The Effect of *Qurs-e-Zarishk Sagheer* (A Compound Unani Formulation) on Liver Enzymes in CCl_4 Induced Hepatotoxicity in Rats

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Abstract

Qurs-e-Zarishk Sagheer (QZS) is a pharmacopoeal compound preparation known to possess hepatoprotective effect. Present study was designed to evaluate its protective and curative potential on liver enzymes in CCl_4 (2 ml/kg of body weight i.p.) induced hepatotoxicity in rats. Ingredients of QZS in powdered form and its hydro alcoholic extract were used in the doses of 700 mg/kg, and 230 mg/kg body weight /day, respectively. Silymarin was used as standard drug in the dose of 100 mg/kg orally/day. Biochemical parameters including Serum Glutamate Oxaloacetate Transaminase, Serum Glutamate Pyruvate Transaminase and TBARS were determined along with the histological studies of liver tissues of all the animals. The elevation of marker enzymes and structural changes in histological reports of liver sections were taken as the indicators of hepatic injury. The study showed gross elevation of liver enzymes and histological changes in CCl_4 treated animals, while the test drug in both doses forms showed significant enzymes lowering activity, which was comparable with that of Silymarin. Biochemical parameters showed better results in respect of extract while hisptopathological observations were almost similar in both groups. The study demonstrated that QZS possesses significant liver enzyme lowering effect in CCl_4 induced hepatic injury indicating hepatoprotective effect.

Keywords: Pharmacopoeal compound, Hepatoprotective, CCl_4, Ethanolic extract

Introduction

Liver is the main organ to metabolize all the foreign compounds and has tremendous capacity to detoxify toxic chemicals of the body that is why it is susceptible to almost as many different diseases, and damage to the liver inflicted by hepatotoxic agents is of grave consequences (Subramanian *et al.,* 1999). In today's world, liver is overwhelmed with toxin problems, alcohol abuse and susceptibility to viral infections, immune disorders or problems of metabolism, leading to serious liver disorders such as cirrhosis, jaundice, tumors, metabolic and degenerative lesions, liver cell necrosis and hepatitis etc. amongst them cirrhosis, fatty liver and hepatitis are important in world health issues (Luper, 1998). The magnitude of derangement of the liver by diseases or hepatotoxins and the extent of hepatic damage are generally assessed by measuring the activity of liver enzymes namely SGOT, SGPT and TBARS (Morrison *et al*., 1983). The decreased levels of transaminases indicate stabilization of plasma membrane and protection of hepatocytes against damage caused by hepatotoxin. The modern synthetic drugs have very little to offer for alleviation of hepatic ailments and some

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of these drugs even adversely affect the liver function (Meyer *et al.*, 2001 and Harsh Mohan, 2002). In recent years, the use of alternative drugs for the treatment of liver diseases has increased all over the world. The natural drugs are believed to be harmless and free from serious adverse reactions and also the limited therapeutic options and disappointing therapeutic success of modern medicine has increased the usage of alternative medicine including their preparations (Stickel *et al.*, 2007). The Unani system of medicine on account of having proven ability of bringing the marker enzymesas to the normal level has a major role in the treatment of liver ailments. It possesses a number of single and compound drugs that are considered highly effective and safe. Few of these drugs have been investigated and shown to possess significant hepatoprotective effect (Ghufran *et al.*, 2002; Anusha *et al.*, 2011; Zafar and Ali., 1998: Akhtar *et al.*, 2009; Handa and Sharma, 2002). *Qurs-e-Zarishk Sagheer* (QZS) is one such compound preparation described to be effective in liver diseases (Khan, 1921) and prescribed commonly by the physicians of Unani medicine. But it has not been investigated for its effect in hepatic diseases specially its effect on marker enzymes of liver function. Therefore the present study was undertaken to evaluate the efficacy of test drug on various liver enzymes in rats subjected to acute hepatotoxicity by the administration of Carbon tetrachloride. The study was designed in such a way that both curative and protective effects can be evaluated.

