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Impact of massive ascorbic acid supplementation on alcohol induced oxidative stress in guinea pigs

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Abstract

The effects of a mega dose of ascorbic acid (AA) on alcohol induced peroxidative damages were investigated in guinea pigs. In the present study, four groups of male guinea pigs were maintained for 30 days as follows. (1) Control group (1 mg AA/100 g body wt); (2) Ethanol group (1 mg AA/100 g body wt. + 9 g ethanol/kg body wt); (3) AA group (25 mg AA/100 g body wt); (4) AA + ethanol group (25 mg AA/100 g body wt. + 9 g ethanol/kg). Results revealed that alcohol induced significant lipid peroxidation, since the lipid peroxidation products malondialdehyde (MDA), hydroperoxides and conjugated dienes were elevated. The activities of scavenging enzymes superoxide dismutase (SOD), catalase were reduced. However, supplementation of AA along with alcohol reduced the lipid peroxidation products in the liver and enhanced the activities of scavenging enzymes. Activities of glutathione peroxidase and reductase were also greater in guinea pigs given alcohol + AA in comparison with those given alcohol alone. Administration of ascorbic acid also reduced the activity of γ-glutamyl transpeptidase (GGT), the marker enzyme of alcohol induced toxicity. The vitamin E level, which was reduced by alcohol intake, was raised by the co-administration of AA and alcohol. These studies suggest that a mega dose of AA helps in the prevention of alcohol induced oxidative stress by enhancing the antioxidant capacity and also by reducing the lipid peroxidation products.
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Keywords: Vitamin C; Alcohol; Guinea pigs; Lipid peroxidation; Antioxidant and vitamin E

1. Introduction

Vitamin C or ascorbic acid (AA) is a naturally occuring free radical scavenger, as such its presence assists various other mechanisms in decreasing the numerous disruptive free radical processes from taking place, including lipid peroxidation (Knight et al., 1993). In AA deficiency, lipid peroxidation occurs progressively in guinea pig tissues, despite the presence of adequate levels of antioxidants like glutathione, protein thiols and

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scavenging enzymes (Chakraborthy et al., 1994). Chakraborthy et al. (1994) have shown that ascorbate protects guinea pig tissues from lipid peroxidation both in vivo and in vitro. Zloch and Ginter (1995) have shown that the need for Vitamin C during chronic alcohol consumption is enhanced, due to the participation of AA in oxidoreducting processes connected with ethanol metabolism, which leads to its irreversible destruction. In chronic ethanol intoxication, increased lipid peroxidation could occur as a consequence of induction of microsomal membrane free radical generation (Boveries et al., 1983; Shaw et al., 1984). The ethanol or its metabolites can alter the balance in the liver towards autooxidation, either acting as prooxidants or reducing the antioxidant level or both (DiLuzio and Hartman, 1967). The pathogenesis of alcohol induced liver disease involves the adverse effect of ethanol metabolites and oxidative tissue injury (Niemela et al., 1995). Recent studies have shown that both vitamin C and E are reduced in alcoholics. Although there have been studies showing peroxidative damage caused by ethanol and the antioxidant capacity of AA, experiments to assess the effect of a mega dose of AA in an intact organism have been scarce. Our earlier studies have shown that the ingestion of a mega dose of AA is beneficial in reducing alcohol-induced hyperlipidemia and AA deficiency in guinea pigs (Suresh et al., 1997). In the present study, we have examined the effect of vitamin C on lipid peroxidation levels and antioxidant defence mechanisms of guinea pigs administered alcohol. Guinea pigs were selected for the study, since like humans they are also incapable of synthesising vitamin C.

