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Hepatoprotective and Antioxidant Activity of Methanol Extract of *Hedychium spicatum* against CCl_4-Induced Liver Injury in Rats.

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ABSTRACT

The present study investigated the antioxidant and hepatoprotective activity of methanolic rhizome extract of *Hedychium Spicatum* (MEHS) in CCl_4-induced hepatotoxicity model in rats. The hepatoprotective and *in vivo* antioxidant activity of methanolic rhizome extract of *Hedychium Spicatum* were evaluated against CCl_4-induced hepatic damage in rats. The MEHS at dose of 100, 200 and 400 mg/kg were administered orally once daily for seven days. Serum enzymatic levels of serum glutamate oxaloacetate transaminase (AST), serum glutamate pyruvate transaminase (ALT), serum alkaline phosphatase (ALP) and total bilirubin were estimated along with estimation of superoxide dismutase (SOD) and malondialdehyde (MDA) levels in liver tissues. Further histopathological examination of the liver sections was carried out to support the induction of hepatotoxicity and hepatoprotective efficacy. The extract revealed significant activities and substantially elevated serum enzymatic levels of AST, ALT, ALP and total bilirubin were found to be restored towards normalization significantly by the MEHS in a dose dependent manner with maximum hepatoprotection at 400 mg/kg dose level. The histopathological results also supported the biochemical evidences of hepatoprotection. Elevated level of superoxide dismutase (SOD) and decreased level of malondialdehyde (MDA) further strengthen the hepatoprotective observations. The results of the present study strongly revealed that MEHS have potent antioxidant activity and hepatoprotective activity against CCl_4-induced hepatic damage in experimental animals.

Keywords: hepatoprotective, antioxidant, carbon tetrachloride, *Hedychium Spicatum,* Silymarin

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INTRODUCTION

Hedychium spicatum Ham. ex Smith. (Zingiberaceae) is an Ayurvedic traditional medicinal plant known as Spiked Ginger Lily (English), Kapuurkachari (Unani), Poolankizangu, Kichilikizangu (Siddha/Tamil), and Ban-haldi (Kumaon). In Ayurveda, it is also denoted with various names such as Shathi, Shati, Gandhashathi, Gandhapalaashi, Kapuurkachari, Suvrataa, Gandhaarikaa, Gandhavadhuu, Gandhamuulikaa. The plant is found in Central Himalaya at 1100 – 2500 m, East India and hills of South India. The Rhizome of the plant is carminative, spasmolytic, hepatoprotective, anti-inflammatory, antiemetic, antidiarrhoeal, analgesic, expectorant, antiasthmatic, emmenagogue, hypoglycaemic, hypotensive, antimicrobial, anthelmintic, insectrepellent. The rhizome shows hypotensive effect in dogs at low doses, lowers blood pressure in high doses. The ethanol (50%) extract is also found to be anti-inflammatory and hypoglycaemic; gave encouraging results in tropical pulmonary eosinophilia in clinical studies. Alcoholic extract of the plant demonstrated vasodilator, mild hypotensive and antiseptic in animals. Essential oil from rhizome is mild tranquilizer in male albino rats. Rhizome gave sitosterol and its glucoside, a furanoid diterpene - hedychenone and 7-hydroxyhedychenone. The essential oil contains cineole, gamma-terpinene, limonene, betaphellandrene, *p*-cymene, linalool and beta-terpineol as major constituents. The oil inhibits the growth of several fungi. The ethanol (95%) extract showed antibacterial activity. The 50% extract showed antimalarial activity *in vitro* against *Plasmodium berghei* strain.

The liver demonstrates a major role in metabolism of xenobiotics by regulating the synthesis, secretion and metabolism of xenobiotics. Various physiochemical functions of the body including oxidation, reduction, hydroxylation, hydrolysis, conjugation, sulfation, acetylation etc. are well balanced by the liver alone. Injury to liver and damage to the hepatic parenchyma are always proved to be associated with distortion of different metabolic functions of liver (Wolf, 1999). Etiologically various infectious agents including viruses and different hepatotoxic chemicals along with environmental pollutants are thought to be responsible for different type of liver damage and hepatic injury. Recent research in free radical biology also suggested the pathophysiological role of free radicals and oxidative stress in liver damage and injury. Revealing the mechanism of actions of potent hepatotoxin such as CCl_4, paracetamol etc. also indicated the role of oxidative stress and free radicals in the pathophysiology of hepatic injury (Oh et al., 2001).