Materials and Methods

Ingredients of QZS (Khan, 1921)

1.	Zarishk	(*Berberis aristata*)	44.5 gm
2.	Behdana	(*Pyrus cydonia*)	44.5 gm
3.	Tukhm-e- Kasni	(*Cichorium intybus*)	10.5 gm
4.	Tukhme Khurfah	(*Portulaca oleracea*)	10.5 gm
5.	Tukhme Kheera	(*Cucumis sativus*)	10.5 gm
6.	Tukhme Kakdi	(*Cucumis melo*)	10.5 gm
7.	Gule surkh	(*Rosa demescena*)	17.5 gm
8.	Rewande cheeni	(*Rheum emodi*)	3.5 gm
9.	Balchad	(*Nardostachys jatamansi*)	3.5 gm

Preparation and Dosing of Test Drug

The ingredients of QZS were purchased from the herbal market of Aligarh and New Delhi. Pharmacognosy section of Department of Ilmul Advia, Aligarh Muslim University, Aligarh authenticated the samples. All the crude drugs of QZS were pulverized to get a fine powder which was homogenized in water before administration to the animals. A 50% ethanol extract was also prepared through Soxhlets Apparatus (Anonymous, 1968; Anonymous, 1987). The extract was dried

over a hot plate until a semi solid preparation was collected. The dried extract was however reconstituted in distilled water before the administration and a homogenous suspension was given to the animals orally with the help of gastric canula. The dose for albino rats was determined after multiplying the human dose by the conversion factor of 7 (Dhawan, 1982). The dose of QZS thus, calculated for an albino rat was found to be 700 mg/kg and 230 mg/kg of powder drug and the extract, respectively.

Chemicals

CCl_4, n-butanol, Acetic acid were purchased from Thomas Baker Pvt. Ltd. Mumbai, Sodium dodecyle sulphate and Thiobarbituric from Otto Kemi Mumbai, 1, 1, 3, 3-tetraethoxypropane from Sigma USA, Silymarin from Sigma-Aldrich, Germany, Folin's reagent from CDH, Mumbai, AST and ALT estimation kits from Span Diagnostic Ltd, Surat; Olive oil and Formalin were purchased from SD Fine chemicals, Chennai. All the reagents used were of analytical grade.

Animals

Albino rats of either sex with weight range of 125-175 gm were used for experiment. The rats were randomly selected and were divided into five groups of 6 animals each. So, total 60 animals were utilized in the two test meant for Protective and Curative study. They were housed in clean polypropylene cages and the room temperature was maintained at 25 ± 2°C with 12 hour light and dark cycle. All the animals received standard diet (Amruta Labs, Pune) and water *ad libitum*. The animals were deprived of food for 12 hours before the treatment. The experimental protocol was approved by the Institutional Ethics Committee.

Experimental Design

The animals were divided into 5 groups of six animals each for the protective and curative test and were treated as follows:

Groups	**Treatment**
Group I (Plain Control)	Vehicle daily for 7 days
Group II (Negative control)	CCl_4 (0.2 ml/100 gm)
Group III (Standard control)	Silymarin (10 mg/100 gm) for 7 days+ CCl_4 (0.2 ml/100 gm)
Group IV (Test groups) (powder)	QZS Powder (70 mg/100 gm) for 7 days+ CCl_4 (0.2 ml/100 gm)
Group V (Test group) (extract)	QZS Extract (23 mg/100 gm) for 7 days+ CCl_4 (0.2 ml/100 gm)

The animals were treated with CCl_4 on second day in the test for preventive effect while in the test designed for curative effect CCl_4 was given on day 6. Other treatments were similar in both the tests. On the 8th day all the rats were sacrificed under ether anesthesia and blood was collected from each animal for serum analysis and liver were removed and fixed in 10% formalin for histopathological studies of the liver to determine the degree of hepatic damage (Devaraj *et al.*, 2011).

