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HEPATOPROTECTIVE ACTIVITY OF AQUEOUS EXTRACT OF *PICRORHIZA KURROA* IN CARBON TETRACHLORIDE (CCL_4) INDUCED HEPATOTOXICITY IN ALBINO WISTAR RATS

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ABSTRACT

Modern clinical studies have confirmed the efficacy and safety of Picrorhiza kurroa for the treatment of liver disease. The roots and rhizomes are used for hepatoprotective activity. Aqueous extract of *Picrorhiza kurroa* hepatoprotective activity was assessed in CCl_4 induced hepatotoxicity in albino wistar rats. Six groups, each group containing of six rats. Group 1 (control) is treated with normal saline (10 ml/kg bw po). Group 2 was serving as CCl_4 treated control with (3 ml/kg bw po) on the last day. Group 3, 4 and 5 received *Picrorhiza kurroa* at different doses (100 mg/kg, 200 mg/kg, 400 mg/kg bw po) respectively and on the final day introduction of CCl_4 was made. As for group 6, silymarin (200 mg/kg bw po) and CCl_4 on the concluding day. On the last day, after 1 hour receiving CCl_4 animals were anaesthetized with ether and blood was collected from the retro orbital plexus to study the liver function. Histopathological study was also conducted to measure the action of *Picrorhiza kurroa* on parameters such as hepatic fatty degeneration and centrizonal necrosis of respective groups. Present study suggested that CCl_4 administration increased the levels of transaminase enzymes. However, the aqueous extract of *Picrorhiza kurroa* significantly reduced the elevated serum levels of transaminase enzymes in CCl_4 intoxicated rats. Similarly histopathological studies showed the same outcome as mentioned above and showed marked reduction in fatty degeneration and centrizonal necrosis in CCl_4 intoxicated rats. Present study suggests that aqueous extract of *Picrorhiza kurroa* has significant hepatoprotective activity against CCl_4 intoxicated rats.

Key Words:- *Picrorhiza kurroa*, CCl_4, Hepatoprotective, Transaminase enzymes, Histopathological study.

INTRODUCTION

Liver is usually large, compound tubular gland. The structural and functional unit of liver is lobule that contains the central vein running longitudinally through these lobules. There are three concentric zones of classical lobule, Zone I, an ellipsoidal area, Zone II, intermediate and Zone III near the end of the lobule.

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Within the lobules, liver cells or hepatocytes are arranged in one-cell-thick plate like layers and hepatocystes make 80% of the cell population of liver (Asif Mir *et al*., 2010; Wynaber DV *et al*., 1995; Aliyu R *et al*., 1995). Hepatic injury caused by hepatocytes death and identified when there is an increase of more than three times of normal serum transaminase enzymes (AST, ALT and ALP) (Victor J *et al*., 2006). Histologist using noted minor differences in the appearance in three concentric zones within the classical lobule (Asif Mir *et al*., 2010; Wynaber DV *et al*., 1995; Aliyu R *et al*., 1995).

The liver which occupies the pivotal position in

body plays an essential role in drug and xenobiotic metabolism and in maintaining the biological equilibrium of the organism. The role played by this organ in the removal of substance from the portal circulation makes it susceptible to a persistent attack by offending foreign (xenobiotic) compound culminating in the liver dysfunction (Usha S *et al*., 2008; Rajesh *et al*., 2001). Herbal drugs are frequently considered to be less toxic and free from side effects than synthetic drugs (Usha S *et al*., 2008; Rawat AK *et al*., 1997). Medicinal plants are widely exploited worldwide for their active ingredients (Vetriselvan S *et al*., 2012). Liver diseases are considered as a serious health problems. Steroids, vaccines and antiviral drugs that are employed as therapy for liver diseases have potential adverse effects when administered for long periods (Anandan R *et al*., 1998). Liver can be damaged by drugs like tetracyclines, sulphonamides and antihypertensive drug methyldopa. One of the best models of injury produced in liver is by CCI_4. Carbon tetrachloride is used as hepatotoxic agent in animals research work to study the hepato-curative action of plants and other compounds (Asif Mir *et al*., 2010; Wynaber DV *et al*., 1995; Aliyu R *et al*., 1995). There is a worldwide trend for use of traditional herbal drugs for the treatment of liver diseases. Several leads from plant sources have been found as potential hepatoprotective agents with diverse chemical structures (Anandan R *et al*., 1998). Use of herbal drugs in the treatment of liver diseases has a long tradition, especially in Eastern medicine (Detlef Schuppan *et al*., 2003).

