

LETTER TO THE EDITOR

EFFECT OF THE ANTIOXIDANTS ALPHA-TOCOPHEROL ACETATE AND SODIUM SELENITE ON HEPATOTOXICITY INDUCED BY ANTITUBERCULAR DRUGS IN RATS

Sir,

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Hepatotoxicity due to antitubercular drugs is found to be mediated through oxidative stress and free radical damage to hepatocytes (1, 2, 3). The antioxidants  $\alpha$ -tocopherol and selenium are known exert beneficial effect in antitubercular drugs induced hepatotoxicity (4, 5). However mere withdrawal itself of antitubercular therapy is known to reverse the hepatotoxic changes (6, 7). Hence the present study was carried out in albino rats to study the effect of these two antioxidants on reversal of hepatotoxic changes as compared to mere withdrawal of anti-tubercular drugs. The effect of individual antioxidant was also compared with the effect of their combination.

Healthy adult albino rats of either sex of Wister strain weighing 150 to 250 g were used after approval of the Institutional Animal Ethics Committee. They were kept on standard pellet diet (Hindustan Lever Ltd) and water *ad libitum*. The oral doses of anti-tubercular drugs [isoniazid (I)-27 mg/kg/day, rifampicin (R)-54 mg/kg/day, pyrazinamide (Z)-135 mg/kg/day] were extrapolated from daily human dose using the conversion table based on body surface area (8). Alpha tocopherol acetate drops were administered in the dose of 36 mg/kg/day orally and sodium selenite powder was administered in the dose 40  $\mu$ g/kg/day

orally. The animals were divided into six groups. Each group contained twelve animals. The groups were treated as follows :

Group I : Vehicle control i.e. gum acacia orally for 30 days.

Group II : (I + R + Z) suspension orally for 30 days.

Group III : (I + R + Z) suspension orally for 30 days + no treatment from day 31 to day 50.

Group IV : (I + R + Z) suspension orally for 30 days + alpha-tocopherol acetate from day 31 to day 50.

Group V : (I + R + Z) suspension orally for 30 days + sodium selenite from day 31 to day 50.

Group VI : (I + R + Z) suspension orally for 30 days + alpha-tocopherol acetate + sodium selenite from day 31 to day 50.

Blood samples of animals from groups I and II were taken for liver function tests by cardiac puncture under ether anaesthesia

and the liver removed for histopathological examination on 30th day. Similarly the blood samples and liver of animals from groups III, IV, V and VI were taken on 50th day. Animals were sacrificed by stunning. Assessment of liver damage was done by estimation of serum alanine aminotransferase (serum ALT) and serum aspartate aminotransferase (serum AST) by Reitman and Frankel Method (9) and serum protein, serum bilirubin and serum alkaline phosphatase (serum ALP) by Biuret method (10), Modified Jendrassik and Grofs method (11) and Kind and King method (12) respectively. Histopathological assessment of liver damage was done by using a method of scoring of the structural changes described by NIH Maryland USA (13).

All the groups were first subjected to analysis of variance by using oneway ANOVA followed by unpaired 't' test for comparison between two groups i.e. group II was compared with group I, group III, group IV, group V and group VI and group

III was compared with the groups IV, V and VI.  $P < 0.05$  was taken as significant.

Group II receiving antitubercular drugs for 30 days showed significant fall in serum protein level and rise in the levels of serum bilirubin, serum ALT, serum AST and serum ALP as compared to vehicle control group. Withdrawal of antitubercular drugs significantly reversed the levels of serum protein, serum bilirubin and serum ALT. Administration of  $\alpha$ -tocopherol acetate, sodium selenite or their combination significantly reversed all the biochemical changes as compared to group II. The reversal in all the biochemical parameters produced by the combination was significant as compared to that produced by mere withdrawal of antitubercular drugs (i.e. group III). On the other hand the individual antioxidant either  $\alpha$ -tocopherol acetate or sodium selenite showed significant reversal in only three biochemical parameters i.e. of serum protein, serum ALT and serum ALP when compared with group III (Table I).

TABLE I: Effect of the antioxidants,  $\alpha$ -tocopherol acetate and sodium selenite on liver function tests in anti tubercular drugs treated rats.

