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FORMULATION AND EVALUATION OF PROPRIETARY POLYHERBAL FORMULATION FOR THEIR HEPATOPROTECTIVE ACTIVITY

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ABSTRACT: The Hepatoprotective activity of Hepjaun syrup **(HA-I)** and Modified Formulations (**HA-II** and **HA-III)** were evaluated and compared statistically after inducing hepatotoxicity in rats by subcutaneous administration of carbon tetrachloride (CCL_4) with olive oil as a diluent in 1:1 %v/v on 2nd and 3rd day. The liver damage was confirmed by estimation of elevated levels of Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Alkaline Phosphatase (ALP), serum bilirubin and liver weights. **HA-I, HA-II, HA-III** pretreatment (500mg/kg) significantly reduces the CCl4 induced elevated serum levels of SGOT, SGPT, SALP and Serum Bilirubin.

KEY WORDS: CCl_4 induced hepatotoxicity, polyherbal formulation, Hepatoprotective activity.

INTRODUCTION

A number of risk factors predispose an individual to hepatic drug injury such as preexisting liver disease, ageing, female sex and genetic inability to perform particular biotransformation. Thus, there is need to develop a effective hepatoprotective formulation which could reduces the emergence of such liver complications caused due to hepatotoxic agents like alcohols, CCl_4, bacterial toxins etc. Medicinal preparations are invariably complex mixtures, based on plant products. Around 1250 plants are currently used in various Ayurvedic preparations. The principle, treatment and philosophy of Ayurveda are one of the best systems which fulfill the essentialities of human beings. But it is very essential to mould this system with modern standards.

The present work was focused on the investigation of hepatoprotective activity of various herbal formulations containing aqueous as well as alcoholic extract of hepatoprotective crude drugs. *HA-I* is one of the well known proprietary polyherbal formulation, containing aqueous extracts of 12 crude drugs. All the ingredients of *HA-I* formulation are *Lawsonia alba (1), Eclipta alba (2), Berberis aristata (3) , Aloe vera (4), Andrographis paniculata(5), Boerhavia biffusa (6), Melia azadirachta (7) , Phyllanthus niruri (8), Croton oblongifolius (9) , Tephrosia purpurea (10), Plumbago zeylanica (11), Picrorrhiza kurroa* (12) which were earlier investigated for their hepatoprotective effects against different experimental hepatotoxicity models. *HA-II* was one formulation prepared using alcoholic extract of all above crude drugs while *HA-III was* another formulation prepared simultaneously which consist of alcoholic extract of seven important hepatoprotective drugs among the above in double concentration. Multi-component drug formulations **Liv 52** and HA-**I** contains the extracts of several medicinal plants and are proprietary polyherbal formulations and are used in liver disorders and screened for various hepatoprotective mechanisms. CCl4 is routinely used hepatotoxic drug for screening purposes. Administration of CCl4 by subcutaneous route cause acute liver damage. Extent of hepatic damage is assessed by increased levels of enzymes like

SGOT, SGPT, ALP and Bilirubin. Formulations **HA-II** and **HA-III** were able to reduce the levels of enzymes SGOT, SGPT, ALP and bilirubin to a considerable level, indicating that they were protective to hepatocytes and maintain normal liver physiology and regeneration of damaged liver cells.

MATERIALS AND METHODS

Procurement of Herbs

Lawsonia alba, Eclipta alba, Berberis aristata, Aloe vera, Andrographis paniculata, Boerhavia biffusa, Melia azadirachta, Phyllanthus niruri, Croton oblongifolius, Tephrosia purpurea, Plumbago zeylanica, Picrorrhiza kurroa were examined as a hepatoprotective drugs gracefully offered by SG Phytopharma Pvt. Ltd., Kolhapur, MS. Ethanol 95 %v/v Gum acacia, Olive oil, Carbon tetrachloride, formalin all the chemicals were also supplied by company SG Phyto pharma Pvt. Ltd. Kolhapur.

Preparation of formulations

Aqueous extract

Formulation **HA-I** is a proprietary polyherbal marketed product (Hepjaun Syrup, SG Phytopharma Pvt. Ltd., Kolhapur, MS) prepared by using aqueous solvent system.

Alcoholic Extract

12 dried and pulverized herbal crude drugs mentioned in Table 1. were extracted using ethanol in Soxhlet extractor.

Alcoholic Extract

7 important hepatoprotective dried and pulverized herbal crude drugs summerised in Table 1. were extracted using ethanol in Soxhlet extractor.

Selection of Animals

Male Wistar rats with weight range from 150-180 gm were obtained from KLESs College of Pharmacy, Hubli-31. All the animals were kept under normal atmospheric condition. All the

Animals received standard diet and water *ad libitum* .The rats were randomly selected and were divided into six groups with five animals in each group.

Induction of hepatotoxicity and drug administration schedule

Hepatoprotective activity was carried out using Male Wistar Rat (150-180 gm). The animals were grouped into 6 (six) groups of 5 (five) animals each group and maintained on standard diet and water *ad libitum* (14).

Normal Group A: Serve as normal control 4 % gum acacia 1ml / kg orally for 4 days with 2ml olive oil given subcutaneously on 2ndand 3rd day.