It is well known that the hepatotoxic effect of carbon tetrachloride is due to the oxidative damage by free radical generation, and antioxidant property is claimed to be one of the mechanisms of hepatoprotective drugs (Recknagel RO, 1967). Carbon tetrachloride (CCl_4) is a widely used experimental hepatotoxicant, it is biotransformed by cytochrome P450 system to produce the trichloromethyl free radical that causes lipid peroxidation and thereby, produce liver damage. It produces the dose dependent hepatotoxicity by directly affecting the liver, causing lipid peroxidation (Ploa GL, 2000). Free radical scavengers have been employed to study the mechanism of CCl_4 toxicity as well as to protect liver cells from CCl_4 induced damage by breaking the chain reaction of LPO (Kamalakkannan N *et al*., 2005). Free radical induced by peroxidation causes development of degenerative disease. Free radicals are involved in several pathological conditions such as atherosclerosis, liver disorders, diabetes and nephrotoxicity, inactivate enzymes and damage important cellular components causing tissue injury through covalent binding and lipid peroxidation (Ajay A *et al*., 2002). Antioxidants are molecules that slow or prevent the oxidation of other chemicals. Oxidation is a redox chemicals reaction that transfers electron from a substance to an oxidizing agent (Wolf G, 2005).

Picrorhiza kurroa (*P. kurroa*; Family Scrophulariaceae), a well-known herb in the Indian traditional Ayurveda system of medicine, has been used to treat disorders of the liver and is an important ingredient of many herbal preparations used for treatment of liver ailments (Sangeeta Sinha *et al*., 2011; Ansari RA *et al*., 1988). Picrorhiza kurroa Royle ex Benth (PK) has been mentioned as an important remedy by Jivak, Charak and Vagbhatt in ancient Ayurvedic literature (Anandan R *et al*., 1998). It has been described under the group of bitter drugs. Picrorhiza kurroa is a small perennial herb that grows in hilly parts of India particular in Himalayas between 3000 and 5000 meters. It is an established herbal remedy for variety of disease ranging from indigestion to hepatitis. Modern clinical studies have confirmed the efficacy and safety of Picrorhiza kurroa for the treatment of liver disease. The roots and rhizomes are used in medicinally important parts. Powder, decoction, infusion, confection, and alcoholic extract of the drug are prescribed in Ayurveda and Homeopathy. Roots contain 2 bitter glycosides, picrorhizin, kutkin, D-menital, benelic acid, kutkisterol. It also contains aromatic substance.

Silymarin is a well-known hepatoprotective agent. Silymarin is a flavones-lignan mixture obtained from seeds of Silybum marianum. Silymarin is a mixture of silybin, isosyllabic, silychristin and silydianin. Silybin A and B are collectively known as silibinin. Randomized, controlled trials have proved efficacy of solitarian in liver diseases. Picrorhiza kurroa, when compared with silymarin, the hepatoprotective effect of Picrorhiza kurroa was found to be similar, or in many cases, superior to the effect of Silibum marianum (Luper S, 1998). The extract of PK appears to offer significant protection against liver damage by CCl_4. It probably acts as free-radical scavenger and inhibitor of lipid peroxidation of liver plasma membrane (Santra A *et al*., 1998). The root contains a number of very bitter glucosides including kitkin and picrorhizin, nine cucurbitacin glycosides, D-mannitol, benetic acid, kutkisterol, vanillic acid and some steroids. Picrorhiza kurroa also contains apocynin, a powerful anti-inflammatory agent, which also reduces platelet aggregation. The actions of Picrorhiza kurroa are antibacterial, antiperiodic, cathartic (in large doses), laxative (in smaller doses) stomachic and bitter tonic, hepatoprotective, anticholestatic (relieves obstruction of bile salts), anti-inflammatory, anti-allergy, antioxidant; modulates the immune system and liver enzyme levels. Picrorhiza kurroa has hepatoprotective effect against CCl_4

