Preliminary investigation on the hepatoprotective activity of Liv.52 Protec (Poultry Feed Supplement)

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Summary

The effect of Liv.52 Protec (poultry feed supplement) was studied against thioacetamide (TAA) (100 mg/kg body weight, subcutaneous) and carbon tetrachloride (CCl4) (1 ml/kg body weight, peroral)-induced hepatic damage in rats at different dose levels (0.5 g, 1 g and 2 g/kg body weight). Also the drug was evaluated in vitro for its antioxidant property against ferric chloride (FeCl3) and tertiary butyl hydroxide (TBH) induced lipid peroxidation in rat liver homogenate. Liv.52 Protec reduced the elevated levels of serum glutamic pyruvate transaminase (SGPT) and serum glutamic oxaloacetate transaminase (SGOT) at a dose of 2 g/kg body weight. It also dose dependent and inhibited ferric chloride (FeCl3) and TBH induced lipid peroxidation in rat liver homogenate.

Introduction

The health and productivity of poultry/birds largely depends on optimum feed utilization, improved body weight, absence of disease and low mortality. Seasonal changes manifest a risk of disease. The liver is the major organ often affected. Ayurveda, an ancient system of Indian Medicine, offers various promising herbal liver tonics and growth promoters. Liv.52 Protec, a poly-herbal formulation, was developed as a hepatic stimulant, growth promoter and production enhancer for poultry with a recommended dose of 250-500 g/tonne of feed for growers, layers and broilers.

The pharmacological activities of individual ingredients present in Liv.52 Protec are reported. *Terminalia chebula* is rich in Vitamin C and mineral nutrients. It is used as tonic and in blood purification. The tannins in the fruits possess antioxidant and protective effects on the liver (Lee et al., 1995). *Eclipta alba* is a source of ascorbic acid used in hepatic and spleen enlargements (Chandra et al., 1987). *Cichorium intybus* is reported for its protective effect on liver against experimentally induced toxins (Gilani and Janbaz, 1994). *Solanum nigrum* is a tonic remedy in various diseases. It inhibited free radical mediated DNA damage in a study (Sultana et al., 1995). *Phyllanthus amarus* is used in traditional systems of medicine for diabetes, jaundice, bronchial infections and diseases of liver. It has reported anti-hepatotoxic activity (Reddy et al., 1993). *Azadirachta indica* is carminative, tonic, astringent, anti bacterial and antifungal and possesses various pharmacological activities (Pandey, 1996). *Terminalia arjuna* is styptic and tonic in cirrhosis of liver (Kiritikar and Basu, 1987). Yashad bhasma, a mineral preparation, is composed of zinc oxide. It promotes weight gain and is used as a nerve tonic, in general debility and in anemia (Nadkarni, 1992). All these herbs are processed in *Aloe vera*, *Zingiber officinale* and *Piper longum* which are stimulant and help the bioavailability of the other herbs (Chopra et al., 1976; Johri and Zushti, 1992).

The present study was taken to evaluate the protective effect of Liv.52 Protec against Thioacetamide (TAA) and carbon tetrachloride (CCl4)-induced liver damage in rats and its antioxidant potential in rat liver homogenate.

Materials and Methods

Herbs of the formulation were procured from local sources, identified and voucher specimens were preserved at the pharmacognosy laboratory, R&D Center, The Himalaya Drug Company, Bangalore. The drug was administered as an oral aqueous suspension.

**In vivo hepatoprotective activity:** Experimental animals were inbred rats of either sex (body weight 200 ± 20 g, Wistar strain) housed in standard conditions of temperature (22 ± 2°C), relative humidity (60 ± 5%) and light (12h-light/dark cycle). They were fed ad libitum with standard pellet diet (Lipton India Ltd., Mumbai) and had a free access to water.