Preparations of Samples for Biochemical Studies

The blood and liver were collected after sacrificing the animals. The blood was kept for 30 minutes without disturbing and was then centrifuged for 15-20 minutes at 5000 rpm to separate the sera. It was stored at 4^0C and ALT, AST (Reitman and Frankel 1957), and TBARS (Okhawa *et al,* 1979) which is an index of lipid peroxides (Lowry *et al.,* 1951) were determined.

Histopathological Observation

The liver of rats was removed immediately after sacrificing them and fixed in 10% formalin. Care was taken to keep the volume of the fixative (Mukherjee, 1988). The tissue was processed and sections were cut. The slides were prepared and stained with haematoxyline and eosin stain and the histopathological features were observed by a photomicroscope under various magnifications.

Statistical Analysis

Data was presented as mean ± Standard Error and analyzed using one way ANOVA test, followed by pair-wise comparison of various groups by LSD. The analysis was carried out by using the software of the website 'analyseit.com'.

Results

CCl_4 in dose of 2 ml/kg produced acute hepatic damage in negative control group when compared with normal control. There was significant rise in the level of enzymes SGOT, SGPT, and also the Thiobarbituric acid reactive substances (TBARS) as compared to plain control. The concentration of Malondialdehyde (MDA), SGOT and SGPT in CCl_4 treated animals in protective group was found to be 4.92±0.45 (ç mole of MDA / mg of protein), 111.7 ± 3.60 (u/ml) and 97 ± 6.61 (u/ml), whereas, the concentration in plain control of protective group was found to be 1.18± 0.095 (ç mole), 26.3 ± 2.94 (u/ml) and 27.7± 3.40 (U/ml), respectively that was significantly less than that in the CCl_4 group ($P<0.001$). The standard Silymarin showed significant reduction in all parameters when compared with CCl_4 treated group. Treatment with two samples of test drug along with CCl_4

intoxication showed decrease in level of marker enzymes. The values for SGOT, SGPT were found to be within normal limit. A similar pattern was also followed in curative group of animals (P<0.001). The animals treated with two samples of test drug (group IV & V) did not show any significant rise in MDA, SGOT & SGPT (Table-A & B and Fig 1&2).

Table A: Protective effect of QZS in CCl_4 mediated hepatic damage

	Groups	TBARS (η mole of MDA / mg Protein)	SGOT (Units/ml)	SGPT (Units/ml)
Group I	Plain Control	1.18 ± 0.095	26.3 ± 2.94	27.7 ± 3.40
Group II	CCl_4 (0.2ml/ 100gm)	4.92 ± 0.45	111.7 ± 3.60	97 ± 6.61
Group III	Silymarin (10mg/100gm)	1.48 ± 0.05	40.3 ± 3.40	44.5 ± 2.06
Group IV	TD *(QZ)* Crude (70mg/100gm)	2.61 ± 0.19 a^3	41 ± 3.39 a^3	44.3 ± 2.08 a^3
Group V	TD *(QZ)* Extract (23mg/100gm)	2.43 ± 0.15 a^3	34.7 ± 3.36 a^3	18.3 ± 1.69 a^3 c^3

(n=6)