The desirable intake of ascorbic acid for maintenance of optimal health is a subject of considerable controversy. It has been suggested by Pauling (1970) and Pauling (1971) that an adequate intake of ascorbic acid should be ~ 3 g/day under ordinary conditions and larger, up to 40 g/day, for a person under stress. Nandi et al. (1973) reported large dose of ascorbic acid (0.5–250 mg in 0.75 ml water) had neither beneficial nor toxic effect on growth and maintenance of rats and guinea pigs fed a nutritionally balanced fortified wheat diet. On the other hand, administration of 50 mg of ascorbic acid/guinea pig per day fed unfortified wheat diet resulted in a significant decrease in the growth and there was 50% mortality within 16 days and 100% mortality within 25 days. Previous reports have shown that supplementation of (25 mg AA/100g body wt.) decreased the cholesterol level in guinea pigs (Bala Nambisan and Kurup, 1975) and also reduced alcohol induced hyperlipidemia (Suresh et al., 1997).

2. Materials and methods

Male guinea pigs (Veterinary College, Mannuthy strain) bred and reared in our animal house were used for the experiment. Weight matched animals were selected. A total of 24 guinea pigs were divided into four groups of six each as follows.

Table 1
Body weight gain of guinea pig (g)[a]

Control	171 ± 7.79
Ethanol	195 ± 10.27[b]
Ascorbic acid	197 ± 11.09[c]
AA + Ethanol	194 ± 10.86[d]

[a] Values expressed as mean ± S.D.
[b] $P < 0.05$ between control and ethanol groups.
[c] $P < 0.05$ between control and ascorbic acid groups.
[d] $P < 0.05$ between control and ethanol + ascorbic acid groups.

Table 2
Liver α-tocopherol (μg/100 g wet tissue)[a]

Liver	
Control	215.12 ± 8.84
Ethanol	165.21 ± 7.86[b]
Ascorbic acid	437.34 ± 19.30[c]
AA + ethanol	372.15 ± 14.45[d,e]

[a] Values expressed as mean ± S.D.
[b] $P < 0.05$ between control and ethanol groups.
[c] $P < 0.05$ between control and ascorbic acid groups.
[d] $P < 0.05$ between control and ethanol + ascorbic acid groups.
[e] $P < 0.05$ between ethanol and ethanol + ascorbic acid groups.

Table 3
Concentration of free fatty acid (mg/100 g tissue) in various guinea pigs tissues[a]

PRIVATE	Liver	Kidney	Brain	Heart
Control	229.56 ± 9.92	242.22 ± 12.97	237.49 ± 9.82	303.44 ± 14.12
Ethanol	294.47[b] ± 12.06	405.76[b] ± 16.18	348.44[b] ± 13.02	746.30[b] ± 20.36
Ascorbic acid	209.24[c] ± 8.88	210.41[c] ± 10.02	217.46[c] ± 9.43	299.33 ± 13.75
Ascorbic acid + ethanol	232.47[e] ± 9.82	382.04[d] ± 14.41	300.86[d,e] ± 11.10	571.30[d,e] ± 15.72

[a] Values expressed as mean ± S.D.
[b] $P<0.05$ between control and ethanol groups.
[c] $P<0.05$ between control and ascorbic acid groups.
[d] $P<0.05$ between control and ethanol + ascorbic acid groups.
[e] $P<0.05$ between ethanol and ethanol + ascorbic acid groups.

Table 4
Concentration of malondialdehyde (mM/100 g tissue) in various guinea pigs tissues[a]

PRIVATE	Liver	Kidney	Brain	Heart
Control	0.323 ± 0.014	1.09 ± 0.07	1.26 ± 0.07	1.86 ± 0.078
Ethanol	0.439[b] ± 0.011	2.53[b] ± 0.37	1.87[b] ± 0.19	2.84[b] ± 0.13
Ascorbic acid	0.299 ± 0.008	0.97 ± 0.07	0.87[c] ± 0.13	1.80 ± 0.07
Ascorbic acid + Ethanol	0.384[d,e] ± 0.022	2.47[d] ± 0.38	1.79[d] ± 0.07	2.62[d,e] ± 0.11

[a] Values expressed as mean ± S.D.
[b] $P<0.05$ between control and ethanol groups.
[c] $P<0.05$ between control and ascorbic acid groups.
[d] $P<0.05$ between control and ethanol + ascorbic acid groups.
[e] $P<0.05$ between ethanol and ethanol + ascorbic acid groups.

