

Hepatoprotective Activity of *Nelumbo nucifera* Geartn. Flower: an Ethnopharmacological Study

G. M. M. Rao^{1*}, P. Pushpangadan¹ and A. Shirwaikar²

¹Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute, Lucknow- 226 001, Uttarpradesh, India.

²Department of Pharmacognosy, College of Pharmaceutical Sciences, Manipal- 576 119, Karnataka, India

Abstract

Nelumbo nucifera Geartn. (Nymphaeaceae), a medicinal plant mentioned in Ayurveda for the treatment of liver disorders, has not been subjected to systematic scientific investigations to assess its hepatoprotective effects. The present study was therefore, undertaken to investigate the hepatoprotective activity of this plant. 50% aqueous ethanolic extract of flowers of *Nelumbo nucifera* Geartn. (Nymphaeaceae) showed a significant dose dependent (200 mg, 400 mg/kg p.o. x 10) protective effect against carbon tetrachloride and paracetamol induced hepatotoxicity in Sprague-Dawley rats. The degree of protection was measured using biochemical parameters like serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum alkaline phosphatase (SAKP) and serum bilirubin (SB). Liver sections were also studied histopathologically to confirm the biochemical results. The results indicated that the 400mg/kg p.o. of 50% aqueous ethanolic extract of flowers of *Nelumbo nucifera* Geartn. exhibited the most significant ($P < 0.01$) activity on both carbontetrachloride and paracetamol induced hepatotoxicity. The pathological changes of hepatic lesions caused by these two hepatotoxicants were improved by treatment with 50% aqueous ethanolic extract of *N. nucifera* Geartn., which are comparable to silymarin, a standard hepatoprotective medicine. Thus the present study provides a scientific support for the traditional use of this plant in the management of liver diseases.

Keywords: *Nelumbo nucifera*, Carbon tetrachloride, Paracetamol, Hepatoprotection.

Introduction

Liver disease is a world wide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. This is one of the reasons for many people worldwide including those in developed countries to return to complementary and alternative medicine (CAM). Many traditional remedies employ herbal drugs for the treatment of liver ailments (Venkateswaran *et al.*, 1997; Latha *et al.*, 1999; Mithra *et al.*, 2000; Dhuley and Naik, 1997). *Nelumbo nucifera* Geartn. (Nymphaeaceae: Kamal) has great reputation in Ayurvedic medicine for treatment of liver disorders. *N. nucifera* is an aquatic herb with orbicular, peltate leaves, pinkish or white flowers, spongy torous fruits, commonly found in tanks, lakes and marshy places, throughout India. Studies on different parts of *N. nucifera* like rhizomes, leaves & seeds showed antioxidant and hepatoprotective activities (Jung *et al.*, 2003; Ming-Jiuan Wu *et al.*, 2003; Lee *et al.*, 2003;

* Corresponding author: E-mail: mmraos@rediffmail.com

Sohn *et al.*, 2003). Antidiabetic and anti-inflammatory effects of rhizomes; antihyperlipidemic effect of leaves, antifertility activity of seeds were also reported by various workers. (Mukherjee^a *et al.*, 1997; Grover *et al.*, 2002, Mukherjee^b *et al.*, 1997; Cour *et al.*, 1995; Mazumder *et al.*, 1992). But no scientific and methodical investigations have so far been carried out on flowers of the plant. The present investigation has, therefore, been designed to study the hepatoprotective activity of 50% aqueous ethanolic extract of *N. nucifera* flowers against paracetamol and carbontetrachloride induced hepatic damage in rats with the objective to verify the traditional claims.

Materials and Methods

Plant material: The fresh flowers of *N. nucifera* were collected from campus garden of National Botanical Research Institute, Lucknow, India in September 2003. The plant material was identified and authenticated taxonomically at National Botanical Research Institute, Lucknow, India. A voucher specimen of the collected sample was deposited in the institutional herbarium and departmental museum for future reference.