Positive Control Group B: Serve as toxicant receiving 4 % gum acacia 1ml /kg orally for 4 days with 1:1 CCl_4 in olive oil 2ml/kg subcutaneously on 2nd and 3rd day.

Test standard Group C: Serve as standard receiving "Liv 52" syrup. 1ml /kg orally for 4 days with 1:1 CCl_4 in olive oil 2ml/kg subcutaneously on 2nd and 3rd day.

Test Group D: Received (**HA-I)** syrup (1ml /kg) orally for 4 days with 1:1 CCl_4 in olive oil 2ml/kg subcutaneously on 2nd and 3rd day.

Test Group E: Received polyherbal formulation prepared using alcoholic extract of all 12 crude drugs **(HA-II)** syrup at 500mg /kg body weight for 4 days with 1:1 CCl_4 in olive oil 2ml/kg subcutaneously on 2nd and3rd day.

Test Group F: Received polyherbal formulation prepared using alcoholic extract of double concentration of 7 imp. Hepatoprotective crude drugs **(HA-III)** at 500mg /kg body weight for 4 days with 1:1 CCl_4 in olive oil 2ml/kg subcutaneously on 2nd and 3rd day.

The Rats were anaesthetized with anaesthetic ether on 5th day and blood was collected from retro-orbital plexus and then sacrificed by cervical dislocation. The liver was carefully isolated and preserved in 10% formalin. The serum was separated by centrifugation and used for

estimation of different bio-chemical parameters like SGOT, SGPT, ALP and Bilirubin. The weight of each liver was recorded and then subjected to histopathological studies.

Dose of hepatotoxicant and Assessment of Hepatoprotective Activity

Acute toxicity studies were carried out using "Up and Down" method (13) and the maximum non–lethal dose was found to be 5000 mg/kg. (5.0gm/kg) body weight. Hence 1/10th of the dose was taken as effective dose (500 mg/kg body weight).

Hepatoprotective activity was assessed by estimation of different parameters viz. morphological, biochemical and histopathological parameters.

Morphological Parameters: changes in color and weight.

Biochemical (serum) Parameters: blood samples were collected and examined for

Serum Glutamate Oxaloacatate Transaminase (SGOT) (IU/L), Serum Glutamate

Pyruvate Transaminase (SGPT) (IU/L), Serum Alkaline Phosphatase (SALP) (IU/L),

and Serum Bilirubin (mg/ml) levels.

Histopathological Parameters: This includes the histological changes in the liver

Architecture like arrangement of hepatic lobules, inflammatory cell infiltration, fatty

Changes etc.

Statistical analysis

One-Way analysis of variance (ANOVA) was applied for each parameter in order to estimate significant intergroup differences.

RESULTS AND DISCUSSION

On subcutaneous administration of CCl4 in dose of 1-ml/kg produced acute hepatic damage in positive control (carbon tetrachloride treated) when compared with normal control. All the groups except the normal group were treated with CCl4 exhibits significant increase in levels of enzymes SGOT, SGPT, SALP and serum bilirubin. Increased livers weight and pale reddish brown color indicate hepatotoxicity(Figure 1).

In case of test groups, formulations **Liv-52, HA-I, HA-II** and **HA-III** at dose of 500mg/kg of body weight were administered simultaneously with CCl4. Estimated levels of enzymes SGOT, SGPT, SALP and bilirubin in test groups were compared with levels of enzymes in normal, positive and standard Liv-52 groups. Significant difference in enzyme levels (figure.1.) in normal, positive and test group indicate the hepatoprotective activity of formulations. Among the test formulations, HA-II formulation exhibits highest hepatoprotective activity followed by Liv-52, HA-I and HA-III. Serum bilirubin levels also confirmed that HA-II have high hepatoprotective activity. (Figure 2.)

In comparison of liver weights, rats treated with HA-II exhibit lowest liver weight among the other formulations this may be due to high hepatoprotective activity of HA-II which prevent liver necrosis. (Figure 3)

CONCLUSION

The polyherbal formulations **HA-II** and **HA-III** designed and optimized as a hepatoprotective agents for the treatment of liver diseases.Formulation **HA-II** which is the alcoholic extract of 12 medicinal plants shows excellent hepatorotective activity as compared to **HA-III** which is an alcoholic extract (double concentration) of seven important hepatoprotective drugs. Hence formulation **HA-II** and **HA-III** can be proposed to be beneficial in jaundice and hepatitis condition, especially formulation **HA-II.**

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Table 1. Composition of formulations HA-I (aqueous extract), HA-II (alcoholic extract) and HA-III (alcoholic extract in double concentration).

