Anti-Hepatotoxic Effect of the Methanolic Anstatica Hierochuntica Extract In Ccl $_4$- Treated Rats

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Abstract

The study was investigated the hepatoprotective activity of methanolic extract of Anastatica hierochuntica using carbon tetra chloride (CCl**4**)-induced hepatotoxicity in rats. The levels of liver enzymes glutamate oxaloacetate transminase , glutamate pyruvate transminase ,alkaline phosphatase ,total Protein, total and direct bilirubin, in addition to Superoxide dismutase, glutathione peroxidase activities ,glutathione malondialdehyde and total antioxidant status levels were evaluated in experimental rats (with or without CCl**4**-inducedhepatotoxicity)following intake of 100 mg/kg p.o alcoholic *Anastatica hierochuntica* extract by using standard procedures compared with standard silymarin at a dose of 100 mg/kg p.o. Results showed that methanolic extract at a dose level of 100 mg/kg had a significant decrease($p<0.05$) in activities of serum liver enzymes, glutamate oxaloacetate transminase , glutamate pyruvate transminase, alkaline phosphatase, total bilirubin and protein were significantly decreased in rats treated with CCl**4** after 4 weeks compared to that of silymarin group in addition to the methanolic extract had antioxidant activity through decreasing activities of superoxide dismutase and glutathione peroxidase to levels in control rats group. Histopathology of a liver tissue of the animals treated with the extract was also studied to monitor the liver status. The liver biopsy of all experimental rat groups treated with the methanolic *Anastatica hierochuntica* extract showed significant restoration of the normal histomorphologic pattern of liver cells. From the above results, it is concluded for the first time that methanolic *Anastatica hierochuntica* extract offers protective effect against CCl**4**-induced hepatotoxicity in experimental rats.

تأثيرالوقائي للكبد للمستخلص الكحولي لنبات كف مريم في الفئران المعاملة برابع كلوريد الكربون

الخلاصة

تضمنت الدراسة تاثير المستخلص الكحولي لنبات كف مريم لمعالجة تسمم الكبد المستحدث في الفئران المعاملة برابع كلوريد الكربون.تم قياس مستويات انزيمات الكبد الناقلة لمجموعة الأمين الأسبارتية ،والناقلة لمجموعة الأمين الألنينية ، وانزيم الفوسفاتيز القاعدي و البروتين الكلي والبيليروبين الكلي والمباشر,اضافة الى مستويات الكلوتاثايون, المالونلديهايد, الكلوتاثايون بيروكسيديز,السوبردايميوتيز وحالة مضادات الاكسدة الكلية في الفئران المعاملة وغير المعاملة برابع كلوريد الكربون بعد تناولها جرعة 100 ملغم / كغم من وزن الفار من المستخلص الكحولي لنبات كف مريم ومقارنتها مع مستوى السيليمارين القياسي بجرعة 100 ملغم / كغم من وزن الفار .اظهرت النتائج ان المستخلص الكحولي للنبات له تاثير معنوي$P<0.05$ على خفض مستويات

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انزيمات الكبد الناقلة لمجموعة الأمين الأسبارتية ، و الأمين الألنينية ، وانزيم الفوسفاتيز القاعدي والبليروبين عند الفئران المعاملة برابع كلوريد الكربون بعد مرور اربعة اسابيع مقارنة بالمجموعة غير المعالجة الى مستويات مقاربة لمجموعة السيطرة. كذلك لوحظ امتلاك المستخلص فعالية مضادة للاكسدة من خلال تاثيره على خفض مستويات انزيمات السوبر دايميوتيز و انزيم الكلوتاثايون بيروكسديز حيث لوحظ ارجاع مستويات هذه الانزيمات الى مستويات مقاربة الفئران الاصحاء التي كانت مماثلة لتلك التي تناولت السيليمارين. وأظهرت عينة من نسيج الكبد لجميع المجموعات التجريبية للفئران المعاملة بجرعة من 100 ملغم / كغم من وزن الفار بالمستخلص الكحولي لنبات كف مريم عملية ترميم كبيرة من النمط الطبيعي لخلايا الكبد. من النتائج أعلاه ، نستنتج ان المستخلص الكحولي لنبات كف مريم يمتلك تأثير وقائي ضد تسمم الكبد المستحدث برابع كلوريد الكربون.

