[*Indian Journal of Experimental Biology* (1979): 7, 662]

Effect of Liv.52 on Hepatic Enzymes

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Liv.52 syrup, an Ayurvedic liver-tonic and extract of *Solanum nigrum* (one of the constituents of Liv.52) were orally administered (0.125 ml/kg b wt./day which is equal to human dose) to weaning rats for 11 weeks. Liv.52 treated rats exhibited better growth during 3rd to 8th week than those administered extract of *S. nigrum* or controls, after which period the body weight in all the groups gradually became more or less equal. After 11 weeks the protein level in the liver of rats treated with Liv.52 was slightly higher and the rats exhibited reduction in hexobarbital induced sleeping time. Mitochondrial succinate dehydrogenase, cytochrome *c* oxidase and ATPase increased in both the Liv.52-treated and *S. nigrum*-treated rats. In Liv.52-fed rats the activities of the drug metabolising enzymes, aniline hydroxylase and aminopyrine N-demethylase were stimulated but lysosomal enzymes, acid phosphatase, alkaline phosphatase, acid deoxyribonuclease, acid ribonuclease and cathepsin B were inhibited. There was no significant change in the activity of these enzymes in rats given the extract of *S. nigrum.* After 48 hours of administration of CCl_4, the activities of the mitochondrial and microsomal enzymes registered a decrease and those of the lysosomal enzymes increased in control as well as Liv.52-fed rats. Feeding of Liv.52 gave protection against a decrease in the activity of the mitochondrial enzymes and aniline hydroxylase consequent to administration of carbon tetrachloride but did not affect the increase in the activity of lysosomal enzymes.

It has been reported that Liv.52 protects the liver from the hepatotoxicity of paracetamol,[1] anti-cancer drugs,[2] antibiotics,[3] oral contraceptives,[4] alcohol,[5,6] allyl alcohol[7] and carbon tetrachloride.[8] Mehrotra and Tandon[9] have conducted a control trial of Liv.52 on 40 adult cases of decompensated cirrhosis of liver for 6 weeks and established that Liv.52 corrects hepatic dysfunction and brings about clinical improvements. Administration of Liv.52 in children suffering from malnutrition has improved liver function tests.[10-13] Therefore, the effect of prolonged oral feeding of Liv.52 and extract of *Solanum nigrum,* one of the constituents of Liv.52 to rats, has been studied on growth rate, hepatic mitochondrial, microsomal and lysosomal enzymes and hexobarbital sleeping time. On the basis of activity of these enzymes it has been examined if Liv.52 provides any protection against hepatotoxicity by carbon tetrachloride.

MATERIAL AND METHODS

Liv.52 syrup was received from the manufacturers. The extract of *S. nigrum* was prepared by homogenising 100 g leaves in distilled water (10% homogenate, wt/vol). It was then kept at 80°C for 1 hour, centrifuged and the clear supernatant concentrated to dryness *in vacuo.*

Male weaning rats (Druckrey strain) drawn from the colony of CDRI animal house were divided into 3 groups. Group I was given Liv.52 (0.125 ml/kg body weight/day), Group II the extract of *S. nigrum* (equivalent to its corresponding amount in Liv.52) and Group III (control) received normal saline for 11 weeks. All the rats had free access to a standard pellet diet and water; 2 rats were housed in one cage. Body weight of rats was recorded after every week. For recording hexobarbital-sleeping time, 125 mg hexobarbital sodium/kg body weight was administered (ip) to each rat. The time interval of losing righting reflex and regaining it was recorded and expressed as sleeping time.

Sublethal dose of carbon tetrachloride (0.7 ml/kg body weight) was given intraperiotoneally for 2 successive days to 6 rats each of the normal and Liv.52 groups. The animals were killed 48 hours

after the first injection of carbon tetrachloride.[14] All rats were fasted for 24 hours before killing by decapitation. Livers were immediately excised, washed with chilled normal saline and 10% homogenate (wt/vol.) in 0.25 M sucrose was prepared. DNA was extracted from 2 ml aliquot of the homogenates according to the method of Schneider[15] and estimated by diphenylamine reaction.[16] Mitochondrial fraction was prepared according to Schneider and Hogeboom[17] and succinate dehydrogenase, cytochrome c oxidase and total ATPase were assayed according to standard methods.[18-20] Microsomal drug metabolising enzymes, aniline hydroxylase[21] and amino-pyrine N-demethylase[22] were assayed in post-mitochondrial supernatant. Glucose-6-phosphatase[23] a microsomal membrane-bound enzyme was assayed in the total homogenates. For acid phosphatase,[24] alkaline phosphatase,[24] acid ribonuclease,[25] acid deoxyribonuclease[25] and cathepsin B[26] total homogenates were frozen and thawed 6 times before enzyme assay. Protein was estimated according to the method of Lowry *et al,*[27] using bovine serum albumin as standard.