(Saraswat B *et al*., 1993). For that reason this study has been conducted to evaluate the hepatoprotective activity of aqueous extract of *Picrorhiza kurroa* on CCL_4 induced hepatotoxic in rats.

MATERIALS AND METHODS

Plant material

Picrorhiza kurroa roots growing in the Himalayan region at an attitude of 2700-4500m were identified and collected under the supervision of a botanist. They were cleaned with distilled water and shade dried at room temperature. The root of *Picrorhiza kurroa* was purchased from Banarus Hindu University, India in the form of aqueous root extract from Dr. Dubey, Department of Natural Sciences (Vinoth kumar P *et al*., 2010). Further identification has also been done.

Preparation of extract

The powdered roots (230g) of *Picrorhiza kurroa* were extracted separately to exhaustion in a soxhlet apparatus using aqueous solvent system. All the extracts were filtered through a cotton plug followed by Whatman filter paper No.1 and then concentrated by using a rotator evaporator at low temperature (40-50°C) and reduced pressure and got 4.87g yield from aqueous fractions respectively. The extracts were preserved in airtight containers and kept at 4-5°C until further use (Vinoth kumar P *et al*., 2010).

Chemicals

All reagents used in the study were of high purity. All chemicals such as carbon tetrachloride (CCl_4), Formalin, Xylene and DMSO were purchased from Sigma Aldrich Chemical (Malaysia). Silymarin used in the experiments was purchased from Sigma Aldrich Company in China.

Experimental animals

Experiments were carried out on healthy adult male albino wistar rats (Vadivel Subramanian *et al*., 2011) weighing 180 ± 20 grams (Joshua PE *et al*., 2010). They were raised in the animal house at the Faculty of Pharmacy of the Masterskill University College of Health Sciences. Animals were housed in polypropylene cages with stainless steel grill top at 25 ± 2^{o}C with 12:12 hours light and dark cycle was followed (Sujeet Singh *et al*., 2011). They were fed a standard diet of pellets and tapped water ad libitum. Rats were routinely acclimatized to laboratory conditions for 7 days prior to experiments (Rahul Somani *et al*., 2011; Pravin V Gomase *et al*., 2011). After acclimation, the animals will be subjected to a gross observation, to ensure that the selected animals are in good state of health. Animals were then randomly selected for final allotment to the study. Prior authorization for the use of laboratory animals in this study was obtained from the University College Animal Ethical Committee (Reg. MUCH/AEC/HS/2012/17). The experimental procedures were carried out in strict compliance with the Animal Ethics Committee's rules and regulations followed in this institute.

Acute toxicity study

Acute toxicity studies (Chan PK *et al*., 1994) were carried on rats as per the guidelines (OECD NO: 423) given by the Organization for Economic Co-operation and Development. Overnight-fasted Albino wistar rats (180 ± 20g) of male sex were used for the study. The animals were divided into five groups of three animals each. The extracts were administered separately to the all the three animals in each group at starting single dose of 5 mg/kg. Animals were observed for the period of 1 h, occasionally for 3 h for severity of any toxic sign and mortality. If no mortality is observed at this dose, the same procedure will be repeated for dose level of 50, 400, 2600 mg/kg of extracts of separate newer groups. The LD_{50} was thus determine, which was selected for the hepatoprotective animals study. The animals were observed up to 7 days after drug administration to find out for any delayed mortality.