Introduction

The liver is a major detoxifying organ in vertebrate body, which involves intense metabolic activities. Certain toxic chemicals and medicines can cause liver damage, which has been recognized as a toxicological problem. Amp experimental and epidemiological studies support the involvement of oxidative stress in the pathogenesis and progression of several chronic diseases (1).Oxygen, indispensable for maintaining life, sometimes becomes toxic, resulting in the generation of most aggressive agents such as reactive oxygen species (ROS). Aerobic organisms employ a battery of defense mechanisms such as antioxidant enzymes superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) to prevent or mitigate oxidative tissue damage (2). SOD removes the superoxide radical to prevent formation of the hydroxyl radical. Catalase deals effectively with a large amount of hydrogen peroxide generated in peroxisomes. GPx is capable not only of utilizing hydroperoxides but also of metabolizing hydrogen peroxide in both the cytosolic and mitochondrial compartments. When the liver cell plasma membrane is damaged, many of the enzymes normally located in the liver cell cytosol are released into the blood stream. Their estimation in the blood is a useful quantitative marker of the extent and type of hepatocellular damage (3). Perturbation of the GSH status of a biological system has been reported to increase the lipid peroxidation (4).

Thiobarbituric acid reactive substances (TBARS) are produced as byproducts of lipid peroxidation that occurs in the hydrophobic core of biomembranes (5).At other sites, intake of compounds that induce

antioxidant enzyme activity or scavenging of free radicals prevents oxidative damage (6). However, herbal medicines are known to play an important role in the treatment of various ailments, including hepatopathy (7). Many traditional practitioners have claimed that numerous medicinal plants and their formulations can be effectively used for the alleviation of different types of liver diseases (8). But most claims are anecdotal and very few have received adequate medical and scientific evaluation. *Anastatica hierochuntica* was widely used as medicinal plant either by itself or in combination with other herbs. The whole plants of Anastatica hierochuntica is commonly called “Kaff maryam” or “Rose of Jericho”, which is a winter annual plant of the Sahara-Arabian deserts, was prescribed in Egyptian folk

medicine and used as a charm for child birth (9). The present investigation was carried out to examine whether the methanolic extract might also have a protective effect against carbon tetrachloride (CCl**4**) induced hepatotoxicity *in vivo*. The exact mechanism of hepatotoxicity of CCl**4** is unclear, most probably resulting from a toxic intermediary that binds covalently to hepatocytes and causes a centrilobular hepatic necrosis. It has been established that CCl**4** is accumulated in hepatic parenchyma cells and metabolically activated by cytochrome P-450 dependent monoxygenases to form a trichloromethyl free radical (CCl**3.**).The latter alkylates cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids in the presence of oxygen to produce lipid peroxides leading to liver damage (10).

Materials and Methods

Reagents.

Reagents for antioxidant enzyme assays were purchased from Sigma Chem. Co. (USA). Kits RANSEL (RS504), RANSOD (SD125) and TAS (N 2332) for determination of the activity of glutathione peroxidase and superoxide dismutase as well as total antioxidative status (TAS), respectively, were purchased from Randox Laboratories Ltd. (UK). Sylimarin as the reference hepatoprotective drug was obtained from Extrasynthese (France). All other chemicals were of reagent grade and were used without further purification**.**

Plant materials

Samples of *Anastatica hierochuntica* plant were purchased from Iraqi local market in Baghdad. The plant material was authenticated by a taxonomist professor Ali Al-Mosowi at the Department of Botany College of science, University of Baghdad, Iraq. The samples were washed with clean tap water to remove dirt on the leaves. The dried plant material was manually powdered and the powder kept in polyethylene bags until used.