1. Control group (1 mg AA/100 g body wt. per day)
2. Ethanol group (1 mg AA/100 g body wt. + 9 g ethanol/kg body wt. per day)
3. Ascorbic acid group (25 mg AA/100 g body wt. per day)
4. AA + ethanol group (25 mg AA/100 g body wt. + 9 g ethanol/kg body wt. per day)

The animals were housed individually in wire netted cage in a room with temperature maintained at 25 ± 1°C and light and dark cycle of 12 h. Guinea pigs were fed with guinea pig feed[1] (Lipton India). Food and water were given ad libitum. AA and ethanol was administered as detailed above. Ascorbic acid, freshly dissolved in distilled water and ethanol diluted in the ratio (1:1) were given orally by gastric tube for 30 days. Control and AA group animals were administered glucose solution equivalent to the caloric value of ethanol in groups 2 and 4. On the 31st day, the animals were sacrificed by the decapitation and tissues were removed to ice cold containers for various estimations. Initial and final weights of the animals were noted. A HPLC method was used for the determination of vitamin E in the liver by the method of Catiganin (1986). Reagents used were all HPLC grade. Liver was weighed and homogenised in methanol, vortexed with hexane for 45 s, centrifuged for 5 min at 2000 rpm and the hexane layer was re-extracted with hexane. Then the hexane layer was evaporated off with a stream of nitrogen, solubilized immediately in methanol and an aliquot injected into a Shimadzu HPLC system and monitored at 296 nm. The column used was a 4.6 × 15 mm reverse-phase C-18 column (5 um Shimadzu), 100% methanol was used as the solvent system at a flow rate of 2 ml/min. Alpha tocopherol elutes at ~2.38 min.

[1] Composition of guinea pig feed: crude protein, 21%; ether extract, 5%; crude fiber, 4%; ash, 8%; calcium, 1%; phosphorus, 0.06%; nitrogen free extract, 53%.

Free fatty acids (FFA) were estimated by the method of Falholt et al. (1973). Malondialdehyde (MDA), hydroperoxide and diene conjugates were estimated according to John and Steven (1978). Activities of superoxide dismutase (SOD) and catalase were determined by the method of Kakkar et al. (1984) and Maehly and Chance (1954), respectively. Glutathione content and the activity of glutathione reductase (GSSG-Red) were determined by the procedure of Patterson and Lazarow (1955) and David and Richard (1983), respectively. The activity of glutathione peroxidase (GSH-PX) was determined by the method of Lawerence and Burk (1976) as modified by Agergurd and Jense (1982). Protein was determined by the method of Lowry et al. (1951). Gamma glutamyl transpeptidase (GGT) activity in the plasma was assayed by Szasz's method (Szasz, 1969).

Statistical analysis was carried out using one way analysis of variance (ANOVA). Differences between treatment means were determined by methods of Snedecor and Cohran (Marcello and Kinberlee, 1993).

3. Results

Weight gain of the control animals was lesser than the weight gain of the animals in the other groups (Table 1).

The concentration of vitamin E increased in the liver of guinea pigs given high dose AA compared to other groups (Table 2). The concentration of vitamin E level decreased in the animals given ethanol alone. On co-administration of ethanol and ascorbic acid, vitamin E level increased when compared with ethanol and control group.

Free fatty acids (Table 3) increased on intoxication of ethanol in liver, kidney, brain and heart. AA administration brought down the FFA levels in all the tissues except heart. Co-administration of ethanol and AA raised the FFA levels in

Table 5
Concentration of hydroperoxides (mM/100 g tissue) in various guinea pigs tissues[a]

