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Protective effect of *Asteracantha longifolia* extract in mouse liver injury induced by carbon tetrachloride and paracetamol

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Abstract

This study was conducted to investigate the protective effect of *Asteracantha longifolia* Linn (Acanthaceae) plant extract on carbon tetrachloride (CCl_4)- and paracetamol-induced acute hepatotoxicity in mice. Hepatotoxicity was induced by the administration of a single intraperitoneal dose of CCl_4 (0.5 mL kg^{-1} CCl_4 in olive oil) in one model and in the other by administration of paracetamol (300 mg kg^{-1} in saline) orally, after a 16-h fast. An aqueous extract of the whole plant (0.9 g kg^{-1}) was used on a pre- and post-treatment basis. *Asteracantha* reduced the alanine aminotransferase (ALT) level by 69.32% ($P < 0.001$) and increased the liver reduced glutathione level by 64.65% ($P < 0.001$) in the pre-treated group, 4 days after the administration of CCl_4. A similar pattern was observed in the pre-treated group 4 h after the administration of paracetamol with a reduction in serum levels of ALT, aspartate aminotransferase and alkaline phosphatase enzymes by 65.04, 55.79 and 45.75% respectively ($P < 0.001$). Plant extract also increased the glutathione concentration of the liver significantly ($P < 0.001$). Histopathological studies also provided supportive evidence for results from the biochemical analysis with marked improvement in liver architecture being observed in the *Asteracantha*-treated groups. Pre-treatment showed better results than post-treatment in both hepatotoxic models. Overall results indicate that the aqueous extract of *Asteracantha longifolia* possesses hepatoprotective effects on CCl_4- and paracetamol-induced hepatotoxicity in mice.

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Introduction

In the practice of traditional ayurvedic medicine in Sri Lanka, a number of herbs have been recognized for their potential benefits in the treatment of liver disorders. *Asteracantha longifolia* Linn (Acanthaceae), commonly known as 'Neeramulliya', is found in Sri Lanka and India. In Sri Lanka it is common in the dry zone and also in the low country in ditches and in marshy lands. A decoction of the plant is used by traditional medical practitioners as a diuretic, as a treatment for renal calculi, hepatic derangements and as an antidysentric. It is also given for gonorrhoea, jaundice and to serve as an aphrodisiac (Jayaweera 1981). The present study was conducted to scientifically prove or disprove the therapeutic efficacy of *Asteracantha longifolia* as a hepatoprotective agent in carbon tetrachloride (CCl_4)- and paracetamol-induced hepatotoxicty.

Despite the fact that the liver is an important organ for the detoxification and deposition of endogenous and exogenous substances, its function may readily be impaired by viruses, hepatotoxins and xenobiotics (Zhu et al 2000). CCl_4, a well-known model compound for the production of hepatic injury, requires biotransformation by hepatic microsomal P_{450} to produce the hepatotoxic metabolite trichloromethyl radical (Recknagel et al 1989). Paracetamol is one of the most widely used non-narcotic analgesic and antipyretic agents in the world because of its overall efficacy and safety (Nelson 1990). It is also frequently misused and its indiscriminate ingestion can lead to poisoning and potentially fatal hepatotoxicity (Black 1984).

Management of paracetamol poisoning is still a major problem in developing countries as the drugs used for it are not readily available, are expensive and ineffective, and are likely to cause adverse effects. There is therefore still a need to search for alternative drugs that are effective, safe, available and inexpensive. Hepatoprotective drugs from plant sources seem to be an attractive alternative.

Materials and Methods

Experimental animals

Healthy male ICR mice, 6–8 weeks old and weighing 30–35 g, were allowed free access to water and pelleted food *ad libitum*. All animals were fasted for 16 h before administration of the hepatotoxin. All protocols used in this study were approved by the ethics committee of the University of Ruhuna, Sri Lanka, guided by the CIOMS international guiding principles of biomedical research involving animals.