Preparation of methanolic extract

The powdered whole plant (100 g) kept in a thimble was extracted with 200 ml 70% methanol in a soxhlet extractor. The extract was concentrated *in vacuum* at 60^0 C using a rotary evaporator. To evaporate the remaining solvent, the extract was kept in an oven at a temperature of 40-50 ^{o}C for 8 hours. The alcoholic extract (250 mg/kg) was formulated as suspension in 1% w/v tracaganth gum and used for the study. Silymarin was used as a standard drug.

Animals

Male Wister strain albino rats (40) weighing 150 – 200 g and they were maintained under standard environmental condition (temperature 25 – 28 C and 12 hr light/dark cycle) and allowed access to standard laboratory feed and water *ad libitum*. The rats were allowed to acclimatize to the laboratory condition for a week before they were used for the experiment. Ethical approval for the use of the animals was obtained from the institutional committee constituted for the purpose.

Acute toxicity studies

Acute oral toxicity was performed according to Ecobichon DJ (11). The male albino rats were fasted over night provided only water, after which the alcoholic extract of *Anastatica hierochuntica* was administered by

gastric intubation to the relevant animals orally at the dose of 5 mg/kg body weight. The animals were then observed for 14 days. When mortality was observed in 2 or 3 animals, the dose administered was recorded as a toxic dose. But when mortality was observed in one animal, then the same dose was repeated again for confirmation. However, if mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2,000 mg/kg body weight. Toxic symptoms for which the animals were observed for 72 hr include some behavioral changes, locomotion, convulsions and mortality.

Hepatoprotective activity

Hepatotoxicity was evaluated as previously described (12). A total of 40 albino rats were divide 4 groups of 10 animals each and treated as follows: ***Group I (Control):*** received subcutaneous administration of 1%w/v of gum tracaganth at the dose of 1ml/kg /day per oral for 14 days. ***Group II:*** received subcutaneous administration carbon tetra chloride (CCl**4** at a dose of 0.1 ml/kg/day) of body weight i.p for 10 days. ***Group III (test):*** served as test and received alcoholic extract of *Anastatica hierochuntica* (100mg/kg p.o) daily for 14 days along with CCl**4** subcutaneous for 10 days. ***Group IV (standard):*** received silymarin (100 mg/kg) per oral for 14 days along with CCl**4** subcutaneously for 10 days. At the end of the 14th day, the blood was collected from all the animals from the rectero orbital plexus and the serum was separated by centrifugation at 2000 rpm for 10 min. The serum was then assayed for hepatic marker enzymes, serum glutamate oxaloacetate transminase (SGOT), serum glutamate pyruvate transminase (SGPT), alkaline phosphatase (ALP), total and direct bilirubin and total protein, glutathione (GSH), lipid peroxidation marker such as malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx) and finally total antioxidant status (TAS).

Assessment of biochemical parameters

The estimation of S.GOT and S.GPT was based on the reference method described in International Federation of Clinical Chemistry (13)**.** The reagent supplied in the kits (Randox Diagnostic Kits) were reconstituted and mixed with the serum. S.GOT and S.GPT were measured at 340 nm and expressed as IU/L. Serum ALP was estimated by mixing the reagent (*p*-nitrophenyl phosphate, magnesium, buffers and stabilizers) with the serum and measuring the absorbance at 405 nm (14). The value obtained was expressed as IU/L. Total protein was measured according to the method of Bradford using bovine serum albumin as a standard (15). The absorbance of the solution was then measured at 555 nm and the estimated total protein was expressed as gm/dl. Total and direct bilirubin were estimated by the method of Jandrassik and Grof at 546 nm and expressed as mg/dl (16). The activities of hepatic marker enzymes (GPx and SOD) and biochemical parameter TAS were assayed in blood using standard kits: RANSEL kit (17), RANSOD kit (18) and TAS kit (19) for determination of the activity of glutathione peroxidase and superoxide dismutase as well as total antioxidative status (TAS) respectively. Colorimetric estimation of reduced glutathione (GSH) in blood was performed as described by Beutler (20). The quantitative

measurement of lipid peroxidation was done by measuring the concentration of thiobarbituric acid reactive substances (TBARS) in serum using the method of Ohkawa *et al*. (21). The amount of malondialdehyde (MDA) formed was quantitated by the reaction with thiobarbituric acid and used as an index of lipid peroxidation. The results were expressed as nmol MDA/ mg protein. Silymarin as the reference hepatoprotective drug was obtained from Extrasynthese (France). All other chemicals were of reagent grade and were used without further purification.