Plants	HA-I	HA-II	HA-III
Lawsonia alba	25	25	----
Eclipta alba	125	125	250
Berberis aristata	75	75	----
Aloe vera	75	75	150
Andrographis paniculata	50	50	100
Boerhavia biffusa	50	50	100
Melia azadirachta	50	50	100
Phyllanthus niruri	75	75	150
Croton oblongifolius	75	75	----
Tephrosia purpurea	50	50	----
Plumbago zeylanica	75	75	----
Picrorrhiza kurroa	50	50	100

Figure 1. Effect of HA-I, HA-II and HA-III compared to LIV-52 (Standard) on CCL_4-induced rise in SGOT (▩), SGPT (▨) and ALP(≣) . The values represents Mean±S.D., n=5,

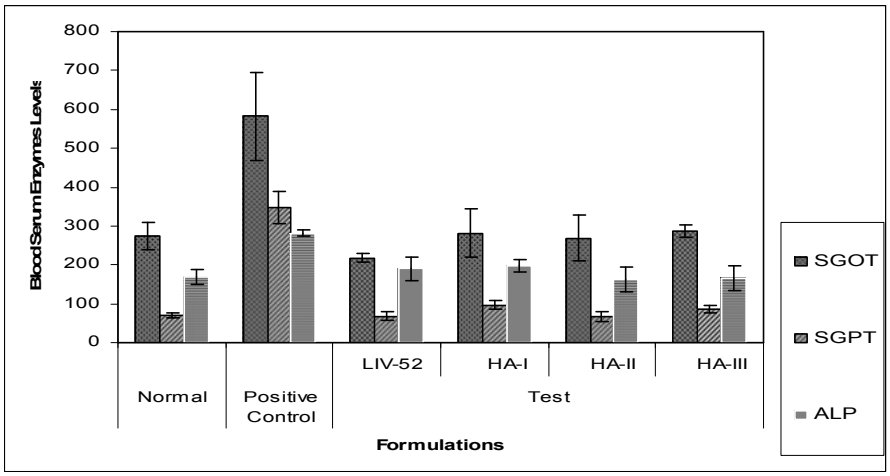


Figure 2. Effect of HA-I, HA-II and HA-III compared to LIV-52 (Standard) on CCL_4-induced rise in Total (▩, T) and Direct (▨, D) Bilirubin. The values represents Mean±S.D., n=5,

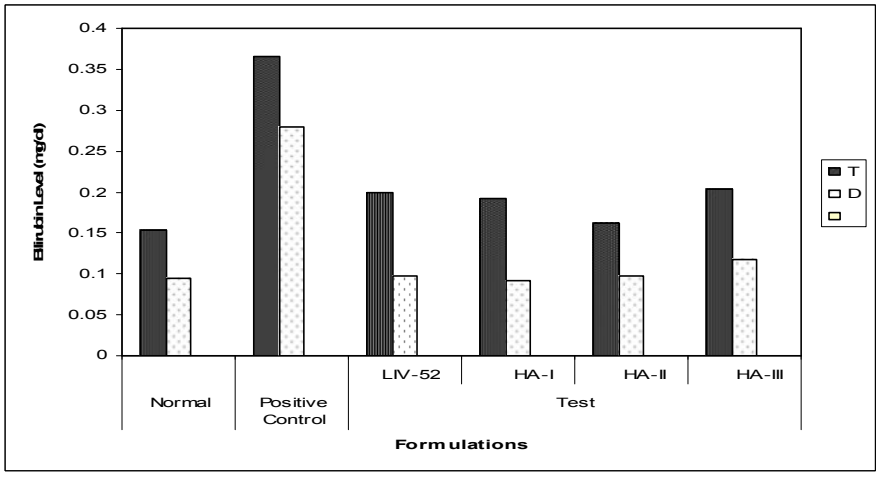
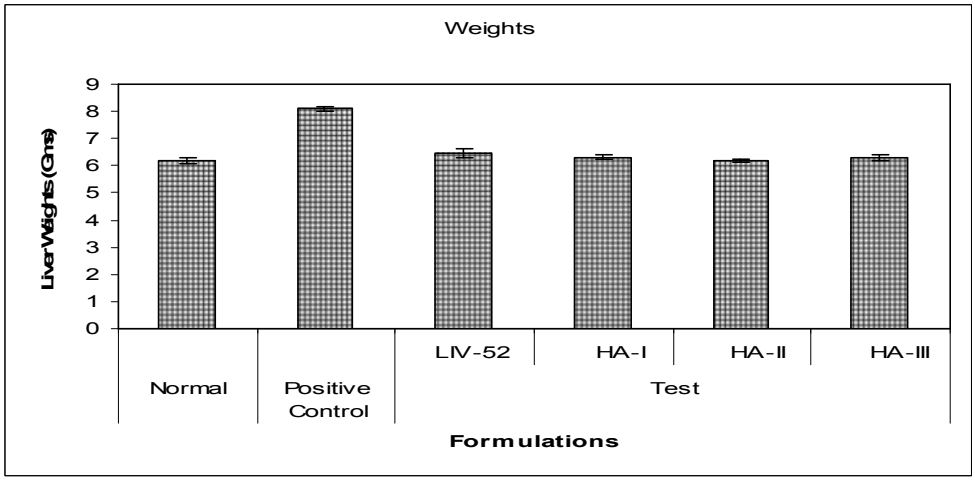


Figure 3. Effect of HA-I, HA-II and HA-III compared to LIV-52 (Standard) on CCL_4-induced rise in liver weights of rats (▦). The values represents Mean±S.D., n=5,



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