Chemicals

Diagnostic kits for serum alanine aminotransferase (ALT, EC 2.6.1.2), aspartate aminotransferase (AST, EC 2.6.1.1) and alkaline phosphatase (ALP, EC 3.1.3.1) were purchased from Randox (UK). Acetaminophen was a gift from the Sri Lanka Pharmaceutical Manufacturing Corporation. 5,5′-Dithiobis (2-nitrobenzoic acid) was purchased from Sigma (St Louis, MO). N-acetyl cysteine (NAC) was obtained from the Teaching Hospital, Karapitiya, Galle, Sri Lanka. All other chemicals were commercially available and of reagent grade.

Preparation of the plant extract

Asteracantha longifolia plants were collected from the Galle district in the Southern province of Sri Lanka. The sample was authenticated by comparison with the herbarium specimen preserved at the National Herbarium in the Botanical Gardens, Peradeniya, Sri Lanka. A voucher specimen was deposited at the Department of Biochemistry, University of Ruhuna, Sri Lanka.

Asteracantha plants were cut into small pieces and dried at 40 °C for two days. The normal therapeutic dose of humans extrapolated to mouse was used (Dhawan & Srimal 1998). 2.625 g of the dried plant material was refluxed in 30 mL of distilled water for 1 h and concentrated to 20 mL. Each mouse was administered a dose of 0.9 g kg^{-1} orally by gavage. The extract was prepared daily from the dried plant material.

Treatment of animals

Control groups

Mice were divided into two groups of 10 animals in each. The first group served as the normal control group and received distilled water orally. The second group was treated with the *Asteracantha* extract for 7 days. Animals were killed 7 days after the administration of the plant extract.

Carbon tetrachloride-induced hepatotoxicity

Mice were randomly divided into six groups (groups 3–8) of 10 animals in each. A single intraperitonial dose of CCl_4 was injected (0.5 mL kg^{-1} in olive oil, CCl_4:olive oil 1:10) in each animal after a 16-h fast. In groups 3 and 4 the animals were killed 24 h and 4 days, respectively, after the administration of CCl_4. Animals in group 5 were administered *Asteracantha* extract half an hour after the administration of a single dose of CCl_4 and were killed 24 h later. The same procedure was carried out for group 6 but instead of killing after 24 h, they were given the extract alone for a further two days at 24-h intervals (post-treatment). They were killed on the fourth day. Groups 7 and 8 were administered *Asteracantha* extract daily for seven days and on the seventh day a single dose of CCl_4 was injected half an hour after the administration of the plant extract. The mice were killed after 24 h and 4 days, respectively.

Paracetamol-induced hepatotoxicity

Mice were randomly divided into four groups (groups 9−12) of 20 animals each. 300 mg kg^{-1} of paracetamol (dissolved in saline and heated at 60 °C) was administered orally after a 16-h fast. Group 9 was given paracetamol alone and was killed 4 h later. Group 10 received the same dose of paracetamol and half an hour later NAC was given orally at 500 mg kg^{-1}. The mice were killed 4 h later. In the group 11 *Asteracantha* extract was administered instead of NAC. *Asteracantha* extract was administered for 7 days in group 12 and on the seventh day paracetamol was administered orally half an hour after the administration of the plant extract. Animals were killed 4 h later.

Assessment of liver damage

Blood was drawn by cardiac puncture under ether anaesthesia to determine serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activity. Liver tissues were excised, weighed and a section of the liver was fixed in 10% buffered formalin for histopathological assessment of liver damage. A liver section was homogenized and used for the determination of the liver reduced glutathione (GSH) level.

Serum ALT, AST and ALP activities were measured using an assay kit from Randox, UK (Reitman & Frankel 1957). The liver GSH level was estimated by the method of Jollow et al (1974). Histological sections of the formalin-fixed liver tissue were stained with haematoxylin and eosin.

