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Adaptation of siblings of female rats given ethanol effect of N-acetyl-L-cysteine

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Summary. Ethanol administration to female rats before and during pregnancy resulted in decreased number of litters and increased activities of serum GOT, GPT and ALP. The hepatotoxicity of ethanol was indicated by the histological alterations both in the mother and siblings. There was increased levels of tissue lipids in mother and litters born to alcoholic rats. The concentration of TBARS in the liver and kidney were significantly increased in alcohol treated rats and their litters. The activities of the anti-peroxidative enzymes SOD and CAT were decreased on alcohol treatment in female rats. The glutathione content in liver and kidney decreased significantly in litters born to alcoholic rats.

We have observed that the treatment with N-acetylcysteine offers protection against the toxic effect of alcohol in female rats during pregnancy and litters born to them. In N-acetylcysteine treated rats the number of litters as well as the average birth weight were close to that of control animals. N-acetylcysteine decreases the activities of serum GOT, GPT and ALP in female rats. We have also observed decreased levels of tissue lipids in mother and litters born to alcoholic rats given N-acetylcysteine when compared to alcoholic rats. The levels of TBARS in liver, kidney were also decreased both in mother and litter born to alcohol + N-acetylcysteine, while the activities of SOD and CAT were increased in liver of alcoholic rats given N-acetylcysteine when compared to alcoholic rats. Histopathological studies also showed the protective effect of N-acetylcysteine in both mother and litter in liver and kidney against alcoholic induced toxicity.

Keywords: Amino acids – Catalase – Ethanol – Glutathione – N-Acetyl-L-cysteine – Superoxide dismutase – Thiobarbituric acid reactive substances

Introduction

Ethanol is a fat soluble non-electrolyte which is readily absorbed from the gastrointestinal tract and diffuses rapidly into circulation and uniformly throughout the body (Goedde and Agarwal, 1989).

Ethanol is almost exclusively metabolized in the body by enzyme – catalysed oxidation processes. The resulting acetaldehyde is further oxidized to acetate, which is then converted to carbondioxide via the citric acid cycle (Salaspuro and Lindros, 1985).

The spectrum of alcoholic liver injury involves hepatic steatosis (fatty liver), perivenular and perisinusoidal fibrosis, alcoholic hepatitis and cirrhosis.

In 1973, Jones, Smith and their colleagues described a pattern of birth defect ranging from spontaneous abortion and infant mortality, through a cluster of abnormalities called "Fetal Alcohol Syndrome" (Jones et al., 1984).

Spontaneous abortion rates were reported to increase, about two-fold in women drinking one to two drinks per day or less during pregnancy (Harlap and Shino, 1980; Kline et al., 1980; Sokol, 1980a).

Studies in non-human primates are very consistent in reporting an increase in spontaneous abortion following alcohol exposure (Scott and Fradkin, 1984; Clarren et al., 1987).

Lowered birth weight is one of the more reliable observed effects associated with in utero alcohol exposure in humans and animals. The average birth weight of such children was 2,000 grams, this may be compared with the median birth weight for infants in the United States of over 3,300 grams. (Sokol, 1980b).

A number of sulphur containing amino acids have been used as possible antidotes to paracetamol and alcohol intoxication in man, with variable success, as some of these are themselves toxic (Prescott et al., 1976). N-acetyl-L-cysteine is the N-acetyl derivative of the amino acid L-cysteine and constitutes the central portion of the glutathione molecules. N-acetylcysteine shows the ability to exert protective effects, including inhibition of genotoxicity of reactive oxygen species, modulation of metabolism coordinated with blocking of reactive metabolites, protection of DNA and nuclear enzymes and prevention of the formation of carcinogen DNA adducts (De-Vries, 1992).

Since N-acetylcysteine has been observed to play a protective role in chronic alcoholism (Jaya and Menon, 1992) and since increased intake of alcohol during pregnancy has been observed to effect the growth and development of the foetus (Harlap and Shino, 1980) and the young, we have studied whether N-acetylcysteine could offer protection to alcoholic mother and siblings.

Materials and methods

Female albino rats Wistar strain (body weight 150–170 g) bred in the Central Animal House, Rajah Muthiah Medical College, were used in this study. The animals were fed on pellet diet (Hindustan Lever Limited, Bombay) and water *ad libitum*.

N-acetyl-L-cysteine was purchased from the Sigma Chemical Company. U.S.A. and all other biochemicals and chemicals used for the experiments are of analytical grade.

The rats were divided into two groups with 18 rats in each group.

Group A: Control rats.
Group B: Rats given 20% ethanol.

Rats in group B were given 20% ethanol (5 ml each) i.e., 7.9 g ethanol/kg body weight as aqueous solution daily using an intragastric tube for 30 days.

After 30 days rats were regrouped and treated as follows:

Group I: Control rats.
Group II: Rats given 20% ethanol.
Group III: Rats given 20% ethanol and given N-acetylcysteine.

N-acetylcysteine was given orally (150 mg/kg body weight) as aqueous solution using an intragastric tube to the rats in Group III on alternate days up to next 30 days. Alcohol treatment to the rats in Group II was continued.

After 30 days the rats were mated. Alcohol and N-acetylcysteine was continued to be given to the rats of Group II and Group III during the period they were pregnant and after wards, till they were sacrificed.

The animals in various groups were delivered. The delivered litter from various groups after weaning period (24 days) were sacrificed by decapitation. The liver and kidney were collected in ice cold containers for various estimations.

The mothers of these litters were also sacrificed by decapitation. The blood was collected and serum separated. The tissues such as liver, kidney were also collected in ice cold containers for various estimations.

For histopathological study both litters and their mother were perfused with formalin (10%) and different tissues were removed.

The activities of serum aspartate transaminase (E.C.2.6.1.1), serum alanine transaminase (E.C.2.6.1.2) determined by the method of King and the activity of serum alkaline phosphatase (E.C.3.1.3.1) were also determined by the method of King (Varley, 1988). The activity of catalase (E.C.1.11.1.6) was determined by the method of Maehly and Chance (1954). The activity of superoxide dismutase (E.C.1.15.1.1) was assayed by the method of Kakkar et al. (1984).

The extraction of serum and tissue lipids was carried out according to the procedure of Folch et al. (1951). The total cholesterol was estimated by the method of Zak et al. (Zak et al., 1953), phospholipids by the method of Zilversmit and Davis (1950) and free fatty acids by the method of Folholt et al. (1973).

