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Research Article

Hepatoprotective Screening of Polyherbal Extract of *Anonna squamosa* and *Nigella ativa*

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ABSTRACT
The present study was carried out to investigate the effects of Polyherbal extract (PHE) on liver fibrosis in rats induced by carbon tetrachloride (CCl_4) and to explore its possible mechanisms. Liver injury was induced in Male Wistar albino rats by injection with 50% CCl_4 subcutaneously twice a week for 8 weeks. At the same time, Polyherbal extract (50, 100 and 150 mg/kg) was administered intragastrically. Upon pathological examination, the Polyherbal extract-treated rats significantly reduced the liver damage and the symptoms of liver injury. Administration of Polyherbal extract (PHE) decreased CCl_4-induced elevation of serum transaminase activities, hyaluronic acid, laminin and procollagen type III levels, and contents of hydroxyproline in liver tissue by approximately 30–60%. It also restored the decrease in SOD and GSH-Px activites and inhibited the formation of lipid peroxidative products during CCl_4 treatment. Moreover, Polyherbal extract (100, 150 mg/kg, ig) decreased the elevation of TGF-β1 by 47.7% and 53.1%, respectively.These results suggested that Polyherbal extract significantly inhibited the progression of hepatic injury induced by CCl_4, and the inhibitory effect of Polyherbal extract on hepatic injury might be associated with its ability to scavenge free radicals, decrease the level of TGF- β1.

Keywords: *Annona squamosa, Nigella sativa,* Liver injury.

INTRODUCTION

Liver diseases represent a major medical problem with significant morbidity. Hepatitis viral infections, including hepatitis B and hepatitis C, represent the major cause of liver fibrosis. Other stimuli for liver diseases include drug-induced, helminthic infection, autoimmune disorders, iron or copper overload and biliary obstruction. Liver diseases can be classified as a wound healing response to a variety of chronic stimuli. It is characterized by an excessive deposition of extracellular matrix proteins of which type I collagen predominates. This excess deposition of extracellular matrix proteins disrupts the normal architecture of the liver that alters the normal function of the organ, resulting in pathophysiological damage to the organ[1]. If left untreated, it can progress to liver cirrhosis ultimately leading to organ failure and death. Current evidence indicates that hepatic diseases even cirrhosis is dynamic and can be bidirectional (involving phases of progression and regression) [2]. Efforts have been made to search for effective anti-fibrotic agents. However, no effective Hepatoprotective therapies are available until now. Therefore, the prevention of liver diseases has a very great significance both in theory and in practice.

Medicinally, natural drugs have made a significant contribution to the treatment of liver diseases.

Use of herbal drugs in the treatment of liver diseases has a long tradition, especially in Indian system of medicine. Herbal medicines used in India are now being manufactured as drugs containing ingredients of standardized quality and quantity. Ayurvedic system of medicine treatment is based on overall analysis of symptoms and signs, as well as the physical condition of the patient. *Nigella sativa* [3], a traditional Ayurvedic herb, practitioners of traditional Indian system of medicine routinely use herbs to treat chronic liver disease and cirrhosis. Many study has been demonstrated its Hepatoprotective effect[4, 5]. Various therapeutic effects, such as antioxidant, antiinflammatory, anticancer, antihistaminic, antibacterial Effects have been described for *Nigella sativa*. Additionally, it has been shown that *Nigella sativa* has protective effect against ischemia reperfusion injury to various organs. Thymoquinone, the active constituent of *Nigella sativa* seeds, is a pharmacologically active quinone, which possesses several properties including analgesic and anti-inflammatory actions. *Anonna squamosa* [6] a traditional Indian herb, has been a component of effective prescriptions for treatment of liver diseases[7], *Anonna squamosa,* have been recognized as the valuable traditional herbs used in the treatment of liver disease[8]. This plant is reputed to possess varied medicinal properties[9]. It is used as an insecticidal agent has been investigated by several workers [10]. Free radical scavenging activity of *Annona squamosa* was reported in the leaf extracts. Hypoglycemic and antidiabetic effect of *Annona squamosa* also was reported in the leaf

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extract. From the bark of *Annona squamosa*, a bioactive acetogenin with anticancer activity have been isolated.