The free radicals normally generated during the normal body metabolic pathways and also they can be acquired from the environment also. Free radicals contain unpaired electrons. The oxygen radicals, such as superoxide radical ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$) and non free radical species, such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^{\bullet}O_2$), are generated in many redox processes of normal physiochemical pathways (Gülçin et al., 2002). Antioxidant defense system comprising different enzymes such as superoxide dismutase, catalase and glutathione peroxidise etc. trap and destroy these free radicals. Vitamin deficiency together with overproduction of free radicals and a reduced level of above mentioned enzymes, is considered as the main culprit for producing oxidative stress (Ellnain-Wojtaszek et al., 2003).

Research on oxidants and antioxidants over the past few years has shown a link between most diseases like cardiovascular diseases, cancer, osteoporosis, degenerative diseases etc and production of reactive oxygen species (ROS) along with oxidative stress.(Halliwell and Gutleridge, 1984) Free radicals mainly act by attacking the unsaturated fatty acids in the biomembranes which causes membrane lipid peroxidation (a hallmark sign of hepatotoxicity), decrease in membrane fluidity and reduction of enzyme and receptor activity and damage to membrane protein which finally triggers the cell inactivation and death.(Dhuley et al., 1993) Therefore, antioxidants can be used to reverse the harmful and pathological action of free radicals. These antioxidants generally restore the normal physiological system by scavenging the free radicals. The antioxidants in use are either derived naturally from plants or synthetically. Due to carcinogenic probability, synthetic antioxidants are not the preferred type of antioxidants.(Ito and Hirose, 1989) Current research in the field of free radical biology therefore accentuates the use of antioxidants from natural origin and in view of this more and more antioxidants of natural origin are being investigated.

CCl_4 is one of the most common hepatotoxin used for experimental induction of liver injury in animal studies (Johnston and Kroening, 1998). Impoverishment of modern system of medicine in terms of a reliable liver protective drug switched on the exploration of traditional systems of medicine including Ayurveda, Siddha, Unani etc. for a probable answer to hepatotoxicity (Subramoniam and Pushpangadan, 1999). Numerous medicinal plants are being researched for a effective hepatoprotective remedy. A number of medicinal preparations in the Indian system of medicine (Ayurveda) have been used as effective hepatoprotective. In view of this several medicinal preparations and a number of medicinal plants mentioned in Ayurveda for treatment of liver disorders are being investigated (Chatterjee, 2000). Moreover traditional folklore and indigenous knowledge of medicinal uses of plants are also now being explored and documented for possible bioactive molecules to be future drugs.

Therefore in this present study an attempt has been made to evaluate the hepatoprotective activity as well as antioxidant activity of *Hedychium Spicatum* rhizome extract with a view towards elucidating the probable mechanism of action.

MATERIALS AND METHODS

Chemistry

Malondialdehyde (MDA) was obtained from Sigma Chemicals Company, St Louis, MO, USA. Silymarin was obtained from Ranbaxy Laboratories, Delhi, India. CCl_4 was obtained from E Merck, Mumbai, India. All other reagents and chemicals used in the experiments were of analytical grade and available commercially via reputed vendors.

Preparation of plant extract

The rhizomes of *Hedychium Spicatum* were collected from Dhankurali village, Jakholi block, Rudraprayag district, India, during the months October and November and authenticated

by Dr. R. L. Painuli, Department of Botany, HNB Garhwal University, Srinagar Garhwal, Uttarakhand (Specimen voucher no. GUH3908). The rhizomes of *Hedychium Spicatum* were dried in shade and coarsely powdered. Coarsely powdered rhizomes (5 kg) was successively extracted in the Soxhlet apparatus using petroleum ether, chloroform, ethyl acetate, methanol and water as solvent for the complete extraction of the phytochemicals. The five extracts were dried in rotary evaporator at 45 °C and the dried extracts were stored in vacuum desiccators containing anhydrous silica gel. All the five extracts were subjected to acute toxicity studies as per the OECD guidelines.

Acute toxicity studies

An acute oral toxicity study was performed according to the OECD guidelines for the testing of chemicals, Test No. 423 (OECD, 2001; Acute oral toxicity-Acute toxic class method). Wistar rats (n = 3) of either sex were selected by a random sampling technique for the acute toxicity study. The animals were fasted overnight prior to the experiment and maintained under standard laboratory conditions. Each extract was administrated orally in increasing dose up to 2000 mg/kg.