The thiobarbituric acid reactive substances (TBARS) content in tissue was estimated by the method of Niehaus and Samuelsson (1968) and reduced glutathione in tissues were determined by the method of Beutler and Kelley (1963). The statistical analysis was done by Student's t-test (Bennett and Franklin, 1954).

Results

Histopathological examination of liver and kidney of rats in the different experimental groups and their siblings are given in the Tables 1 and 2.

The liver samples of animals administered 20% alcohol showed portal inflammation, congestion of the vessel, nuclear disintegration, focal degeneration, micronecrosis, increased cytoplasmic vacuolation and focal kupffer cell hyperplasia. Sinusoid widening in the area of vessel congestion was also observed in these animals (Figs. 1 to 4).

On the other hand, animals given N-acetylcysteine exhibited limited portal inflammation and vascular congestion. There was also lower degree of sinusoidal widening (Fig. 5).

In case of the siblings (litters) from alcoholic mothers, we observed congestion of vessel, focal sinusoidal widening, portal inflammation, fatty changes and limited nuclear disintegration. Siblings of animals given N-acetylcysteine

Table 1. Histopathological changes of liver of control, alcohol and alcohol + NAC treated rats and their litters

Microscopic observation	Normal mother	Litter born to normal mother	Alcoholic mother	Litter born to alcoholic mother	Alcohol + NAC treated mother	Litter born to alcohol + NAC treated mother
Nuclear disintegration	Absent	Absent	Well present	Limited	Decreased	Decreased
Congestion of vessel	Absent	Absent	Present	Present	Reduced	Reduced
Portal inflammation	Absent	Absent	Present	Present	Limited	Limited
Focal kupffer cell hyperplasia	Absent	Absent	Present	Variably present	Absent	Variably present
Micronecrosis	Absent	Absent	Present	Variably present	Reduced	Variably present
Cytoplasmic vacuolation	Absent	Absent	Present	Variably present	Reduced	Variably present
Focal degeneration	Absent	Absent	Present	Present	Absent	Absent
Fatty change	Absent	Absent	Present	Variably present	Absent	Absent

Table 2. Histopathological changes in kidney of control, alcohol and alcohol + NAC treated rats and their litters

Microscopic observation	Normal mother	Litter born to normal mother	Alcoholic mother	Litter born to alcoholic mother	Alcohol + NAC treated mother	Litter born to alcohol + NAC treated mother
Cloudy swelling	Absent	Absent	Present	Present	Reduced	Reduced
Congestion of vessels	Absent	Absent	Present	Present	Reduced	Reduced
Micronecrosis	Absent	Absent	Present	Present	Reduced	Reduced

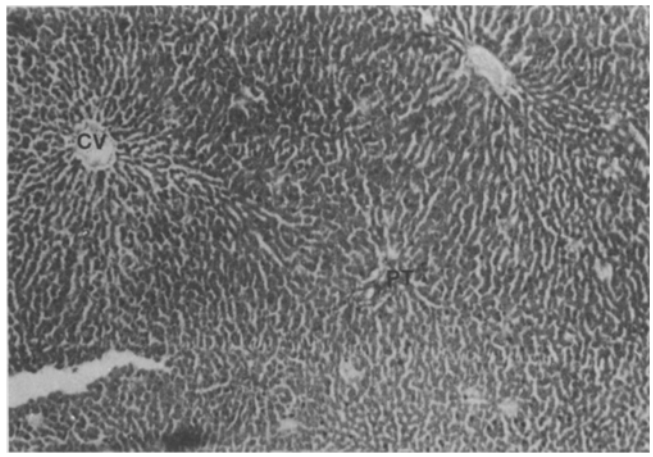

Fig. 1. Control animals liver: H & E × 10. Liver showing normal portal triad (*PT*) and central vein (*CV*). The hepatocytes are orderly arranged

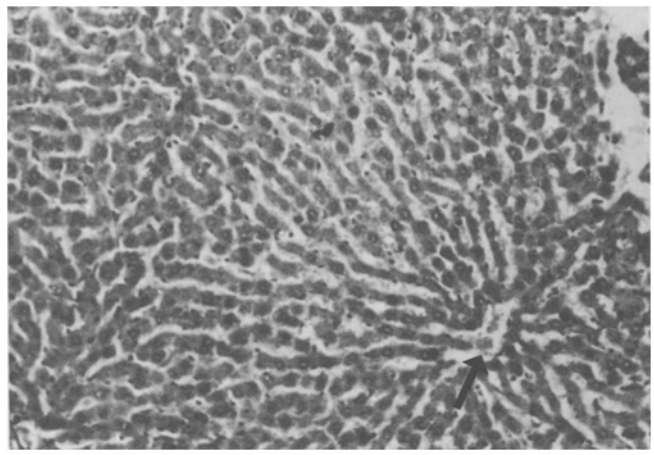

Fig. 2. Alcohol treated animals liver: H & E × 10. Sinusoids are dialated and there is congestion of vessels (➞)

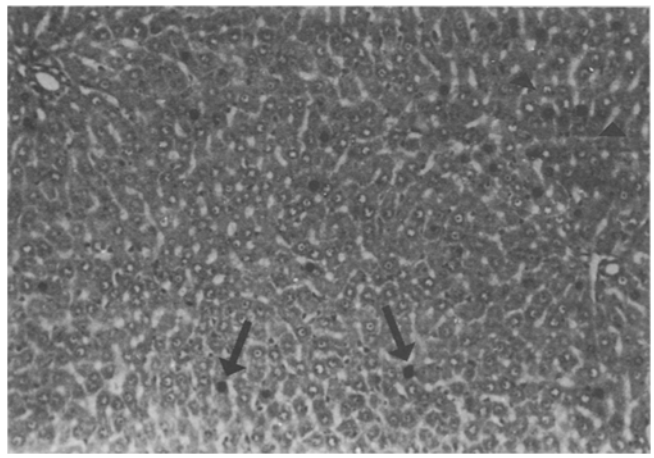

Fig. 3. Alcohol treated animals liver: H & E × 10. ▲ Focal areas of fatty change; regenerative changes are also noted (➞)

showed only limited congestion in the vessel, less degree of portal inflammation and sinusoidal widening (Figs. 6 to 8).

Kidney also showed changes in alcohol treated animals. There was cloudy swelling, vessel congestion and focal area of changes in both the mother and siblings. In the case of N-acetylcysteine treated animals all these changes were to a lesser degree (Figs. 9 to 16).