In traditional Oriental medicine, it is conventional to combine different herbs in order to achieve a variety of treatment purposes simultaneously, or to enhance a single effect without causing severe side effects [11]. In order to obtain a more effective remedy for the treatment of liver diseases other than exclusively using *Nigella sativa* or *Annona squamosa*, we combined these two herbs based on both traditional references and the results of our previous work mentioned above. As a result of preliminary tests of extracts with several different combination-ratios, we found that the combination in the ratio of 1:1 of two plants, *Nigella sativa* and *Annona squamosa*, respectively, exhibited the most significant hepato-protective activity among the combinations tested. Polyherbal extract (PHE) was composed of *Nigella sativa* or *Annona squamosa*. To further evaluate the antifibrotic activity of Polyherbal extract, the present study was designed to investigate the effects of Polyherbal extract administration on carbon tetrachloride (CCl_4)-induced rats liver fibrosis in vivo. Furthermore, the actions of Polyherbal extract (PHE) on markers of oxidative stress and fibrogenesis were investigated.

Colchicine is an alkaloid agent that has been widely used in clinical practice for the treatment of acute gout and other immunologic diseases. Long-term colchicine treatment in patients with hepatic fibrosis appears to exert an anti-inflammatory, anti-fibrotic and immunomodulatory effect. In experimental studies, colchicines reduces acute liver injury[12], inhibits collagen secretion, and increases collagen degradation, thereby it reduces liver fibrosis. In this study, colchicine was used as a suitable positive control.

MATERIALS AND METHODS

Animals

Male Wistar albino rats (weighing 160–200 g) were procured from Venkateshwara Enterprise, Bangalore and they kept in under standard environmental conditions (12 h light/dark cycles at 25–28 ^{0}C, 60–80% relative humidity) in clean and dry cages and maintained in well-ventilated animal house. Animals were fed with standard diet and water *ad libitum*. The study was approved by the Institutional Animal Ethics Committee.

Preparation of drug

The seeds of *Nigella sativa* obtained from Prgati Ayurvedic Drug store Belgaum and matured fruit of *Annona squamosa* from local market of Belgaum and they were authenticated from Botanical Survey of India, Pune (Maharastra). The extracts of the both antidiabetic plants were mixed and polyherbal formuation was prepared (Table 1). Five hundred grams of each plant (chopped into small pieces) was extracted individually were, soaked overnight in 1 l of water. This suspension was filtered and the filtrates were pooled and the solvents were evaporated in a rotavapor at 40–50 ^{0}C under reduced pressure and lyophilized.

Reagents

Colchicine was obtained from Sigma Chemical Co. (St. Louis, MO, USA). CCl_4 was purchased from E Merck, India. Commercial kits used for determining aspartate aminotransferase (AST), alanine aminotransferase (ALT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) were obtained from Ranbaxy diagnostic, New Delhi, India. The hyaluronic acid (HA), laminin (LN) and procollagen type III (PC III) radioimmunoassay kits were purchased from Ranbaxy diagnostic, New Delhi, India. ELISA kits of transforming growth factor-beta1 (TGF-β1) were obtained from Sigma Chemical (St. Louis, MO).

Drug administration

The procedure for CCl_4-induced model of liver diseases was based on the method[13], with some modifications. Rats were subcutaneously injected with 50% CCl_4 mixed with vegetal oil, twice a week for 8 weeks. The amount of CCl_4 administered was 1 ml/kg. The rats were randomly divided into five groups. Group 1 was normal control, Group 2 was CCl_4 control, Groups 3, 4 and 5 were CCl_4 and Polyherbal extract (50, 100 and 150 mg/kg) treated rats, Groups 6 was CCl_4 and colchicines (0.1 mg/kg) treated rats, served as positive control. Groups 2–6 received CCl_4 subcutaneously twice weekly for 8 week. Group 2 was fed the basal diet throughout the experiment and was designed as a model group. Groups 3–5 and 6 were treated with Polyherbal extract (50, 100 and 150 mg/kg) and colchicine (0.1 mg/kg, ig) per day respectively at the beginning of injection of CCl_4. The control group was administered with the same volume of vehicle.

