British Journal of Pharmaceutical Research
8(2): 1-7, 2015, Article no.BJPR.18427
ISSN: 2231-2919

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An Experimental Study to Evaluate Hepatoprotective Activity of Herbal Formulation in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors NAK, MN and IAK designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author AP managed the literature searches, analyses of the study performed, managed the experimental process and author AAK performed histological examination. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2015/18427
Editor(s):
(1) Vasudevan Mani, Universiti Teknologi MARA (UiTM), Selangor, Malaysia.
Reviewers:
(1) Bhaskar Sharma, Suresh Gyan Vihar University, Jaipur Rajasthan, India.
(2) Mutlu Çayırlı, Mevki Military Hospital, Turkey.
(3) Anonymous, Lagos State University, Lagos, Nigeria.
Complete Peer review History: http://sciencedomain.org/review-history/10605

Original Research Article

Received 21st April 2015
Accepted 4th July 2015
Published 19th August 2015

ABSTRACT

Objective: To study the hepatoprotective activity of herbal formulation against CCl_4 induced hepatoxicity in rats.
Materials and Methods: Twenty four Charles Foster albino rats of either sex, weighing 150-200 g were divided into four groups of 6 animals each. The animals of group I and II were administered distilled water in the dose of 1 mL/kg, orally, daily for 8 days. Group III and IV rats were administered Silymarin (100 mg/kg/day) and herbal formulation (150 mg/kg/day) orally, daily for 7 days respectively. On 7th day, carbon tetrachloride (CCl_4) was administered in the dose of 2mL/kg as 1:1 mixture with liquid paraffin i.p. to induce hepatotoxicity in animals of group II, III and IV along with routine treatment. On 8th day all animals were sacrificed and blood as well as liver was collected for biochemical parameters and histological examination.
Results: There was significant increase in serum glutamic oxaloacetic transaminase (80.2±4.5 vs 26±3.1), serum glutamic pyruvic transaminase (68.7±5.6 vs 24.5±2.6), alkaline phosphatase (53.1±5.6 vs 9.4±2.8) and total bilirubin (2.64±0.09 vs 1.03±0.05) in CCl_4 treated group (P< 0.01)

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as compared to control group. These parameters were not statistically different in Silymarin and herbal formulation treated groups (SGOT: 47.3±4.2 vs 48.6±3.2, SGPT: 44.2±4.3 vs 46.5±3.5, S.ALP: 23.1±7.4 vs 27.9±2.5 and total bilirubin: 1.68±0.02 vs 1.52±0.05 in Silymarin and herbal formulation group respectively) as compared to control group. But Silymarin and extract treated groups showed significant decrease in these parameters as compared to CCl_4 treated group ($P< 0.01$). Histology of the liver sections confirmed that the extract prevented hepatic damage induced by CCl_4.

Conclusion: The hydro-alcoholic extract of herbal formulation showed significant hepatoprotective activity against CCl_4 induced hepatoxicity in rats.

Keywords: Extract of herbal formulation; Carbon tetrachloride; Silymarin; Hepatoprotective Activity.

1. INTRODUCTION

Liver is one of the most vital organ of the body. The metabolism of various food nutrients, administered drugs and ingested chemical takes place mainly in liver. It converts several toxic substances into nontoxic form and helps in their elimination from the body. The liver has gigantic task of maintaining the body's metabolic homeostasis [1]. This is the most vulnerable organ to be affected by virus, xenobiotics, hepatotoxic drugs (paracetamol, rifampin, isoniazid), excess alcohol ingestion and environmental pollution. Liver diseases are among the most serious ailments. Annually, about 20,000 deaths occur due to liver disease [2]. Hepatotoxic drugs can injure the hepatocyte by generating free-radical or metabolic intermediate that causes peroxidation of membrane lipids and results in liver cell injury. In modern medicine, there is no drug which can prevent or cure hepatic cell injury [3,4].

Numerous medicinal plants and their formulations are used in liver disorders in ethno-medical practice as well as traditional system of medicine in India [5]. In developing countries, more than 80% people depend on traditional system of medicine [6]. Medicinal plants are used in large proportion by Indians because of low cost, easy access and ancestral belief as well as experiences [7]. However a number of herbal formulations have not been still evaluated scientifically for their hepatoprotective effect. Therefore, the study was planned to investigate the hepatoprotective effect of the herbal formulation against CCl_4 induced hepatoxicity in rats.

2. MATERIALS AND METHODS [8]

2.1 IAEC Approval

Approval for the study protocol was taken from Institutional Animal Ethics Committee and all experiments were carried out in accordance to the rules and regulations of IAEC & CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Acute toxicity was carried out and lethal dose was more than 2 g/kg body weight.