Histopathological examination

Animals were sacrificed by cervical dislocation and the blood was collected from the rectero-orbital plexus. The liver was removed, sliced and washed in saline and the pieces were preserved in 10% formasal (10% formaldehyde diluted with normal saline)to evaluate histopathological changes. Sections of pieces of the liver (about 4-6 mm in thickness) were processed and embedded in paraffin wax, stained with haematoxylin and eosin, mounted and observed under light microscope for histological changes (22).

Data analysis

As appropriate, measurements were carried out in triplicates and descriptive statistics (Mean ± SD) were used in presentation of the results. Data comparison was carried out using one way analysis of variance (ANOVA). At 95% confidence interval, 2-Tailed p-values less than 0.05 were considered to be significant.

Results

The marker liver enzyme levels are provided in the Table 1 .There was significant increase in the levels of the marker enzymes (S.GOT, S.GPT and S.ALP) as well as total and direct bilirubin in the animals treated with CCl**4** when compared with the control animals. For the animals given the extract (100 mg/kg), the levels of these enzymes and bilirubin were relatively normal when compared with CCl**4** treated group ($p<0.05$).The serum total protein concentration of the CCl**4** treated group was significantly decreased to 3.1 ± 0.3 g/dl Vs. 5.5 ± 0.2 gm/dl in control animal group but administration of alcoholic plant extract increased the levels of total protein to 4.7±0.2gm /dl.The results in table 2 indicate that 14 days after CCl**4** administration, there was a significant increase in blood GPx (62.72 ± 9.5 U/mL) compared to the healthy group (35.15 ± 6.35U/ml). In the groups where sylimarin was given, the levels of GPx (34 ± 2.43 U/ml) were significantly lower ($p < 0.05$) than in the CCl**4**-treated group (62.72±9.5 U/ml).The levels of SOD (140 ± 13U/ ml) and TAS (0.38 ± 0.24mmol / L) were significantly reduced after intoxication with CCl**4** ($p < 0.05$). Acute exposure to a daily single intraperitoneal dose of CCl**4** for 10 days resulted in a severe decrease of GSH content in blood (38 ± 6.5 µmol/L) to (120 ± 16 µmol/L) of normal values. The depletion of GSH by CCl**4** was associated with an increase in lipid peroxidation as measured by the level of TBARS (2.53 ± 0.23*mmol MDA/ mg protein). Intake of sylimarin or *Anastatica hierochuntica* extract for 7 consecutive days afforded different degrees of protection against such depletion .The most significant protection effect of plant extract was found at the levels of SOD and GPx (from 140 ± 13 to 430 ± 43 U/ mL),

for SOD and (from (62.72 ± 9.5 U/mL to (36.76 ± 2.30 U/mL for GPx ,TAS (from 0. 38 ± 0. 24 to 0.62 ± 0.18 mmol/ L and MDA (from 6.53 ± 0.23 to 3.22 ± 0.35 nmol MDA mg/ protein (Table 2).The effect of *Anastatica hierochuntica* extract was quite close to that of sylimarin on the level of MDA bases and the TAS value. This protective effect of *Anastatica hierochuntica* methanolic extract was confirmed by histological examination as shown in Figure 1.

Histological examination of animals in group I showed a normal hepatic architecture (Figure 1a). Animals in group II (CCl**4** treated) demonstrated severe hepatotoxicity as evidenced by profound steatosis, centrilobular necrosis and ballooning degeneration, Massive fatty changes, gross necrosis, broad infiltration of lymphocytes and of Kupffer cells around the central vein and loss of cellular boundary (Figure 1b). In group III and IV, the animal livers exhibited an almost normal architecture barring a little deformation of hepatocytes with pyknosis and clearing of cytoplasm (Figure 1c & 1d).

