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HEPATOPROTECTIVE ACTIVITY OF *POLYHERBAL FORMULATION* ENCOMPASSING ORIGINAL *THERAPEUTIC PLANTS* IN RATS

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ABSTRACT:
Rats and primary cultures of hepatocytes were used as the in vivo and in vitro models to evaluate the hepatoprotective activity of the polyhedral formulation, comprising of Ajowan, Cardamom, Clove, Mace, Nutmeg. Carbon tetrachloride(CCl_4) was selected as hepatotoxin. Liv-52 was the reference hepatoprotective agent. In the in vivo study, serum transaminase (SGOT and SGPT), alkaline phosphatase (ALP), total bilirubin, albumin together with total protein and histopathological criteria for the evidences of liver injury. Carbon tetrachloride caused the alterations in all the biochemical parameters and centrilobular necrosis. These biochemical observations were supplemented by histopathological examination of liver sections. Various pathological changes such as architectural intactness, centrilobular observed in rats treated only with carbon tetrachloride, but the groups treated with the carbon tetrachloride and hepatoprotective formulations (Liv-52 and RVSPHF567) were protected to a moderate extent from such pathological changes. It was concluded from the study that the prepared formulation has shown comparable hepatoprotective activity against Carbon tetrachloride induced hepatotoxicity in rats in comparison to Liv-52.

KEYWORDS: *Hepatoprotective activity, Liv-52, Carbon tetrachloride.*

INTRODUCTION:

Liver is the largest not only in organ size but also in functions. Liver has more than 500 separate functions including synthesis, secretes, excretes, stores, generates, metabolises, protects and detoxicates various substances,etc. Any type of injury (due to systemic drugs, food preservatives, agrochemicals and addiction to alcohol) or impairments of its functions may lead to many complications in one's health. There is a no rational therapy available for liver disorder, and it is a still challenge to modern medicine[1]. Many environmental and therapeutic agent produce hepatic

injury when inhaled, ingested or administered parentally. The therapeutic agents damaging the liver are in general, not true hepatotoxins but cause injury by sensitization reactions[2].

Hepatic injury can be life threatening when the entirely or most of the liver is exposed to any hepatotoxin, including Carbon tetrachloride[8]. It is used to study hepatotoxic potentialbecause it is life threatening when an entire liver or most of the liver is exposed to carbon tetrachloride; this requires metabolic activation, particularly by liver cytochrome P-450enzyme, to form reactive toxic metabolites that in turn causeliver injury in experimental animals and humans[2].

The location of liver is defined mainly by the biotransformation of carbon tetrachloride, which is cytochrome P-450-dependent. Free radical initiates the process of lipid peroxidation, which generally leads to the inhibition of enzyme activity[3, 4]. Liv-52 is an indigenous multiherbal hepatotonic that has been widely used as a hepatoprotective agent in various liver disorders[4-7] and moreover, it has shown protective effects in hepatotoxicity. The present study was conducted to compare the hepatoprotective activity of one marketed formulation (Liv- 52) with the formulated polyherbal formulation against Carbon tetrachloride induced acute hepatitis in rats [1, 9-14].

MATERIALS AND METHODS:

Drugs and Chemicals:

The crude drugs such as Ajowan, Cardamom, Clove, Cumin, Mace, and Nutmeg meant for the formulation of thepolyherbal formulation (RVSPHF567) were purchasedalong with standard drug LIV-52 (The Himalaya Drug Company). All other chemicals used in this study were of analytical grade.

Animals:

24 inbred adult Wistar strain albino rats having a bodyweight range of 120-200gm were used for the experiment. The animals were obtained from R.V.S. College of Pharmaceutical sciences, Animal house, Coimbatore, India. The animal room was well ventilated and the animals had

12±1 hour night schedule, throughout the experimental period. The animals were housed in large spacious hygienic cages and they were given food and water during the courseof experiment with temperature between 25 and 27□C.

EXPERIMENTAL DESIGN (Carbon Tetrachloride – Induced Hepatotoxicity): The animals were randomised and divided into four groups of six animals each. The rat dose was calculated on the basis of the surface area ratio[9].