PRIVATE	Liver	Kidney	Brain	Heart
Control	11.55 ± 0.97	10.94 ± 1.42	8.53 ± 0.70	14.23 ± 0.76
Ethanol	$18.94^{b} \pm 1.44$	$19.89^{b} \pm 1.36$	$11.90^{b} \pm 1.35$	$18.23^{b} \pm 1.18$
Ascorbic acid	$7.06^{c} \pm 0.70$	$5.04^{c} \pm 0.63$	$6.04^{c} \pm 0.69$	$10.25^{c} \pm 0.76$
Ascorbic acid + Ethanol	$16.8^{d,e} \pm 1.39$	$12.33^{e} \pm 0.74$	$10.53^{d} \pm 0.71$	$16.36^{d,e} \pm 0.95$

[a] Values expressed as mean ± S.D.
[b] $P < 0.05$ between control and ethanol groups.
[c] $P < 0.05$ between control and ascorbic acid groups.
[d] $P < 0.05$ between control and ethanol + ascorbic acid groups.
[e] $P < 0.05$ between ethanol and ethanol + ascorbic acid groups.

Table 6
Concentration of conjugated dienes (mM/100 g tissue) in various guinea pigs tissues[a]

PRIVATE	Liver	Kidney	Brain	Heart
Control	71.55 ± 2.78	16.94 ± 0.98	12.53 ± 1.04	14.23 ± 1.34
Ethanol	$180.94^{b} \pm 5.45$	$22.89^{b} \pm 1.64$	$17.90^{b} \pm 1.49$	$19.23^{b} \pm 1.77$
Ascorbic acid	$65.06^{c} \pm 2.28$	$11.04^{c} \pm 0.82$	$8.24^{c} \pm 0.92$	$11.25^{c} \pm 1.06$
Ascorbic acid + ethanol	$78.12^{d,e} \pm 2.95$	$19.33^{d,e} \pm 1.45$	$15.53^{d} \pm 1.48$	$17.36^{d} \pm 1.41$

[a] Values expressed as mean ± S.D.
[b] $P < 0.05$ between control and ethanol groups.
[c] $P < 0.05$ between control and ascorbic acid groups.
[d] $P < 0.05$ between control and ethanol + ascorbic acid groups.
[e] $P < 0.05$ between ethanol and ethanol + ascorbic acid groups.

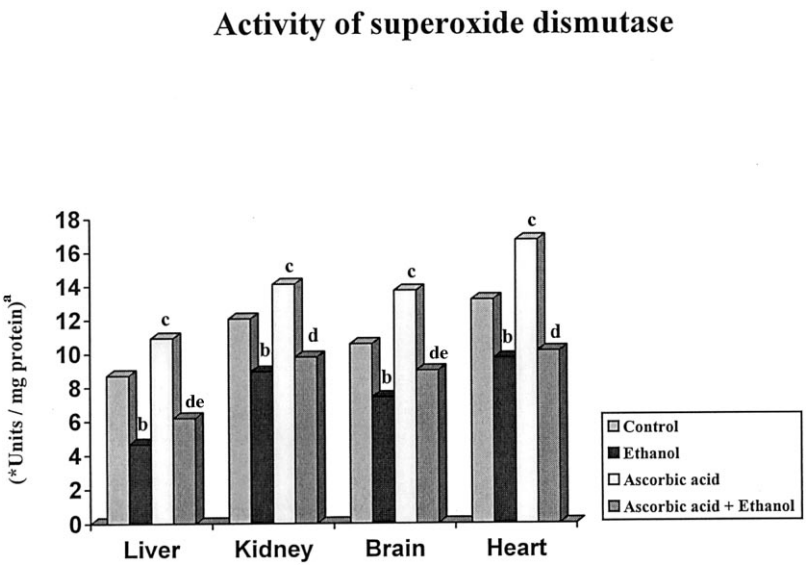

Fig. 1. Activity of superoxide dismutase. 1*Units, enzyme concentration required to inhibit the chromogen production (OD at 560 nm) by 50% in 1 min. Details as same as in Table 2.