There was a significant decrease in the number of litters born to alcoholic rats when compared with corresponding control group. The birth weight was also decreased significantly in alcoholic rats when compared with corresponding control group. In case of animals given N-acetylcysteine + alcohol, the birth weight and number of litters born was nearly same as control animals (Table 3).

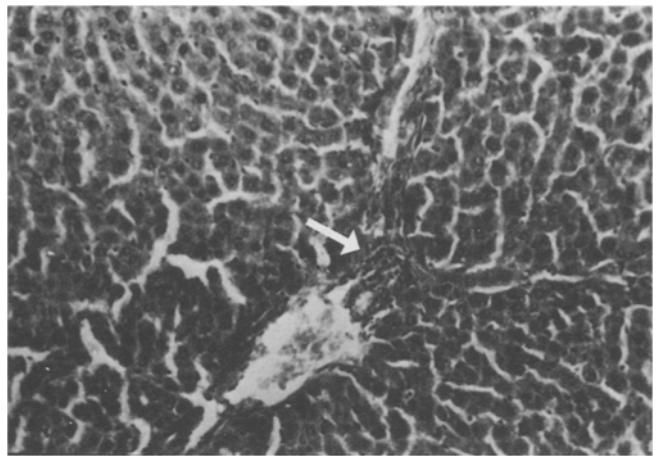

Fig. 4. Alcohol treated animals liver: H & E × 10. Portal triad showing inflammatory cell infiltrate ➞

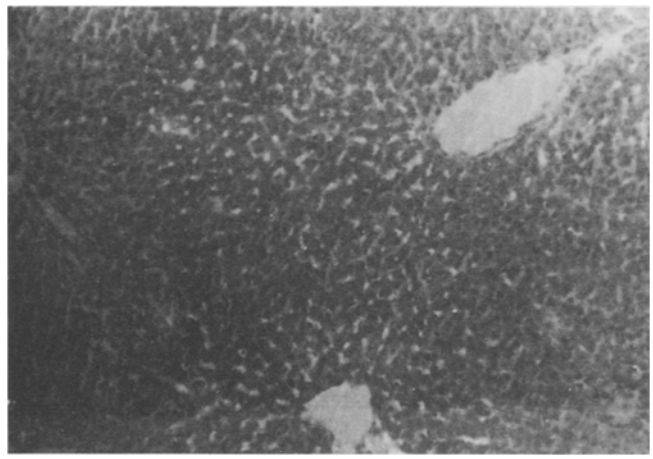

Fig. 5. Alcohol + NAC treated animals liver: H & E × 10. The histological effect of alcohol are less pronounced

Table 3. Average number of litters born and average weight

Group	Average number of litters born	Average weight of litters
1 Control	13.0 ± 1.414	5.0 ± 0.316
2 Alcohol	6.4 ± 1.01[a]	3.7 ± 0.244[a]
3 Alcohol + NAC	9.8 ± 0.748[b,B]	4.5 ± 0.316[NS,B]

Values are mean ± S.D. from 6 rats in each group. Group 2 and 3 have been compared with group 1; [a] $p < 0.001$; [b] $p < 0.01$. Group 2 has been compared with group 3; [B] $p < 0.01$. *NS* Not significant.

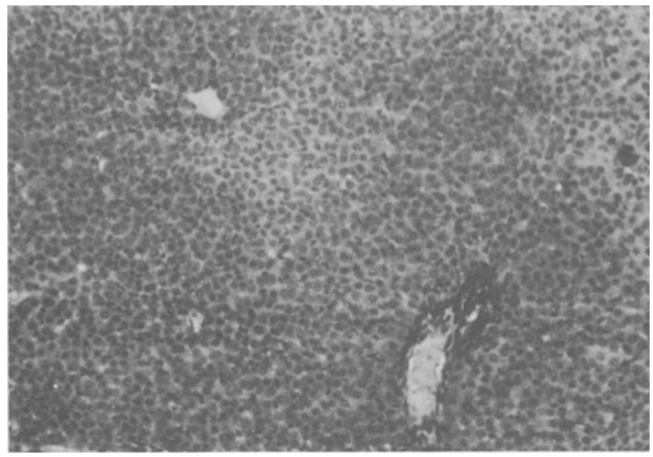

Fig. 6. Liver of litter born to control animal: H & E × 10

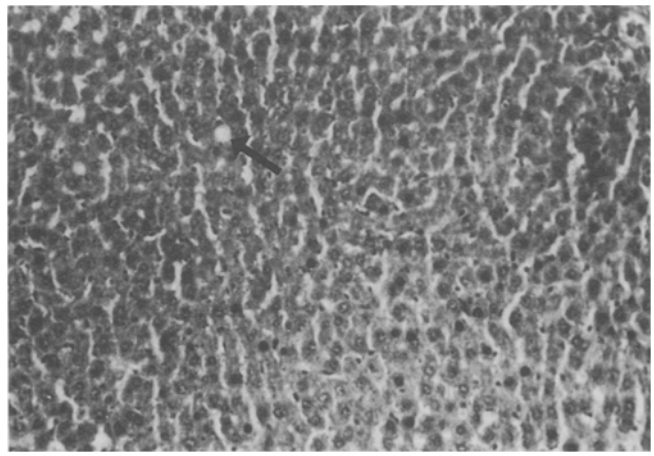

Fig. 7. Liver of litter born to alcohol treated animals: H & E × 10. Occasional foci of fatty change ➡

Table 4. Activities of serum GOT, GPT and ALP of control, alcohol and alcohol + NAC treated rats

Group	GOT μmoles of O.A.A. lib/min/litre of serum	GPT μmoles of pyruvate lib/min/litre of serum	ALP K.A. units/100 ml of serum
1 Control	136 ± 4.06	44 ± 3.23	9.71 ± 0.22
2 Alcohol	263 ± 9.11[a]	131 ± 7.89[a]	20.46 ± 0.61[a]
3 Alcohol + NAC	220 ± 7.07[a,A]	99 ± 4.12[a,A]	11.26 ± 0.26[a,A]

Values are mean ± S.D. from 6 rats in each group. Groups 2 and 3 have been compared with group 1; [a] $p < 0.001$. Group 2 has been compared with group 3; [A] $p < 0.001$.