**Table 1:** Effect of Liv.52 Protec on TAA and CCI4 induced hepatotoxicity in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>TAA</th>
<th>CCI4</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>SGPT</td>
<td>SGOT</td>
</tr>
<tr>
<td>Control</td>
<td>29.00 ± 1.76°C</td>
<td>108.29 ± 9.50°C</td>
</tr>
<tr>
<td>Positive control</td>
<td>187.50 ± 13.77°C</td>
<td>523.33 ± 53.50°C</td>
</tr>
</tbody>
</table>

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Rats were divided into different groups, with eight animals in each. The control and positive control group rats received water orally as vehicle (10 ml/kg) for 14 days. The treatment group rats were administered orally an aqueous suspension of Liv.52 Protec at a dose of 0.5 g, 1 g or 2 g/kg body weight, for 14 days. At the end of the treatment, the rats, except for the control group, received either TAA (100 mg/kg body weight, subcutaneous) or CCl4 (1 ml/kg body weight, per oral).

After 24 hours of administration of hepatotoxin, blood was collected from all the groups from the retro-orbital plexus, under light ether anesthesia. Serum was separated and subjected for the estimation of serum glutamic pyruvate transaminase (SGPT) and serum glutamic oxaloacetate transaminase (SGOT).

**Assessment of liver parameters:** Biochemical parameters SGPT and SGOT were measured as markers for evaluation of hepatoprotective effect of Liv.52 Protec, and expressed as IU/L.

**In vitro lipid peroxidation:** The drug was prepared as 1% suspension in 5% dimethyl sulphoxide (DMSO). The lipid peroxidation study was carried out as per the method by Braughler et. al., 1986. Concentrations of 1 mg, 2 mg, 4 mg and 8 mg/ml of the drug were taken with 1ml of the liver homogenate and the lipid peroxidation was induced using 2 mM TBH (200ml) and 2 mM (FeCl3) (200 ml) solution. Tocopherol was used as standard reference. Lipid peroxidation was estimated by thiobarbituric acid reaction and expressed as per cent inhibition of malondialdehyde formation with respect to control (Okhawa, et. al., 1979).

**Statistical analysis:** Results of biochemical estimations have been indicated in terms of mean ± SEM. Data has been analyzed by ANOVA followed by Dunnet's t-test. Minimum level of significance was fixed at P<0.05.

### Results and Discussion

Administration of the hepatotoxic compounds TAA and CCl4 caused a significant rise in the SGPT and SGOT levels indicating hepatic damage. The reduction in elevated levels of liver enzymes SGPT and SGOT were observed in Liv.52 Protec treated groups (Table 1).

The drug showed dose dependent protection against tertiary butyl hydroxide (TBH) and ferric chloride (FeCl3) induced lipid peroxidation. The maximum protection was 51.53% in TBH induced tissue damage, and 70.6% in FeCl3 induced tissue damage, at 8 mg/ml (Table 2).

Thioacetamide and CCl4 are commonly employed experimental hepatotoxins. The nucleolar changes induced by TAA have led to the hypothesis that it interferes with the movement of RNA from the nucleus to the cytoplasm, possibly leading to necrosis of liver cells (Zimmerman, 1978).

Carbon tetrachloride induces liver damage by producing free radical intermediates. CCl4, converts trichloromethyl radical (CCl3) by the cytochrome P-450 system which in turn converts to peroxy radical (CCl3O2.) which causes lipid peroxidation (Recknagael, 1967; Reynolds and Molslen, 1974).

Tertiary butyl hydroxide as OH- and FeCl3 as Fe+3 stimulate lipid peroxidation through various mechanisms, such as the generation of hydroxyl radical (Gutteridge et. al., 1979), the decomposition of lipid peroxides (Braughler et. al., 1987), or reduction of ions by endogenous reducing substances, a process necessary for the initiation of lipid peroxidation.

All these findings suggest that the hepatoprotection offered by Liv.52 Protec could be due to the membrane stabilizing and free radical scavenging activity.

### Table 2: Effect of Liv.52 Protec on in vitro lipid peroxidation against TBH and FeCl3

<table>
<thead>
<tr>
<th>Concentration of the drug (mg/ml)</th>
<th>% inhibition</th>
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<tbody>
<tr>
<td></td>
<td>TBH</td>
</tr>
<tr>
<td>1</td>
<td>11.17</td>
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<tr>
<td>2</td>
<td>31.48</td>
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<td>4</td>
<td>41.54</td>
</tr>
<tr>
<td>8</td>
<td>51.53</td>
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### Acknowledgement

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