2.2 Chemicals

The serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP) and total bilirubin (TB) kits were purchased from Siemens, Mumbai. Carbon tetrachloride (CCl_4) was purchased from Thomas Baker Pvt. Ltd. Mumbai and Silymarin from Sigma-Aldrich, Germany.

2.3 Preparation of Extract

All the ingredients (Table 1) of herbal formulation were procured from Dawakhana Tibbiya College, A.M.U., Aligarh and identified by Prof. S. H. Afaq, Department of Ilmul Advia, A.K.T.C., A.M.U., Aligarh, U.P., India. All the ingredients were coarsely powdered and then subjected to extraction. The extraction of powder in hydro-alcohol was done continuously for 6 hours using Soxhlet apparatus. The extract was filtered using Whatman No. 1 filter paper, evaporated on water bath at 40 - 60ºC until it dried completely and stored in refrigerator for further use.

The dose of extract for rats was calculated by multiplying its clinical doses described in Unani literature with conversion factor 7 [9].

2.4 Animal

Twenty four Charles Foster albino rats of either sex, weighing 150-200 g were procured from Central Animal House of the institute. They were divided into four groups of 6 animals each. They were kept under standard laboratory conditions

and provided commercial diet pellets as well as water *ad libitum*. The room temperature was maintained at 25±1ºC.

Table 1. Ingredients of herbal formulation

Ingredients	Amount (in gm)
Rheum emodi wall	5
Iris ensata thumb	15
Creteria lacca	5
Cinnamomum cassia blume	5
Rosa damascena mill	10
Crocus sativum linn	3

2.5 Treatment

Group I (Control): Distilled water orally in the dose of 1 mL/kg, daily for 7 days.

Group II (Negative control): Distilled water orally in the dose of 1 mL/kg, daily for 7 days.

Group III (Positive control): Silymarin (100 mg/kg) orally daily for 7 days.

Group IV (Test group): Hydro-alcoholic extract of herbal formulation in dose of 150 mg/kg suspended in distilled water orally daily for 7 days.

On 7th day, carbon tetrachloride (CCl_4) was administered in the dose of 2 mL/kg as 1:1 mixture with liquid paraffin i.p. to induce hepatotoxicity in animals of group II, III and IV along with routine treatment. On 8th day all animals were sacrificed including group I [10].

2.6 Sample Collection

The blood was collected and kept undisturbed for 30 minutes. The blood was centrifuged at 5000 rpm for 15-20 minutes to separate serum. The sera of all animal were estimated for SGOT, SGPT [11], total bilirubin [12] and alkaline phosphatase [13].

2.7 Histological Examination

The liver of all rats were removed and fixed in 10% formalin [14]. The tissue was stained with haematoxyline and eosin and histological changes were observed by photomicroscope under high power magnification.

2.8 Statistical Analysis

All the results were expressed as mean ± SEM. One-way Analysis of Variance (ANOVA) followed by post-Hoc Dunnett's test was used for the statistical analysis of data and $P < 0.05$ was considered significant.

3. RESULTS

Administration of CCl_4 to the rats in group II resulted in significant increase in SGOT (80.2±4.5 vs 26±3.1), SGPT (68.7±5.6 vs 24.5±2.6), alkaline phosphatase (53.1±5.6 vs 9.4±2.8) and total bilirubin (2.64±0.09 vs 1.03±0.05) when compared with group I ($P<0.01$). Administration of silymarin and hydro-alcoholic extract of herbal formulation in group III and group IV respectively prevented the rise in SGOT, SGPT, and TB levels when compared with group II ($P<0.01$). The levels of liver function test enzymes in the animals treated with the extract were significantly deceased as compared non-treated animals following CCl_4 administration (SGOT: 80.2±4.5 vs 48.6±3.2, SGPT: 68.7±5.6 vs 46.5±3.5, S.ALP: 53.1±5.6 vs 27.9±2.5 and total bilirubin: 2.64±0.09 vs 1.52±0.05 in CCl_4 and herbal formulation group respectively) and were comparable with the standard drug silymarin treated rats (SGOT: 47.3±4.2 vs 48.6±3.2, SGPT: 44.2±4.3 vs 46.5±3.5, S.ALP: 23.1±7.4 vs 27.9±2.5 and total bilirubin: 1.68±0.02 vs 1.52±0.05 in Silymarin and herbal formulation group respectively) (Table 2).

3.1 Histological Examination of Liver

Group I: Normal control group showed central blood vessels and radiating cords of hepatocytes as well as the vascular sinusoids with no evidence of fatty changes, necrosis or inflammation (Fig. 1).