Preliminary Phytochemical Studies: The flowers of *N. nucifera* were washed with distilled water to remove dirt and soil, and were shade dried. Routine pharmacognostic studies including organoleptic tests, macroscopic and microscopic observations were carried out to confirm the identity of the materials. The dried materials were powdered and passed through a 10-mesh sieve. The coarsely powdered material (1000gm) was extracted thrice with ethanol (50% v/v). The extracts were filtered, pooled and concentrated under reduced temperature (-5 °C) on a rotary evaporator (Buchi, USA) and then freeze-dried (Freezone[®] 4.5, Labconco, USA) at high vacuum (133×10^{-3} m Bar) and at temperature -40 ± 2 °C (yield 15.2%, w/w). Preliminary qualitative phytochemical screening of extract gives the positive test for alkaloids (Chao and Marderosian, 1973), lipids (Joshi and Garg, 1981), flavonoids, “steroids and triterpenoids”, “coumarin and aryl esters” (Srivastava and Shukla, 1998), and proteins, fatty oils, resins and tannins. The optimum conditions for experiments were decided on the basis of initial pilot experiments performed on three rats per treatment.

Animals and treatment: Sprague-Dawley rats weighing 140 - 160 g of either sex were purchased from the animal house of the Central Drug Research Institute, Lucknow. They were kept in departmental animal house in well cross - ventilated room at 27 ± 2 °C, and relative humidity 44 - 56%, light and dark cycles of 10 and 14 h, respectively, for one week before and during the experiments. Animals were provided with standard rodent pellet diet (Amrut, India) and the food was withdrawn 18 - 24 h before the experiment thought, water was allowed *ad libitum*. All the experiments were performed in the morning according to current guidelines for the care of the laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals (Zimmerman, 1983). All the chemicals used were of the analytical grade from standard companies and the water represents the double distilled water. The standard orogastric cannula was used for oral drug administration.

Carbon tetrachloride induced hepatotoxicity studies: Rats were divided into five groups (six per group). Group I, the normal control group animals were administered a single daily dose of liquid paraffin (1 ml/kg body weight, i.p), Group II animals received carbon tetrachloride (1ml/kg body weight, i.p.). Group III animals received silymarin, the known hepatoprotective compound (Sigma Chemicals Company, USA), at a dose of 100 mg/kg p.o., along with carbon tetrachloride (1ml/kg body weight, i.p.). Group IV & V received orally 200 and 400 mg/kg body weight plant extract, respectively, in the form of aqueous suspension daily once in a day. The extract was given simultaneously with carbon tetrachloride. Treatment duration was 10 days. Dosages of carbon tetrachloride were administered as 30% solution in liquid paraffin after every 72 h. Animals were sacrificed 24 h. after the last injection by mild ether anaesthesia.

Blood samples were collected for evaluating the biochemical parameters and liver tissue samples for histological studies.

Paracetamol induced hepatotoxicity studies: Paracetamol was obtained from Suzen pharmaceuticals Pvt. Ltd. Hyderabad. Animals were divided into five groups (six per group). Group I served as normal control given vehicle 50% aqueous sucrose solution, Group II animals (toxin control groups) received vehicle (50% aqueous sucrose solution) for 10 days in succession followed by single oral administration of paracetamol 3 g/kg p.o., 1 h after ethanolic extract administration. Group III animals received silymarin, the known hepatoprotective compound (Sigma Chemicals Company, USA), at a dose of 100 mg/kg p.o. in 50% aqueous sucrose solution once daily for 10 days in succession followed by single administration of paracetamol 3 g/kg p.o., 1 h after silymarin administration. Group IV & V were given the pre-treatment of 50% aqueous ethanolic extracts of *N. nucifera* (200 & 400 mg/kg p.o) in 50% aqueous sucrose solution once daily for 10 days in succession followed by single administration of paracetamol 3 g/kg p.o., 1 h after ethanolic extract administration. The normal control group (Group I) received vehicle alone. Animals were sacrificed 24 h. after the last injection by mild ether anaesthesia. Blood samples were collected for evaluating the biochemical parameters and liver tissue samples collected for histological studies.

Biochemical estimations: Biochemical parameters like serum enzymes: serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum alkaline phosphatase (SAKP) and serum bilirubin (SB) were assayed according to standard methods (Malloy and Evelyn, 1937; Reitman and Frankel, 1957; King and Armstrong, 1980) respectively.