Discussion

Liver diseases remain as one of the serious health problems. However we do not have satisfactory liver protective drugs in allopathic medical practice for serious liver disorders. Herbal drugs play a role in the management of various liver disorders in addition to other natural healing processes of the liver (23).Previous studies have demonstrated the use of carbon tetrachloride to successfully induce hepatotoxicity in experimental animals (24). In experimental hepatopathy, the toxin carbon tetrachloride is biotransformed by cytochrome P-450 to produce the trichloromethyl free radical, which causes peroxidative degradation in the adipose tissue resulting in fatty infiltration of the hepatocytes. Trichloromethyl free radicals elicit lipid peroxidation of membrane lipids in the presence of oxygen generated by metabolic leakage from mitochondria. All these events culminate in loss of integrity of the cell membranes and damage of hepatic tissue (22). Histopathological examination of liver section of normal rats showed normal hepatic cells with cytoplasm and nucleus whereas CCl**4** treated group showed that the liver cells are intoxicated with CCl**4** and the normal architecture of the liver was completely damaged. The treatment of the rats with methanolic plant extract exhibited protection against liver damage by CCl**4** which is confirmed by the results of biochemical studies. The increase in the levels of transaminase reflects a clear indication of cellular leakage and loss of functional integrity of the cell membrane (26). Assessment of liver function can be made by estimating the activities of serum GOT and GPT, which are originally present in higher concentrations in cytoplasm. In hepatopathy, these enzymes leak into blood stream in conformity with the extent of liver damage (27). The elevated levels of marker enzymes (SGOT, SGPT, ALP), T.Bilrubin in treated CCl**4** rats in the present study corresponded to the extensive liver damage. A reduction in total serum protein (TSP) (Table 1) observed in the CCl**4** treated rats may be associated with the decrease in the number of hepatocytes which in turn may result into decreased hepatic capacity to synthesize protein and consequently decrease in the liver weight (Table 1). But, when the methanolic plant

extract was given along with CCl**4**, the significant increase in TSP was observed indicating the hepato protection activity of extract and also accounting for the increase in the liver weight most probably through the hepatic cell regeneration. Treatment with the 100mg/kg methanolic plant extract of the plant significantly reduced the elevated liver enzymes and bilirubin level, indicating hepatoprotective action. Hepatotoxic effect of CCl**4** is due to oxidative damage by free radical generation and antioxidant property is claimed to be one of the mechanisms of hepatoprotective (28).In our work, the CCl**4**-mediated hepatotoxicity was taken as the experimental model for liver injury. By estimating the activities of blood marker enzymes (GPx and SOD) and other biochemical parameters (TAS, GSH and MDA), an assessment of the liver function can be made. The changed activities of these liver marker enzymes observed in CCl**4**-treated rats in our study correspond to the extent of liver damage induced by the toxin.

The tendency of these enzymes to return towards a near normal level in groups treated with sylimarin or *Anastatica hierochuntica* methanolic extract is a clear manifestation of their anti-hepatotoxic effect. Decline in GSH content in the serum of CCl**4**-intoxicated rats, and its subsequent return towards near normally in sylimarin and in *Anastatica hierochuntica* extract treated rats reveal the antioxidant effect of the plant. Explanation of the possible mechanism underlying the hepatoprotective properties of the *Anastatica hierochuntica extract* includes the prevention of GSH depletion and destruction of free radicals (28). These two factors are believed to attribute to the hepatoprotective properties of *Anastatica hierochuntica.* Elevated levels of MDA observed in CCl**4**-treated rats indicate excessive formation of free radicals and activation of the lipid peroxidation system, resulting in hepatic damage. The significant decline in the concentration of these constituents in the liver homogenate of sylimarin and *Anastatica hierochuntica* extract administered rats indicates anti-lipid peroxidative effect of Anastatica hierochuntica. In order to provide a better understanding of the possible role of the methanolic extract of Anastatica hierochuntica in the hepatoprotective effect observed in this study, we carried out a preliminary Phytochemical screening of the extract of the plant and found it to contain flavonoids and glycosides. Earlier report indicated that the flavonoids are phenolic compounds exert multiple biological effects, including antioxidant properties and free radical scavenging abilities (9). Over expression of fibrogenic cytokines as well as increased transcription and synthesis of collagen can be down regulated, at least in experimental models by the use of antioxidants and a study has demonstrated that natural phenolics inhibit satellite cell activation by perturbing signal transduction pathway and cell protein expression. The co- administration of hepatoprotective agents may induce the hepatocytes to resist the toxic effects of carbon tetrachloride. Therefore, the protective activity of the extract may be due to its antioxidant property exerted by flavonoids in this plant.

