005*</td>
</tr>
<tr>
<td>Beta carotene treated</td>
<td>5.18±0.72*</td>
<td>0.027±0.002*</td>
</tr>
</tbody>
</table>

n=6

Values are mean ± SD. Significant difference among control, thioacetamide and beta carotene treated groups by t-test P<0.01*, P<0.05**.
Effect of thioacetamide and beta carotene treatment on plasma total and direct bilirubin and plasma ALT activity in control and treated rats

Table 2, figure 2 showed a marked increase in total bilirubin level in TAA-treated group as compare to control (3.6±0.1 p<0.01) whereas, in TAA+beta-carotene treated group, beta carotene supplementation brought those increased levels to the normal concentrations as compare to control (0.72±0.01 p<0.01). Beta carotene treated group showed normal range of total bilirubin as compare to control (0.60±0.01 p<0.01). Increased level of direct bilirubin was shown by TAA-treated group as compare to control (3.8±0.03 p<0.01) whereas beta-carotene supplementation in TAA+beta carotene group brought those higher levels to normal levels as compare to control (1.37±0.001 p<0.01). Alone beta-carotene had no effect on direct bilirubin level (table 2, figure 3).

Plasma Alanine aminotransferase level was markedly increased in TAA-treated group as compare to control (960.3±30.19 p<0.01). Alanine amino transferase level was decreased in TAA + beta carotene group as compare to control (269±10.1 p<0.01). Alone beta-carotene showed no effect on plasma ALT level as compare to control (210±9.6 p<0.01) Table 2, figure 4.

Table-2: Effects of thioacetamide and beta-carotene treatment on total and direct bilirubin and ALT activity in control and treated rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>TAA-treated</th>
<th>TAA+betacarotene treated</th>
<th>Beta carotene-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bilirubin (unit/L)</td>
<td>0.60±0.04*</td>
<td>3.6±0.2*</td>
<td>0.71±0.01*</td>
<td>0.60±0.01*</td>
</tr>
<tr>
<td>Direct bilirubin (unit/L)</td>
<td>1.35±0.03*</td>
<td>3.8±0.03*</td>
<td>1.37±0.001*</td>
<td>1.35±0.03*</td>
</tr>
<tr>
<td>Alanine-amino transferase (unit/L)</td>
<td>210±9.6*</td>
<td>960.3±30.19*</td>
<td>269±10.1*</td>
<td>210±9.6*</td>
</tr>
</tbody>
</table>

n=6

Values are mean ± SD. Significant difference among control, TAA-treated, TAA+beta-carotene treated and beta carotene treated groups by student’s t-test **P<0.05, *P<0.01.
Figure 2: Effect of thioacetamide and beta carotene treatment on total bilirubin in control & treated rats

Figure 3: Effect of thioacetamide and beta carotene treatment on direct bilirubin in control & treated rats

Figure 4: Effect of thioacetamide and beta carotene treatment on alanineaminotransferase in control & treated rats
Effect of thioacetamide and beta-carotene treatment on hepatic concentration of glutathione reductase

Hepatic concentration of glutathione reductase was significantly reduced in TAA-treated group as compared to control (0.052±0.001 p<0.01).

TAA+beta-carotene group, after beta-carotene supplementation, showed increased level of glutathione reductase as compared to control (0.83±0.01 p<0.01) Table.3, figure.5. Glutathione reductase was normal in beta-carotene group as compared to control (0.91±0.02 p<0.01).

Table.3: Effect of thioacetamide and beta carotene treatment on hepatic concentration of glutathione reductase, superoxide dismutase, malondialdehyde and catalase

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>TAA-treated</th>
<th>TAA+beta carotene treated</th>
<th>Beta carotene-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione Reductase (unit/gm of tissue)</td>
<td>0.91±0.02</td>
<td>0.052±0.01*</td>
<td>0.83±0.01*</td>
<td>0.91±0.02*</td>
</tr>
<tr>
<td>Superoxide dismutase unit/gm of tissue</td>
<td>890±2.0</td>
<td>500±2.3*</td>
<td>772±5.6*</td>
<td>890±2.2*</td>
</tr>
<tr>
<td>Malondialdehyde mmol/gm of tissue</td>
<td>58.1±3.4</td>
<td>130.2±2.1*</td>
<td>62.0±1.6*</td>
<td>58.0±1.7*</td>
</tr>
<tr>
<td>Catalase nmol/gm of tissue</td>
<td>7.2±0.01</td>
<td>42.3±0.01*</td>
<td>7.7±0.14*</td>
<td>7.2±0.01*</td>
</tr>
</tbody>
</table>

n=6

Values are mean ±SD. Significant difference among control, TAA-treated, TAA+beta carotene treated and beta carotene-treated groups by student’s t-test **P<0.05, *P<0.01.