Statistical analysis

The results were evaluated by one-way analysis of variance and Tukey's multiple comparison test. A probability (P) value of less than 0.05 was considered significant.

Results

The effects of CCl_4, paracetamol and the extract of *Asteracantha longifolia* on the activities of serum enzyme

levels of ALT, AST and ALP and liver GSH in mice are summarized in Tables 1 and 2.

A significant increase ($P < 0.001$) in the activities of serum enzyme levels and a decrease ($P < 0.001$) in liver GSH occurred within 24 h of exposure of mice to a single dose of CCl_4. The LD50 of CCl_4 was found to be $0.8\,mL\,kg^{-1}$. The results in Table 1 demonstrate that pre- or post-treatment with the extract under investigation can moderate CCl_4-mediated alterations in serum enzyme levels and liver GSH and that the plant extract itself has no effect on these parameters in the control group (group 2). As shown in Table 1, pre-treatment seems to be more effective than post-treatment. *Asteracantha* post-treated and pre-treated groups showed a faster recovery compared to the CCl_4 control group 4 days after the administration of CCl_4.

As shown in Table 2, the activities of serum ALT, AST and ALP 4 h after the administration of paracetamol alone were significantly increased ($P < 0.001$). In addition, liver GSH levels were significantly decreased ($P < 0.001$) compared to the normal control.

Four hours after the administration of paracetamol, *Asteracantha* pre- and post-treated mice showed a significant decrease ($P < 0.001$) in serum enzyme levels and a significant increase ($P < 0.001$) in the liver GSH, a pattern similar to that observed in the CCl_4-treated mice (Table 2). The LD50 for paracetamol was found to be $350\,mg\,kg^{-1}$. Although the extent of protection was not as potent as for NAC, *Asteracantha* also proved to be an effective hepatoprotective agent.

Histopathological examination also provided supportive evidence for the results obtained from the enzyme analysis. Microscopically, liver slices from control animals stained with haematoxylin and eosin showed normal parenchymal architecture with cords of hepatocytes, portal tracts and terminal veins without noticeable alterations.

Table 1 Effect of *Asteracantha longifolia* on CCl_4-induced alterations in the activity of serum enzymes and hepatic levels of reduced glutathione in mice.

Group	Enzyme activity			GSH ($\mu g\,(g\,liver)^{-1}$)
	ALT ($U\,L^{-1}$)	AST ($U\,L^{-1}$)	ALP ($U\,L^{-1}$)	
1	5.1 ± 0.7	12.4 ± 0.8	14.2 ± 2.1	2916.0 ± 222.4
2	5.1 ± 0.5^{ns}	15.0 ± 1.4^{ns}	11.0 ± 1.4^{ns}	2703.8 ± 104.6^{ns}
3	974.8 ± 28.8	1347.4 ± 90.3	68.5 ± 3.6	1015.2 ± 137.5
4	31.6 ± 2.4	45.2 ± 5.2	27.3 ± 5.3	1447.0 ± 68.7
5	$1003.6 \pm 8.7^{ns*}$	947.2 ± 25.7*	49.4 ± 6.9*	1878.5 ± 432.6*
6	21.5 ± 2.4*	22.3 ± 2.4**	$26.4 \pm 3.8^{ns*}$	2378.8 ± 109.4**
7	$933.3 \pm 41.1^{ns*}$	902.9 ± 80.6*	33.2 ± 6.3**	$1642.1 \pm 367.5^{ns*}$
8	9.7 ± 0.9**	12.1 ± 1.6*	$16.7 \pm 2.5^{ns*}$	2382.7 ± 107.3**