At 24 h after final injection of CCl_4, all rats were anaesthetized with diethyl ether; samples of blood were drawn from the abdominal aorta and collected in polyethylene tubes. The serum samples obtained by centrifugation (3000×g, 4 ^{0}C) for 10 min and were kept frozen at -80 ^{0}C until assayed as described below. After the animals were killed, the liver and spleen were promptly removed, and weighed. A portion of the liver was fixed for histopathology, and the remaining tissues were stored at -80 ^{0}C until required.

Analysis of liver function

The serum activities of ALT and AST were evaluated by spectrophotometry using commercially available kits.

Measurement of hydroxyproline content in liver

Liver collagen concentration was determined by measuring hydroxyproline content in fresh liver samples according to the method of Mitchell and Taylor[14] with minor modifications. Samples of liver tissue obtained at the end of the experiment were weighed, hydrolyzed in 6.0 M HCl, and analyzed for total hydroxyproline content. Results were expressed as mg/g wet tissue.

Measurement of serum fibrotic markers and serum TGF-β1

The levels of HA, LN and PCIII in the serum were assayed with radioimmunoassay. The operations were performed according to the manufacturer's instruction. *TGF-β1* level in serum was determined by using rat enzymelinked immunoadsordent assay (ELISA) kit.

Histopathological examination

Immediately after sacrifice, the liver tissue was removed and a portion of the tissue was instantly fixed in 10% phosphate buffered formalin, processed by routine histology procedures, embedded in paraffin, cut in 5 μm pieces and mounted on the slide. The samples were stained with hematoxylin and eosin (HE) for histopathological examination. Two pathologists who had no knowledge of their sources examined the stained slides independently. Histological grade of hepatic fibrosis was determined by a semi-quantitative method according to the following scoring systerm[15]: 0, no fibrosis, normal liver and absence of fibrosis; I, fibrosis present (collagen fiber present that extends from portal triad or central vein to

peripheral region); II, mild fibrosis (mild collagen fiber present with extension without compartment formation); III, moderate fibrosis (moderate collagen fiber present with some pseudo lobe formation); IV, severe fibrosis (severe collagen fiber present with thickening of the partial compartments and frequent pseudo lobe formation).Each sample was observed at 100× magnification. The degree of fibrosis was expressed as the mean of 10 different fields in each slide.

Estimation of antioxidant enzyme and lipid peroxidation

Liver tissues were washed with normal saline to remove any red blood and clots, and then were homogenized on ice with Tris–HCl (5 mmol/L containing 2 mmol/L EDTA, pH 7.4). Homogenates were centrifuged at 1000×g for 15 min at 4 ^{0}C. Aliquots samples of the supernatants were analyzed for antioxidant enzymes and lipid peroxidation. The assays were performed according to each manufacturer's recommended protocol. For the determination of antioxidant enzymes, we measured the activities of SOD and GSH-Px based on their ability to inhibit the oxidation of oxyamine by the xanthine-xanthine oxidase system. The results are expressed as the units for SOD or GSH-Px per milligram of liver tissue. Lipid peroxidation in the liver was determined by measuring the level of MDA, an end product of lipid peroxidation, using a thiobarbituric method[16]. The level of hepatic MDA was expressed as μmol/g protein.

Statistical analysis

Data were expressed as means ± S.D. Statistical significance of the difference between groups was determined by one-way analysis of variance and the *t*-test. The frequency data were compared using Ridit procedure. $P < 0.05$ indicated a statistically significant difference.

Table 1: Effect of Polyherbal extract on indices of liver and spleen of CCl_4-induced liver injury in rats (n = 8; mean± S.D.)