1 = P < 0.05, 2 = P<0.01, 3 = P<0.001

a= against CCl_4, b=against plain control, c=against Silymarin

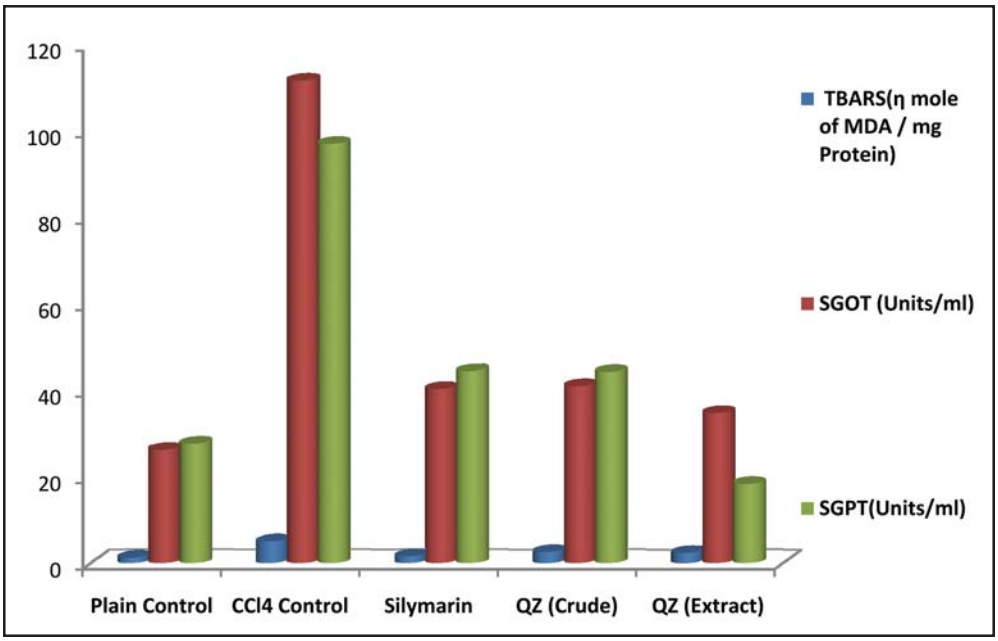


Figure 1

Table B: Curative effect of QZS in CCl_4 mediated hepatic damage

Groups	TBARS (η mole of MDA / mg Protein)	SGOT (Units/ml)	SGPT (Units/ml)
Plain Control	1.96 ± 0.13	30.7 ± 2.40	24.7 ± 2.58
CCl_4 (0.2ml/100gm)	5.46 ± 0.45	114.8 ± 3.43	100.2 ± 4.05
Silymarin (10mg/100gm)	1.68 ± 0.10	46.2 ± 2.10	41.8 ± 2.74
TD *(QZ)* Crude (70mg/100gm)	2.07 ± 0.15 a^3	60.3 ± 3.84 a^3	54.3 ± 2.19 a^3
TD *(QZ)* Extract (23mg/100gm)	2.52 ± 0.20 a^3	46.5 ± 4.24 a^3	48.7 ± 3.84 a^3

(n=6)

