HEPATOPROTECTIVE EFFECT OF A POLYHERBAL FORMULATION (AYUSH-LIV.04) AGAINST ETHANOL AND CCl₄ INDUCED LIVER DAMAGE IN RATS

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ABSTRACT

A polyherbal formulation was evaluated for its hepatoprotective activity against ethanol and CCl₄ induced liver damage in rats. The rats were divided into five groups of six animals each and serve as control, toxic, post-treated, herbal control, Liv.52 treated groups respectively. The results showed that the activities of liver marker enzymes in serum namely AST, ALT, ALP, ACP and serum bilirubin level (total) were increased in toxic group animals. But the activities of these enzymes were significantly lowered in post-treated group of rats. Thus, the results suggest antihepatotoxicity of “Ayush-Liv.04”.

Keywords: polyherbal formulation, hepatic damage, CCl₄, ethanol, Liv.52, serum enzymes and bilirubin.

INTRODUCTION

The liver is an important organ, actively involved in many metabolic reactions and is the frequent target of number of toxicants (1). The metabolic disorders associated with this organ are numerous and varied highly during recent years (2). Alcohol dependency is also a major health and socio-economic problem associated with liver disorders throughout the world (3),(4). Almost all ingested alcohol is metabolised in the liver and excessive alcohol consumption leads to acute and chronic liver disease. Most of the alcohol consumed is eventually metabolised by the liver and the products generated and accumulated during alcohol metabolism are more toxic than alcohol itself (5).

Hence, in the absence of enough number of “liver protective drugs” in the modern medicine, “Ayurveda” an Indian traditional medicinal method recommends several herbal plants for the treatment of liver disorders (6). In recent years, there is growing interest in polyherbal formulation for the treatment of various diseases and it has enormously increased world wide (7), (8).

A vast number of medicinal plants have traditionally been used in the ayurvedic system of medicine for the treatment of liver disorders and have been scientifically proven to have hepatoprotective properties (9),(10). Polyherbal formulations have shown curative effect on various diseases and disorders in rats. Hence, the present study involves the use of a polyherbal formulation “Ayush -Liv.04” which consists of four herbal plants and a copper
containing milk stone against ethanol and CCl₄ induced hepatotoxicity in albino rats.

MATERIALS AND METHODS

Preparation of plant extract

A polyherbal formulation consists of four medicinal plants and a copper containing stone (milk tuttam) were prepared as crude extract by mixing the plant parts and the composition is shown in Table-I. The plants used in the present study were collected from Centre for Indian Medical Heritage (CIMH), a medicinal plant conservation park, located near the westerrhats of Palghat, Kerala. Aqueous extract (6.25%) was prepared with boiling water (<100ºC) at normal pressure (11). Then, the aqueous extract was used for the studies on laboratory animals.

Animal treatment

Albino wistar rats (160-200g) were bred in the central animal house, P.S.G. Institute of Medical College, Coimbatore-14. The animals were fed with a pellet diet [Hindustan Lever Ltd; Mumbai] and water ad libitum. The animals were maintained under 12 hrs light and at the temperature of 28°C±2°C for 45 days.

Experimental design and animal sensitization

Animals were divided into five groups of six animals in each group. Group-I, II, III, IV and V served as control, toxic, post-treated, herbal control, and Liv.52 treated respectively. Group-II, III and group-V animals were sensitized by 40% ethanol (v/v) (2.0 ml / 100g body wt / day; orally) (12) twice per day for 21 days and a single dose of CCl₄ in liquid paraffin (1:1 ratio) on 20th day (0.2 ml / kg body wt; ip) (13). Group-III and group-IV rats were post-treated with “Ayush-Liv.04” (4.0ml / 100g body wt / day; orally) (14),(15) twice daily for 21 days. Group-V rats were post-treated with “Liv.52”, a commercially available hepatoprotective drug (The Himalaya drug company, Bangalore, India) twice daily for 21 days (2.0 ml / 100 g body wt / day; orally) (16).

Assessment of liver function

After 48 hours of the last day of treatment, rats of all groups were sacrificed by cervical decapitation under mild chloroform anesthesia. Blood was collected from the carotid arteries in the neck blood vessels and centrifuged at 3000 rpm for 10 minutes to separate the serum, which was kept at 4°C to assay the activities of serum enzymes. Then biochemical assays were carried out.