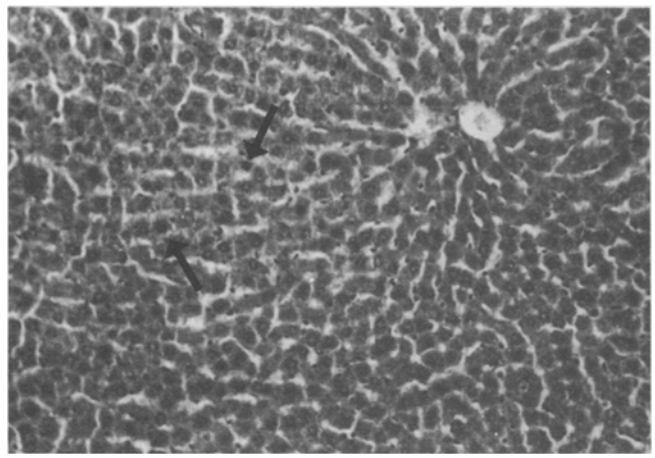

Fig. 8. Liver of litter born to alcohol + NAC treated animal: H & E × 20. Few foci of regenerative changes ➡

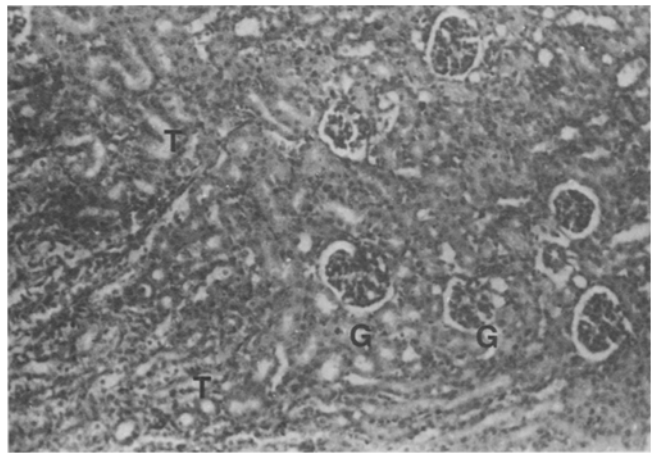

Fig. 9. Control animals kidney: H & E × 10. Showing glomeruli (*G*) and tubules (*T*)

Table 5. Levels of serum cholesterol, phospholipids and free fatty acids of control, alcohol and alcohol + NAC treated rats

Group	Cholesterol mg/100 ml serum	Phospholipids mg/100 ml serum	Free fatty acids mg/100 ml serum
1 Control	95.0 ± 4.17	86.0 ± 3.27	79.0 ± 2.11
2 Alcohol	164.165 ± 6.02[a]	150.26 ± 3.16[a]	154.72 ± 7.36[a]
3 Alcohol + NAC	129.0 ± 3.11[a,A]	123.0 ± 5.97[a,A]	120.0 ± 4.18[a,A]

Values are mean ± S.D. from 6 rats in each group. Groups 2 and 3 have been compared with group 1; [a]$p < 0.001$. Group 2 has been compared with group 3; [A]$p < 0.001$.

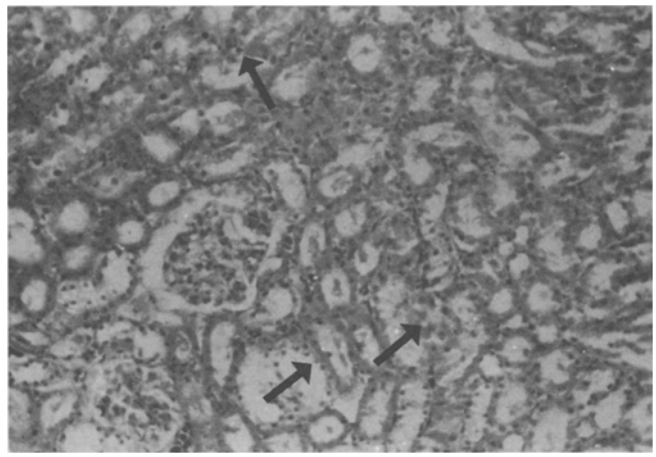

Fig. 10. Alcohol treated animals kidney: H & E × 10. Cloudy swelling in the cells lining the tubules ➞

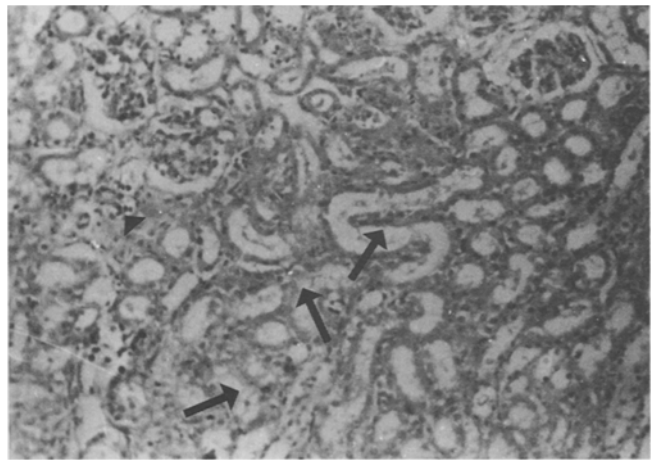

Fig. 11. Alcohol treated animals kidney: H & E × 10. ▲ Cloudy swelling of the cells lining the tubules. The stroma show congestion ➞

Table 6. Cholesterol and free fatty acid content in the liver and kidney of control, alcohol and alcohol + NAC treated rats

	Cholesterol mg/100 g tissue		Free fatty acids mg/100 g tissue	
	Liver	Kidney	Liver	Kidney
1 Control	312.16 ± 9.11	397.63 ± 7.83	689.11 ± 17.96	403.91 ± 12.01
2 Alcohol	498.11 ± 15.06[a]	494.62 ± 11.63[a]	1592.11 ± 41.15[a]	879.62 ± 40.06[a]
3 Alcohol + NAC	413.26 ± 7.21[a,A]	445.02 ± 30.07[c,A]	1345.20 ± 26.11[a,A]	518.23 ± 20.63[a,A]

Values are mean ± S.D from 6 rats in each group. Groups 2 and 3 have been compared with group 1; [a] $p < 0.001$; [b] $p < 0.01$; [c] $p < 0.02$. Group 2 has been compared with group 3; [A] $p < 0.001$.