References

[1] Sunita,T.,Vupta V and Sandeep B. (2008).Comparative study of antioxidant potential of tea with and without additives, *Indian. J. Physiol. Pharmacol.* 44**:** 215–219.

[2] Halliwell B. and. Gutteridge J. M. C(1989).*Free Radicals in Biology and Medicine*, 2nd ed., Oxford University Press, Oxford, pp. 78–93.

[3] Mitra S. K. Venkataranganna, M. V. Sundaram R(2007) . Protective effect of HD03, a herbal formulation, against various hepatotoxic agents in rats, *J.Ethnopharmacology*. 63**:** 181–186.

[4] Uday, B. Das D.and Banerjee, K. R. (2002).Reactive oxygen species: Oxidative damage and lipids and its Role in athrogenesis,*Curr. Sci*. **77:** 658–666.

[5] Fraga, C. Leibovitz B. and Tappel A. (1987). Halogenated compounds as inducers of lipid peroxidation in tissue slices, *Free Rad. Biol. Med*. 3**:** 119–123.

[6] Hochstein P. And Atallah A. S. (1988) .The nature of oxidants and antioxidant systems in the inhibition of mutation and cancer, *Mutation Res*. 202**:** 363–375.

[7]Venukumar MR, Latha MS (2002).Hepatoprotective effect of the methanolic extract of *Curculigo orchioides* in CCl 4-treated rats. *Indian J Pharmacol*.; 34:269-275**.**

[8] Dash DK, Yeligar VC, Nayak SS, Ghosh T, Rajalingam D, Sengupta P, Maiti BC, Maity TK (2007).Evaluation of hepatoprotective and antioxidant activity of Ichnocarpus frutescens (Linn.) R.Br. on paracetamol-induced hepatotoxicity in rats. Trop J Pharm Res.; 6(3): 755-765.

[9]Amal A. Mohamed, Ashraf A. Khalil, and Hossam E. Beltagi (2010).Antioxidant and antimicrobial properties of kaff maryam *(Anastatica hierochuntica*) and doum palm *(Hyphaene thebaica)* ENERO-MARZO, 61 :67-75.

[10] Recnagel RO, Glende EA, Jr Dolak JA, Walter RL (1989). Mechanism of carbon tetra chloride toxicity. Pharmacol Ther, 43:139-54.

[11] Ecobichon DJ (ed). The basis of toxicology testing. 2nd ed. CRC Press, New York. 1997, 43-60.

[12] Jaiprakash B, Aland R, Karadi RV, Savadi RV (2003). Hepatoprotective activity of fruit pulp of *Balanites aegyptiaca. Indian Drugs*, 40: 296-297.

[13]Schwartz M.K, de Cecile N, Curnow DH, Fraser CG, Porter CJ, Worth HG, inder O(1985). International Federation of Clinical Chemistry, Education Committee and Union of Pure and Applied Chemistry, Division of Clinical Chemistry: Definition of the terms certification, licensure and accreditation in clinical chemistry. J Clin Chem Clin Biochem. 23(12): 899-901.

[14]Kind, P.R.N. and E.J. King, (1954).Estimation of plasma phosphatase by determination of hydrolyzed phenol with amino antipyrine. J. Clin. Pathol, 7: 322.