***In vivo* hepatoprotective activity**

Test Animals

Wistar rats (180–240 g) of either sex procured from the central animal house, were used for the study. The animals were housed in large, clean polypropylene cages in a temperature-controlled room (22 ± 2 ◦C with relative humidity (44–55%) under 12-h light and dark cycles. All the animals were acclimatized to laboratory environment for a week prior to experiments. Animals were provided with a standard rodent pellet diet and clean drinking water *ad libitum*. The care and use of laboratory animals were strictly in accordance with the guidelines prescribed by the Institutional Animal Ethical Committee constituted under the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

Experimental design

A total of 36 rats were divided into 6 groups of 6 rats each.

- Group I served as normal control and received only the vehicle (1 ml/kg/day of 1% CMC; p.o.).
- Group II received CCl_4 1 ml/kg (1:1 of CCl4 in olive oil) i.p. once daily for 7 days.
- Group III received CCl_4 1 ml/kg (1:1 of CCl4 in olive oil) i.p. and silymarin 100 mg/kg orally (p.o.) for 7 days.
- Groups IV, V, VI were administered MEHS at 100, 200, and 400 mg/kg body weight p.o. respectively and dose of 1 ml/kg i.p. of CCl_4 (1:1 of CCl_4 in olive oil) for 7 days.

All rats were sacrificed by cervical dislocation 24 h after the last treatment. Just before sacrifice, blood was collected from the retro-orbital sinus plexus under mild ether anesthesia. Collected blood was allowed to clot and serum was separated at 3500 rpm for 15 min for carrying out further biochemical investigations. One part of liver was dissected out and used for biochemical and histopathological studies.

Measurement of serum biochemical parameters

The activities of serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and total bilirubin were determined using the Hitachi 912 clinical chemistry automatic analyzer (Roche Diagnostic GmbH, Mannheim, Germany).

Assessment of lipid peroxidation and superoxide dismutase (SOD)

In chilled normal saline excised livers were perfused to remove all the blood cells. Then they were cut down into small pieces, placed in 0.1M phosphate buffer (pH 7.4), and homogenized using remi homogenizer to obtain 20% homogenate. The homogenate thus obtained was centrifuged at 3000 rpm for 15 min and the supernatant was collected in an Eppendorf tube. This supernatant was again centrifuged at 12,000 rpm for 30 min. The final supernatant was used for the determination of malonaldehyde (MDA) as a lipid peroxidation marker (Nourooz-Zadeh et al., 1995). Superoxide dismutase (SOD) was also assayed by the method described previously (Misra and Fridovich, 1972).

Histopathology

The liver tissue was dissected out and fixed in 10% formalin solution. It was then dehydrated in ethanol (50-100%), cleared in xylene and embedded in paraffin wax. Afterwards thick sections (5–6 mm) were made and then stained with hematoxylin and eosin dye for photomicroscopic observation. Scoring on scale of 1-4 was done for the liver sections under microscope as given below (Hirayama et al., 1979, Ala-Kokko et al., 1987).

- 0 = Normal liver histology.
- 1 = Tiny and short septa of connective tissue without influence on the structure of hepatic lobules.
- 2 = Large septa of connective tissue, flowing together and penetrating into the parenchyma. Tendency to develop nodules.
- 3 = Nodular transformation of the liver architecture with loss of structure of hepatic lobules.
- 4 = Excessive formation and deposition of connective tissue with subdivision of the regenerating lobules and with development of scars.

Statistical analysis

The data were expressed as mean ± SD. Statistical differences at $p < 0.001$ between the groups were analyzed by one-way ANOVA followed by Turkey as *post hoc* using GraphPad Instat software package.

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RESULTS

Acute toxicity studies

All the extracts of *Hedychium Spicatum* rhizomes did not cause any mortality upto 2000 mg/kg dose level. Hence 1/20 th, 1/10th and 1/5 th of the maximum dose (i.e., 100, 200 and 400 mg/kg, p.o.) were selected for the present study.

In vivo hepatoprotective activity

Effect of MEHS on the measurement of serum biochemical parameters

The hepatoprotective effects of MEHS on serum biochemical parameters in CCl_4-intoxicated rats are shown in **table 1**. Rats treated with CCl_4 (Group II) showed a significant increase in serum AST, ALT, ALP and total bilirubin levels compared to control animals (Group I). Pre-treatment with MEHS at 100, 200 and 400 mg/kg for 7 days (Groups IV, V and VI) showed significant hepatoprotection in terms of serum AST, ALT, ALP and total bilirubin levels compared to the toxic control group (Group II). Pretreatment with the standard hepatoprotective agent-Silymarin (Groups III) also decreased all measured serum biochemical activities towards normalness.