Mice were treated as follows: group 1, untreated; group 2, *Asteracantha* ($0.9\,g\,kg^{-1}$, p.o.) for 7 days; group 3, a single dose of CCl_4 ($0.5\,mL\,kg^{-1}$ in olive oil, i.p.) and killed 24 h later; group 4, a single dose of CCl_4 and killed 4 days later; group 5, a single dose of CCl_4 followed by one dose of plant extract and killed 24 h later; group 6, a single dose of CCl_4 + 3 daily doses of plant extract and killed 4 days later; group 7, 7 daily doses of plant extract + a single dose of CCl_4 and killed 24 h later; group 8, 7 doses of plant extract + a single dose of CCl_4 and killed 4 days later. ns: not significant compared to group 1. ns*: not significant compared to corresponding CCl_4 control group. *$P < 0.05$ and **$P < 0.001$ compared to corresponding CCl_4 control group. Results given as mean ± s.e.m.

Table 2 Effect of *Asteracantha longifolia* on paracetamol-induced alterations in the activity of serum enzymes and hepatic levels of reduced glutathione in mice.

Group	Enzyme activity			GSH ($\mu g\,(g\,liver)^{-1}$)
	ALT ($U\,L^{-1}$)	AST ($U\,L^{-1}$)	ALP ($U\,L^{-1}$)	
9	588.1 ± 38.6	609.4 ± 60.1	89.6 ± 7.1	346.2 ± 64.6
10	13.0 ± 1.7*	30.6 ± 5.6*	47.5 ± 4.9*	3363.3 ± 157.5*
11	290.5 ± 45.7*	238.0 ± 44.9*	$78.2 \pm 8.4^{ns*}$	2225.5 ± 314.7*
12	205.6 ± 54.2*	269.4 ± 61.5*	48.6 ± 4.6*	1613.7 ± 210.0*

Mice were treated as follows: groups 1 and 2 given in Table 1 are common to both CCl_4 and paracetamol treatments; group 9, a single oral dose of paracetamol ($300\,mg\,kg^{-1}$ in saline after 16-h fast) and killed 4 h later; group 10, paracetamol + a single dose of NAC ($500\,mg\,kg^{-1}$) and killed 4 h later; group 11, a single dose of paracetamol + one dose of plant extract and killed 4 h later; group 12, 7 doses of plant extract over 7 consecutive days + a single dose of paracetamol and killed 4 h later. ns*: not significant from the paracetamol control group. *$P < 0.001$ compared to paracetamol control group. Results given as mean ± s.e.m., n = 20.

Liver sections of mice challenged with CCl_4 alone showed mainly centrilobular necrosis with focal fatty change and ballooning degeneration in the surviving hepatocytes (Figure 1A). In animals treated with *Asteracantha*, there was a marked reduction in the hepatocellular necrosis whereas the pre-treated group 4 days after the administration of CCl_4 showed no histological evidence of necrosis (Figure 1B). The treatment regimen with *Asteracantha* extract alone did not induce hepatotoxicity in mice.

Macroscopically, the liver appeared dark and congested in paracetamol-intoxicated mice. Histologically, the liver showed confluent necrosis with vacuolation and ballooning degeneration in the surviving hepatocytes (Figure 1C). A remarkable improvement in the histopathological changes was observed in *Asteracantha*-treated mice compared to the paracetamol control group, a pattern similar to that observed in the CCl_4-induced liver injury model. Animals pre-treated with the plant extract showed no necrosis but diffused vacuolar degeneration 4 h after the administration of paracetamol (Figure 1E).

Histopathological observations clearly complement results from biochemical analysis and overall results indicate that pre-treatment is superior to post-treatment in both models of hepatotoxicity studied.

Discussion

Viral hepatitis is a common disease in the world, especially in developing countries. However, there are no effective drugs for the treatment of this disease. In recent years scientists have carried out a considerable amount of research on traditional medicine in an attempt to develop new drugs for hepatitis. Compounds that can either decrease the necrotic damage to hepatocytes via enhanced defence mechanisms against toxic insult or improve the repair of damaged hepatocytes are considered potentially useful in the treatment of human hepatitis (Perrissoud & Testa 1982).