Groups (n = 12)	Serum total protein (gm/dl)	Serum bilirubin (mg/dl)	S.ALT (units/ml)	S.AST (units/ml)	S.ALP (KA units)
Group I	7.2±0.42	0.86 ± 0.03	37.33±2.64	31.5± 6.43	11.5±1.63
Group II	4.7±0.36 <sup>#</sup>	2.73 ± 0.43 <sup>#</sup>	178.66±5.33 <sup>###</sup>	87± 9.07 <sup>###</sup>	42.4±10.88 <sup>#</sup>
Group III	5.8±0.25 <sup>*</sup>	1.4 ± 0.14 <sup>*</sup>	73±9.81 <sup>***</sup>	61.83± 15	36.78±8.13
Group IV	6.76±0.31 <sup>***@</sup>	0.98 ± 0.09 <sup>**</sup>	39±7.63 <sup>***@</sup>	34.5± 8.39 <sup>**</sup>	12.41±1.06 <sup>*@</sup>
Group V	6.73±0.28 <sup>**@</sup>	1.05 ± 0.08 <sup>**</sup>	40.66±9.96 <sup>***@</sup>	30± 6.28 <sup>***</sup>	12.81±0.94 <sup>*@</sup>
Group VI	6.86±0.28 <sup>***@</sup>	0.85 ± 0.1 <sup>**@</sup>	37.33±7.81 <sup>***@</sup>	23.33± 6.21 <sup>***@@</sup>	11.78±1.4 <sup>*@</sup>

(All values are Mean ± SEM for each group)

One-way ANOVA revealed significant difference between the groups.

Unpaired 't' test :-

<sup>#</sup>=group II Vs group I

<sup>\*</sup>=group II Vs group III, group IV, group V, group VI

<sup>@</sup>=group III Vs group IV, group V, group VI

<sup>#,\*,@</sup>= $P < 0.05$  <sup>##,\*\*,@@</sup>= $P < 0.01$  <sup>###,\*\*\*,@@@</sup>= $P < 0.001$

Administration of antitubercular drugs for 30 days to group II produced changes of degeneration, necrosis and fibrosis on histological examination of rat livers. Mere withdrawal of antitubercular drugs significantly reversed the changes of degeneration, necrosis and fibrosis but there was no evidence of significant regeneration. Administration of either of the two antioxidants or their combination significantly reversed the scores of degeneration, necrosis and fibrosis with evidence of significant regeneration. However the reversal produced by the individual antioxidants was not significant when compared with the group which received no treatment after 30 days of antitubercular therapy i.e. group III except that produced by  $\alpha$ -tocopherol acetate in the score of regeneration. Combination of the two antioxidants effected significant reversal in scores of degeneration and regeneration even when compared with the reversal produced by mere withdrawal of antitubercular drugs i.e. group III (Table II).

This study thus reveals that both  $\alpha$ -tocopherol acetate and sodium selenite can reverse the hepatotoxicity induced by antitubercular drugs in rats, the combination of the two antioxidants is more effective than either of the individual antioxidant and that the therapeutic benefit of either of the two antioxidants, especially their combination is more effective than mere withdrawal of antitubercular therapy in reversing the hepatotoxicity.  $\alpha$ -tocopherol and selenium spare each other and reduce each others requirements as antioxidants.  $\alpha$ -tocopherol prevents loss of selenium from the body. Selenium facilitates digestion and absorption of  $\alpha$ -tocopherol, it also helps in retention of  $\alpha$ -tocopherol in the blood and plasma proteins (14). Thus, it may be presumed that the combined use of  $\alpha$ -tocopherol and selenium exerts more powerful antioxidant action that achieved by the individual use of either  $\alpha$ -tocopherol or selenium.

TABLE II: Effect of the antioxidants,  $\alpha$ -tocopherol acetate and sodium selenite on histopathology score in antitubercular drugs treated rats.

Groups (n = 12)	Degeneration	Necrosis	Fibrosis	Regeneration
Group I	0	0	0	0
Group II	2.83±0.4 <sup>###</sup>	2.5±0.34 <sup>###</sup>	1.83±0.3 <sup>###</sup>	0
Group III	1.33±0.33 <sup>*</sup>	0.83±0.31 <sup>**</sup>	0.66±0.33 <sup>*</sup>	0.66±0.33
Group IV	0.51±0.22 <sup>***</sup>	0.5±.22 <sup>***</sup>	0.16±0.1 <sup>***</sup>	1.83±0.4 <sup>***@</sup>
Group V	0.5±0.22 <sup>***</sup>	0.5±0.22 <sup>***</sup>	0.33±0.21 <sup>***</sup>	1.66±0.5 <sup>***</sup>
Group VI	0.33±0.2 <sup>***@</sup>	0.33±0.2 <sup>***</sup>	0.16±0.1 <sup>***</sup>	1.83±0.31 <sup>***@</sup>

(All values are Mean ± SEM for each group)

One-way ANOVA revealed significant difference between the groups.

Unpaired 't' test :-

#=group II Vs group I

\*=group II Vs group III, group IV, group V, group VI

@=group III Vs group IV, group V, group VI

#,\*,@=P<0.05    ##,\*\*,@@=P<0.01    ###,\*\*\*,@@@=P<0.001

A study of 199 patients with pulmonary tuberculosis accompanied by liver diseases receiving antitubercular therapy showed that the inclusion into the treatment schedule of  $\alpha$ -tocopherol acetate exerted

hepatoprotective and antioxidant benefits (15). Thus  $\alpha$ -tocopherol, selenium or their combination could be beneficial in prevention and treatment of antitubercular drugs induced hepatotoxicity.

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