Table 1: Effects of MEHS on serum biochemical parameters in CCl_4-intoxicated rats.

Group	Treatment	AST (SGOT) (IU/L)	ALT (SGPT) (IU/L)	ALP (IU/L)	Serum Billirubin (mg/dl)	MDA	SOD
Group I	Control	170 ± 2.3^{a}	55 ± 4.5^{a}	190 ± 3.4^{a}	0.40 ± 0.04^{a}	90 ± 0.01^{a}	8.0 ± 0.05^{a}
Group II	CCl_4 1 ml/kg (i.p.)	610 ± 28.5	121 ± 8.1	541 ± 6.7	0.87 ± 0.04	120 ± 1.3	3.2 ± 0.08
Group III	Silymarin 100 mg/kg + CCl_4 (prophylactic)	230 ± 13***	61 ± 1.7***	190 ± 2.8***	0.32 ± 0.05***	101 ± 1.5***	8.0 ± 0.06***
Group IV	MEHS 100 mg/kg + CCl_4 (prophylactic)	420 ± 3.2***	74 ± 2.6***	251 ± 7.2***	0.58 ± 0.04***	121 ± 1.7***	5.0 ± 0.24***
Group V	MEHS 200 mg/kg + CCl_4 (prophylactic)	310 ± 17***	68 ± 1.9***	205 ± 3.6***	0.60 ± 0.08***	109 ± 0.59***	5.4 ± 0.18***
Group VI	MEHS 400 mg/kg + CCl_4 (prophylactic)	240 ± 5.8***	56 ± 3.7***	200 ± 6.2***	0.48 ± 0.06***	119 ± 0.87***	7.0 ± 0.19***

MEHS: Methanolic extract of *Hedychium spicatum*. Data are expressed as mean ± SD (n = 6).
One-way ANOVA Tukey *post hoc*: *** $p < 0.001$

Effect of MEHS on MDA and SOD levels

Lipid peroxidation was increased in the toxic control group, as revealed by elevated MDA levels, when compared with the normal control group. Pre-treatment with MEHS at 100, 200 and 400 mg/kg significantly decreased the MDA levels, which were almost similar to those of rats receiving the standard drug Silymarin. A level of antioxidant enzyme, SOD was significantly increased in MEHS treated groups. The extract at the dose of 400 mg/kg demonstrated maximum hepatoprotection as shown in **table 1.**

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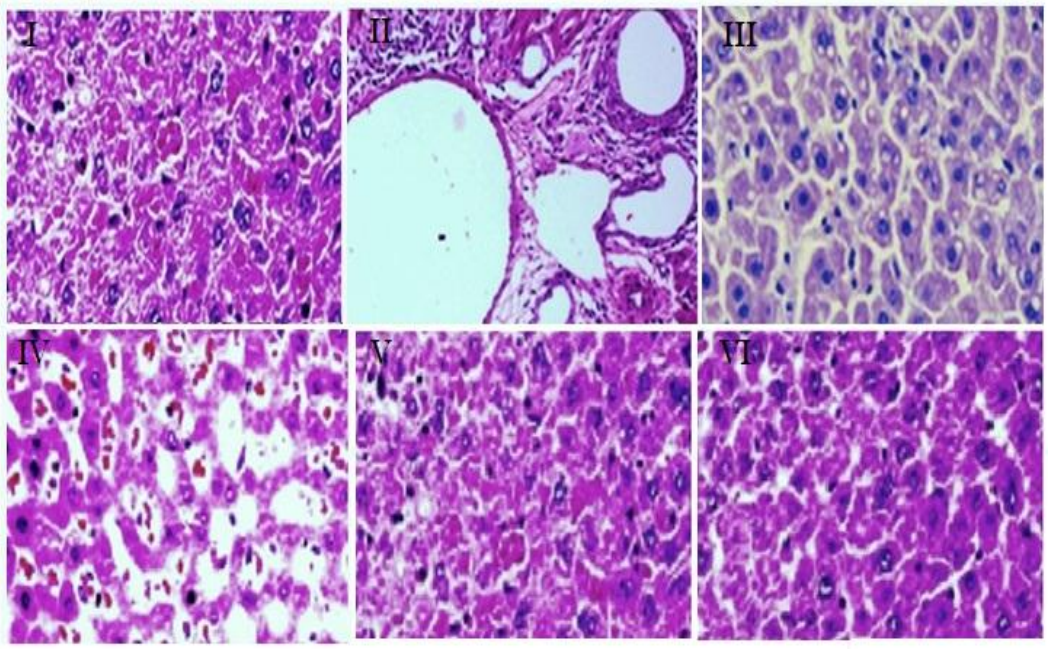
Histopathology