1 = P < 0.05, 2 = P<0.01, 3 = P<0.001

a= against CCl_4, b=against plain control, c=against Silymarin

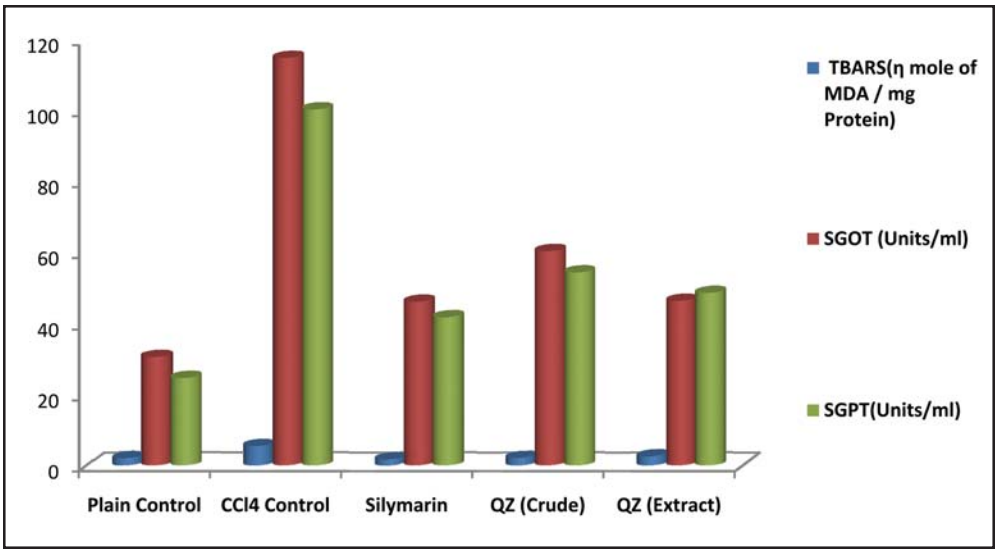


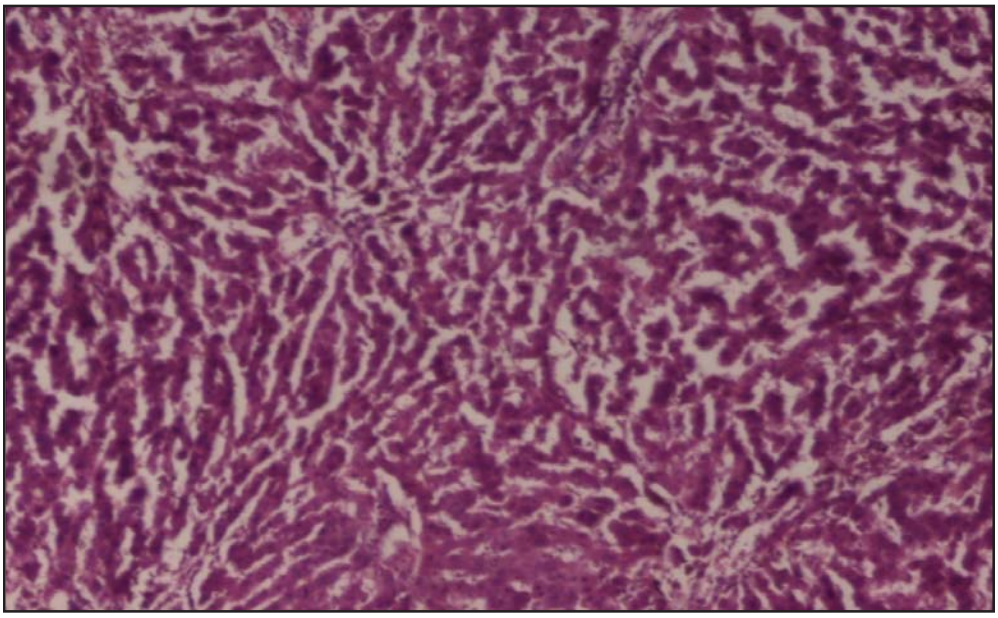
Figure 2

Histopathology

The histopathological studies of the liver showed centrilobular necrosis and vascular congestion with mononuclear cell infiltration in CCl_4 control rats. CCl_4 treatment caused marked congestion of central vein and portal triads, indicating fibrosis (Fig 4), in comparison with normal control where, central blood vessels and radiating cords of hepatocytes as well as the vascular sinusoids were observed

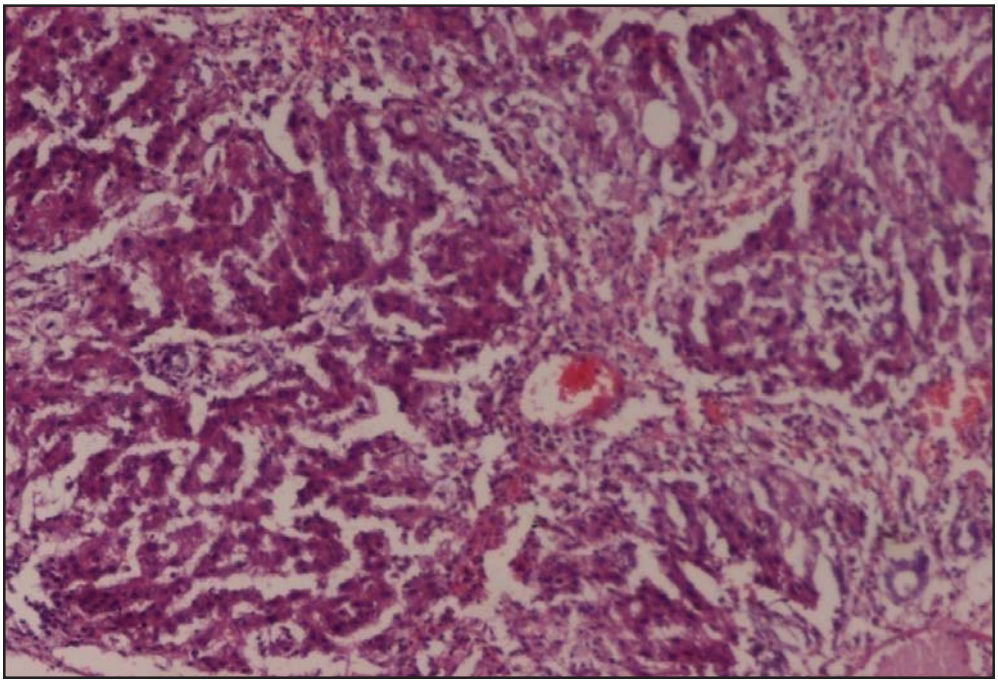
with no evidence of fatty changes, necrosis or inflammation (Fig. 3). The animals treated with Silymarin showed almost normalization of fatty accumulation and necrosis (Figure 5). Animals treated with crude powder exhibited intact