Group I: Distilled water (10 ml/kg, once a day orally for 3 days). (Control)

Group II: Carbon Tetrachloride (Sin. dose of 25%, 2 ml/kgand paraffin oil 1:1 IP.). (Toxicant Control)

Group III: Hepatotoxicity induced animals (1 ml/kg, 3times, Oral). (Liv-52 Treated)

Group IV: Hepatotoxicity induced animals 1 ml/kg, 3 times, Oral). (RVSPHF567)

Treatment duration was 3 days; Animals were sacrificed at the end of the experiment. Blood were collected by sinus orbital puncture, allowed to clot and the serum is separated. The liver was dissected out and used for biochemical and histopathological studies.

BIOCHEMICAL ANALYSIS:

The blood samples were collected by sinus orbital puncture using sterile capillary tube, cardiac puncture. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm for 15 min at 30°C and utilized for the estimation of various biochemical parameters, namely, SGOT, SGPT, ALP, Total Bilirubin, Total Protein, Albumin (Raichem standard kits from Raichem, Raichem Diasys, Nicolas piramal, E. Merck, Zydus Pathline respectively)[15]. The results were expressed as International Units / litre (IU / L) for SGOT, SGPT, ALP, Total Bilirubin and grams / desilitre (g /dl).

HISTOPATHOLOGICAL EXAMINATION:

The rats were sacrificed on the last day of the study by cervical dislocation; blood was collected and liver was removed and washed with saline, observed macroscopically and then were sliced. Liver pieces were preserved in 10% formalin for histopathological study. The sections were approximately 4-6 micron in thickness. They were stained with hematoxylin and eosin and photographed. The liver pieces were processed and embedded in paraffin wax.

STATISTICAL ANALYSIS:

With the help of PSTAT software, statical evaluation was carried out using one-way analysis of variance (ANOVA) and F-ratio was computed to detect the significant changes between the groups. The unpaired students 't' test was used to compare group I with Group II, Group III and Group IV to find the significant chances of the individual groups. For comparison with the control group and carbon tetrachloridetreated group, $P < 0.001$ was considered as significant value.

RESULTS AND DISCUSSION:

The results of carbon tetrachloride induced hepatotoxicity are shown in (Table 1). Carbon tetrachloride intoxication in normal rats significantly elevated the serum levels of SGOT, SGPT, ALP, Total Bilirubin (total and direct), whereas there was a significant decrease in level of total proteins and albumins that indicated acute hepatocellular damage and biliary obstruction. The rats treated with Liv-52and RVSPHF567 showed a significant reduction in all biochemical parameters, whereas increase in the level of total proteins and albumin were observed.

S.No	Treatment	SGOT IU/L	SGPT IU/L	ALP IU/L	BILIRUBIN IU/L	TOTAL PROTEIN (g/dl)	ALBUMIN (g/dl)

1.	Control (10ml/kg Distilled	81.28 ± 3.22	45. 36 ± 2.45	424 ± 7.03	0.41 ± 0.05	9.7 ± 0.78	8.34 ± 0.36
2.	Carbon Tetrachloride (2ml/kg)	380.06 ± 7.14	226.80 ± 2.92 [a]	975 ± 14.9 [a]	1.96 ± 0.23 [a]	5.4 ± 0.52 [a]	3.24 ± 1.16 [a]
3.	Carbon Tetrachloride	89.40 ± 3.33 [a]	49.89 ± 3.65 [a]	509 ± 6.15[b]	0.74 ± 0.20 [b]	8.3 ± 0.41 [b]	5.64 ± 0.23
4.	Carbon Tetrachloride (2ml/kg) + Polyherbal formulation (RVSPHF567) (2ml/kg)	86.96 ± 6.72[c]	47.62 ± 3.12 [c]	446 ± 8.92 [c]	0.57 ± 0.10 [c]	9.5 ± 0.46 [c]	7.74 ± 0.51 [c]
5.	ONEWAY ANOVA	F= < 981.25 df = 23 p < 0.01	F= < 885.84 df = 23 p < 0.01	F= <637.25 df = 23 p < 0.01	F= < 20.46 df = 23 p < 0.01	F= < 17.59 df = 23 p < 0.01	F= < 22.58 df = 23 p < 0.01

Values are mean ± SEM; n = 6, ap < 0.001 in comparison with control, bp < 0.001 in comparison with carbon tetrachloride (2ml/kg), cp < 0.001 in comparison with carbon tetrachloride (2ml/kg).