RESULTS AND DISCUSSION

Liv.52-fed rats exhibited a (Fig.1) slightly better growth in the initial phase (i.e. from 3rd to 8th week) as compared to those administered the extract of *S. nigrum* or controls, but in the final stage (8 to 11 weeks) the body weight in all the groups was more or less the same. Liver weight in all the 3 groups was almost the same, but liver protein in the Liv.52 treated rats registered increase as compared to rats treated with *S. nigrum* or control group (Table 1). On the basis of DNA estimations, it was evident that there was no shrinkage in liver (2.95 mg and 2.90 mg DNA/g liver of control and Liv.52-fed rats respectively). Hexobarbital sleeping time was also reduced in Liv.52 treated group (42 ± 6 min) as compared to control or *S. nigrum*-fed group (63 ± 8 and 59 ± 4 min respectively).

Effect of Liv.52 and Extract of *S. nigrum* on the growth rate of rats

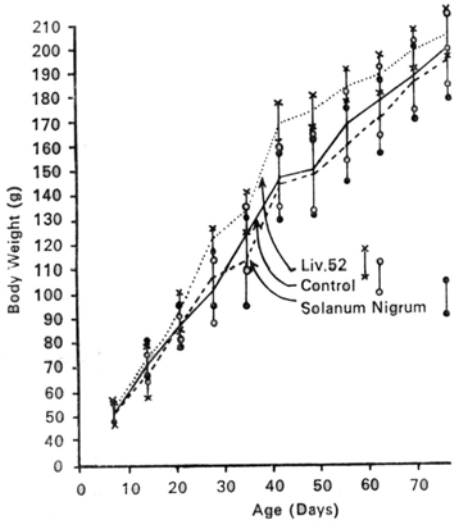

Table 1: Effect on body weight and liver*
(Values are mean ± SE from 6 animals in each group)

	Control	Liv.52	S. nigrum
Body wt. (g)	183.3 ± 29.1	196.7 ± 29.1	200.6 ± 16.1
Liver wt. (g)	5.6 ± 0.78	5.65 ± 1.5	5.69 ± 0.68
Liver protein (mg/g tissue)	167.8 ± 10.3	196.7 ± 10.2	187.7 ± 5.2
Hexobarbital-induced sleeping time (min)	63 ± 8	42 ± 6	59 ± 4

* After 11 weeks of drug regimen.

Activities (Table 2) of succinate dehydrogenase, cytochrome c oxidase and ATPase in the hepatic mitochondrial fraction showed an increase in rats fed Liv.52 or the extract of *S. nigrum* as compared to those of control group. However, the magnitude of increase was more in rats given the extract of *S. nigrum* than those administered Liv.52. the activity of these enzymes registered decrease when the control or Liv.52-fed animals were treated with carbon tetrachloride. However, the levels of these enzymes in Liv.52-fed rats treated with carbon tetrachloride were almost equal to the levels of these enzymes in normal healthy rats.