Figure 3: Plain control (Water only)



Central blood vessels and radiating cords of hepatocytes as well as the vascular sinusoids with no evidence of fatty changes, necrosis or inflammation.

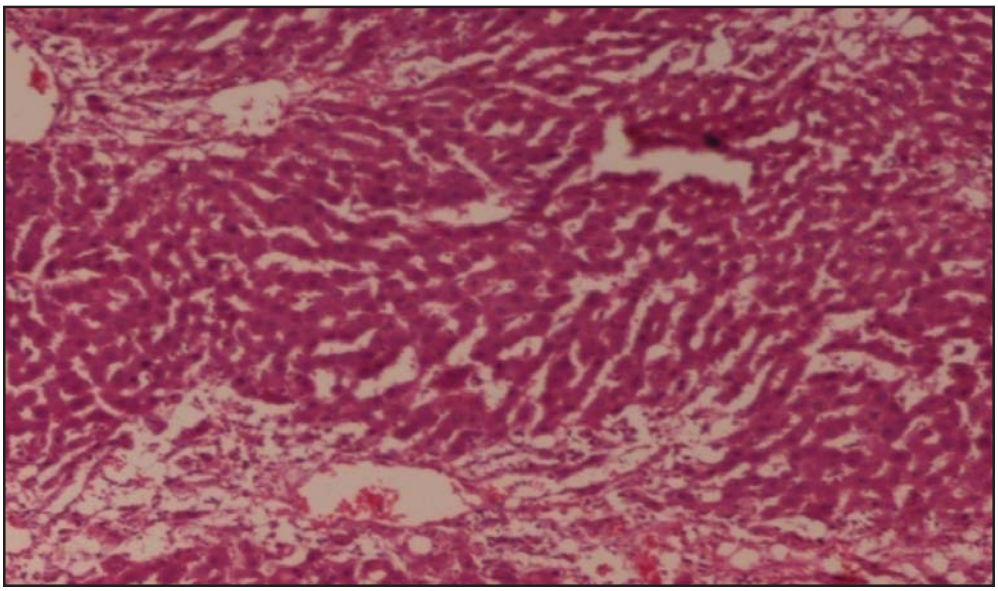
Figure 4: Negative Control (CCl_4 only)