Experimental design

Six groups, each group containing of six rats allotted to different treatment groups. Group 1 (control) is treated with normal saline (10 ml/kg bw po) as vehicle only. Group 2 was serving as CCl_4 treated control with receiving CCl_4 (3 ml/kg bw po) on the (10^{th}) last day. Group 3, 4 and 5 received *Picrorhiza kurroa* at different doses (100 mg/kg, 200 mg/kg, 400 mg/kg bw po) respectively and on the final day introduction of CCl_4 was made to each group. As for group 6, all the rats received silymarin (200 mg/kg bw po) and CCl_4 on the concluding day. This study was carried out continuously for 10 days.

Collection of blood

On the (10^{th}) last day, after 1 hour receiving CCl_4 animals were anaesthetized with ether and blood was collected from the retro orbital plexus and serum is separated by centrifugation at 2500 rpm at 30^{0}C (Mahuya S *et al*., 2011).

Biochemical estimation

To study the liver function, the transaminase enzymes (AST, ALT, and ALP) levels in the serum were essayed spectrophotometrically using Sigma Diagnostic

kits (USA) (Vadivel Subramanian *et al*., 2011; Rahul Somani *et al*., 2011).

Histopathological studies

Livers from animals from different groups were isolated and fixed in 10% phosphate buffered formalin for at least 24 h. Then the paraffin sections were prepared and cut into 5 µm thick section in a rotary microtome and mounted on the slide. The sections were then stained with haemotoxylin-eosin dye. After staining, the sections were observed under light microscope for histopathological changes, i.e. necrosis, fatty degeneration, lymphocytes and Kupffer cells infiltration, and photographs were taken (Vadivel Subramanian *et al*., 2011).

Statistical Analysis

Data were expressed as the mean ± standard deviation (SD). The data were analyzed using one way analysis of variance (ANOVA) followed by Tukey's test as post hoc test for multiple comparisons. Data were considered significant at different level of P value; $P < 0.05$, $P < 0.01$ and $P < 0.001$(Agnel Arul John Nayagam *et al*., 2011).

RESULTS

Acute toxicity studies

All the rats that received aqueous extract of *Picrorhiza kurroa* either at high dose up to 2600 mg/kg or low dose were found to be harmless. No mortality or toxic symptoms were observed throughout the entire length of the study. Aqueous extract of Picrorhiza kurroa showed a steady compliance towards the rats and proved to be safe.

Table 1. Effect of aqueous extract of *Picrorhiza kurroa* on liver enzymes in CCl_4 induced hepatotoxicity in albino wistar rats

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control	54.456 ± 9.522	24.750 ± 13.427	32.613 ± 2.233
CCl_4	291.065 ± 28.219 [a]	141.024 ± 19.969 [a]	88.723 ± 14.295 [a]
PK 100mg	252.935 ± 28.829 [a]	119.047 ± 19.805 [a]	83.645 ± 11.179 [a]
PK 200mg	215.908 ± 28.904 [a, d]	100.587 ± 18.393 [a, d]	74.728 ±8.549 [a]
PK 400mg	175.326 ± 30.367 [a, c]	93.081 ±11.373 [a, d]	74.097 ± 5.522 [a]
Silymarin	130.186 ± 31.074 [b, c]	70.225 ±18.964 [b, c]	69.031 ± 13.784 [a, e]

The results are expressed as the Mean ± SD of six rats/group; One way ANOVA followed by Turkey's multiple test.
a = Results significantly different from Control group, $P < 0.001$
b = Results significantly different from Control group, $P < 0.01$
c = Results significantly different from CCl_4 group, $P < 0.001$
d = Results significantly different from CCl_4 group, $P < 0.01$
e = Results significantly different from CCl_4 group, $P < 0.05$

Figure 1. Effect of aqueous extract of *Picrorhiza kurroa* on serum AST level in CCl_4 induced hepatotoxicity in albino wistar rats