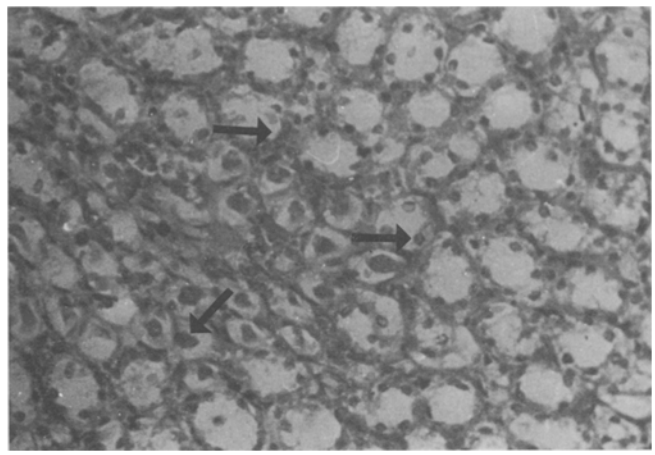

Fig. 12. Alcohol treated animals kidney: H & E × 10. Lumen of the tubules showing hyaline casts ➞

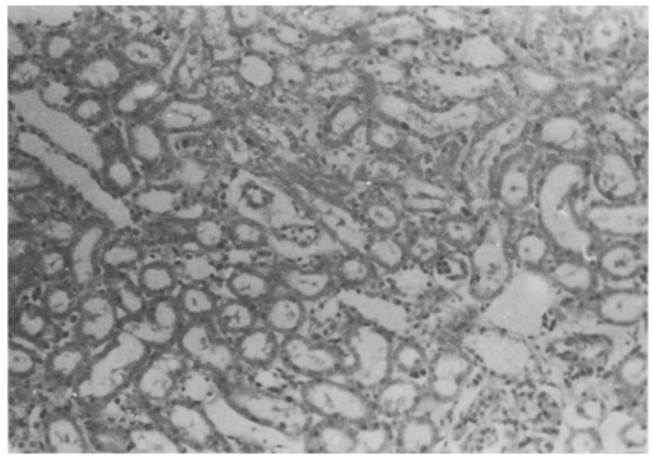

Fig. 13. Alcohol + NAC treated kidney: H & E × 10. Cloudy swelling is less pronounced

The activities of serum GOT, GPT and ALP showed a significant decrease in N-acetylcysteine treated alcoholic rats when compared with corresponding alcohol treated group (Table 4).

The administration of alcohol to female rats caused increase in the serum cholesterol, phospholipids and free fatty acids levels. Treatment of N-acetylcysteine resulted in decrease level of serum lipid (Table 5).

The cholesterol and free fatty acids in liver and kidney was increased in alcohol treatment. When treated with N-acetylcysteine there was decrease in cholesterol and free fatty acids in all the tissues when compared with corresponding alcohol treated group (Table 6).

The cholesterol, phospholipids and free fatty acids in liver and kidney increased in litters born to alcohol treated rats. The litters born to N-acetylcysteine treated rats showed a significant decrease in cholesterol,

Table 7. Cholesterol, phospholipid and free fatty acid content in liver and kidney of litters born to control, alcohol and alcohol + NAC treated rats

Group	Cholesterol mg/100 g tissue		Phospholipid mg/100 g tissue		Free fatty acid mg/100 g tissue	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
1 Litters born to control	144.28 ± 4.32	151.54 ± 6.11	166.34 ± 5.03	259.69 ± 6.26	651.44 ± 18.16	587.36 ± 14.67
2 Litters born to alcohol	274.34 ± 5.63[a]	242.02 ± 5.11[a]	1982.97 ± 31.01[a]	2425.26 ± 21.11[a]	2251.34 ± 20.62[a]	3427.68 ± 28.16[a]
3 Litters born to alcohol + NAC	186.11 ± 3.97[a,A]	211.57 ± 4.19[a,A]	1579.00 ± 21.19[a,A]	1722.79 ± 18.97[a,A]	1860.22 ± 19.71[a,A]	1767.23 ± 81.32[a,A]

Values are mean ± S.D. from 6 rats in each group. Groups 2 and 3 have been compared with group 1; [a] $p < 0.001$. Group 2 has been compared with group 3; [A] $p < 0.001$.

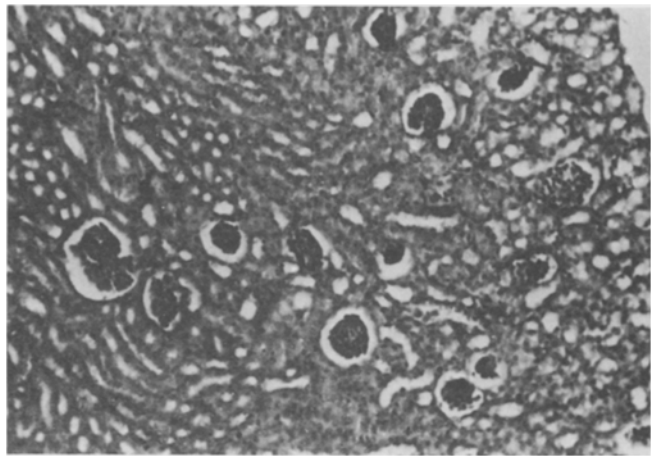

Fig. 14. Kidney of litter born to control animal: H & E × 10. Normal glomeruli and tubules

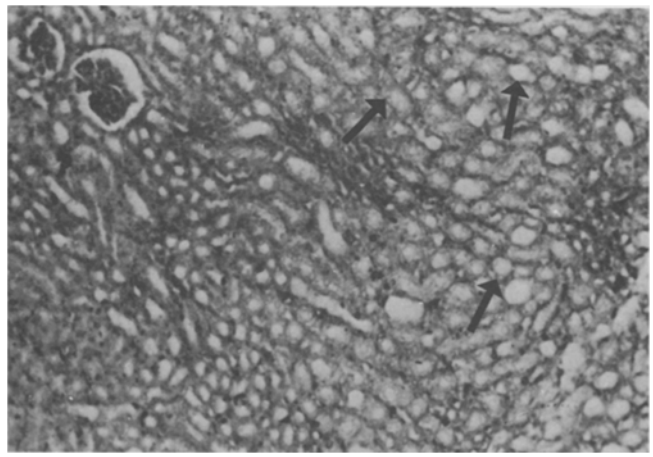

Fig. 15. Kidney of litter born to alcohol treated animal: H & E × 10. Cell lining the tubules showing cloudy swelling ➞

Table 8. TBARS in liver and kidney of control, alcohol, alcohol + NAC treated rats

Group	TBARS (mM/100 g of tissue)	
	Liver	Kidney
1 Control	0.67 ± 0.19	1.09 ± 0.06
2 Alcohol	2.52 ± 0.08[a]	2.99 ± 0.19[a]
3 Alcohol + NAC	0.83 ± 0.07[NS,A]	2.46 ± 0.90[a,B]

Values are mean ± S.D. from 6 rats in each group. Groups 2 and 3 have been compared with group 1; [a] $p < 0.001$. Group 2 has been compared with group 3; [B] $p < 0.01$; [A] $p < 0.001$. *NS* Not significant.

