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Protective Effect of Liv.52 and Liv.100, Ayurvedic Formulations on Lipid Peroxidation in Rat Liver Homogenate – An *in vitro* Study

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ABSTRACT

Liv.100 is an improvised herbal formulation of Liv.52. Liv.52 is an important component of the Ayurvedic system of medicine. This report highlights on the protective effect of Liv.52 and Liv.100 against in vitro peroxidation induced by hydrogen peroxide in rat liver homogenate. Addition of the two herbal formulations reduced the peroxidation effect of hydrogen peroxide in the dose – and time dependent manner. The protective effect of the drugs is attributed to the enhanced supply of reduced glutathione that inhibit the deleterious process of lipid peroxidation. The results suggest on the anti-oxidant potential of Liv.52 and Liv.100

Liver being the major site for detoxification is the primary target for environmental or occupational toxic exposure[1]. Recently, free radical induced lipid peroxidation becomes a necessity to prevent, cure or delay the aforesaid pathologies[2,3]. Protection of the cell membrane becomes a necessity to prevent, cure or delay the aforesaid pathologies.

Liv.100 (The Himalaya Drug Co. Pvt. Ltd., India) is a modified formulation of Liv.52, a powerful and popular hepatic stimulant[4]. The present study is an attempt made to elucidate the anti-peroxidative property of Liv.100 on *in vitro* peroxidation of rat liver homogenate. Liv.52 and Liv.100 are also compared for their efficacy to inhibit lipid peroxidation. To the best of our knowledge, this is the first report on the herbal formulation Liv.100.

MATERIALS AND METHODS

Drug – Liv.52, one of the oldest Ayurvedic formulation still in effective clinical use, contains (%). *Capparis spinosa*, 24, *Cichorium intybus*, 24 *Solanum nigrum*, 12, *Cassia occidentalis*, 6 *Tamarix gallica*, 6 and Mandur bhasma, 10. Liv.100 is an improvised and indigenous preparation of Liv.52. The drugs were gifted by The Himalaya Drug Company.

Chemicals – Thiobarbituric acid, 1, 1, 3, 3 tetra ethoxy propane, reduced glutathione and DTNB (5, 5-dithio-bis (2-nitro benzoic acid) were procured from Sigma chemicals Co., USA. All the other reagents were of analytical grade.

Animals – Male albino rats weighing 125 to 150 g were purchased from Fredrick Institute of Plant Protection and Toxicology, (FIPPAT), Padappai, Madras. The animals were housed in plastic bottom cages and allowed free access to standard laboratory chow (Hindustan Lever, Bombay) and water. The animals were starved for 12 hr prior to the experiment.

Tissue homogenate – Rats were sacrificed by cervical dislocation, liver was immediately excised and aN homogenate (1 g, w/v) was prepared using phosphate buffer saline (pH 7.4) in cold

condition. It was centrifuged (2000 g) for 10 min. Supernatant was collected and finally suspended in PBS to contain approximately 0.8-1 mg protein in 0.1 ml suspension to perform *in vitro* experiment.

Experimental design - Different concentrations of Liv.52 and Liv.100 were added to 3 ml liver homogenate, gently mixed and incubated at 37ºC for 20 min. This was followed by the addition of hydrogen peroxide, mixed gently and further incubated at 37ºC. At different time intervals, incubation mixture was taken out for the estimation of thio barbituric acid-reactive substances [TBA-RS] as described by Ohkawa *et al.*[5], with slight modifications[6] and reduced glutathione[7]. Protein was estimated by the method of Lowry *et al.*[8]

Statistical evaluation – The results, are the mean ± SD of six animals. Level of significance has been evaluated by using student's t test.

RESULTS

Protective effect of Liv.52 and Liv.100 on hydrogen peroxide induced lipid peroxidation – To the fixed volume of liver homogenate were added different concentrations of hydrogen peroxide [0.1- 4 mM] and incubated at 37ºC. Incubation mixture (0.1 ml) was taken out at different time intervals to estimate TBARS. The results depicted the dose-dependent response of hydrogen peroxide on lipid peroxidation in rat liver homogenate. The pilot study gave the optimum dose to be 1 mM and ideal time for maximum induction of peroxidation to be 20 min for TBA-RS estimation in our laboratory conditions.

To assess the protective effect of Liv.52 and Liv.100, the experiment comprised of two groups – The control group contained buffer and different concentrations of Liv.52 and Liv.100 and the experimental group varied from the control group with the addition of hydrogen peroxide [1 mM final concentration]. The mixtures were incubated at 37ºC for 20 minutes. A significant (p<0.001) dose-dependent effect was noted with the two drugs and did not show any augment in TBA-reactive substances upto 6.6 mg/ml (Table 1).

Table 1: Protective effect of Liv.52 and Liv.100 on hydrogen peroxide (1 mM) induced lipid peroxidation in rat liver homogenate (Values are mean ± SD of 6 animals in each group)

		Drug concentration mg/ml				
		0	0.8	1.6	3.3	6.6
Without H_2O_2 "TBARS" nm/100 mg protein	A	116.81 ± 3.25	116.63 ± 0.99	116.01 ± 0.72	115.86 ± 0.70	115.74 ± 0.65
	B	116.77 ± 2.20	116.37 ± 1.8 ±2	115.98 ± 0.68	115.42 ± 0.75	115.37 ± 0.91
With H_2O_2 "TBARS" nM/100 mg protein	A	228.63 ± 3.21	198.59 ± 0.72*	144.69 ± 0.61*	137.32 ± 0.91*	117.05 ± 0.49*
	B	229.15 ± 4.21	182.32 ±0.76*	135.71 ± 0.85*	123.09 ± 1.10*	115.50 ±0.54*

A = Liv.52; B = Liv.100 *p<0.001

Effect of Liv.52 and Liv.100 on reduced glutathione (GSH; Fig. 1) – The experimental design followed was the same as described earlier.