Figure .5: Effect of thioacetamide and beta carotene treatment on glutathione reductase in control & treated rats
Effect of thioacetamide and beta-carotene treatment on hepatic concentration of MDA

Level of MDA was markedly increased in TAA group as compare to control (130±2.1 p<0.01). Beta-carotene administration in TAA+beta-carotene group decreased the concentration of MDA as compare to control (62.0±1.6 p<0.01) while rats of beta-carotene group showed normal range of MDA level as compare to control (58.0±1.7 p<0.01) (Table-3, figure 6).

![Figure 6: Effect of thioacetamide and beta carotene treatment on Malondialdehyde in control & treated rats](image)

Effect of thioacetamide and beta-carotene treatment on hepatic concentration of superoxide dismutase in control and treated rats.

Table-3, figure-7: showed a significant decrease in SOD activity in TAA group as compare to control (500±2.3 p<0.01). TAA+beta-carotene group, after beta-carotene supplementation, showed a significant reduction in SOD activity (772±5.6 p<0.01) as compare to control. SOD activity was normal in beta-carotene group (890±2.2 p<0.01) as compare to control.

![Figure 7: Effect of thioacetamide and beta carotene treatment on Superoxide dismutase in control & treated rats](image)
Effect of thioacetamide and beta-carotene treatment on hepatic concentration of catalase.

Concentration of catalase was significantly increased in TAA group (42.3±0.01 p<0.01) as compare to control. Administration of beta-carotene in second phase in TAA+beta-carotene group brought these higher levels to normal limits (7.7±0.14 p<0.01) as compare to control. Activity of catalase was normal (7.2±0.01 p<0.01) in beta-carotene group as compare to control (Table-3, figure-8).

![Figure 8](image)

**Figure 8:** Effect of thioacetamide and beta carotene treatment on Catalase in control & treated rats:

Effect of thioacetamide and beta-carotene treatment on intra-erythrocytes sodium and potassium in control and treated rats

Decreased levels of intra erythrocyte sodium was observed in TAA group (2.9±0.01 p<0.01), whereas beta-carotene supplementation significantly increased intra erythrocyte sodium in TAA+beta-carotene group (3.78±0.4 p<0.01) as compare to control. Beta-carotene group showed normal level of intra-erythrocyte sodium as compare to control (3.76±0.5 p<0.01) (Table-4, figure-9).

Decreased intra erythrocyte potassium level was observed in TAA group as compare to control (42.3±1.5 p<0.01). Whereas intra- erythrocyte potassium level in TAA+beta-carotene group was restored after beta-carotene supplementation in second phase to TAA treated rats (52.0±5.2 p<0.01) as compare to control. Increased intra-erythrocyte potassium level was found in beta-carotene group as compare to control (62.3±3.5) Table -4, figure -10.
Table 4: Effect of thioacetamide and beta-carotene treatment on plasma and intra-erythrocyte sodium and potassium in control and treated rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>TAA-treated</th>
<th>TAA+beta-carotene</th>
<th>Beta-carotene treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraerythrocyte Na⁺ mmol/L</td>
<td>3.78±0.06</td>
<td>2.9±0.01*</td>
<td>3.78±0.4*</td>
<td>3.76±0.05*</td>
</tr>
<tr>
<td>Intraerythrocyte K⁺ mmol/L</td>
<td>53.28±1.1</td>
<td>42.33±1.5*</td>
<td>52.03±5.26*</td>
<td>62.3±3.5*</td>
</tr>
<tr>
<td>Plasma Na⁺ mmol/L</td>
<td>140.8±1.2</td>
<td>122±1.0*</td>
<td>111±0.7*</td>
<td>106±0.8*</td>
</tr>
<tr>
<td>Plasma K⁺ mmol/L</td>
<td>5.16±0.3</td>
<td>4.3±0.2*</td>
<td>5.16±0.2*</td>
<td>5.2±0.09*</td>
</tr>
</tbody>
</table>

Values are mean ± SD. Significant difference among control, thioacetamide, thioacetamide and beta-carotene and beta-carotene treated groups by t-test **P<0.05, *P<0.01.