Histopathological studies: For the histopathological study, the livers were immediately removed after autopsy and the tissues were fixed in 10% formalin for a period of at least 24 h. The paraffin sections were then prepared (Automatic Tissue Processor, Lipshaw) and cut into Five micrometer thick sections in a rotary microtome. The sections were then stained with haemotoxylin-eosin for photomicroscopic observations of the liver histological architecture of the control and treated rats.

Statistical analysis: The difference among means has been analysed by Student's *t*-test (Woolson, 1987). Results of biochemical estimations are expressed as mean \pm S.E.M.

Results

Carbon tetrachloride induced hepatotoxicity The effects *N. nucifera* on carbon tetrachloride-induced hepatotoxicity in rats are shown in Table 1. Administration of carbon tetrachloride to rats caused severe damage as indicated by a significant increase in hepatic enzymes (AST, ALT, SAKP) and serum bilirubin, compared to normal control rats. Pretreatment of rats with extract (200 & 400 mg/kg p.o) of *N. nucifera* exhibited a significant ($P < 0.05$, $P < 0.01$) reduction in the carbon tetrachloride induced increase in the levels of AST, ALT, SAKP and SB (Table 1). The protective effect was also compared with silymarin a standard hepatoprotective agent. The levels elevated in AST, ALT, SAKP and SB were almost significantly decreased by treatment of the *N. nucifera* extract. However the fact that 400 mg/kg showed a highly significant activity ($P < 0.01$), indicates that the protective effect was in a dose dependent manner. Histological observations basically support the results obtained from serum enzyme essays. The liver of carbon tetrachloride-intoxicated rats showed massive fatty changes, gross necrosis, and broad infiltration of lymphocytes and Kupffer cells around the central vein and loss of cellular boundaries. The histological pattern of liver of rats pre-treated with the extracts (subsequently given Carbon tetrachloride) of *N. nucifera* showed more or less normal lobular pattern with a mild degree of fatty change, necrosis and lymphocyte infiltration almost comparable to the normal control and silymarin treated groups (Figs. 1-5).

Table 1: Effect of 50% aqueous ethanolic extract of *N. nucifera* flowers on Carbon tetrachloride induced liver damage in rats.

Group	Dose	AST	ALT	SAKP	SB
Normal	--	75.00 ± 3.12	70.20 ± 0.4	34.75 ± 1.43	0.31 ± 0.01
Control(CCl ₄)	1ml/kg	172.30 ± 12.13	145.40 ± 11.18	65.00 ± 4.12	5.31 ± 1.10
Silymarin	100mg/kg	78.01 ± 7.10 ^b	71.30 ± 6.8 ^b	36.71 ± 3.98 ^b	1.30 ± 0.20 ^b
<i>N.Nucifera</i>	200 mg/kg	116.01 ± 9.80 ^a	95.00 ± 9.1 ^a	48.12 ± 1.32 ^a	1.20 ± 0.81 ^a
<i>N.Nucifera</i>	400 mg/kg	80.10 ± 6.80 ^b	74.12 ± 8.80 ^b	39.10 ± 2.01 ^b	1.50 ± 0.81 ^b

Student's *t*- test was performed. Each value represents the mean ± S.E.M. for six rats.

Significantly different from carbon tetrachloride treated group. P^a<0.05, P^b<0.01

Paracetamol induced hepatotoxicity Biochemical parameters AST, ALT, SAKP and SB were significantly elevated in the groups of rats given paracetamol (3 g/kg p.o.) (Table 2). The pre-treatment (200 & 400 mg/kg p.o) of 50% aqueous ethanolic extract of *N. nucifera* exhibited inhibition of paracetamol induced increase in the levels of all the four biochemical parameters, resulting in significant restoration towards their control values. The results are comparable to silymarin a standard hepatoprotective agent. It is very clear from the Table 2 that maximum protection against paracetamol induced hepatic aberrations was achieved with the higher dose of the extract.

Table 2: Effect of 50% aqueous ethanolic extract of *N. nucifera* flowers on paracetamol induced liver damage in rats.