Group II: CCl_4 treated animals showed centri-lobular (acidophilic) necrosis and vascular congestion (Fig. 2).

Group III: Silymarin treated rats showed mild vascular congestion and peri-vascular infiltrate of mono nuclear cells and fibroblast. No fatty degeneration was observed (Fig. 3).

Group IV: Hydro-alcoholic extract treated rats showed well preserved liver architecture, only mild vascular congestion and peri-vascular infiltrate of mono nuclear cells and fibroblast and regenerating hepatocytes were observed. The hepatic architecture was found similar to that observed in silymarin treated group (Fig. 4).

Table 2. Effect of herbal formulation and silymarin on biochemical parameters of liver function in CCl_4 induced toxicity

Groups	Treatment	SGPT (IU/L)	SGOT (IU/L)	S.ALP (IU/L)	Total bilirubin (mg/dL)
GROUP-I Control	Distilled water	24.5±2.6	26±3.1	9.4±2.8	1.03 ±0.05
GROUP-II Negative control	Distilled water + CCl_4	68.7±5.6*	80.2±4.5*	53.1±5.6*	2.64±0.09*
GROUP-III Positive control	Silymarin 100 mg/kg + CCl_4	44.2±4.3**	47.3±4.2**	23.1±7.4**	1.68±0.02**
GROUP-IV Test group	Hydro-alcoholic extract 150 mg/kg +CCl_4	46.5±3.5**	48.6±3.2**	27.9±2.5**	1.52±0.05**

*Values are mean ± SEM; N = 6; *P≤0.01 compared with Group I; **P≤0.05 compared with Group II, SGOT - serum glutamicoxaloacetic transaminase; SGPT- serum glutamic pyruvic transaminase, S.ALP- Serum alkaline phosphatise*

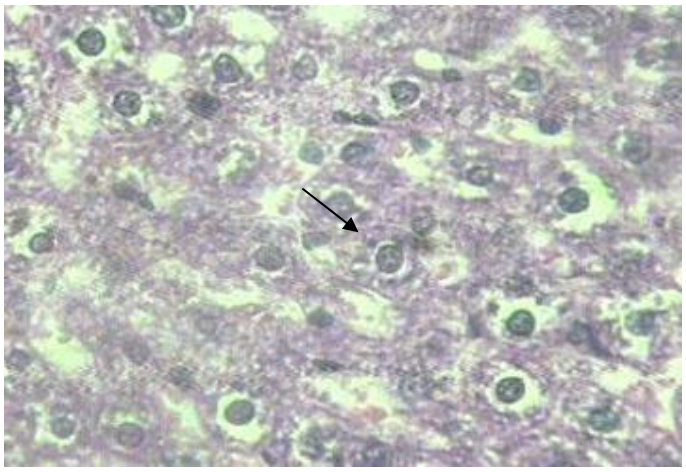

Fig. 1. Group I- shows central blood vessels and radiating cords of hepatocytes as well as the vascular sinusoids with no evidence of fatty changes, necrosis or inflammation

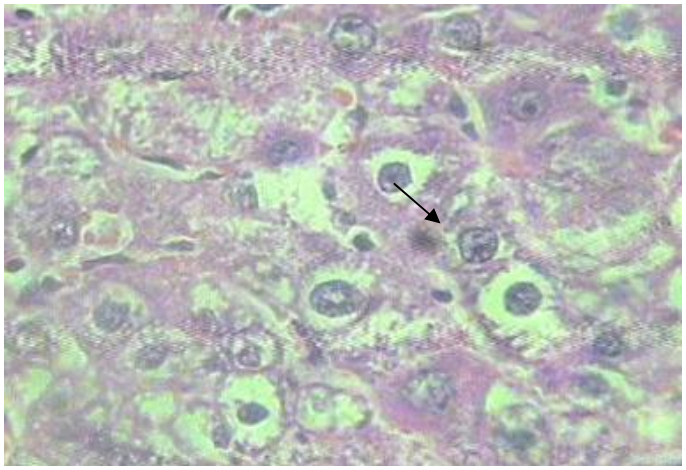

Fig. 2. Group II- shows centri-lobular (acidophilic) necrosis and vascular congestion