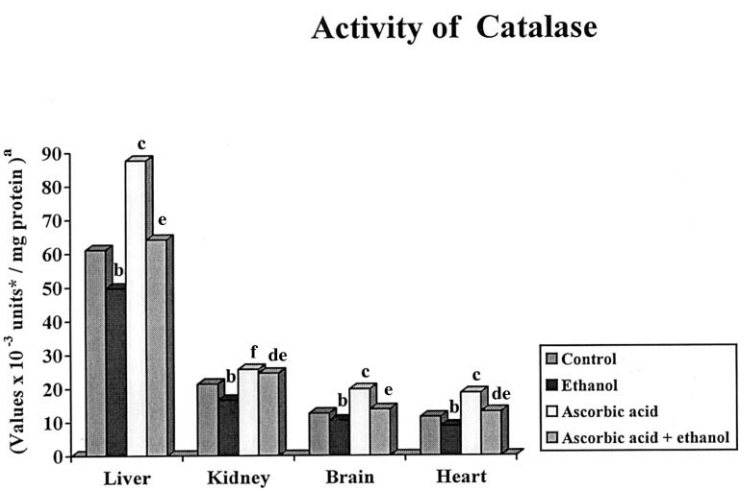

Fig. 2. Activity of catalase. Unit, velocity constant/s. Details same as in Table 2.

kidney, brain and heart, in comparison with controls. However, this elevation in FFA content was much lower than that induced by ethanol.

The level of MDA, hydroperoxides and conjugated dienes was found to be increased in all the tissues administered ethanol when compared to other groups (Tables 4–6). The concentration of hydroperoxides and conjugated dienes significantly decreased in all the tissues of guinea pigs given mega dose of vitamin C. But significant reduction of MDA was seen only in the brain. In the ethanol + AA treated group MDA, hydroperoxide and conjugated dienes were reduced in the liver in comparison with those administered ethanol. In the kidney, hydroperoxide and conjugated dienes were reduced. In the heart, malondialdehyde and hydroperoxide were reduced. But in the brain no significant alterations were observed.

The activity of SOD and catalase was found to be increased significantly in all the tissues studied

Table 7
Glutathione levels in (mM/100 g tissue) various guinea pigs tissues[a]

PRIVATE	Liver	Kidney	Brain	Heart
Control	265.97 ± 10.03	143.47 ± 6.46	72.62 ± 3.67	202.89 ± 8.44
Ethanol	334.80[b] ± 16.15	287.83[b] ± 13.07	118.53[b] ± 7.03	274.80[b] ± 10.76
Ascorbic acid	297.71[c] ± 12.04	180.95[c] ± 8.86	89.80[c] ± 3.92	238.70[c] ± 11.73
Ascorbic acid + ethanol	277.10[e] ± 10.51	168.16[d,e] ± 7.55	79.87[e] ± 3.90	217.06[e] ± 10.62

[a] Values expressed as mean ± S.D.
[b] $P<0.05$ between control and ethanol groups.
[c] $P<0.05$ between control and ascorbic acid groups.
[d] $P<0.05$ between control and ethanol + ascorbic acid groups.
[e] $P<0.05$ between ethanol and ethanol + ascorbic acid groups.

in guinea pigs administered mega doses of vitamin C, when compared with all other groups (Figs. 1 and 2). The activity of SOD and catalase was decreased in all the tissues given ethanol alone. Administration of ethanol + AA increased the activity of SOD in liver and brain when compared with ethanol treated group, whereas catalase activities were increased in all the tissues studied. The concentration of glutathione increased in all the tissues of ethanol and AA group. The maximum increase was observed in animals given ethanol. In the AA + ethanol group, glutathione content was lower than in the ethanol group (Table 7).

The activity of glutathione reductase was decreased in the liver and serum of ethanol administered group when compared to other groups (Fig. 3). The activity of glutathione reductase was augmented in the tissues of guinea pigs given a mega dose of vitamin C. Administration of ethanol + AA significantly increased the enzyme activity when compared with the ethanol group.