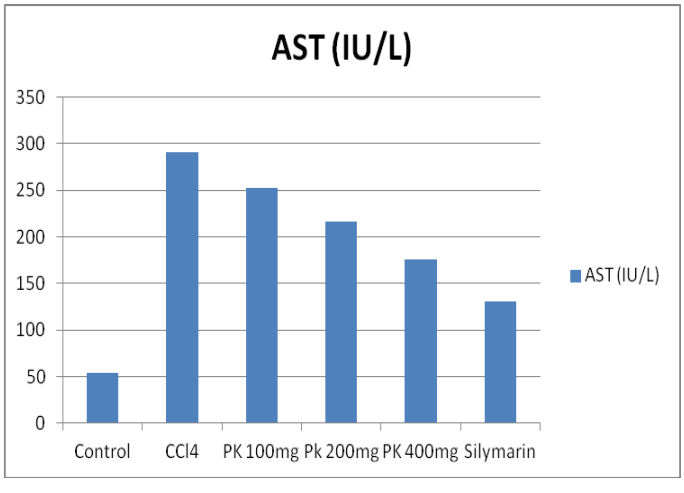


Figure 2. Effect of aqueous extract of *Picrorhiza kurroa* on serum ALT level in CCl_4 induced hepatotoxicity in albino wistar rats

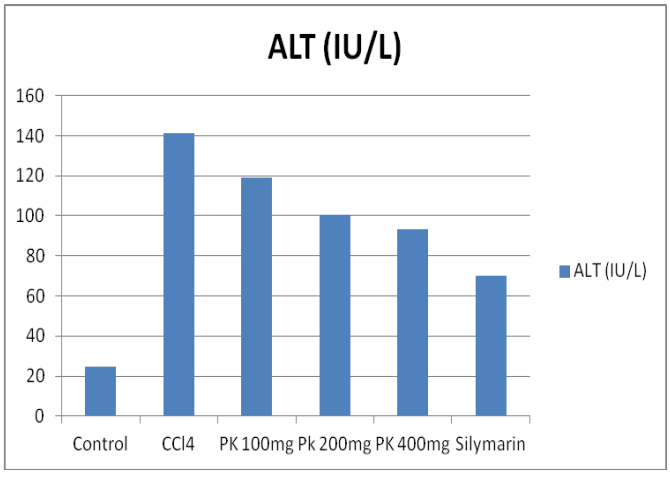


Figure 3. Effect of aqueous extract of *Picrorhiza kurroa* on serum ALP level in CCl_4 induced hepatotoxicity in albino wistar rats

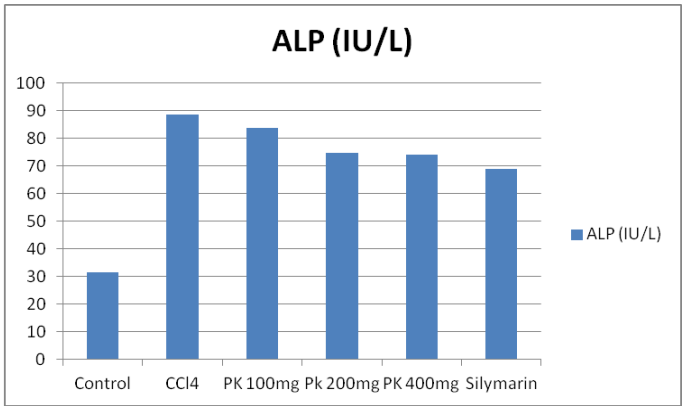


Figure 4a. Control group (10 ml/kg normal saline) liver section revealing normal hepatic parenchyma with a central vein at the top corner in100x