Group	Dose	AST	ALT	SAKP	SB
Normal	--	75.00 ± 3.12	70.20 ± 0.4	34.75 ± 1.43	0.31 ± 0.01
Control (paracetamol)	3g/kg	151.01 ± 12.10	145.00 ± 11.50	69.10 ± 5.13	4.21 ± 0.80
Silymarin	100 mg/kg	80.04 ± 7.50 ^a	76.01 ± 7.31 ^b	40.10 ± 2.98 ^b	0.61 ± 0.40 ^b
<i>N.Nucifera</i>	200 mg/kg	100.01 ± 8.10 ^b	105.01 ± 4.65 ^a	50.23 ± 2.01 ^a	1.53 ± 0.33 ^a
<i>N.Nucifera</i>	400 mg/kg	84.23 ± 6.13 ^a	73.01 ± 10.91 ^b	38.21 ± 3.24 ^b	0.65 ± 0.31 ^b

Student's *t*- test was performed. Each value represents the mean ± S.E.M. for six rats.

Significantly different from paracetamol treated group. P^a<0.05, P^b<0.01.

Histopathological examinations of livers damaged with paracetamol showed centrilobular necrosis, ballooning degeneration, inflammatory infiltration of lymphocytes and fatty changes. The liver sections of rats treated with the extracts after paracetamol challenge showed well preserved architecture, almost similar to silymarin treated groups (Fig. 5-10).

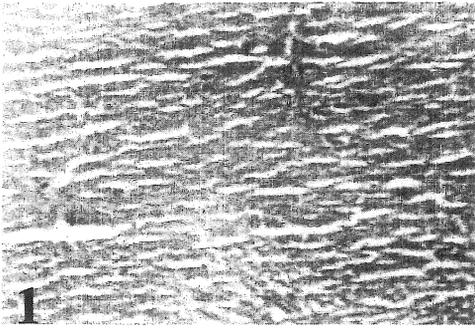


Fig.1. Section of liver of normal control rat showing hepatic cells with nuclei, cytoplasm, central vein and portal tract.

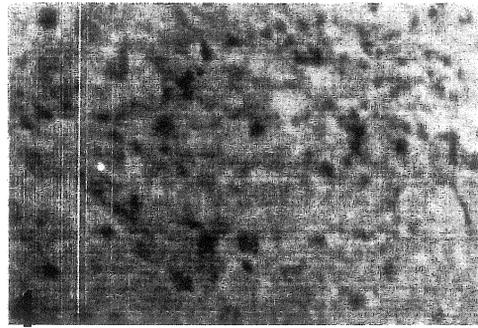


Fig.4. Section of 200mg/kg of *N.nucifera* extract treated liver showing marked improvement over Carbon tetrachloride control group.

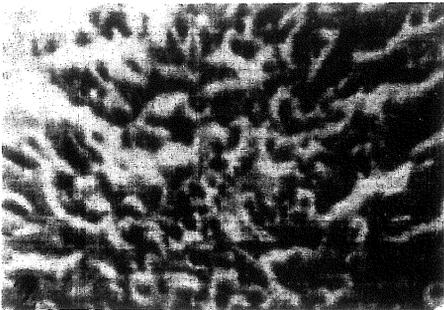


Fig.2. Section of carbon tetrachloride (Carbon tetrachloride control group, 1 ml/kg) rat liver showing marked necrosis, severe fatty degeneration and extensive vacuolisation with disappearance of nuclei

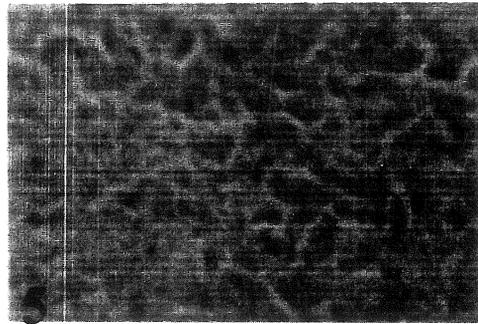


Fig.5. Section of 400mg/kg of *N.nucifera* extract treated liver showing marked improvement over Carbon tetrachloride control group.

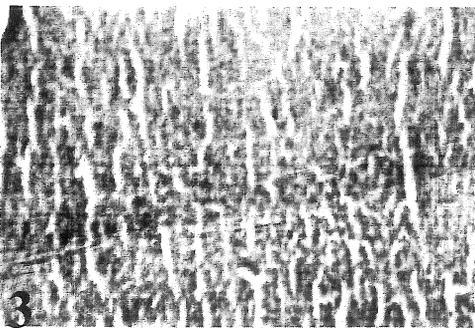


Fig.3. Section of Silymarin(100mg/kg) + Carbon tetrachloride-treated liver, showing marked improvement over Carbon tetrachloride control group.