[15] Bradford, M.M., (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal.
Biochem, 72: 248-254.

[16] Jandrassik L, Grof P. (1938).Quantitative determination of total and direct bilirubin in serum and plasma. Biochem Z.; 297: 81-89

[17] Uzel, N., Sivas, A. and Uysal, M. (1987). Erythrocyte lipid glutathione peroxidase. J. Lab Clin. Med, 70: 158-169.

[18]Nishikimi M, Appaji N, Yagi K(1972).The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun, 46:849-53.

[20] Dury R. and Wallington E. (1980). Carleton Histological Technique (5 editions PP 57-150), New York: Oxford University Press.

21-Beutler, E., Duran, and B.M. Kelly, (1963).Improved method for the determination of blood glutathione. J. Lab. Clin. Med., 61: 882.

[22]Ohkawa, H., N. Onishi and K. Yagi, (1979). Assay of lipid peroxidation in animal tissue by thiobarbituric acid reaction. Anal. Biochem., 95: 351–354.

[23]Subramoniam A, Evans DA, RajasekaranSP(1998). Hepatoprotctive activity of Trichopus zeylanicus extract against paracetamol induced damage in rats. Ind J Expt Biol,36: 385-389.

[24]Okuno H, Hazama H, Muraze T, Shiozaki Someshima YT(1986).Drug metabolizing activity in rats with chronic liver injury, induced by carbon tetra chloride relationship with hydroxyproline content. Japan J Pharmacol, 41:363-371.

[25] Recnagel RO, Glende EA, Jr Dolak JA, Walter RL(1989).Mechanism of CCl_4 toxicity.Pharmacol Ther, 43:139-54.

[26] Saraswat B, Visen PK, Patnaik GK, Dhawan BN.(2003).

[19]Hinesburg I, Dorman HJD, Hiltunen R (2006). Antioxidant activities of extracts from selected culinary herbs and spices. Food Chem, 97:122-9.

Anticholestic picroliv, active hepatoprotective principle of Picrorhiza kurrooa, against carbon tertrachloride induced cholestatis. Indian J Exp Biol, 31: 316-318.

[27]Venkataranganna MV,Sundaram R, Gopu madhavan S.(1998).Protective effect of HD-06 an herbal formulation, against various hepatotoxic agents in rats. J EthnoPharmacol, 63: 181-186.

[28]Hewawasam RP, Jayatilaka KAPW,PathiranaC,MudduwaLK(2004) Protective effect of Epaltes divaricata extract on carbon tetrachloride induced hepatotoxicity in mice. Indian J Med Res, 120: 30-33.

Table 1: Effect of oral administration of methanolic *Anastatica hierochuntica* extract on serum biochemical liver marker in CCl4 intoxicated albino rats.

Groups	n	Liver function markers				
		GOT(IU/L)	GPT(IU/L)	ALP(IU/L)	T.protein gm/dl	T.Bilrubin (mg/dl)
Vehicle control	10	60.2± 0.2	62.34± 3.11	195.7±15.4	5.5± 0.3	0.64± 0.03
CCl4-treated	10	130.6± 4.3*	155.61±4.0**	315.2±14.0**	3.1± 0.3	0.97± 0.05
Alcoholic extract (100m g/kg)	10	68.4± 0.54**	71.57± 0.71***	200.4±12.3**	4.70 ±0.2*	0.63± .06*
Silymarin (100 mg/kg)	10	65.8± 3.1**	70.9± 4.2***	196.2±13.6**	5.4 ±0.20*	0.64 ±0.02*

Table 2: Effect of oral administration of methanolic *Anastatica hierochuntica* extract on serum antioxidant enzymes (SOD and GPx), Glutathione, malondialdehyde, and total antioxidant status in CCl4 intoxicated albino rats.