Groups	Doses (mg/kg)	Liver index (%)	Spleen index (%)
Normal	–	3.54 ± 0.92	0.27 ± 0.40
Model	–	6.90 ± 1.73$^{\#\#}$	0.69 ± 0.22$^{\#\#}$
Polyherbal extract	50	6.39 ± 1.70	0.56 ± 0.13
	100	4.76 ± 1.58*	0.46 ± 0.14
	150	3.47 ± 1.03**	0.37 ± 0.14**
Colchicine	0.1	4.68 ± 1.30*	0.43 ± 0.14**

$^{\#\#}P < 0.01$ compared with normal control group; $^{*}P < 0.05$, $^{**}P < 0.01$ Compared with model group.

RESULTS

Effect of PHE on liver and spleen indices

A significant increase in liver index and spleen index was recorded in only CCl_4-treated animals after 8 weeks. Polyherbal extract (150 mg/kg) treatment prevented the increase of liver and spleen indices after CCl_4 administration, the same result as colchicines (0.1 mg/kg, ig) (Table 1).

Effect of* Polyherbal extract *on liver function

Analysis of serum ALT and AST activities was carried out in order to evaluate the extent of liver injury after chronic CCl_4 treatment. ALT and AST activities at the end of the experiment are shown in Table 2. A significant increase in the activities of the two enzymes (ALT 1.7-fold and AST 2.4-fold) was observed in the model group compared with those of the normal control group. Treatment with Polyherbal extract (100 and 150 mg/kg) or colchicines (0.1 mg/kg) caused significant decrease in serum transaminase activities.

Effect of* Polyherbal extract *on hydroxyproline content in liver

Analysis of hepatic hydroxyproline content was carried out as an index of liver fibrosis. Elevated hydroxyproline levels were measured in CCl_4-treated animals with respect to normal control group (Table 2). Treatment with Polyherbal extract (100 and 150 mg/kg),

hydroxyproline content in liver tissue was significantly reduced by 42.9% and 40.3%, respectively compared with CCl_4 control group. The same results as colchicine at dosage of 0.1 mg/kg.

Effect of Polyherbal extract on serum HA, LN and PCIII

As shown in Table 3, serum levels of HA, LN and PC III, the surrogate markers of liver fibrogenesis, increased significantly in hepatic fibrotic rats in model group. dministration with Polyherbal extract or colchicine effectively decreased the serum HA, LN and PC III levels, with the degree of protection being 40–73%.

Effect of* Polyherbal extract *on liver histological examination

CCl_4 control group had a high degree of injury. Treatment with Polyherbal extract (100 and 150 mg/kg) or colchicine (0.1 mg/kg) significantly improved histological scores in comparison with model group (Table 4). Representative photographs of the liver morphology are shown in Fig. 1. The control group showed normal lobular architecture with central veins and radiating hepatic cords (Fig. 1A). In contrast, CCl4 administration elicited extensive changes in liver morphology, including marked fatty degeneration, necrosis, obvious collagen deposition, hepatocyte ballooning and infiltration of inflammatory cells in liver interstitial (such as macrophages and lymphocytes) after 8 weeks of treatment (Fig. 1B). Polyherbal extract (100 and 150 mg/kg) treatment markedly alleviated the degree of liver fibrosis indicated by decreased collagen deposition and lowed inflammation (Fig. 1C and D).

Effect of* Polyherbal extract *on hepatic lipid peroxidation and SOD, GSH-Px activity

Lipid peroxidation is considered as an important factor for the hepatotoxicity of CCl_4. Data on lipid peroxidation in terms of MDA formation of whole liver homogenate of control and experimental animals are presented in Table 5. A marked increase (74%) in MDA production was found in the liver of CCl_4- treated animals relative to normal control group. Polyherbal extract treated rats at doses of 100 and 150 mg/kg significantly reduced MDA level by 33% and 33.7% compared to the CCl_4 control, respectively. Colchicine (0.1 mg/kg) treatment decreased the MDA level, but had no statistical significance compared with CCl_4 control group.