Biochemical analysis

The liver marker enzymes in serum alanine transaminase (ALT), aspartate transaminase (AST) (17), serum acid phosphatase (ACP), serum alkaline phosphatase (ALP) (18) and serum bilirubin (total) (19) levels were estimated in control and experimental rats.

Statistical analysis

Results were expressed as mean ± SD and the data obtained were analysed by one way analysis of variance (ANOVA) (20).

RESULTS AND DISCUSSION

The results are presented in Table-II. CCl₄ is an extensively studied toxicant and its metabolites such as trichloromethyl peroxy radicals (CCl₃O₂⁻) are involved in the pathogenesis of liver damage (21). Group-II rats treated with 40% ethanol and a single dose of CCl₄ developed significant hepatocellular damage as evidenced from a significant (P<0.05) increase in the serum AST, ALT, ALP, ACP and bilirubin levels, when compared with control rats. Similar results have been reported by
Kumar *et al.* (2005) (22). After the treatment with “Ayush- Liv.04”, group-III rats have significantly reduced the elevated serum levels of these enzymes and bilirubin. The findings of the present study are also in accordance with the study of Garg *et al.* (1994) (23) who reported the elevated levels of these serum enzymes in thioacetamide induced liver damage in rats. Similarly, Mukherjee *et al.* (1997) (24) reported an increase in serum bilirubin level in CCl₄ induced hepatotoxicity and this increase was lowered by an extract prepared from *Swertia chirata* on rats.

There was no significant (P<0.05) difference between normal control rats and herbal control rats. This indicates that herbal formulation does not have any deleterious effect on the rats. Similarly, when Liv.52 treated rats (group-V) were compared with post-treated rats (group-III), there was no significant (P<0.05) difference between these groups of rats. This indicates that the hepatoprotective nature of the present herbal formulation is almost equal to the standardised Liv.52 drug.

**CONCLUSION**

The present study provides experimental evidence for the protection of liver by “Ayush-Liv.04” without any side effects. Thus, our study has demonstrated the efficacy of the polyherbal formulation as an effective hepatoprotective agent similar to Liv.52 drug.

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TABLE – I
Composition of plant parts used for preparation of polyherbal formulation, Ayush-Liv.04

| S.No | Name of the Plant                  | Plant part used    | Composition (%) |
|------|------------------------------------|--------------------|-----------------|
| 1.   | *Eclipta alba*                     | Whole plant        | 20              |
| 2.   | *Clitoria ternatea*                | Leaves             | 20              |
| 3.   | *Asparagus racemosus; Linn*        | Tuberous roots     | 30              |
| 4.   | *Alpinia galanga*                  | Rhizomes           | 20              |
| 5.   | Milk tuttam (Copper containing stone) | Powdered stone    | 10              |
TABLE II
EFFECT OF THE POLYHERBAL EXTRACT ON THE LIVER PARAMETERS IN RATS

| Groups | AST (µ moles of pyruvate liberated / litre) | ALT (µ moles of pyruvate liberated / litre) | ALP (µ moles of phenol liberated / litre) | ACP (µ moles of phenol liberated / litre) | Serum bilirubin (mg %) |
|--------|------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|----------------------|
| Group I | 34.7 ± 49.8                              | 51.9 ± 35.19                             | 64.7 ± 3.21                              | 9.55 ± 5.89                              | 1.27 ± 0.19          |
| Group II | 208.8 ± 12.6a*                           | 192.0 ± 52.19a*                          | 128.8 ± 39.46a*                          | 29.15 ± 8.79a*                           | 1.62 ± 0.39a*       |
| Group III | 68.9 ± 35.5b*                            | 59.8 ± 12.32b*                           | 92.6 ± 31.77b*                           | 15.83 ± 10.54b*                          | 1.33 ± 0.25b*       |
| Group IV | 34.8 ± 26.2cns                            | 52.53 ± 24.09cns                         | 64.3 ± 13.48cns                          | 9.73 ± 2.48cns                           | 1.27 ± 0.22cns      |
| Group V  | 35.5 ± 26.3dns                            | 65.88 ± 15.65dns                         | 66.3 ± 20.54dns                          | 13.18 ±ss 7.72dns                        | 1.38 ± 0.52dns      |

Values are mean ± SD from 6 rats in each group
P values:  *- p<0.05    **- p<0.01    ns - Not significant

Statistical Comparisons:

a - Group II is compared with Group I
b - Group III is compared with Group II
c - Group IV is compared with Group I
d - Group V is compared with Group III

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