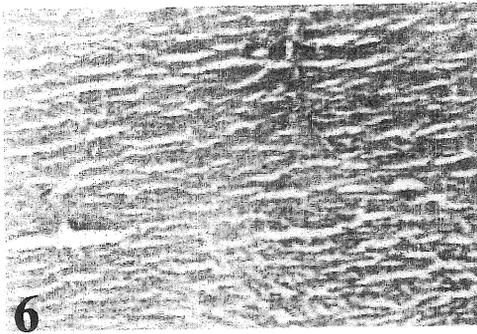


Fig. 6. Section of liver of normal control rat showing hepatic cells with nuclei, cytoplasm, central vein and portal tract.

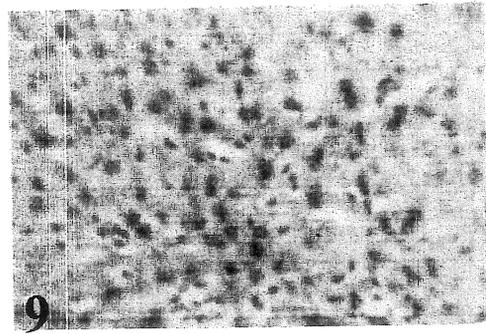


Fig. 9. Section of 200mg/kg of *N.nucifera* extract treated liver showing marked improvement over paracetamol control group.

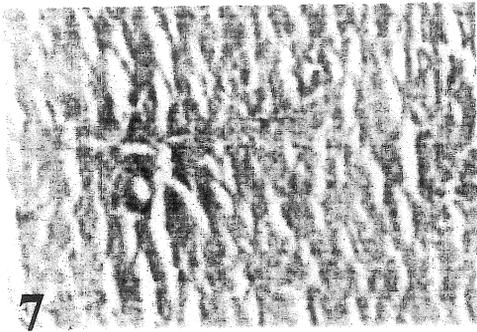


Fig. 7. Section of paracetamol (paracetamol control group, 3g/kg) rat liver showing marked necrosis, severe fatty degeneration and extensive vacuolisation with disappearance of nuclei

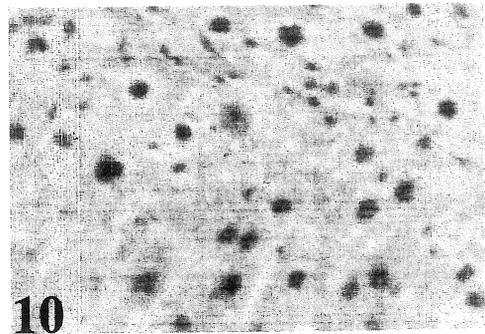


Fig. 10. Section of 400mg/kg of *N.nucifera* extract treated liver showing marked improvement over paracetamol control group.

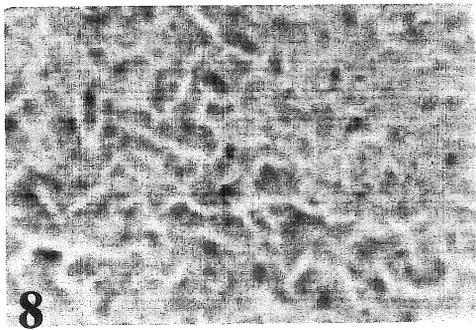


Fig. 8. Section of Silymarin (100 mg/kg) + paracetamol-treated liver, showing marked improvement over paracetamol control group.