Figure 9: Effect of thioacetamide and beta carotene treatment on Intraerythrocyte sodium in control & treated rats

Figure 10: Effect of thioacetamide and beta carotene treatment on Intraerythrocyte potassium in control & treated rats
Effect of thioacetamide and beta-carotene treatment on plasma sodium and potassium in control and treated rats.

Plasma sodium was decreased in TAA group (122.3±1.0 p<0.01) as compare to control whereas it was also decreased in TAA+beta-carotene group (111±0.7p<0.01). Beta-carotene group also showed a significant reduction in plasma sodium (106±0.8 p<0.01) (table-4, figure-11). Table-4, figure-12, showed decreased plasma potassium in TAA group  (4.3±0.2 p<0.01) as compare to control whereas beta-carotene treatment in TAA+beta-carotene group significantly increased plasma potassium up to the normal range as compare to control (5.16±0.2 p<0.01). Beta-carotene group showed normal concentration of plasma potassium (5.2±0.09 p<0.01) as compare to control (Table-4).

![Figure 11: Effect of thioacetamide and beta carotene treatment on Plasma sodium in control & treated rats](image1)

![Figure-12: Effect of thioacetamide and beta carotene treatment on Plasma potassium in control & treated rats](image2)
**Histology of liver in control, thioacetamide-treated, thioacetamide+beta-carotene-treated and beta-carotene-treated rats**

After 12 week administration of thioacetamide in TAA-treated rats, histological examination showed last stage of liver cirrhosis, amount of fibrosis was (+++++) maximum. Supplementation of beta-carotene in TAA+beta-carotene group reduces the amount of fibrous tissue and the stage of nodule formation was (+1) minimum (Figure 13).

<table>
<thead>
<tr>
<th>Group</th>
<th>Amount of fibrosis</th>
<th>Disorganization of liver architecture</th>
<th>Stage of nodule formation and disorientation of vascular architecture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Thioacetamide</td>
<td>+++++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Thioacetamide+beta-carotene</td>
<td>+++</td>
<td>+</td>
<td>+1</td>
</tr>
</tbody>
</table>

Figure 2A. Normal liver histology from control rats  
Figure 2B. Shows histological abnormalities after 12 week administration of thioacetamide in TAA treated rats.  
Figure 2C. Shows effect of beta carotene treatment which reduces degree of fibrosis in TAA+beta carotene-treated rats.
DISCUSSION
In our study, chronic administration of thioacetamide resulted in development of severe cirrhosis in experimental rats which was confirmed by the histological examination as well as by biochemical estimations. Elevated levels of liver biomarker, bilirubin and ALT activity (table-2), indicated improper functioning of liver. Histological examination of liver (figure-13) shows high degree of fibrosis, disorganization of liver architecture and severe stage of nodule formation in thioacetamide treated rats. Bruck also reported induction of liver cirrhosis confirmed by liver histopathology, gross macroscopic appearances and activation of HSC (Bruck et al., 1977). Supplementation of thioacetamide treated rats with ß-carotene resulted in the reduction of elevated levels of bilirubin and ALT activity (table -2), indicating role of beta-carotene in attenuation of liver cirrhosis. Activation of hepatic stellate cell is an important step in liver fibrogenesis. Which results in hepatic stellate cell differentiation into myofibroblast-type cell which become secretory source for a number of extracellular matrix components. Many cytokines and platelet-derived growth factor are considered to play a role in activation of hepatic stellate cells (Friedman, 1993). A role of retenoids in activation of hepatic stellate cells has been reported. In normal conditions, hepatic stellate cells serve as a store house for vitamin A but in case of liver injury vitamin A content becomes reduced. As depletion of vitamin A has been found in liver disorder induced by alcohol (Leo and Lieber, 1982). Davis reported inhibition of activation of HSC by retinol and retinoic acid (Davis, 1990). Okuno reported attenuation of hepatic fibrosis by orall coadministration of beta-carotene with carbon tetrachloride (Okuno, 1997). Beta- carotene actually maintains the concentration of vitamin A in liver and reduces synthesis of collagen by hepatic stellate cells. Scavenging of free radicals formed by hepatotoxin is done by beta-carotene (Hendriks et al., 1993). Seifert et al reported role of retinoids in exacerbation of liver fibrosis by activating latent TGF-beta in experimental rats (Seifert et al., 1995). Goodman reported the conversion of beta-carotene into retinol by intestinal mucosa. In present study, normalization in the levels of bilirubin, ALT activity, in the level of antioxidant enzymes (table-3), in the level of MDA, in the electrolyte homeostasis indicates that beta-carotene ameliorates liver cirrhosis in rats.

REFERENCES