Paracetamol- and CCl_4-induced liver damage in experimental animals are commonly used models for the screening of hepatoprotective drugs (Slater 1965; Plaa & Hewitt 1982). In the present study, we compared the effect of *Asteracantha longifolia* on these two mechanistically different liver injury models in an attempt to examine its alleged hepatoprotective activity.

Both paracetamol and CCl_4 share the common property of being converted to their respective reactive metabolites, N-acetyl parabenzo quinoneimine (NAPQI) and the halogenated free radical trichloromethyl radical, by hepatic cytochrome P_{450} (Packer et al 1978; Van de Straat et al 1987). Covalent binding of the trichloromethyl radical to cell protein is considered the initial step in a chain of events that eventually leads to lipid peroxidation of the cell membrane and endoplasmic reticulum. The peroxidative products induce hypofunction of the membrane and finally cytosolic enzymes appear in the blood (Recknagel et al 1989).

In agreement with this explanation, the present investigation shows significantly high serum ALT, AST and ALP values ($P < 0.001$) 24 h after the administration of CCl_4. A percentage reduction in the serum enzyme levels of ALT, AST and ALP was observed in the pre- and post-treated groups 24 h and 4 days after the administration of CCl_4 (Table 1).

In contrast to the toxic activation of CCl_4 via the P_{450} 2E1 pathway, the detoxification pathway is by glutathione conjugation of trichloromethyl radical with a P_{450} 2E1-mediated CCl_4 metabolite. GSH is a critical determinant of tissue susceptibility to oxidative damage and the depletion of hepatic GSH has been shown to be associated with an enhanced toxicity to chemicals, including CCl_4. Significant impairment in hepatic GSH status associated with substantial hepatocellular damage induced by CCl_4 further suggests the determinant role of hepatic GSH in the development of CCl_4 toxicity (Ko et al 1995). Cell injury induced by xenobiotics occurs only if mitochondrial GSH is depleted.

In the present study a significant decrease ($P < 0.001$) in liver GSH was observed 24 h and 4 days after the administration of CCl_4. In pre- and post-treatment with *Asteracantha*, the level of GSH increases significantly ($P < 0.001$) 4 days after the administration of CCl_4.

The increase in hepatic GSH level in mice pre-treated with *Asteracantha* may result from the enhancement of either de-novo GSH synthesis or GSH regeneration or both. As a consequence of the action of *Asteracantha* in GSH metabolism, the hepatic GSH level can be sufficiently maintained to counteract the increased formation of free radicals, as in the case of CCl_4 toxicity.

Paracetamol (acetaminophen) is metabolized to the reactive metabolite NAPQI and once formed this metabolite conjugates and depletes the cellular GSH level and then binds extensively to the sul-fhydryl groups of the cellular proteins (Tirmenstein & Nelson 1990). Since the toxicity is enhanced by factors that cause GSH depletion, enhanced NAPQI formation or reduction in the antioxidative capacity of the liver, it could be suggested that the partial hepatoprotection afforded by some of the plants used may be ascribed to the opposing action of one or more of these factors (Ali et al 2001).

In the present experiment, the significant reduction observed in the liver GSH level ($P < 0.001$) 4 h after the administration of paracetamol alone was improved significantly ($P < 0.001$) when the animals were pre- and post-treated with the *Asteracantha* extract (Table 2).

Significant reductions in the activities of serum ALT, AST and ALP enzymes were also observed in the pre- and post-treated groups compared with those of the paracetamol control group, showing reduced leakage of enzymes from the hepatocytes (Table 2).

The acetaminophen toxicity following NAPQI generation is chiefly due to oxidative stress and can effectively be ameliorated by antioxidants, whereas the hepatic damage due to halogenated free radicals may be due to lipid peroxidation as well as alkylation (Bus & Gibson 1979; Harman 1985; Dogterom et al 1988).