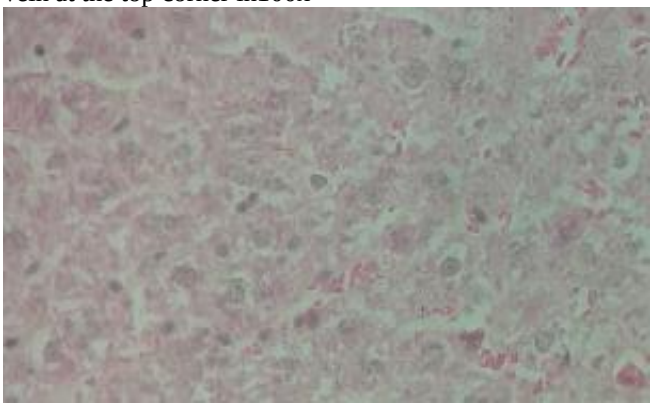


Figure 4b. CCl_4 control group (3 ml/kg CCl_4) showing increase in fibrous tissues and inflammatory cells around the congested blood vessel. Marked hepatocellular fatty degeneration also seen in 400x

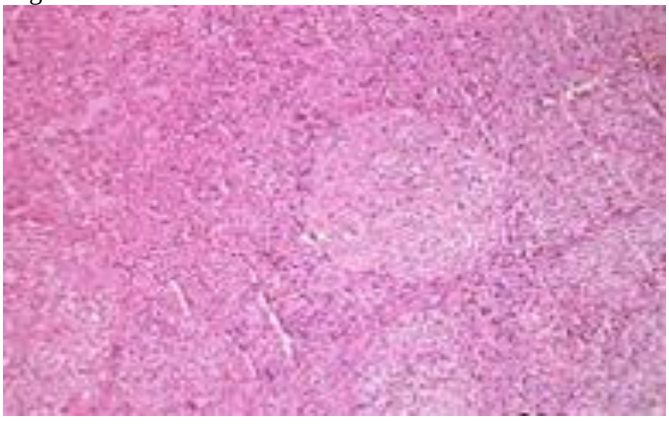


Figure 4c. Treated group (CCl_4 3 ml/kg + *Picrorhiza kurroa* 100 mg/kg) liver section revealing swollen hepatocytes with decreased sinusoidal spaces in 400x

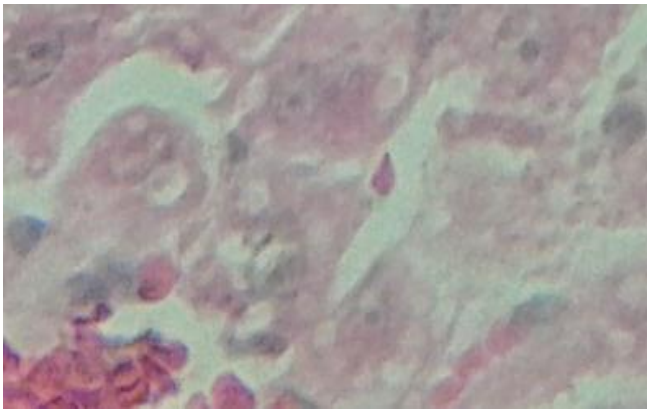


Figure 4d. Treated group (CCl_4 3 ml/kg + *Picrorhiza kurroa* 200 mg/kg) liver section revealing swollen hepatocytes with decreased sinusoidal spaces in 400x

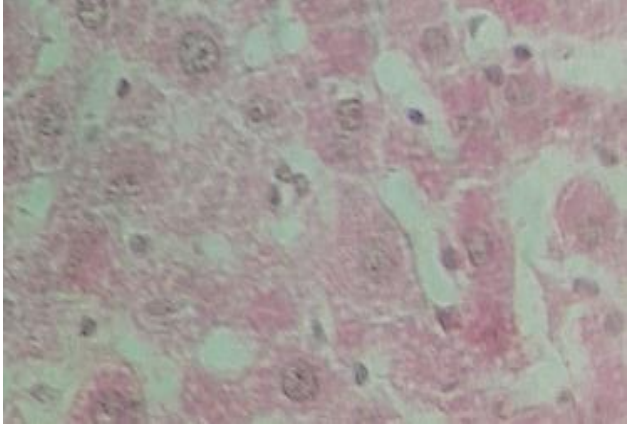


Figure 4e. Treated group (CCl_4 3 ml/kg + *Picrorhiza kurroa* 400 mg/kg) liver section revealing of spotty necrosis in 400x