Discussion and Conclusions

In recent years, there are many researches on traditional medicines attempting to develop new drugs for hepatitis (Rao *et al.*, 2003, Rao *et al.*, 2004). In the present study, we used two mechanistically different models including carbontetrachloride and paracetamol to evaluate the hepatprotective activity of the 50% aqueous ethanolic extracts of flowers of the *N. nucifera* efficiently against the toxicity produced by these hepatotoxicants. It has been assured that carbon tetrachloride is the best characterised system of xenobiotic-induced hepatotoxicity and is frequently employed as a model to study hepatotoxic/hepatoprotective activity of the drugs. It is metabolized in the body to a highly reactive trichloromethyl radical ($\text{CCl}_3\cdot$) which attacks membrane phospholipids stimulating lipid peroxidation and cell lysis (Brent and Rumack 1993). This damage to the structural integrity of the liver is observed from elevated serum levels of hepatospecific enzymes, i.e. AST, ALT and SAKP as well as SB. Paracetamol is a common antipyretic agent, which is safe in therapeutic doses but can produce fatal hepatic necrosis in man, rats and mice with toxic doses (Mitchell *et al.*, 1973; Kuma and Rex, 1991; Eriksson *et al.*, 1992). Protection against paracetamol-induced toxicity has been used as a test for potential hepatoprotective activity by several investigators (Visen *et al.*, 1993; Singh and Handa, 1995; Ahmed and Khater, 2001). An obvious sign of hepatic injury is leakage of cellular enzymes into plasma (Wilkinson, 1962; Schmidt and Schmidt, 1967; Schmidt, 1975). When the liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released into blood stream. Their estimation in the serum is a useful quantitative marker for the extent and type of hepatocellular damage (Ansari *et al.*, 1991).

Hepatic cells participate in a variety of metabolic activities and contain a host of enzymes. In tissues, AST and ALT are found in higher concentrations in cytoplasm and AST in particular also exists in mitochondria (Wells, 1988). In liver injury, the transport function of the hepatocytes is disturbed, resulting in the leakage of plasma membrane (Zimmerman and Seef, 1970), thereby causing an increased enzyme level in serum. If injury involves organelles such as mitochondria, soluble enzymes like AST normally located there, will also be similarly released. The elevated activities of the serum enzymes are indicative of cellular leakage and loss of the functional integrity of cell membranes in liver (Drotman and Lawhorn, 1978). Administration of carbon tetrachloride and paracetamol significantly raises the serum level of enzymes like AST and ALT in rats (Nazirogulu *et al.*, 1999) as observed in our results. Determination and evaluation of these parameters in the serum and tissue samples of experimental animals are used to assess hepatotoxicity and inhibitory effects of the test drugs on this process are employed as an indicator of antihepatotoxic or hepatoprotective activity. Oral administration of extract (200 and 400 mg/kg p.o) of *N. nucifera* caused a decrease in the activity of the above enzymes, which may be a consequence of the stabilization of plasma membrane as well as repair of hepatic tissue damage caused by carbon tetrachloride and paracetamol. This is supported by the view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes (Thabrew *et al.*, 1987). In conclusion, mechanisms by which the two hepatotoxicants (carbon tetrachloride and paracetamol) produced liver injuries are different. However, both Carbon tetrachloride induced and paracetamol induced models must rely on the cytochrome P-450 system to produce reactive metabolites, $\text{CCl}_3\cdot$ and *N*-acetyl-*p*-benzo-quinoneimine (Vermuelen, *et al.*, 1992). The results coincided with our investigations that the 400 mg/kg body weight showed the best protective effect which was not only against the paracetamol induced hepatotoxicity but also against the carbon tetrachloride induced liver injuries. Therefore, the possible hepatoprotective mechanisms of extract of *N. nucifera* may be due to the following factors: (1) preventing the

process of lipid peroxidation; (2) inhibiting the cytochrome P-450 activity; (3) stabilizing the hepatocellular membrane; and (4) enhancing the protein synthesis. However, further studies are necessary to isolate the active component and to clarify in more detail the pathway concerning the protective mechanism of flowers of *Nelumbo nucifera* Geartn. against chemically or virally induced liver injuries.

Acknowledgements

The authors are extremely thankful to Suzen Pharmaceuticals Private Limited, Hyderabad, for sending the paracetamol as gift sample. Financial assistance to the first author in the form of SRF from Council of Scientific Industrial Research (CSIR), India is gratefully acknowledged.