The histopathological observations showing a faster regeneration of hepatic cells in mice seem to suggest the possibility of these plant extracts being able to condition

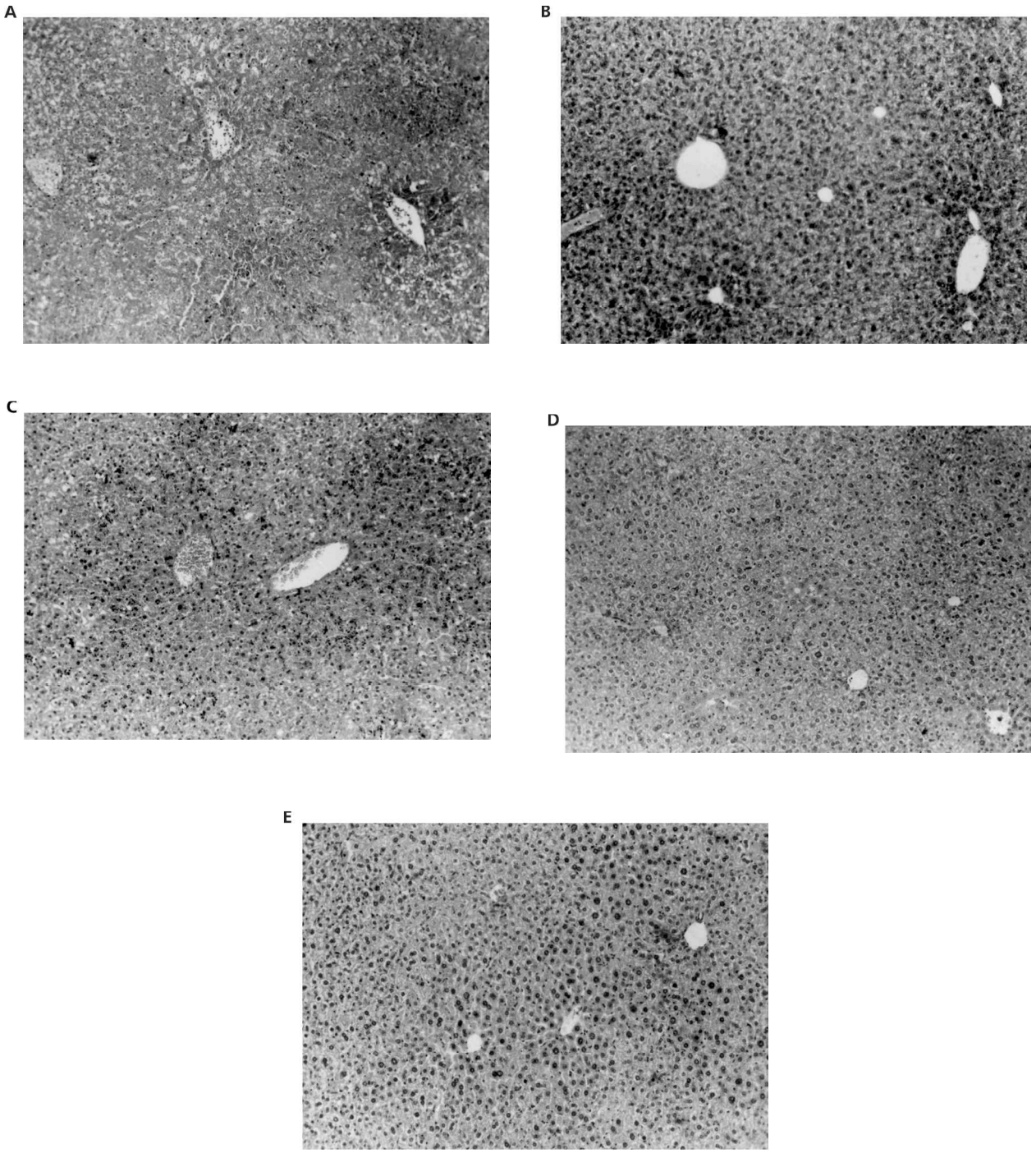

Figure 1 Assessment of CCl_4- and paracetamol-induced hepatotoxicity by histopathology. Haematoxylin and eosin-stained liver sections. (A) CCl_4 control group 4 days after the administration of CCl_4. (B) Pre-treatment with *Asteracantha longifolia* and killed 4 days after the administration of CCl_4 shows no histological evidence of necrosis. (C) Paracetamol control group shows confluent necrosis with congestion 4 h after the administration of paracetamol. (D) Paracetamol + NAC 4 h after the administration of paracetamol does not show necrosis but vacuolar degeneration is visible. (E) Pre-treatment with *Asteracantha longifolia* and killed 4 h after the administration of paracetamol shows no necrosis but diffused vacuolar degeneration is visible. 100 × magnification.

the hepatic cells to a state of accelerated regeneration, thus decreasing the leakage of ALT, AST and ALP into the circulation (Jayatilaka et al 1989, 1990). The observed protective effect of *Asteracantha* against the hepatotoxins may be attributed to the presence of flavonoids, terpenoids, sterols, betulin and aliphatic esters among the plant constituents (Misra et al 2001). The flavonoids are known to be antioxidants, free radical scavengers and

antiperoxidants leading to hepatoprotection. However, no attempt was made in the present experiment to study the active principles of this extract which are responsible for the liver protective activity.

In several plants with hepatoprotective properties the crude drug effect is known to be mediated by the action of a number of different active components, the potencies of the individual active principles being less than that of the crude extract (Jayatilaka et al 1989).