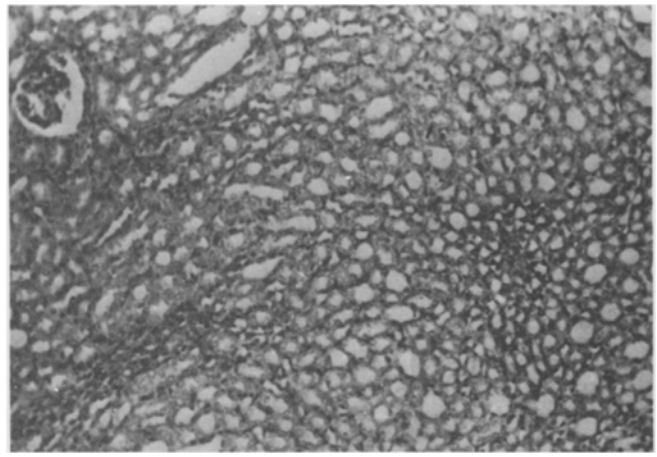

Fig. 16. Kidney of litter born to alcohol + NAC treated animal: H & E × 10. Cloudy swelling is very much reduced

Table 9. TBARS in liver and kidney of litters born to control, alcohol and alcohol + NAC treated rats

Group	TBARS (mM/100 g tissue)	
	Liver	Kidney
1 Control	4.638 ± 0.462	1.449 ± 0.108
2 Litters born to alcohol	10.183 ± 0.628[a]	6.990 ± 0.263[a]
3 Litters born to alcohol + NAC	7.345 ± 0.526[a,A]	4.320 ± 0.114[a,A]

Values are mean ± S.D. from 6 rats in each group. Groups 2 and 3 have been compared with group 1; [a] $p < 0.001$; Group 2 has been compared with group 3; [A] $p < 0.001$.

Table 10. Activities of superoxide dismutase (SOD) and catalase (CAT) in liver of control, alcohol and alcohol + NAC treated rats

Group	Superoxide dismutase units/mg protein	Catalase × 10^{-3} units/mg protein
1 Control	6.73 ± 0.21	59.14 ± 1.43
2 Alcohol	3.42 ± 0.20[a]	39.73 ± 2.11[a]
3 Alcohol + NAC	5.21 ± 0.13[a,A]	49.02 ± 1.62[a,A]

Values are mean ± S.D. from 6 rats in each group. Groups 2 and 3 has been compared with group 1; [a] $p < 0.001$. Group 3 has been compared with group 2; [A] $p < 0.001$.

phospholipids and free fatty acids in all the tissues studied when compared with litters born to alcohol treated group (Table 7).

The concentration of TBARS in liver and kidney increased in alcohol treatment. Treatment with N-acetylcysteine resulted in a significant decrease in the liver and kidney (Table 8).

The concentration of TBARS in liver and kidney was increased in litters born to alcohol treated rats. While it decreased significantly in litters born to N-acetylcysteine treated rats (Table 9).

Table 11. Glutathione content in liver and kidney of litters born to control, alcohol and alcohol + NAC treated rats

Group	Glutathione (mg/100g tissue)	
	Liver	Kidney
1 Litters born to control rats	32.28 ± 2.017	35.34 ± 1.46
2 Litters born to alcohol treated rats	8.74 ± 1.06[a]	12.76 ± 1.07[a]
3 Litters born to alcohol + NAC	20.35 ± 1.74[a,A]	19.095 ± 1.11[a,A]

Values are mean ± S.D. from 6 rats in each group. Groups 2 and 3 have been compared with group 1; [a]$p < 0.001$. Group 2 has been compared with group 3; [A]$p < 0.001$.

The activities of SOD and CAT in the liver decreased significantly in alcohol treated group when compared to corresponding control group. Treatment with N-acetylcysteine to the alcohol rats resulted in an increase in the SOD and CAT activities (Table 10).

Glutathione content in the liver and kidney decreased significantly in litters born to alcohol treated rats. On the other hand, litters born to N-acetylcysteine treated rats there was an increase in glutathione content in all the tissues studied (Table 11).

Discussion

Our studies show that chronic intake of ethanol in female rats results in both biochemical and histopathological alterations in the mother and their litters.

Histopathological studies shows that administration of N-acetylcysteine to alcohol treated rats reduces congestion in the vessels, cytoplasmic vacuolation, decrease nuclear disintegration and micronecrosis. There was only some vascular congestion and limited portal inflammation. Similar changes were also observed in the young ones. Studies have shown that N-acetylcysteine is an effective antidote for cyclophosphamide (Nelson et al., 1973), isophosphamide (Kline et al., 1973), acetaldehyde (Sprince et al., 1975) poisoning and in conjugation with pyridoxine an antagonist to the hepatotoxic effect of carbontetrachloride. N-acetylcysteine is also a known antioxidant. Thio compounds such as cysteine are known to increase the survival rate of animals given a lethal dose of ethanol to reduce ethanol induced sleeping time and to prevent ethanol induced fatty liver.

Jaya and Menon (1992) have observed that administration of N-acetylcysteine to male rats given alcohol and paracetamol results in decreased liver stenosis and liver necrosis.

We have also observed that alcohol produces changes in the kidney of both mother and sibling. There was cloudy swelling and congestion of vessel and some amount of micronecrosis in both mother and sibling. Administration of N-acetylcysteine showed a decreased in these changes.

We have observed that in female rats given alcohol, the number of litters born was less when compared to control animals. The birth weight of the siblings were also decreased. Studies have shown that in pregnant mothers

consuming alcohol the weight of the child is decreased than normal (Sokol, 1980). We have also observed that in alcoholic rats given N-acetylcysteine, the number of litters as well as the average birth weight were close to that of the control animals, suggesting that N-acetylcysteine can to a greater degree decrease the harmful effect of ethanol consumption in female pregnant rats.

Biochemically, we have observed that N-acetylcysteine an N-acetyl derivative of the sulphur containing amino acid L-cysteine act as a protective agent against the toxic effect of alcohol in female rats. The activities of serum GOT, GPT and ALP, the index of hepatic dysfunction, was increased in the ethanol treated rats. Sato et al. (1981) have observed increased serum GOT and glutathione dehydrogenase activities in ethanol fed rats. On administration of N-acetylcysteine to alcohol treated rats the levels of serum GOT and GPT decreased.