Centrilobular (Acidophilic) necrosis and vascular congestion

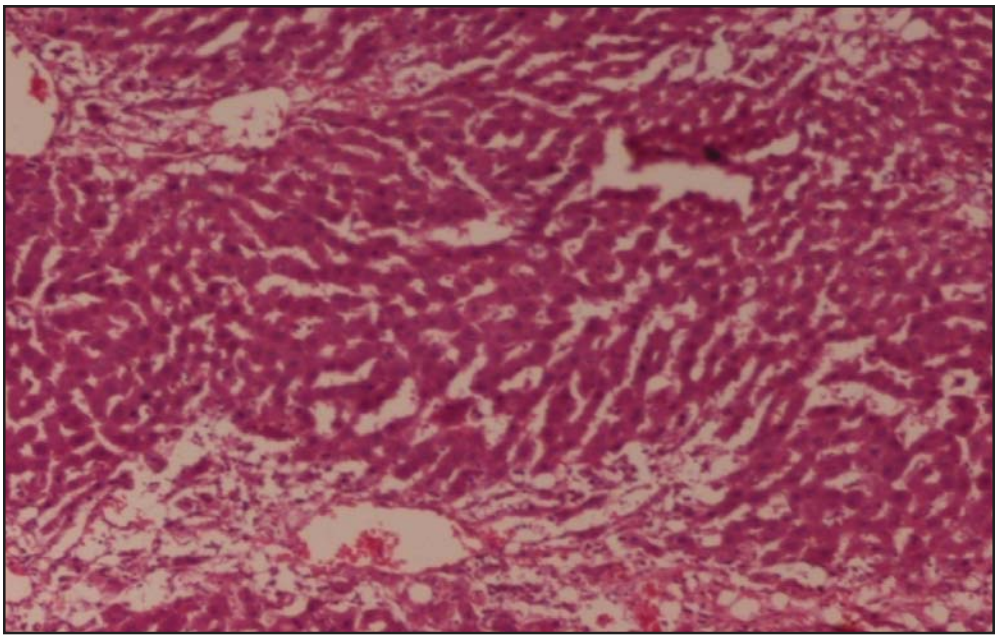
hepatocytes, some congestion in portal triad. The group received extracts form of test drug showed minimal degree of edema, normalization of fatty changes as well as normalization of necrosis of the liver. A substantial protection against hepatic damage was achieved by the two samples of test drug (Fig. 6 & 7). Both the

Figure 5: Standard (Silymarin) + CCl4



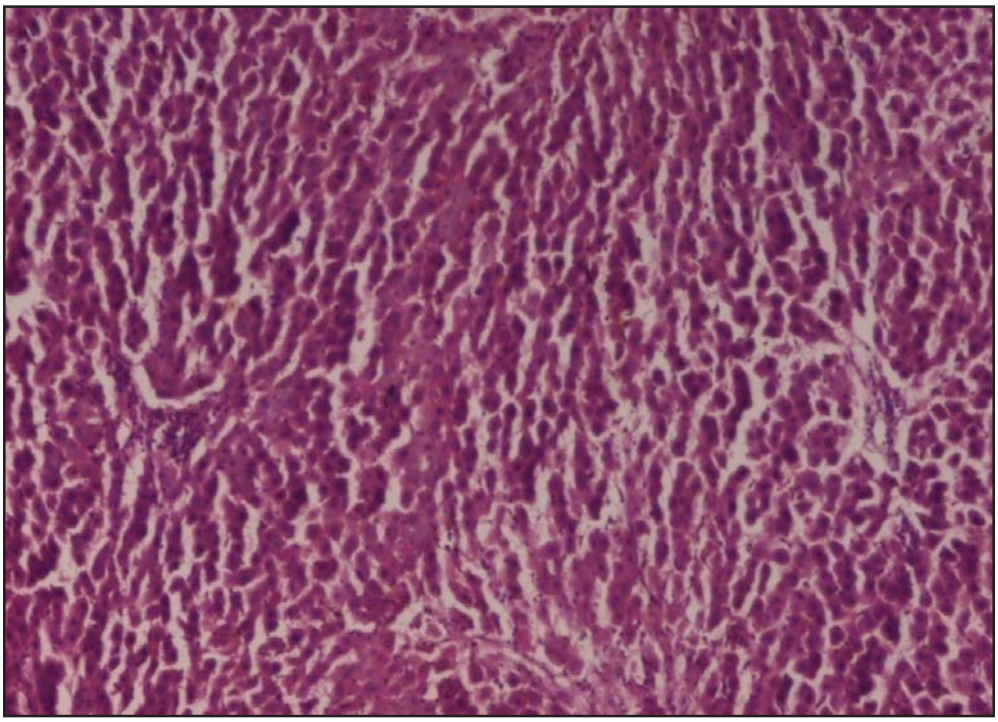
Mild vascular congestion and peri-vascular infiltrate of mono nuclear cells and fibroblast. No fatty changes