Table 2: Mitochondrial, microsomal and macrosomal enzymes (Values in parentheses indicate per cent increase (+) or decrease (—) in activity)					
Enzymes* (Units/g liver)	Control		Liv.52		S. nigrum Untreated
	Untreated	CCl_4 treated Mitochondrial	Untreated	CCl_4 treated	
Succinate Dehydrogenase[a]	17.70±1.40	13.83±1.40	21.90±0.70	18.76±0.70	23.40±1.10
Cytochrome c oxidase[b]	0.43±0.07	0.298±0.01	0.65±0.08	0.415±0.007	0.75±0.13
Total ATPase[c]	6.37±0.43	3.51±0.3 Microsomal	8.69±0.97	6.62±0.03	11.05±0.36
Aniline hydroxylase[d] (x 10^2)	8.3±0.9	2.28±0.84 (—73)	12.8±1.3	6.61±0.91 (—45)	6.7±0.8
Aminopyrine N-demethylase[e]	185±10	55±7.9 (—70)	475±50	145±21 (—69)	195±10
Glucose-6-phosphatase[e]	5.56±0.24	4.19±0.22 Lysosomal	5.58±0.37	4.15±0.18	not done
Acid phosphatase[f]	21.2±2.17	34.53±4.7 (+63)	16.65±0.77	28.9±1.47 (+73)	23.5±1.9
Alkaline phosphatase[f]	7.22±0.56	13.45±2.29 (+88)	5.62±0.53	10.18±1.9 (+81)	not done
Acid deoxyribonuclease[g] (x 10^{-3})	0.60±0.1	not done	0.37±0.04	Not done	0.60±0.03
Acid ribonuclease[g] (x10^{-3})	2.67±0.34	6.42±1.6 (+140)	1.91±0.1	4.72±1.61 (146)	not done
Cathepesin B[h]	2.86±0.12	2.33±0.104 (—18)	1.86±0.11	1.68±0.08 (—9)	2.71±0.34
* One enzyme unit is expressed as: [a]µmole potassium ferricyanide reduced/min; [b]2.303 log OD omin/OD min x conc. of cytochrome c; [c]µmole Pi liberated/min, [d]pmole p-aminophenol formed/min; [e]nmol formaldehyde formed/min; [f]µmole phenol liberated/min; [g]OD/min; [h]µmole of tyrosine released/min.					

Activity (Table 2) of microsomal aniline hydroxylase and aminopyrine N-demethylase increase in rats fed Liv.52 as compared to the control but glucose-6-phosphatase activity was not significantly affected. However, feeding of extract of *S. nigrum* had no effect on the activity of these enzymes. When carbon tetrachloride was administered to control and Liv.52-fed rats, all these enzymes were significantly reduced, but the percentage of inactivation of aniline hydroxylase and aminopyrine N-demethylase treated with carbon tetrachloride was 73 and 70% respectively in rats which were not given Liv.52 while the corresponding inactivation of these enzymes in Liv.52-fed rats was 45 and 69% respectively. This shows that Liv.52 provides a certain amount of protection to microsomal aniline hydroxylase in carbon tetrachloride-induced hepatotoxicity.

The activity of hepatic lysosomal enzymes *viz*. acid phosphatase, alkaline phosphatase, acid deoxyribonuclease, acid ribonuclease and cathepsin B registered decrease in rats fed Liv.52 as compared to control while feeding of *S. nigrum* had no effect on these enzymes. The activity of all the enzymes except cathepsin B registered an increase on administration of carbon tetrachloride in both the control and Liv.52-fed group of rats (Table 2). The magnitude of increase in the activity of these enzymes was of the same order in both control and Liv.52 groups. Surprisingly the activity of cathepsin B which is a lysosomal membrane-bound enzyme registered decrease on treatment with carbon tetrachloride (control, 18% and Liv.52, 9%).

Earlier work[28] has shown that Liv.52 when administered (0.5 ml/100 g body weight/day) stimulated growth of young rats even in presence of corticosteroids, but the mechanism of growth-promoting effect of Liv.52 is still not clear. The foregoing results show that even in extremely low doses of 0.125 ml/kg body weight/day Liv.52 promotes growth of rats up to 8 weeks and that after 11 weeks protein levels are higher in animals treated with Liv.52. Growth-promoting effect of Liv.52 at such a low dose as observed in present study is remarkable and suggests that it acts like an anabolic hormone because 40 times higher doses administered by earlier workers did not elicit better stimulation of growth. This is further substantiated by present observation that administration of Liv.52 increases the protein level in the liver also.

Several workers have reported that Liv.52 gives protection against the hepatotoxicity of drugs.[1-4] These studies have been based on haematology and assay of serum enzymes in human subjects and histological examinations of liver in experimental animals. Results of the present study show that oral feeding of Liv.52 stimulates the activity of hepatic mitochondrial and microsomal enzymes but the activity of hepatic lysosomal enzymes is considerably reduced.

Extract of *S. nigrum*, which is a constituent of Liv.52 stimulates mitochondrial enzymes, but has no effect on the hepatic lysosomal or microsomal enzymes. On the basis of the changes in the activity of hepatic enzymes, it seems that Liv.52 provides certain amount of protection against carbon tetrachloride-induced hepatotoxicity at the levels of microsomal drug-metabolising enzymes and mitochondrial enzymes, but there seems to be no protection against the increase in the activity of lysosomal enzymes on treatment with carbon tetrachloride.

ACKNOWLEDGEMENT
One of the authors (AS) is a recipient of junior research fellowship of CSIR, New Delhi. Excellent technical assistance of Shri S. Yadav is gratefully acknowledged.

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