The activity of serum alkaline phosphatase was also increased during administration. Alkaline phosphatase is excreted by the liver via bile and hence when liver is affected the serum enzyme level increases due to defective excretion (Burtis and Ashwood, 1989). The administration of N-acetylcysteine to these female rats caused decreased levels of serum ALP. This shows the protective effect of N-acetylcysteine during liver injury.

Ethanol is a powerful inducer of hyperlipemia in both animals and humans (Avogaro and Cazzolatu, 1975). It also causes a change in the metabolism of lipoproteins. Marked alterations in lipid metabolism have been reported in chronic ethanol feeding. Remla et al. (1991) reported that administration of ethanol to rats caused changes in the metabolism of serum and tissue lipids. Several authors have reported that both acute and chronic ethanol consumption induced hypertriglyceridemia (Kaffarnik et al., 1978). Brodie et al. (1961) showed that single oral doses of ethanol in rats induced an accumulation of liver triglycerides.

Our results show that the metabolism of serum and tissue lipids are greatly affected by ethanol intake in female rats. Serum cholesterol, phospholipids and free fatty acids are increased. This shows the hyperlipidemic action of ethanol. Antonenkov et al. (1983) have reported that the chronic ingestion of ethanol results in moderate hypercholesterolemia, hypertriglyceridemia and increased concentration of lipids in the liver. The administration of N-acetylcysteine to alcoholic rats result in decreased concentration of serum lipid.

Our results also show, that cholesterol levels increase during alcohol treatment in liver and kidney of female rats. The increase in cholesterol level may probably result in lipid deposition in these tissues. Increased intake of alcohol to the female rats increases the levels of free fatty acids in liver and kidney. The increased fatty acid accumulation may directly be due to lipid breakdown and indirectly due to the oxidation of ethanol by the liver to acetate and its conversion to fatty acids, which is a means to remove excess of hydrogen generated by ethanol. This increased level of free fatty acid may cause greater generation of NADPH or NADH, which may result in the activation of NADPH dependent microsomal peroxidation. Administration of N-acetylcysteine to alcoholic female rats decreased the levels of tissue choles-

terol and free fatty acids. This shows the hypolipidemic action of N-acetylcysteine in ethanol fed rats.

Our results also shows, that there is an increased levels of cholesterol, phospholipids and free fatty acids in liver and kidney of litters born to these alcoholic rats. This shows the adverse effect of alcohol which can affect the siblings. The levels of cholesterol, phospholipids and free fatty acids were decreased considerably in liver and kidney of litters born to alcoholic pregnant rats given N-acetylcysteine. This suggests that N-acetylcysteine can to a greater degree decrease the harmful effects of ethanol consumption during pregnancy.

Increased free radical generation and lipid peroxidation may be the important mechanisms by which ethanol and its metabolite, acetaldehyde brings about hepatotoxicity in rats. The increased peroxidation can result in changes in the cellular metabolism of the hepatic and extra hepatic tissues.

Free radical generation and catalytic iron have been implicated in the pathogenesis of alcohol induced liver injury (Shaw and Jayatilleke, 1992). Several enzymatic functions of the endoplasmic reticulum are affected as a consequence of peroxidative events and among these are the activities of glucose-6-phosphatase, cytochrome P_{450} and calcium sequestration capacity. Moreover, a release of hydrolytic enzymes from lysosomes and a decrease in fluidity of plasma membrane can contribute to liver damage consequent to the stimulation of lipid peroxidation.

Our results show, that the concentration of lipid peroxidation product (TBARS) increased when alcohol was administered to female rats, in liver and kidney. The administration of N-acetylcysteine caused significant decrease in the levels of TBARS in the liver and kidney of alcohol treated rats. This shows the antiperoxidative property of N-acetylcysteine. The protective action of N-acetylcysteine a thiol compound to ethanol intoxication may be interpreted as a replenishment of reduced glutathione which may have decreased in alcoholism and/or lipid peroxidation by its antioxidant property.

We have also observed a significant increase in the levels of TBARS contents in the liver and kidney of litters born to these alcoholic rats. It shows the toxic effect of alcohol which may have affected the foetus as well as the siblings. There is a significant decrease in the levels of TBARS content in litters born to N-acetylcysteine treated alcoholic rats, suggesting that N-acetylcysteine can protect the foetus and siblings from the adverse effects of alcohol by decreasing the production of free radicals.

Both SOD and CAT are ubiquitous and are found in all oxygen consuming organisms. Superoxide dismutases (SODs) convert superoxide to hydrogen peroxide (Halliwell, 1994). These enzymes are metallo-proteins, widely distributed in cells with active oxidative metabolism and play an important role against oxidative damage in protecting cells. The hydrogen peroxide formed by the action of superoxide dismutase is degraded by catalase and glutathione peroxidases (Fridovich, 1986).

Our results show decreased activities of these enzymes (SOD and CAT) in hepatic tissue during alcohol treatment in female rats. The inhibition of catalase activity by ethanol may cause the accumulation of H_2O_2 or products

of its decomposition. Further decreased activity of SOD in ethanol treated rats results in the substrate for SOD i.e., the superoxide radical accumulating and this has been observed to be more aggressive than its products H_2O_2 or O_2.

Loss of SOD and CAT activities also results in oxygen intolerance and triggers of a number of deleterious reactions. It is clear that the cytotoxicity of molecular oxygen is held in check by the delicate balance between rates of the partially reduced oxygen species and the rates of their removal by the different defence mechanisms (Paul et al., 1989), any shift in this delicate balance can lead to cellular damage.

The activities of these enzymes SOD and CAT was near normal in the case of N-acetylcysteine administered female rats.

Glutathione (γ-glutamylcysteinylglycine) is a tripeptide consisting of glutamic acid, cysteine and glycine. A number of potentially toxic electrophilic xenobiotics are conjugated to the nucleophilic GSH. GSH is therefore an important defence mechanism against certain toxic compounds. If the levels of GSH in a tissue such as liver are lowered, then the tissue can be shown to be more susceptible to injury by various chemicals that would normally be conjugated to GSH.

Our studies show that the glutathione in liver and kidney of litters born to alcoholic rats decreased. Liver glutathione concentration after chronic ethanol consumption have been reported to increase (Duguay et al., 1980), decrease (Guerri et al., 1980) and remain unchanged (Sato et al., 1981). The decrease in glutathione content may be due to increased utilization of glutathione, to scavenge the toxic intermediate which may be formed from ethanol. In the litters born to N-acetylcysteine treated alcohol rats the levels of GSH returned to near normal, suggesting the protective role of N-acetylcysteine against alcohol toxicity.