References

- Ahmed, M.B., Khater, M.R. (2001). Evaluation of the protective potential of *Ambrosia maritima* extract on acetaminophen-induced liver damage. *J. Ethnopharmacol.* 75: 169-174.
- Anonymous, (1986) Useful Plants of India, CSIR, New Delhi, p. 394
- Ansari, R.A., Tripathi, S.C., Patnaik, G.K., Dhawan, B.N. (1991). Anti-hepatotoxic properties of picroliv, an active fraction from rhizomes of *Picrorhiza kurroa*. *J. Ethnopharmacol.* 34: 61-68.
- Brent, J.A., Ruack, B.H. (1993). Role of free radicals in toxic hepatic injury II, *Clin. Toxicol.* 31: 173-196.
- Chao, J. M., Marderosian, A. H. (1973). Identification of ergoline alkaloids in the genus *Argyria* and related genera and their chemo taxonomic implications in the Convolvulaceae. *Phytochem.* 12: 2435 – 2440.
- Dhuley, G.P., Naik, S.R. (1997). Protective effect of Rhinax, a herbal formulation against CCl₄ induced liver injury and survival in rats. *J. Ethnopharmacol.* 56: 159-164.
- Drotman, R.B., Lawhorn, G.T. (1978). Serum enzymes as indicators of chemical-induced liver damage. *Drug Chem. Toxicol.* 1: 163-171.
- Eriksson, L., Broome, U., Kahn, M., Lindholm, M. (1992). Hepatotoxicity due to repeated intake of low doses of paracetamol. *J. Int. Med.* 231:567-570.
- Grover, J.K., Yadav, S., Vats, V. (2002). Medicinal plants of India with anti-diabetic potential. *J. Ethnopharmacol.* 81(1): 81-100.
- Joshi, R., Garg, B. D. (1981). Analysis of the fatty acid composition of *Argyria speciosa*, *J. Sci. Res.* 3: 195-198.
- Jung, H.A., Kim, J.E., Chung, H.Y., Cho, J.S. (2003). Antioxidant principles of *Nelumbo nucifera* stamens. *Arch. Pharm. Res.* 26(4): 279-85.
- King, E.J., Armsrong, A.R. (1980). Calcium, magnesium, phosphorus and phosphatase. In: Varley, B., Gowenlock, A.H., Bell, M. (Eds.), *Pract. Clin. Biochem.* vol. 1. Heinemann, London, p. 850.
- Kuma, S., Rex, D. (1991). Failure of physicians to recognise acetaminophen hepatotoxicity in chronic alcoholics. *Arch. Int. Med.* 151: 1189-1191.

- La, C.B., Molgaard P, Yi Z. (1995). Traditional Chinese medicine in treatment of hyperlipidaemia. *J. Ethnopharmacol.* 46(2):125-129.
- Latha, U., Rajesh, M.G., Latha, M.S. (1999). Hepatoprotective effect of an ayurvedic medicine, *Ind. Drugs* 36: 470-473.
- Lee, S.E., Hwang, H.J., Ha, J.S., Jeong, H.S., Kim, J.H. (2003). Screening of medicinal plant extracts for antioxidant activity. *Life Sci.* 73: 167-179.
- Malloy, J.R., Jollow, Evelyn, K.A., (1937). The determination of bilirubin with the photometric colorimeter. *J. Biol. Chem.* 119: 481-490.
- Mazumder, U.K., Gupta, M., Pramanik, G., Mukhopadhyay, R.K., Sarkar, S. (1992). Antifertility activity of seed of *Nelumbo nucifera* in mice. *Indian J. Exp. Biol.* 30(6):533-4.
- Mitchell, J.R., Jollow, D.J., Potter, W.Z., Gillette, J.R., Brodie, B.N. (1973). Acetaminophen-induced hepatic necrosis. I. Role of drug metabolism. *J. Pharmacol. Exp. Therapeutic.* 187: 185-194.
- Mitra, S.K., Seshadri, S.J., Venkataranganna, M.V., Gopumadhavan, S., Venkatesh Udupa, U., Sarma, D.N.K. (2000). Effect of HD-03- a herbal formulation in galactosamine-induced hepatopathy in rats. *Indian J. Physiol. Pharmacol.* 44: 82-86.
- Rao, G.M.M., Rao, Ch.V., Sudhakar, M., M M Pandey, Rawat, A.K.S., Shirwaikar, A., and Joshi, A.B. (2004). Anti-inflammatory and Antinociceptive activities of *Coccinia indica* Fruit juice powder in animals. *Nat. Prod. Sci.* 10: 20-23.
- Rao, G.M.M., Vijayakumar, M., Rao, Ch.V., Rawat, A.K.S., Mehrotra. S. (2003). Hepatoprotective effect of *Coccinia indica* against carbon tetrachloride induced hepatotoxicity. *Nat. Prod. Sci.* 9: 13-17.
- Mukherjee^a, P.K., Saha, K., Pal, M., Saha, B.P. (1997). Effect of *Nelumbo nucifera* rhizome extract on blood sugar level in rats. *J. Ethnopharmacol.* 58:207-13.
- Mukherjee^b, P.K., Saha. K., Das. J., Pal. M., Saha. B.P. (1997). Studies on the anti-inflammatory activity of rhizomes of *Nelumbo nucifera*. *Planta Med.* 63:367-369.
- Nazirogulu, M., Cay, M., Ustundag, B., Aksakal, M., Yekeller, H. (1999). Protective effects of vitamin E on carbon tetrachloride-induced liver damage in rats. *Cell Biochem. Function* 17:253-259.
- Reitman, S., Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *American J. Clin. Path.* 28: 53-56.
- Schmidt, E., Schmidt, F.W. (1967). Guide to Practical Enzyme diagnosis Boehringer Manaheion GmbH, West Germany, p. 15.
- Schmidt, E., Schmidt, F.W., Mohr, J., Otto, P., Vido, I., Wrogeman, K., Herfarth, C. (1975). Liver Morphology and enzyme release. Further studies in the isolated perfused rat liver. In: Keppler (Ed.), Pathogenesis and Mechanism of liver cell necrosis. Medical and Technical publishing Co. Ltd., Lancaster, p. 147.
- Singh, A., Handa, S.S. (1995). Hepatoprotective activity of *Apium graveolens* and *Hygrophilla auriculata* against paracetamol and thioacetamide intoxication in rats. *J. Ethnopharmacol.* 49: 119-126.