Groups	n	GPX U/ml	SOD U/ml	GSH µmol/L	TAS (mmol /L)	MDA mmol /mg protein
Vehicle control	10	35.15 ±6.35	452 ± 20	120 ± 16	1.23 ± 0.13	3.83 ± 0.28
CCl4-treated	10	62.72 ± 9.5	140 ± 13	38 ± 6.5	0. 38 ± 0. 24	6.53 ± 0.23*
Alcoholic extract (100 mg/kg)	10	36.76 ± 2.3**	430 ±43***	98 ± 7.1**	0.62 ± 0.18*	3.22 ± 0.35*
Silymarin (100 mg/kg)	10	34 ± 2.43**	398± 27***	100 ± 6.4**	0.64 ± 0.09*	3.32 ± 0.25*

*** Statistically significant difference *vs*. CCl4 group ($p < 0.05$).GPx – glutathione peroxidase, SOD – superoxide dismutase, TAS – total antioxidative status, GSH – reduced glutathione.**

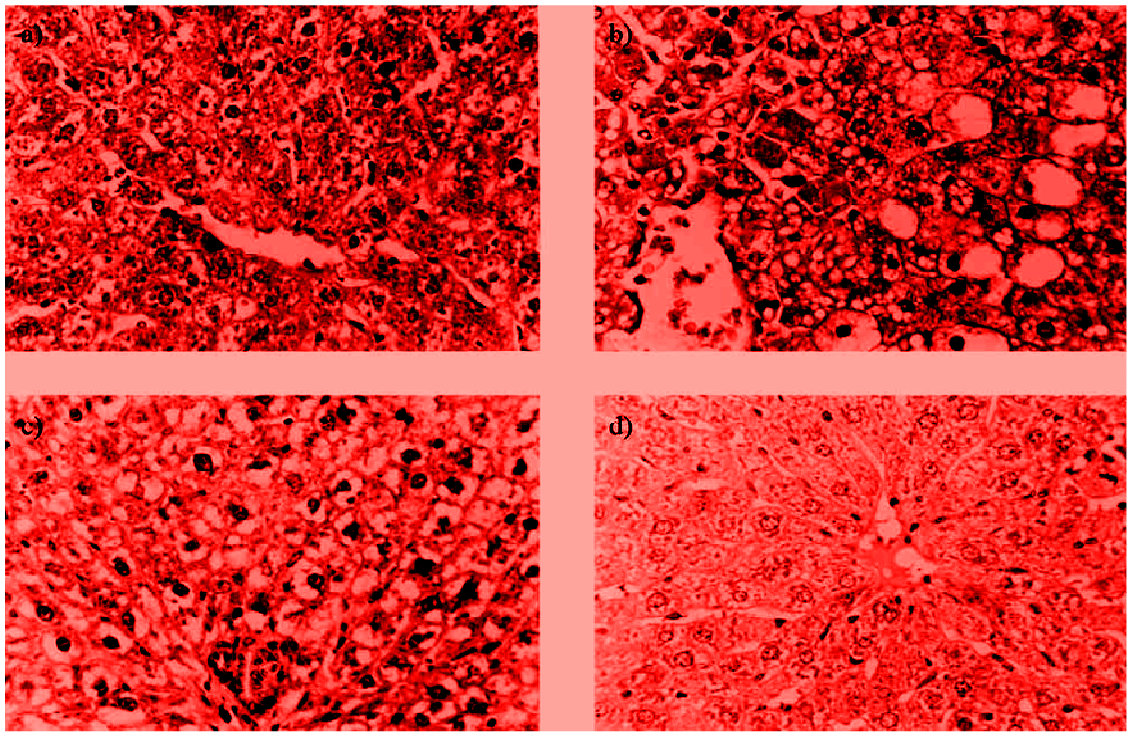

Figure 1: Histological section of rat liver. Groups of rats: a) group 1, control (gum tracaganth for 14 days); b) group 2, CCl_4 for the 10th day); c) group 3, *Anastatica hierochuntica* extract (100 mg /kg for 14 days plus CCl_4, as above on the 10th day).d group 4 sylimarin (100 mg/ kg for 14days plus CCl_4,as above on the 10th day).