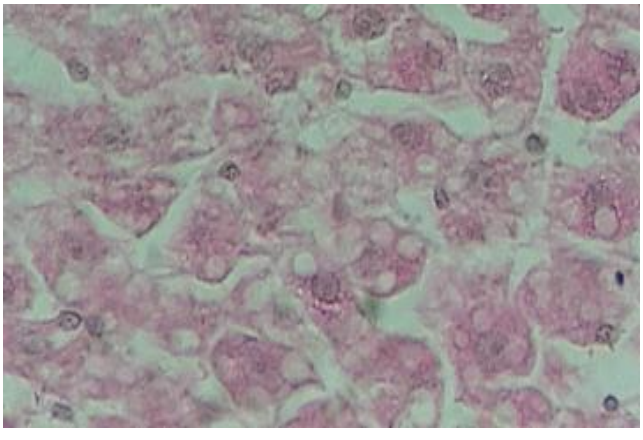
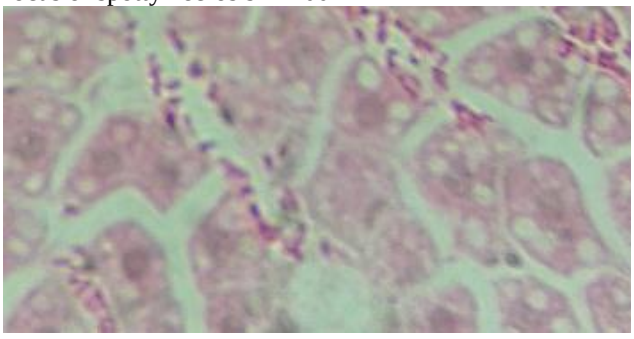


Figure 4f. Treated group (Silymarin 200 mg/kg + *Picrorhiza kurroa* 400 mg/kg) liver section revealing comparatively normal hepatic parenchyma with a single focus of spotty necrosis in 400x



Effect of aqueous extract of *Picrorhiza kurroa* on serum levels

Figure 1 showed the effect of *Picrorhiza kurroa* on AST level in serum of CCl_4 induced hepatotoxicity in male albino wistar rats. The control had shown the AST level in serum of 54.456 ± 9.522 IU/L but after CCl_4 treatment, it increased to 291.065 ± 28.219 IU/L. Whereas after administration of *Picrorhiza kurroa* at the doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg bw po in Ccl_4 intoxicated rats, the AST level reduced to 252.935 ± 28.829 IU/L, 215.908 ± 28.904 and 175.326 ± 30.367 respectively.

Effect of aqueous extract of *Picrorhiza kurroa* on serum ALT level in CCl_4 induced hepatotoxicity in albino wistar rats

Figure 2 showed the outcome of *Picrorhiza kurroa* on ALT level in serum of CCl_4 induced hepatotoxicity in male albino wistar rats. The control had shown the ALT level in serum of 24.750 ± 13.427 IU/L but after CCl_4 treatment, it increased to 141.024 ± 19.969 IU/L. Whereas after administration of *Picrorhiza kurroa* at the doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg bw po in CCl_4 intoxicated rats, the ALT level reduced to 119.047 ± 19.805 IU/L, 100.587 ± 18.393 IU/L and 93.081 ± 11.373 IU/L respectively.

Effect of aqueous extract of *Picrorhiza kurroa* on serum ALP level in CCl_4 induced hepatotoxicity in albino wistar rats

Figure 3 showed the outcome of *Picrorhiza kurroa* on ALP level in serum of CCl_4 induced hepatotoxicity in male albino wistar rats. The control had shown the ALP level in serum of 32.613 ± 2.233 IU/L but after CCl_4 treatment, it increased to 88.723 ± 14.295 IU/L. Whereas after administration of *Picrorhiza kurroa* at the doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg bw po in CCl_4 intoxicated rats, the ALP level reduced to 83.645 ± 11.179 IU/L, 74.728 ± 8.549 and 74.097 ± 5.522 respectively.