- Sohn, D.H., Kim, Y.C., Oh, S.H., Park, E.J., Li, X., Lee, H.B. (2003). Hepatoprotective and free radical scavenging effects of *Nelumbo nucifera*. *Phytomedicine* 10: 165-167.
- Srivastava, A., Shukla, Y. N. (1998). Aryl esters and a coumarin from *Argyria speciosa*. *Indian J. Chem.* 37 B: 192 – 194.
- Thabrew, M.I., Joice, P.D.T.M., Rajatissa, W. (1987). A comparative study of the efficacy of *Pavetta indica* and *Osbekia octandra* in the treatment of liver dysfunction. *Planta Med.* 53: 239-241.
- Venkateswaran, S., Pari, L., Viswanathan, P., Menon, V.P. (1997). Protective effect of a herbal formulation against erythromycin estolate-induced hepatotoxicity in rats. *J. Ethnopharmacol.* 57: 161-167.
- Vermuelen, N.P.E., Bessems, J.G.M., Van de Streat, R. (1992). Molecular aspects of paracetamol induced hepatotoxicity and its mechanism based prevention. *Drug Metab. Rev.* 24: 367-407.
- Visen, P.K.S., Shukla, B., Patnaik, G.K., Dhawan, B.N. (1993). Andrographolide protects rat hepatocytes against paracetamol-induced damage. *J. Ethnopharmacol.* 40: 131-136.
- Wells, F.E. (1988). Tets in liver and biliary tract disease. In: Gowenlock, H.A. (Ed.), Varley's Practical Clinical Biochemistry. CRC Press, Florida.
- Wilkinson, J.H. (1962). An Introduction to Diagnostic Enzymology. Edward Arnold, London, p. 84.
- Woolson, R.F. (1987). Statistical methods for the analysis of biomedical data, John Wiley and Sons Inc., New York.
- Wu, M.J., Wang, L., Weng, C.Y., Yen, J.H. (2003). Antioxidant activity of methanol extract of the lotus leaf (*Nelumbo nucifera* Gertn.). *Am. J. Chin. Med.* 31: 687-98.
- Zimmerman, H.J., Seef, L.B. (1970). Enzymes in hepatic disease, In: Goodly, E.I. (Ed.), Diagnostic Enzymology, Lea and Febiger, Philadelphia.
- Zimmerman, M. (1983). Ethical guidelines for investigation of experimental pain in conscious animal. *Pain.* 16: 109-110.

Received: 17.11.2004
Accepted: 23.12.2004