Thus, our studies shows that N-acetylcysteine can offer protection both to the siblings and mother in experimental alcoholism.

References

Antonenkov VD, Popova SV, Panchenko LF (1983) Influence of ethanol and dofibrate on the activity of lipid catabolism enzymic systems in the rat liver. Farmakol Toksikol 46: 86–90

Avogaro P, Cazzolatu G (1975) Changes in the composition and physiochemical characteristics of serum lipoproteins during ethanol induced lipemia in alcohol subjects. Metab Clin Exp 24: 1231–1242

Bennett CA, Franklin NL (1954) Statistical analysis of chemistry and the chemical industry. John Wiley and Sons, Inc, London New York

Beutler E, Kelley BM (1963) The effect of sodium nitrate on red cell glutathione. Experientia 19: 96–97

Brodie BB, Maling HM, Horning MG, Maickel RP (1961) In: Garritini S, Paoletti R (eds) Drugs affecting lipid metabolism. Elsevier, London

Burtis CA, Ashwood ER (1989) Clinical chemistry, 2nd edn. W.B. Saunders, Philadelphia

Clarren SK, Bowden DM, Astley SJ (1987) Pregnancy outcome after weekly oral administration of ethanol during gestation in the pig-tailed macaque (Macacanmnistrina) Teratology 35: 345–354

De-Vries N, De Flora S (1993) N-acetyl-L-cysteine. J Cell Biochem [Suppl] 17F: 270–277
Duguay L, Coutu D, Hetu C, Joly JG (1980) Chronic ethanol consumption impairs liver regeneration. Inst Natl Sante Rech Med 95: 105–110
Folch J, Lees M, Stanley GHS (1951) A simple method for the isolation, purification of total lipids from animal tissue. J Biol Chem 226: 497–509
Folholt K, Folholt, Lund B (1973) An easy colorimetric method for routine determination of free fatty acids in plasma. Clin Chem Acta 46: 105
Fridovich I (1986) Biological effects of superoxide radical. Arch Biochem Biophys 247: 1–11
Goedde WH, Agarwal DP (1989) Alcoholism biomedical and genetics aspects, 1st edn. Pergamon Press, New York, pp 3–57
Guerri C, Grisolia S (1980) Influence of prolonged ethanol intake on the levels and turnover of alcohol and aldehyde dehydrogenases and glutathione. Adv Exp Med Biol 126: 365–384
Halliwell B (1994) Free radicals, antoxidants and human disease: curiosity, causes or consequence? Lancet 344: 721–724
Harlap S, Shino PH (1980) Alcohol, smoking and incidence of spontaneous abortions in the first and second trimester. Lancet ii: 173–176
Jaya DS, Menon PVG (1992) Metabolism of lipids and glycoconjugates in alcohol treated rats. Ph.D. Thesis, Kerala University, Trivandrum, pp 215–249
Jones KL, Chernoff FG, Kelley CD (1984) Out come of pregnancy in women who "bring" drink during the first trimester of pregnancy. Clin Res 32: 114A
Kaffarnik H, Schneider J, Schubotz R, Hausmann L, et al (1978) Plasma lipids, triglycerids/fatty acid pattern and plasma insulin in fasted healthy volunteers during continuous ingestion of ethanol. Influence of lipolysis inhibited by nicotinic acid. Atherosclerosis 29: 1–7
Kakkar P, Bos B, Visvanathan PN (1984) A modified spectrophotometric assay of SOD. Ind J Biochem Biophys 21: 130–132
Kline I, Gang M, Woodman RJ, et al (1973) Evaluation of antileukemic agents in advanced leukemic L1210 in mice. Cancer Chemo Ther Rep 3: 1–69
Kline J, Shrout P, Steinz, Susser M, Warburton D (1980) Drinking during pregnancy and spontaneous abortion. Lancet ii: 176–180
Maehly AC, Chance B (1954) In: Glick D (ed) Methods of biochemical analysis, vol. i. Interscience Inc, New York, p 357
Nelson LW, Botta JA Jr, Weikel JH Jr (1973) Estrogenic activity of norethindrone in the immature female beagle. Commun Chem Pathol Pharmacol 5: 879–882
Nichens WG Jr, Samuelsson B (1968) Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. Eur J Biochem 6: 126
Paul LA, Fulton AM, Heppner GH (1989) Reactive oxygen mediated damage to murine mammary tumor cells. Mutat Res 215: 223–234
Prescott LF, Park J, Proud Foot AT (1976) Cysteamine, L-methionine and D-penicillamine in paracetamol poisoning. J Int Med Res 4: 112–117
Salaspuro M, Lindros K (1985) In: Seitz HK, Kommerell B (eds) Metabolism and toxicity of acetaldehyde. Alcohol related diseases in gastroenterology. Springer, Berlin Heidelberg New York Tokyo, pp 106–123
Remla A, Menon PVG, Kurup PA (1991) Effect of ethanol administration on metabolism of lipids in heart and aorta in isoproterenol induced myocardial infarcation in rats. Ind J Exp Biol 29: 244–248
Sato C, Matsuda Y, Lieber CS (1981) Mechanism of the preventive effect of ethanol on acetaminophen-induced hepatotoxicity. Gastroenterology 80: 140–144
Scott WJ Jr, Fradkin R (1984) The effect of prenatal ethanol in cynomolgus monkey (Macafascicularis). Teratology 29: 49–56
Shaw S, Jayatilleke E (1992) The role of cellular oxidases and catalytic iron in the pathogenesis of ethanol-induced liver injury. Life Sci 50: 2045–2052

Sokol RJ (1980a) Alcohol and spontaneous abortion (letter to the editor) Lancet ii: 1079
Sokol RJ (1980b) Alcohol and abnormal outcomes of pregnancy. Can Med Assoc J 125: 143–148
Sprince H, Parker CM, Smith GC, et al (1975) Protective action of ascorbic acid and sulfur compounds against acetaldehyde toxicity. Implications in alcoholism and smoking. Agents Actions 5: 164–173
Varley H (1988) Practical clinical biochemistry, 4th edn. CBS Publishers, New Delhi, pp 296–297
Zak B, Boyle AJ, Zlatkis A (1953) A method for the determination of serum cholesterol. J Clin Med 41: 486–492
ZilverSmit DB, Davis AK (1950) Microdetermination of plasma phospholipids by trichloroacetic acid precipitation. J Lab Clin Invest 35: 155–160

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