Histopathological observations revealed that the normal architecture of liver was completely lost in rats treated with CCl_4 with the appearance of vacuolated hepatocytes and degenerated nuclei **(Figure 1).** Vacuolization, fatty changes and necrosis of hepatocytes were severe in the central lobular area. CCl_4 intoxication led to excessive formation of deposition of connective tissue and development of scars (score of 4). MEHS at 100 mg/kg dose did show significant hepatoprotective activity. Liver sections of rats treated with MEHS (100 mg/kg) revealed nodular transformation of liver architecture with loss of structure of hepatic lobules (score of 3). Large septa of connective tissue flowing together and penetrating into the parenchyma (score of 2) were observed in liver sections of rats treated with MEHS 200 mg/kg. Liver sections of rats treated with MEHS 400 mg/kg bear witness more or less normal lobular pattern with short septa of connective tissue and a mild degree of fatty change, and necrosis (score of 1) almost comparable to the control and silymarin treated groups.

Figure 1. Representative photomicrographs of liver sections from Group I to Group VI.



DISCUSSION

The present study demonstrates the hepatoprotective and antioxidant effects of MEHS against CCl_4–induced liver injury in rats. The liver mainly detoxifies toxic chemicals and drugs and becomes the main target organ for all possible toxic xenobiotics. Being a potent hepatotoxin, CCl_4 is the, most extensively used chemical agent to investigate hepatoprotective activity on various experimental animal models. CCl4 is known to cause hepatotoxicity. The experimental hepatic damage caused by CCl_4 histologically also resembles viral hepatitis (James

and Pickering, 1976). CCl_4 is biotransformed in liver by cytochrome P_{450} enzymes to CCl_3 radical which is a very active radical. This active CCl_3 radical reacts with oxygen to produce trichloromethylperoxyl radical ($CCl_3O_2^{\cdot}$), which is then covalently binds with cellular macromolecules and biomembranes to cause lipid peroxidation of the lipid membranes of the adipose tissue. Peroxide products finally trigger production and leakage of biomarkers like MDA (Malonaldehyde). This whole cascade of biochemical events ultimately causes loss of cellular integrity and hepatic damage (Thabrew et al., 1987). Lipid peroxidation is an important parameter of oxidative stress along with other free radical damage occurred in the biochemical cascade. Therefore, antioxidant efficacy is regarded as one of the utmost important parameter indicative of the possible mechanism of hepatoprotection.

AST, ALT and ALP are the serum hepatobillary enzymes present normally in the liver in high concentrations. Upon necrosis or hepatic damage these enzymes will be leaked into the circulation; raising serum concentration of these enzymes (Drotman and Lawhan, 1978). Elevated serum AST, ALT and ALP levels in CCl_4 treated animals indicated cellular breakage and loss of functional integrity of cell membranes in liver (Wolf, 1999, Drotman and Lawhan, 1978).

In the present study, increased MDA levels in liver indicated increased lipid peroxidation induced by CCl_4 (Group II animals). This enhanced lipid peroxidation finally triggered hepatic tissue damage. Reduced estimation of SOD in CCl_4 treated animals also suggested failure of antioxidant defense mechanism to block peroxidation damage.

In view of this, the increased serum level of AST, ALT and ALP enzymes in CCl_4 treated animals (Group II) confirmed hepatic damage. As a breakdown product of heme in red blood cells, bilirubin is regarded as a clinical and pathophysiological indicator of necrosis of liver tissues. Pretreatment with MEHS in different animal groups (Group IV/V/VI) resulted a significant decrease in serum AST, ALT, ALP and total bilirubin levels as compared to CCl_4 treated group (Group II). Prophylactic use of the extract resulted in an inhibition of the degree of hepatic necrosis and concomitantly decreased the leakage of intracellular enzymes by stabilizing hepatic cellular membranes. The results are further confirmed by the histopathological observations. Inhibition of lipid peroxidation to a significant degree is also a predominant mechanism of hepatoprotection as suggested by the significant decrease in MDA levels. Increase in the SOD level was also suggestive of repairment of antioxidant defense system, which plays an important role in hepatoprotection. Based upon the results of this present study, it can be concluded that the methanol rhizome extract of *Hedychium spicatum* has proven itself as a significant hepatoprotective as well as a considerable antioxidant.