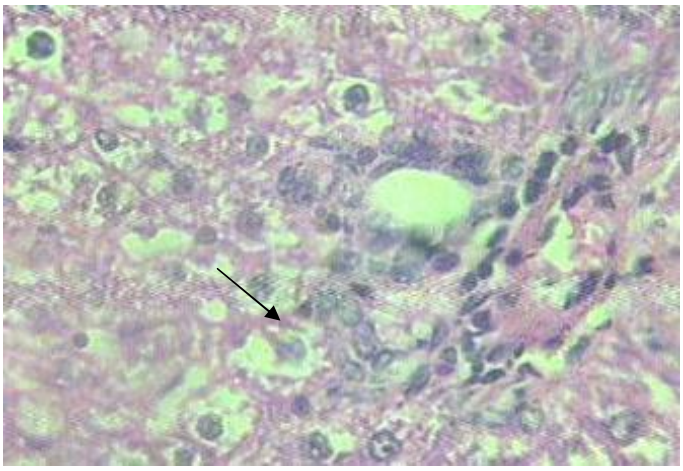

Fig. 3. Group III-shows mild vascular congestion and peri-vascular infiltrate of mono nuclear cells and fibroblast. No fatty degeneration was observed

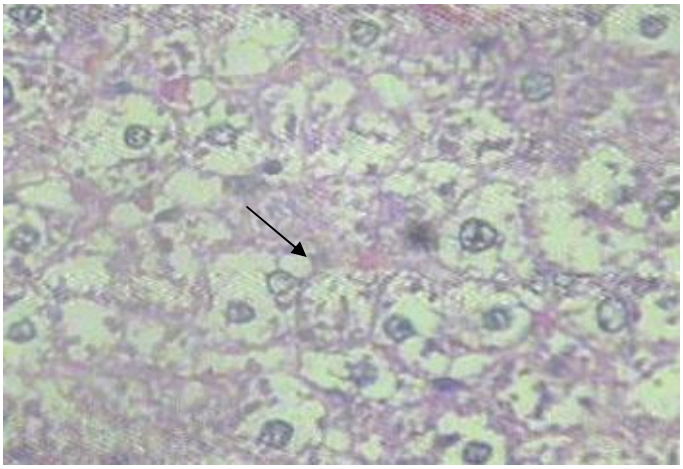

Fig. 4. Group IV-shows well preserved liver architecture, only mild vascular congestion and peri-vascular infiltrate of mono nuclear cells and fibroblast and regenerating hepatocytes were observed

4. DISCUSSION

The damage produced by CCl_4 is described to be similar to the pathological changes seen in infective hepatitis and in many other liver diseases [15]. The liver function test was used to assess the extent of liver damage and the protection induced by the test drug. Silymarin (100 mg / kg) was used as standard hepatoprotective agent for confirming integrity of test system and to compare the efficacy of the test drug as it has been used in the treatment of hepatic diseases [16]. The hepatoprotective properties of Silymarin have been related to inhibition of lipid peroxides formation or scavenging of free radicals [17]. Carbon tetrachloride (CCl_4) produces hepatotoxicity in a wide variety of mammals. It has been emphasized that CCl_4 by itself is not biologically active but gets converted to its metabolite – trichloromethyl radical (CCl3*) by microsomal enzymes namely CYP2E1, CYP2B1 or CYP2B2 and CYP3A and this is toxic to hepatocytes. This radical react with oxygen to form trichloromethylperoxy radical CCl3OO*, which initiates the chain reaction of lipid peroxidation. This brings changes in permeability of mitochondria, endoplasmic reticulum and plasma membranes resulting in elevated levels of transaminases, alkaline phosphatase and

bilirubin. CCl_4 is commonly used for inducting liver toxicity experimentally. This toxic chemical causes peroxidative degradation in liver tissue resulting in coagulative necrotic changes in hepatocytes. CCl_4 produces change around central vein in the liver (Fig. 2) and other oxidative damages resulting in leakage of marker enzymes like SGOT, SGPT and ALP in serum and increase in total bilirubin levels [18,19]. The herbal extract lowered various biochemical markers which shows that they prevent the oxidative damage. Administration of extracts showed significant hepatoprotective activity, which was comparable with silymarin. The qualitative phytochemical studies of hydro-alcoholic extract of herbal formulation also showed positive for flavonoids. Previous studies suggests that plant flavonoids possess antioxidant properties [20] and hence proved useful in the treatment of hepatic damage. The results indicate that the hydro-alcoholic extract of herbal formulation has significant hepatoprotective activity which may be due to higher content of flavonoids like rutin, luteolin and apigenin. Other investigators have screened the hepatoprotective activity of rutin, which is also claimed to have free radical scavenging property and it inhibits lipid peroxidation against CCl_4-induced hepatic toxicity [21].

5. CONCLUSION

The hydro-alcoholic extract of herbal formulation showed significant hepatoprotective activity against CCl_4 induced hepatoxicity in rats.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/10605