The mechanism by which *Asteracantha* exerts its protective action against the hepatotoxin-induced alterations in the liver is not clear. The fact that post-treatment with the plant extract is also capable of bringing about a recovery indicates that the protective action of *Asteracantha* may not simply be due to an antioxidative property.

The overall results of the present study indicate that, under the present experimental conditions, the aqueous extract of *Asteracantha longifolia* possesses hepatoprotective and antioxidative properties against CCl_4- and paracetamol-induced hepatotoxicities, which rationalizes the use of *Asteracantha* for the treatment of patients with liver diseases in Sri Lanka.

While the present investigation has scientifically confirmed the usefulness of the aqueous extract of *Asteracantha longifolia* as an effective hepatoprotectant, further studies are needed to elucidate its exact mechanism of action.

References

Ali, B. H., Basher, A. K., Rashid, R. A. (2001) Effect of the traditional medicinal plants *Rhazya stricta*, *Balanitis aegyptiaca* and *Haplophylum tuberculatum* on paracetamol induced hepatotoxicity in mice. *Phytotherapy Res.* **15**: 598–603

Black, M. (1984) Acetaminophen toxicity. *Annu. Rev. Med.* **35**: 577–593

Bus, J. S., Gibson, J. E. (1979) Lipid peroxidation and its role in toxicology. *Rev. Biochem. Toxicol.* **1**: 125–149

Dhawan, B. N., Srimal, R. C. (1998) Acute toxicity and gross effects. In: *Laboratory manual for pharmacological evaluation of natural products*. United Nations Industrial Development Organization and International Centre for Science and High Technology, pp 17–20

Dogterom, P., Nagelkerke, J. F., Van Stevenink, J., Mulder, G. J. (1988) Inhibition of lipid peroxidation by disulfiram and diethyl dithio carbamate doesn't prevent hepatotoxin induced cell death in isolated rat hepatocytes: A study with allyl alcohol tertiary-butyl hydroperoxide, diethyl maleate, bromoisovaleryl-urea and carbon tetrachloride. *Chem. Biol. Interact.* **66**: 251–265