At the end of the experiment the changes inSODand GSH-Px activities were assayed in rat livers in order to evaluate endogenous antioxidant defences (Table 5). A significant decrease in SOD and GSH-Px activity (54.7% and 48.2%, respectively) was observed in the liver of CCl_4-treated rats relative to control rats administered with vehicle alone. Administration with Polyherbal extract (100 and 150 mg/kg) or colchicine (0.1 mg/kg) significantly elevated the activities of both enzymes. Compared with colchicine group, the efficacy of Polyherbal extract (100 and 150 mg/kg) restored the activities of SOD is more significant.

Effect of* Polyherbal extracts *on production of TGF-β1 in hepatic fibrosis rats

As shown in Table 2, when the rats were challenged with CCl_4, the level of TGF- β 1 was elevated significantly (2.1-fold) compared with normal control group. Polyherbal extract (100 and 150 mg/kg, ig) significantly decreased the TGF- β1 level (by 47.7% and 53.1%) in serum when compared with

Table 2: Effect of Polyherbal extract on liver function, hydroxyproline content and production of TGF-β1 in hepatic fibrosis rats (n = 8; mean±S.D.)

Groups	Doses (mg/kg)	ALT (U/L)	AST (U/L)	Hydroxyproline (mg/g protein)	TGF-β1 (μg/L)
Normal	–	39.94 ± 12.68	32.23 ± 9.34	1.16 ± 0.21	11.21 ± 2.84
Model	–	105.55 ± 32.54$^{\#\#}$	111.17 ± 28.24$^{\#\#}$	3.08 ± 0.68$^{\#\#}$	34.41 ± 8.69$^{\#\#}$
Polyherbal extract	50	76.20 ± 18.42 *	86.38 ± 21.98	2.23 ± 0.56 *	28.12 ± 7.65
	100	56.99 ± 14.45 **	52.92 ± 15.81 **	1.76 ± 0.54 **	18.01 ± 4.84 **
	150	53.67 ± 15.53 **	53.94 ± 16.31 **	1.84 ± 0.41 **	16.13 ± ±5.27 **
Colchicine	0.1	72.30 ± 32.61 **	67.93 ± 19.45 **	1.72 ± 0.46 **	18.02 ± 5.42 **

$^{\#\#}P < 0.01$ compared with normal control group; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with model group.

Table 3: Effect of Polyherbal extract on serum HA, LN and PCIII levels of CCl_4-induced liver injury in rats (n = 8; mean± S.D.)

Groups	Doses (mg/kg)	HA (μg/L)	LN (μg/L)	PCIII (μg/L)
Normal	–	107.49 ± 34.84	102.95 ± 33.76	93.53 ± 29.60
Model	–	257.45 ± 73.62$^{\#\#}$	225.21 ± 64.90$^{\#\#}$	252.69 ± 56.68$^{\#\#}$
Polyherbal extract	50	181.68 ± 50.62*	159.46 ± 41.51*	192.15 ± 62.47
	100	150.79 ± 32.37**	146.87 ± 26.55**	156.01 ± 47.70**
	150	152.57 ± 41.95**	138.23 ± 38.11**	153.90 ± 40.45**
Colchicine	0.1	189.34 ± 56.71*	152.63 ± 45.57**	178.22 ± 54.99*

$^{\#\#}P < 0.01$ compared with normal control group; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with model group.

Table 4: Effect of on the pathologic grading of CCl_4-induced liver injury in rats

Groups	Dose (mg/kg)	Pathologic grading of hepatic fibrosis					P value
		0	I	II	III	IV	
Normal	–	8	0	0	0	0	-
Model	–	0	0	2	4	2	0.000
Polyherbal extract	50	0	1	2	3	1	0.264
	100	0	2	4	2	0	0.026*
	150	0	4	2	2	0	0.016*
Colchicine	0.1	0	3	3	2	0	0.021*

Results are 10 fields of vision. $^{\#\#}P < 0.01$ compared with normal control group; $^{*}P < 0.05$ compared with model group.

Table 5: Effects of Polyherbal extract on MDA level, SOD and GSH-Px activities in liver homogenates of liver fibrosis rats induced by CCl_4 (n = 8; mean±S.D.)