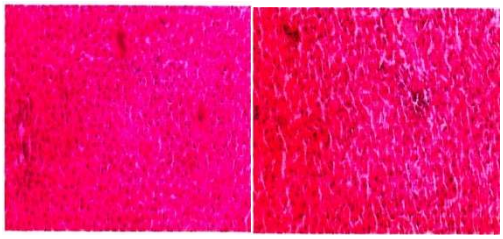

Figure-1 ***GROUP 1 – Normal Control induced group***

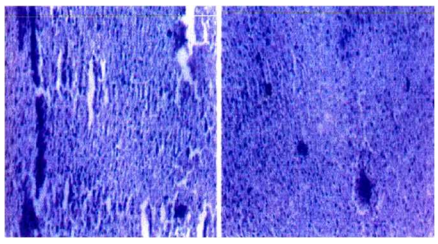

Figure-2 ***GROUP 2 – Carbon tetrachloride***

In the histopathological examination of liver sections ofcontrol group (Figure 1), Centrilobular and architecturalintactness without any apparent damage like fatty metamorphosis, necrosis and fatty infiltration. In the carbon tetrachloride intoxicated group (Figure 2), damages to the

architectural intactness characterized by the presence of hepatocyte nuclei, fatty metamorphosis, oedema and biliary pigments were observed. In the histopathological profile of Liv-52 treated groups (Figure 3), there was no evidence of fatty metamorphosis, nor any oedema or necrosis. In the groups treated with the polyherbal formulation RVSPHF567 (Figure 4), revealed the restoration of normal structural and architectural intactness, no evidence of necrosis detectable. This formulation was able to control this necrotic change that was comparable to that of Liv-52 treated group. Thus, the biochemical observations correlate well with the histopathology results of the liver samples. Thus, these observations confirmed the potent hepatoprotective activity of Liv-52 and RVSPHF567 are comparable to each other.

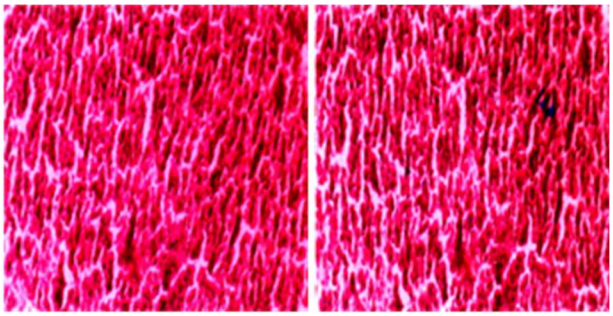

Figure-3 ***GROUP 3 – LIV-52 treated group***

Carbon tetrachloride is one of the most commonly used hepatotoxins in experimental study of liver disease[16]. The lipid peroxidative degradation of bio membrane is one ofthe principle causes of hepatotoxicity of carbon tetrachloride[18, 19]. The reduction is attributed to the initial damage produced and localized in the endoplasmic reticulum leading to its functional failure with a decrease in protein synthesis. The hepatotoxic effect of carbon tetrachloride is largely due to its active metabolite trichloromethyl radical[20] that binds to the macromolecule and induces peroxidative degradation of the membrane lipids of endoplasmic reticulum that is rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxide, which in turn produces a toxic aldehyde that causes damage to liver21. This was evident by an increase in the level of lipid peroxidation in the carbon tetrachloride group and there was a significant decrease in lipid peroxidation in the groups treated with carbon tetrachloride and polyherbal formulation22.

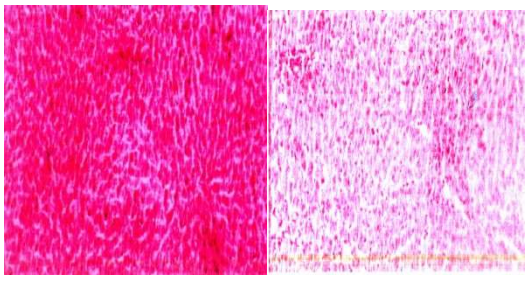

Figure-4 ***GPOUP 4 – Polyherbal formulation treated***

CONCLUSION:

Overall, the results of the present study indicate that Liv-52 and RVSPHF567 demonstrated a significant hepatoprotective activity against carbon tetrachloride induced hepatotoxicity in rats. Moreover, Liv-52 and RVSPHF567 has shown significant comparable hepatoprotective activity.

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