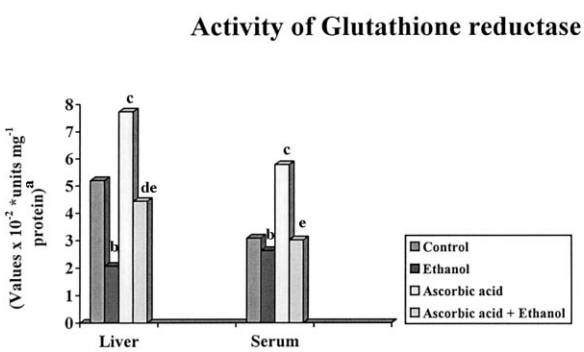

Fig. 3. Activity of glutathione reductase. 1*Units, 1 μmol NADPH oxidized/min. Details same as in Table 2.

The activity of glutathione peroxidase showed an increase in ascorbic acid group and ethanol group (Fig. 4). Co-administration of ethanol along with ascorbic acid significantly increased the activity of glutathione peroxidase when compared with the ethanol group

The activity of GGT in serum was significantly increased in guinea pigs administered ethanol (Fig. 5). But on supplementation of AA along with ethanol their activities were lower than in those given alcohol alone.

4. Discussion

Antioxidants are essential in preventing the cellular damage caused by free radicals and free radical modified lipid peroxidation. In normal metabolism there is a balance between the generation of free radicals and antioxidant defence mechanism. Chronic ingestion of alcohol upsets this balance, and there are ample evidence (DiLuzio and Hartman 1967) to demonstrate the oxidative stress induced by ethanol. In the present study the activity of GGT was elevated, indicating the toxicity induced by alcohol. Various markers of oxidative stress such as TBARS, H_2O_2, diene conjugates were also elevated. This is in agreement with the studies of Lutnicki et al. (1992). FFA concentration was increased in the ethanol group. This may be due to the oxidative degradation of phospholipids and these FFA may serve as

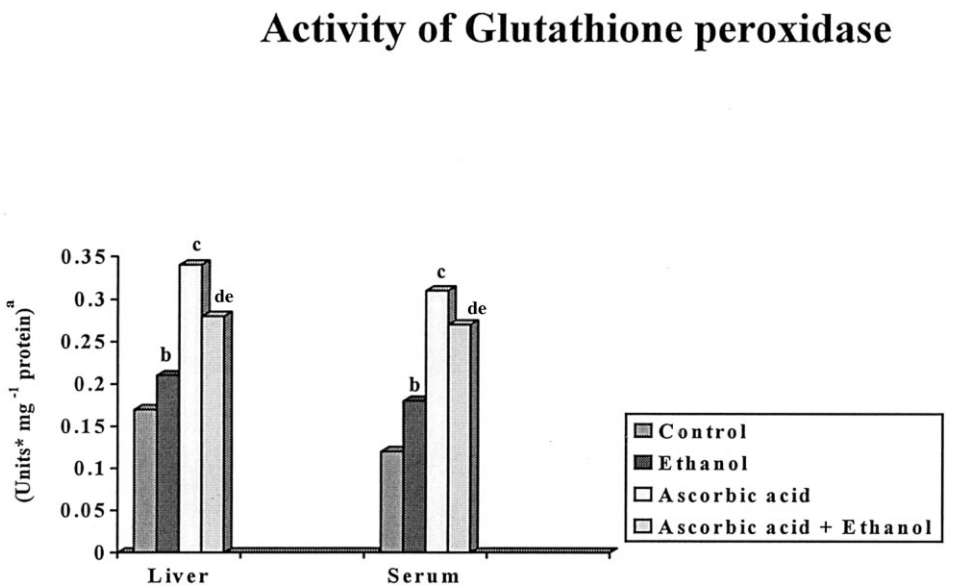

Fig. 4. Activity of glutathione peroxidase.*1 Unit, 1 μmol NADPH oxidized/min. Details same as in Table 2.

substrates for lipid peroxidation. The activity of SOD and catalase was found to be significantly decreased in the tissues of guinea pigs exposed to ethanol. These results agree with the reports that activity of the antioxidant enzymes was suppressed by ethanol administration (Sacks et al., 1978; Nathan et al., 1979).