Figure 6: QZS (powder) + CCl_4 treated



Intact hepatocytes, congestion in Portal Triad, edema minimal

Figure 7: QZS (Extract) + CCl_4 treated



Edema+, no inflammatory cell no cholestasis, Kupffer cells present

dosage forms prevented CCl_4-induced changes in liver. The Silymarin and test drug treated groups showed excellent protection and cure to liver architecture.

Discussion

The present study was under taken to determine the concentration of serum marker enzymes of liver and that of TBARS against hepatic injury produced by carbon tetrachloride in rats. Administration of CCl_4 increased the concentration of SGOT, SGPT and TBARS significantly as compared to their normal values. The enzymes leaking out from damaged liver cells into circulating blood represent the damage to hepatic cells. The extent of hepatic damage was assessed by the elevation in the release of cytosolic transaminases (SGPT and SGOT) in circulation, as the CCl_4 administration is reported to cause marked elevation in serum enzymes. A high level of SGOT indicating cellular damage is frequently observed in cases of viral hepatitis, myocardial infarction and muscle injury etc (Muriel *et al.*, 1992). The release and thereby the high concentration of SGPT in the blood followed the similar pattern as that of SGOT. However since it is mostly present in hepatocytes therefore, SGPT is considered more reliable and comparative better parameter to detect the liver injury (Agarwal *et al.*, 2006). Carbon tetrachloride (CCl_4) mediates changes in liver function that ultimately leads to destruction of hepatocellular membrane. Cytochrome P-450 activates CCl_4 to form various free

radicals (trichloromethyl, Cl_3C-CCl_3 (hexachloroethane), $COCl_2$ (phosgene), which are involved in pathogenesis of liver damage in chain reactions causing peroxidation of lipids, covalent binding to macromolecules, disruption of metabolic mechanisms in mitochondria, decrease in phospholipids, increase in triglyceride, inhibition of calcium pump of microsomes thus leading to liver necrosis (Maryam *et al.*, 2011) and subsequently liver fibrosis and cirrhosis (Waer *et al.*, 2012). Since, the pathological lesions develop in CCl_4 treated animals closely resemble the symptoms of acute viral hepatitis and cirrhosis in human therefore it serves as an excellent model to assess the efficacy of any drug having hepatoprotective potential (Desai, 2011). QZS has shown very significant reduction in the concentration of enzymes in both the models which is comparable with the results of standard drug (Sylimarin). The extract form has demonstrated slightly better results as compared to powder form in respect of biochemical parameters but no significant difference was observed in histological findings. In the histological study it was observed that QZS caused greater retention of hepatic architecture, reduction in fatty degeneration and necrosis in comparison of CCl_4 treated animals. The two dosage forms of QZS were able to reduce the level of enzymes especially SGOT in both the experiments, indicating that they protected the hepatocytes and maintained the normal liver physiology and the functional status of the liver, and further caused stabilization of plasma membrane and even regeneration of damaged liver cells. Since the test drug has shown both protective and curative effect as evidenced from the biochemical and histological findings therefore it can be proposed to be beneficial in different forms of hepatitis. The likely mechanism of hepatoprotective and hepatocurative effect appears to be antioxidant activity as both the dosage forms demonstrated significant antioxidant effect (TBARS).

Conclusion

The study demonstrated a significant protective and curative effect produced by QZS (both the extract and crude powder forms) in CCl_4 induced hepatic damage as it significantly decreased the level of marker enzymes of liver function, decreased the level of Thiobarbituric acid reactive substances and protected the liver tissue from any major damage. It also demonstrated a significant antioxidant effect possessed by the test drug; antioxidant effect may be one of the mechanisms of its hepatoprotective effect.

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