Harman, A. W. (1985) The effectiveness of antioxidants in reducing paracetamol induced damage subsequent to paracetamol activation. *Res. Commun. Chem. Pathol. Pharmacol.* **49**: 215–228

Jayatilaka, K. A. P. W., Thabrew, M. I., Pathirana, C., De Silva, D. G. H., Perera, D. J. B. (1989) An evaluation of the potency of *Osbeckia octandra* and *Melothria maderaspatana* as antihepatotoxic agents. *Planta Med.* **55**: 137–139

Jayatilaka, K. A. P. W., Thabrew, M. I., Perera, D. J. B. (1990) Effect of *Melothria maderaspatana* on carbon tetrachloride induced changes in rat hepatic microsomal drug metabolizing enzyme activity. *J. Ethnopharmacol.* **30**: 97–105

Jayaweera, D. M. A. (1981) *Medicinal plants used in ceylon*. Part 1, National Science Council of Sri Lanka, Colombo, pp 8–9

Jollow, D. Z., Mitchel, J. R., Zampaglione, N., Gillete, J. R. (1974) Bromobenzene induced liver necrosis: Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. *Pharmacology* **11**: 151–169

Ko, K. M., Ip, S. P., Poon, M. K. T., Wu, S. S., Che, C. T., Ng, K. M., Kong, Y. C. (1995) Effect of lignan enriched *Fructus schisandrae* extract on hepatic glutathione status in rats: Protection against carbon tetrachloride toxicity. *Planta Med.* **61**: 134–137

Misra, T. N., Singh, H. S., Pandey, B. K., Singh, R. P., Pandey, R. P. (2001) Constituents of *Asteracantha longifolia*. *Fitoterapia* **72**: 194–196

Nelson, S. D. (1990) Molecular mechanisms of the hepatotoxicity caused by acetaminophen. *Semin. Liver Dis.* **10**: 267–278

Packer, J. E., Slater, T. F., Wilson, R. L. (1978) Reaction of the CCl_4 related peroxy free radical (CCl_3–O–O·) with amino acids. Pulse radiolysis evidence. *Life Sci.* **23**: 2617–2620

Perrissoud, D., Testa, B. (1982) Hepatic pharmacology: Mechanisms of action and classification of antinarcotic hepatoprotective agents. *Trends Pharmacol. Sci.* **2**: 356–357

Plaa, G. L., Hewitt, W. R. (1982) Quantitative evaluation of indices of hepatotoxicity. In: Zakim, D., Boyer, T. D (eds) *Toxicology of the liver*, Raven, New York, pp 103–120

Recknagel, R. O., Glende, E. A. Jr., Dolak, J. A., Waller, R. L. (1989) Mechanisms of carbon tetrachloride toxicity. *Pharmacol. Ther.* **43**: 139–145

Reitman, S., Frankel, S. (1957) A colorimetric method for the determination of serum levels of glutamic oxaloacetic acid and pyruvic acid transaminases. *Am. J. Clin. Pathol.* **10**: 394–399

Slater, T. F. (1965) Biochemical studies of the liver. In: *Biochemical mechanisms of liver injury*. Academic Press, London, pp 1–44

Tirmenstein, M. A., Nelson, S. D. (1990) Acetaminophen induced oxidation of protein thiols: Contribution of impaired thiol-metabolizing enzymes to the breakdown of adenine nucleotides. *J. Biol. Chem.* **265**: 3059–3065

Van de Straat, R., de Vries, J., Debets, A. J. J., Vermueulein, N. P. E. (1987) The mechanism of paracetamol induced hepatotoxicty by 3,5-dialkyl substitution. The role of glutathione depletion and oxidative stress. *Biochem. Pharmacol.* **36**: 2065–2071

Zhu, M., Yeung, R. Y., Lin, K. F., Li, R. C. (2000) Improvement of phase 1 drug metabolism with *Schisandra chinensis* against CCl_4 hepatotoxicity in a rat model. *Planta Med.* **66**: 521–525