Histopathological studies provided supportive evidence for the biochemical analysis

Histopathological profile of liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein (Figure 4a). Group II animals exhibited increase in fibrous tissues and inflammatory cells around the congested blood vessel. Marked hepatocellular fatty degeneration also seen (Figure 4b). The liver sections of the rats treated with aqueous extract of *Picrorhiza kurroa* followed by CCl_4 intoxication showed a sign of protection as it was apparent by the absence of necrosis and vacuoles (Figure 4c, 4d and 4e). The liver sections of the rats treated with silymarin followed by CCl_4 intoxication showed a sign of protection as it was apparent by the absence of necrosis and vacuoles (Figure 4f).

DISCUSSION

This study was done to evaluate the hepatoprotective activity of aqueous extract of *Picrorhiza kurroa* on CCl_4 induced hepatotoxic in male albino wistar rats. Adding to this, this study was also to evaluate the hepatoprotective activity of aqueous extract of *Picrorhiza kurroa* against standard drug silymarin and determining the dose that is producing almost similar activity to silymarin.

The LD_{50} of *Picrorhiza kurroa* was found to be safe up to 2600 mg/kg in rats. Therefore, it would be safe to use this extract (*Picrorhiza kurroa*) as a hepatoprotective agent. The significant ($P < 0.01$) increase in levels of serum AST (291.065 ± 28.219), ALT (141.024 ± 19.969) and ALP (88.723 ± 14.295) confirmed the hepatotoxicity in the group of rats administered with CCl_4 as shown in table 1.

Pretreatment of group of rats with aqueous extract of *Picrorhiza kurroa* at dose level of 100 and 200 mg/kg showed significant ($P < 0.001$) dissimilar from control group, which proves at this doses the extract has hepatoprotective activity but not adequate activity by restoring at the levels of AST (291.065 ± 28.219, 252.936 ± 28.829), ALT (119.047 ± 19.805, 100.587 ± 18.393) and ALP (83.645 ± 11.179, 74.728 ± 8.549) respectively.

Groups of rats pretreated with aqueous extract of *Picrorhiza kurroa* at dose level of 400 mg/kg showed more significant ($P < 0.001$) different from CCl_4 control group proved by improvement in levels of the AST (175.326 ± 30.367), ALT (93.081 ± 11.373) and ALP

(74.097 ± 5.522) respectively. The animals treated with the silymarin (200 mg/kg) showed same significant ($P < 0.001$) decline in rise in the serum enzymes level, AST (130.186 ± 31.074), ALT (70.225 ± 18.964) and ALP (69.031 ± 13.784) in contrast to CCl_4 control group.

The aqueous extract of *Picrorhiza Kurroa* used in the study conserved the structural integrity of the hepatocellular membrane in a dose dependent manner as evident from the protection provided as compared to the enzyme levels in CCl_4 control group rats. The aqueous extract of *Picrorhiza kurroa* at dose level 400 mg/kg showed major hepatoprotection in contrast to the CCl_4 control group and silymarin pretreated group rats.

CONCLUSION

In this study, hepatoprotective activity of the aqueous extract of *Picrorhiza kurroa* was studied. The aqueous extract of *Picrorhiza kurroa* at the dose of 400 mg/kg showed very major and similar hepatoprotective activity to silymarin as recognized by significant ($P<0.001$) decline in transaminase enzyme levels and conserved the structural integrity of the hepatocellular membrane as apparent from the protection provided as compared to the CCl_4 control group rats.

Identification of natural compound of plant will help to develop new therapeutically agents. The results obtained from present study shows that this plant is a good natural source for hepatoprotective activity. The aqueous extract is showing good activity, this suggests that this plant is a cost effective natural treatment. Further clinical trials should be done in order to develop a well-known formulation that will be useful for public. Since the cost of treatment is rising, developing a cost effective remedies will definitely give a better option and opportunity to treat chronic diseases. Finally traditional remedies should be more looked into since the side effects are lesser then when compared to conventional drugs.

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