Groups	Doses (mg/kg)	MDA (mol/g protein)	SOD (U/mg protein)	GSH-Px (U/mg protein)
Normal	–	25.26 ± 7.94	275.02 ± 65.14	436.99 ± 76.53
Model	–	43.94 ± 11.74$^{\#\#}$	124.60 ± 34.92$^{\#\#}$	226.25 ± 73.87$^{\#\#}$
Polyherbal extract	50	36.07 ± 11.34	195.90 ± 39.87*	269.00 ± 61.15
	100	29.48 ± 8.69*	234.40 ± 50.50**†	371.66 ± 69.46**
	150	29.55 ± 9.31*	229.93 ± 42.80**†	354.52 ± 37.46**
Colchicine	0.1	33.94 ± 7.14	181.24 ± 42.00*	322.94 ± 76.91*

$^{\#\#}P < 0.01$ compared with normal control group; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with model group; $^{\dagger}P < 0.05$ compared with colchicine (0.1 mg/kg) group.

CCl_4 control group. The efficacy is similar to that of colchicine at dosage of 0.1 mg/kg.

DISCUSSION

Hepatic diseases are present in various chronic hepatic diseases. It is well known that constant fibrosis can lead to the development of hepatocellular carcinoma[17]. Interrupting and/or reversing hepatic fibrosis may be a new approach for improving its progression to hepatocellular carcinoma. However, the therapy for reversing liver fibrosis is not yet well established. Recently, research for new drugs has refocused on natural products. The present study demonstrated that Polyherbal extract (PHE) of *anonna squamosa and nigella sativa* had therapeutic effects on liver diseases induced by CCl_4 exposure in rats. The animal model of CCl_4-induced liver injury was established firstly. The histological results showed that the normal structure of lobules was destroyed and pseudolobules formed. Moreover, the increased hydroxyproline content in liver and serum HA, LN and PCIII also confirmed the hepatic fibrogenesis in rats. Treatment with Polyherbal extract (PHE) effectively decreased these biomarkers. The inhibitory effect of Polyherbal extract (100 mg/kg) on the content of hydroxyproline in liver tissue was appeared to be more potent than that of Polyherbal extract (150 mg/kg) and appeared not dose dependent. This emphasizes the fact that herbal medicines often contain multiple active substances and many active substances have regulatory activities. The synergistic effects of the potential active components on the liver fibrosis will be further studied. Hepatocellular necrosis leads to elevation of the serum marker enzymes, which are released from the liver into blood. The increased levels of ALT and AST are conventional indicators of liver injury[18]. The present study revealed a significant increase in the activities of ALT and AST on exposure to CCl_4, indicating considerable hepatocellular injury. Administration of Polyherbal extracts (100 and 150 mg/kg) for 8 weeks attenuated the increased activities of the serum enzymes and caused a subsequent recovery towards normalization similar to that of colchicine treatment. The hepatoprotective effect of Polyherbal extract was further concluded by marked improvement in the histopathological examinations. Polyherbal extract administration not only protected against hepatocyte damage and reduced collagen deposition, but also ameliorated oxidative stress. Results from these experiments clearly showed that Polyherbal extract could inhibit the progression of liver injury induced by CCl_4 in rats.

Chronic CCl_4 treatment is frequently used in rats to produce an experimental model to study hepatic fibrosis[19, 20]. Oxidative stress and its consequent lipid peroxidation have been currently considered to be involved in the generation of CCl_4-induced liver fibrosis[21]. When CCl_4 is used to treat rats in studies of liver injury, it is metabolized by cytochrome P450 in liver cells to yield the trichloromethyl-free radical, which either extracts a hydrogen atom from unsaturated membrane lipids to initiate lipid peroxidation or reacts with the sulfhydryl compounds, triggering a chain of lipid peroxidation. These changes lead to cell injury, and chronic liver injury result in liver fibrosis [22]. Oxidative stressors are also commonly detected in livers from patients with alcohol abuse, hepatitis C virus infection, iron overload, or chronic cholestasis[23]. To confirm the effect of Polyherbal extract on