In normal condition GSH content decreased after 20 min as an outcome of auto-oxidation, but in the drug (1 mg/ml) treated groups, rate of lowering of GSH content was found to be minimum and the level was maintained upto 40 min. In presence of 0.2 mM hydrogen peroxide, the effect of the drugs was found to be promising in maintenance of the levels of glutathione at normal (Fig.1a and 1b).

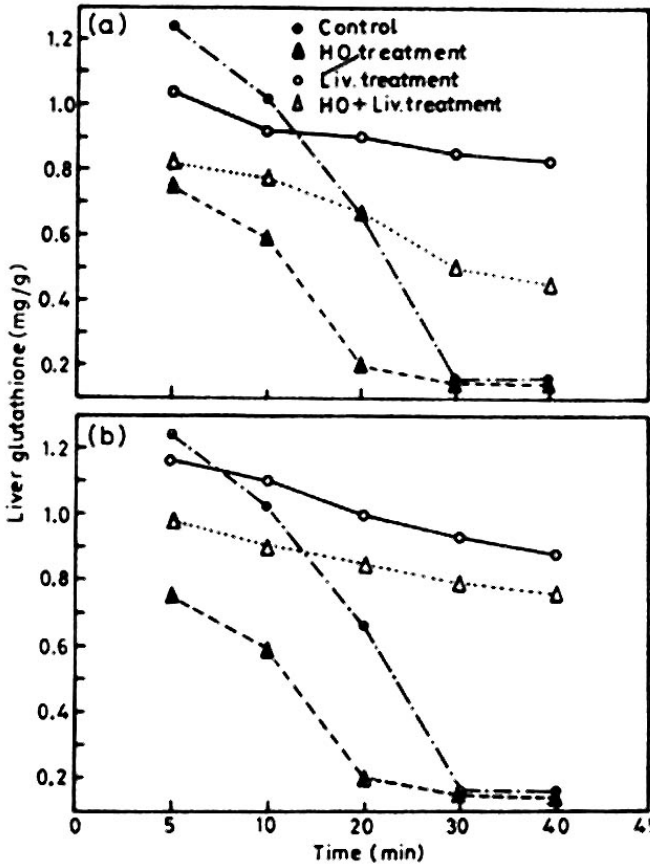

Fig. 1: Effect of hydrogen peroxide (0.2 mM) and (a) Liv.52 and (b) Liv.100 on changes in reduced glutathione content in liver homogenate (Each value is mean from 6 animals)

DISCUSSION

Lipid peroxidation is a complex and natural deleterious process. The effects of free radicals on human beings have recently been considered as their close relation to toxicity, diseases and aging[9,10]. Liver is under constant threat of oxidants; especially hydrogen peroxide.

Hydrogen peroxide forms an important tool for the study of lipid peroxidation in terms of TBARS formation. To being with, standardization of hydrogen peroxide to induce lipid peroxidation liver homogenate was carried out in order to establish the optimum dose and duration or estimation. From the observations, the optimum dose and duration for estimation. From the observations, the optimum dose and duration selected was 1 mM and 20 minutes for the estimation of lipid peroxidation in terms of "TBARS" formation. The peroxidative property of hydrogen peroxide can be justified as the formation of free radicals with ferrous iron and with oxygen radicals [Habber Weiss reaction]. These free radicals could further attack the phospholipid of membrane causing lipid peroxidation[11]. The fact remains that the generation of free radical imposes depletion of anti-oxidants such as Vitamin A, E, C and glutathione (reduced). The optimum dose of hydrogen peroxide used for the estimation of reduced glutathione is 0.2 mM. At concentrations greater than 1 mM it releases detectable amount of TBARS" but reduces the glutathione level to the minimum. This shows that there may be accumulation of oxidized glutathione (GSSG) is followed by lipid peroxidation, hence the estimation of reduced glutathione becomes difficult. Reduced glutathione may serve a better index or marker than quantitation of "TBARS" formation. The same was expressed by Savita *et al.*[12] The present results clearly exhibit the dose and time-dependent protective response afforded by Liv.52 and Liv.100 against hydrogen peroxide induced lipid peroxidation. An inverse relationship is established between formation of TBARS and reduced glutathione content. Earlier reports claim Liv.52 have caused significant enhancement in the GSH level in animals treated with it and also normalize radiation-induced alterations in GSH levels[13]. These findings further support our observations and the results of the current study are comparable to the observations of Savita *et al.*[12], a work with a different herbal extract.

To conclude, the protective effect of the drugs, Liv.52 and Liv.100, against lipid peroxidation induced TBARS formation in homogenate can be attributed to the enhanced supply of reduced glutathione that suppressed the deleterious process. The above finding suggests that Liv.52 and Liv.100 are good scavengers of free radicals. The results also encourage further *in vivo* studies to throw more light on the therapeutic efficacy of these drugs, especially Liv.100.

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