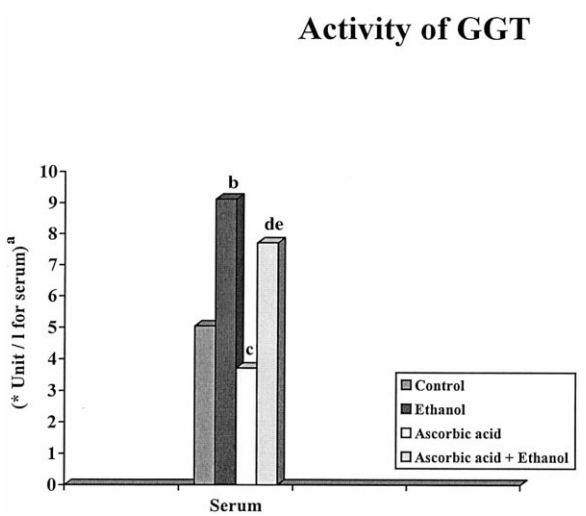

Fig. 5. Activity of gama glutamyl transpeptidase. *Units, the molar absorption coefficient of 4 nitro aniline at 405 nm is 9900/mo/cm. Details same as in Table 2.

Supplementation of AA along with alcohol decreased the GGT level indicating the reduction in the alcohol-induced toxicity. Elevated lipid peroxidation products formed by alcohol exposure were also reduced. Administration of ethanol enhanced glutathione content, and it was brought down to normal level by AA supplementation. This is in agreement with the result of Heter et al. (1981) who observed that the chronic ethanol feeding increased glutathione content. The increase in the glutathione content may be due to a feed back activation of glutathione synthesis (Smith and Boyd, 1984; Jenkinson et al., 1988). Weight of the control animals was less than the weight of animals in the other groups. The enhanced weight gain of animals given alcohol may be due to hyperlipidemia, since alcohol ingestion causes hyperlipidemia (Suresh et al., 1997). Control animals were given only minimum doses of ascorbic acid. Hence the weight gain in the AA group may be due to the intake of a mega dose of ascorbic acid.

In vitro studies have shown that high concentration in ascorbate induced cytotoxicity and low concentration had a stimulatory effect on growth (Chakraborthy et al., 1994). This induction of toxicity is attributed to the generation of H_2O_2. But in the present study we have observed that

administration of AA at a dose of 25 mg/100 g body weight enhanced the activity of catalase, the enzyme responsible for the removal of the peroxide radical.

Glutathione peroxidase, another scavenging enzyme is also increased in guinea pigs given ethanol + AA in comparison with those given alcohol. The increased glutathione may be channelled as a substrate for glutathione peroxidase. Enhancement in the glutathione peroxidase activity in the liver of guinea pigs administered high dose of ascorbic acid has been reported earlier (Zloch and Ginter, 1995).

Consistent with several human studies, (Glascott et al., 1996) guinea pigs administered alcohol also demonstrated low vitamin E levels. But supplementation of vitamin C elevated not only vitamin C content but also vitamin E level. This is in agreement with the reports of Bendich et al. (1983) who have demonstrated that supplementation of 10 g vitamin C/kg body wt. significantly increased the level of vitamin E. But there are also contrary reports of lower vitamin E levels in vitamin C supplemented experimental animals (Helen and Vijayammal, 1997). The in vivo interaction of two vitamins may be dose dependent. It has been hypothesised that vitamin C is involved in the regeneration of vitamin E in cell free system (Packer et al., 1979; Bendich et al., 1983; Buettner, 1993). It may be also due to low utilisation of vitamin E in the presence of mega dose of vitamin C, which enhanced the levels of scavenging enzymes. However, present observations suggest that a mega dose of ascorbic acid spare the use of vitamin E under stressful conditions. So it can be concluded that AA nullifies to an extent, the alcohol induced oxidative stress by enhancing the antioxidant capacity of guinea pigs and also by reducing the lipid peroxidation products.

Acknowledgements

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