oxidative stress in liver injury, we examined the oxidative stress parameters, including SOD, GSH-Px, and MDA. Increased liver MDA levels and depressed SOD and GSH-Px activities were observed in the CCl_4-treated rats. The increase in MDA levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals[24]. Administration of Polyherbal extract (PHE) intragastrically could significantly elevate the activity of SOD and GSH-Px, two antioxidant enzymes, and markedly decreased MDA level, the products of lipid peroxidation, in liver fibrosis rats. These results of present study indicate that Polyherbal extract might inhibit lipid peroxidation and effectively recover the anti-oxidative defense system in liver fibrosis. It is possible that the mechanism of hepato-protective effects of Polyherbal extract may be due to its antioxidant action. From the tables it is clear that the Polyherbal extract showed greater antioxidant activity which is comparable with the positive control, colchicine. Furthermore, oxidative stress, particularly lipid peroxidation induces collagen synthesis to aggravate liver fibrosis[25]. Hence, the reduction of extracellular matrix in Polyherbal extract -treated group, as evidenced by HA, LN, PCIII and hdroxyprline, was probably related to its anti-oxidant activity.

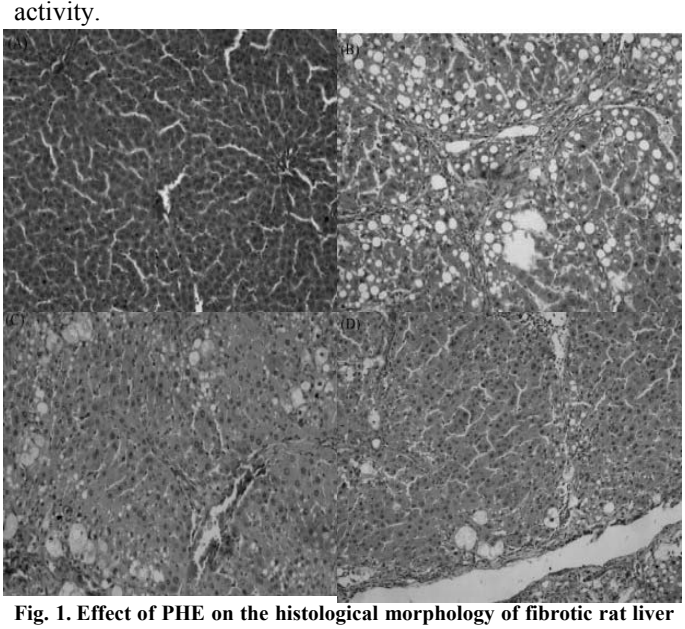

Fig. 1. Effect of PHE on the histological morphology of fibrotic rat liver by HE staining (×100). (A) Normal control. (B) CCl_4 control. (C) CCl_4 + PHE 80 mg/kg. (D) CCl_4 + PHE 160 mg/kg.

Cytokines play an important role in the development of liver fibrosis. One of the major fibrogenic cytokine whose role in patients with cirrhosis and in animal-induced liver fibrosis has been clearly established is TGF-β1 [26]. The evidence for TGF- β1 having a central role in liver fibrosis is sizable. First, it's over expression has been correlated with the degree of fibrosis in both animal models as well as in human disease. Third, transgenic mice that over-express TGF- β1 develop acute hepatic fibrosis[27]. Due to the pleiotropic fibrogenic effects of TGF- β1, strategies aimed at disrupting TGF- β1 synthesis and/or signaling pathways can markedly decrease fibrosis in experimental models[28]. Therefore, the effect of Polyherbal extract on the level of TGF-β1was assessed using ELISA. The results showed that Polyherbal extract treatment significantly decreased the elevated level of TGF-β1 in fibrotic rats. These findings suggest that the inhibitory effects of Polyherbal extract on liver fibrosis might be related to its action on the production of TGF-β1.

In conclusion, results from the present study demonstrated that Polyherbal extract was effective in the prevention of CCl_4-induced liver fibrosis in rats. The primary mechanisms of this therapeutic effect could be due to its radical scavenging action, antioxidant activity, decreasing the level of TGF-β1.

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