CONCLUSION

The present study clearly demonstrated the 'in vivo' effectiveness of the extract in terms of lipid peroxidation inhibitory capacity and further confirmed the significant hepatoprotective activity of the methanol extract of rhizomes of *Hedychium spicatum* along with its antioxidant mechanisms of action.

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REFERENCES

[1] ALA-KOKKO, L., STENBACK, F. & RYHANEN, L. 1987. Preventive effect of malotilate on carbon tetrachloride induced liver damage and collagen accumulation in the rat. *Biochemical Journal,* 246**,** 503-509.

[2] CHATTERJEE, T. K. 2000. *Herbal Options,* Kolkata, Books and Allied (P) Ltd.

[3] DHULEY, J. N., RAMAN, P. H., MAJUMDAR, A. M. & NAIK, S. R. 1993. Inhibition of lipid peroxidation by piperine during experimental inflammation in rats. *Indian J Exp Biol,* 31**,** 443-445.

[4] DROTMAN, R. & LAWHAN, G. 1978. Serum enzymes are indications of chemical induced liver damage. *Drug Chem Toxicol,* 1**,** 163–171.a

[5] ELLNAIN-WOJTASZEK, M., KRUCZYNSKI, Z. & KASPRZAK, J. 2003. Investigation of the free radical scavenging activity of Ginkgo biloba L. leaves. *Fitoterapia,* 74**,** 1-6.

[6] GÜLÇIN, I., OKTAY, M., KÜFREVIOGLU, Ö. I. & ASLAN, A. 2002. Determination of antioxidant activity of lichen Cetraria islandica (L) Ach. *Journal of Ethnopharmacology,* 79**,** 325-329.

[7] HALLIWELL, B. & GUTLERIDGE, M. 1984. Review article. Oxygen toxicity, oxygen radicals, transition metals and disease *Biochemical Journal,* 219**,** 1-4.

[8] HIRAYAMA, C., MOROTAMI, I. & HIROSHIGE, K. 1979. Quantitative and metabolic changes of hepatic collagens in rats after carbon tetrachloride poisoning. *Biochemical Journal,* 118**,** 229-232.

[9] ITO, N. & HIROSE, M. 1989. Antioxidants--carcinogenic and chemopreventive properties *Adv Cancer Res,* 53**,** 247-302.

[10] JAMES, G. W. L. & PICKERING, R. W. 1976. The protective effect of a novel compound RU-18492 on galactosamine induced hepaptotoxicity in rats. *Drug Research,* 26**,** 2197-2199.

[11] JOHNSTON, D. E. & KROENING, C. 1998. Mechanism of early carbon tetra chloride toxicity in cultured rat hepatocytes.;:. *Pharmacol Toxicol,* 83**,** 231-239.

[12] MISRA, H. P. & FRIDOVICH, I. 1972. Role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry,* 247**,** 3170.

[13] NOUROOZ-ZADEH, J., TAJADDINI-SARMADI, J., MCCARTHY, S., BETTERIDGE, D. J. & WOLFF, S. P. 1995. Elevated levels of authentic plasma hydroperoxides in NIDDM. *Diabetes,* 44**,** 1054-1058.

[14] OH, T. Y., LEE, J. S., AHN, B. O., CHO, H., KIM, W. B., KIM, Y. B., SURH, Y. J., CHO, S. W. & HAHM, K. B. 2001. Oxidative damages are critical in pathogenesis of reflux esophagitis: implication of antioxidants in its treatment. *Free Radical Biology and Medicine,* 30**,** 905-915.

[15] SUBRAMONIAM, A. & PUSHPANGADAN, P. 1999. Development of phytomedicines for liver disease. *Indian J Pharmacol,* 31**,** 166-175.

[16] THABREW, M. I., JOICE, P. D. T. M. & RAJATISSA, W. A. 1987. Comparative study of the efficacy of Paetta indica and Osbeckia octandra in the treatment of liver dysfunction. *Planta Medica,* 53**,** 239-241.

[17] WOLF, P. L. 1999. Biochemical diagnosis of liver diseases. *Indian Journal of Clinical Biochemistry,* 14**,** 59-90.