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Hepatoprotective and Antioxidant Activities of the Fractions of *Coccinia grandis* against Paracetamol-induced Hepatic Damage in Mice

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ABSTRACT

The leaves of *Coccinia grandis* L. Voigt (Cucurbitaceae) are used in Indian traditional medicine for the treatment of gout, rheumatism, jaundice, bronchitis, fever, skin eruptions, wounds, etc. We evaluated the hepatoprotective and antioxidant activities of the petroleum-ether, chloroform, ethyl acetate and residual fractions of the hydromethanol extract of the leaves of *C. grandis* against paracetamol-induced hepatic damage in *Swiss* albino mice. Hepatotoxicity was induced by oral administration of paracetamol (2 g/kg) for 7 days. Silymarin (25 mg/kg, p.o.) was used as the standard drug. Activities of liver marker enzymes (aspartate aminotransferase, alanine aminotransferase, acid phosphatase, and alkaline phosphatase), uric acid, and biluribin levels were determined in serum, while end products of lipid peroxidation, *viz.* lipid hydroperoxides and malondialdehyde, tissue protein, enzymatic, and non-enzymatic antioxidants were measured in liver homogenate. Oral administration of the fractions at a dose of 200 mg/kg b.w. for 7 days resulted in a significant reduction in serum biochemical parameters and liver malondialdehyde (MDA) and an increase in enzymatic and non-enzymatic antioxidants when compared with paracetamol-damaged mice. Profound fatty degeneration, fibrosis, and necrosis observed in the hepatic architecture of paracetamol-treated mice were found to acquire near-normalcy in drug co-administered mice. The effect produced by the chloroform fraction was almost comparable with the silymarin-treated group. The leaves of *C. grandis* protected the liver against paracetamol damage, which may be due to its antioxidant mechanism.

Keywords: hepatotoxicity, lipid peroxidation, silymarin, traditional medicine

INTRODUCTION

Paracetamol (also known as Acetaminophen) is a widely used analgesic and antipyretic agent available as an over-the-counter medication. Although considered safe at therapeutic doses, in overdose, paracetamol causes acute centrilobular hepatic necrosis that can be fatal (Burke *et al*. 2006). Paracetamol at therapeutic doses is rapidly metabolized in the liver principally through conjugation with sulfate and glucuronide, and then excreted by the kidneys. Only a small portion (5-10%) is oxidized by hepatic cytochrome P450 enzyme system (specifically CYP2E1 and CYP1A2) to generate highly reactive and cytotoxic intermediate, *N*-acetyl-*p*-benzoquinoneimine (NAPQI), which is quickly conjugated by hepatic glutathione to yield a harmless water-soluble product, mercapturic acid (Dahlin *et al.* 1984). Paracetamol toxicity in hepatocytes initiates a sequence of events that eventually leads to cell death. Toxic doses of paracetamol will deplete the levels of hepatic glutathione (GSH) followed by covalent binding of NAPQI to hepatic parenchymal cell proteins and DNA with resultant liver injury (Jollow *et al.* 1973; Potter and Hinson 1986). Evidence indicates that lipid peroxidation resulting from oxidative stress, generation of reactive oxygen and nitrogen species, mitochondrial dysfunction, and disruption of calcium homeostasis are the mechanisms that may contribute to the initiation and progression of acetaminophen-induced hepatotoxicity (Wendel *et al.* 1979; Arnaiz *et al.* 1995).

Historically plants have been used in folk medicine to treat various diseases and are rich natural sources of antioxidants. Many researchers have examined the effect of plants used traditionally by indigenous people to support liver function and treat diseases of the liver. *Coccinia grandis* L. Voigt., belonging to the family Cucurbitaceae, is commonly known as "ivy gourd" in English. It is a tropical plant found in many countries of Asia and Africa. The roots, stems, leaves, and whole plant of *C. grandis* are used in the treatment of jaundice, bronchitis, skin eruptions, burns, insect bites, fever, indigestion, nausea, eye infections, allergy, syphilis, gonorrhoea, etc. (Kirthikar and Basu 1987; Wasantwisut and Viriyapanich 2003). The leaves of this species are widely used in Indian folk medicine for reducing the amount of sugar in urine of patients suffering from diabetes mellitus. Venkateswaran and Pari (2003) suggested the use of this plant in the treatment of diabetes. The crude hydromethanol extract of the leaves of *C. grandis* has been reported for its xanthine oxidase inhibitory and hypouricaemic activities (Umamaheswari *et al.* 2007) The various fractions of the hydromethanol extract of the leaves of *C. grandis* possessed significant *in vitro* antioxidant activity (Umamaheswari and Chatterjee 2008a), *in vitro* xanthine oxidase inhibitory activity (Umamaheswari and Chatterjee 2008b) and anti-cataract activity against naphthalene-induced cataractogenesis in rats (Umamaheswari and Chatterjee 2008c). To our knowledge, there are no available reports on the hepatoprotective activity of *C. grandis* leaves. The objective of the present work was to evaluate the hepatoprotective and antioxidant activities of the various fractions of *C. grandis* hydromethanol leaf extract against paracetamol-induced hepatic damage in mice.

MATERIALS AND METHODS

Experimental animals

Swiss albino mice of either sex weighing between 20-25 g pro-

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cured from the University Animal house were used for the study. The animals were housed in polypropylene cages inside a well-ventilated room. The room temperature was maintained at 23 ± 2°C with a 12 h light/dark cycle. The animals were fed with commercial rat feed pellets and provided with drinking water *ad libitum.* All animal procedures described were reviewed and approved by the University Animal Ethical Committee.

Drugs and chemicals

Paracetamol was obtained as a gift sample from Eurochem Laboratories Ltd., Alathur, Tamil Nadu, India. Silymarin was obtained from Microlabs, Bangalore, India. Oxidized glutathione, epinephrine and DTNB were obtained from Sisco Research Laboratories Pvt., Ltd., Mumbai, India. 2-2′ dipyridyl and *O*-dianisidine were obtained from Himedia Laboratories Ltd., Mumbai, India. All other chemicals used in the study were obtained commercially from S.D. Fine Chemicals, Mumbai and Qualigens Chemicals, Mumbai and were of analytical grade.

Plant material

Leaves of *C. grandis* were collected from approximately 6-month old plants in vegetative season from Coimbatore district, Tamil Nadu, during May, 2006. The plant was identified and authenticated by Dr. G. V. S. Murthy, Joint Director, Botanical Survey of India, Tamil Nadu Agricultural University Campus, Coimbatore, India, where a voucher specimen (No. BSI/SC/5/23/06-07/ Tech.1951) of the plant has been deposited in the herbarium.

Preparation of extract and fractionation

Fresh leaves of the plant were dried in the shade at room temperature and powdered mechanically and sieved through a No. 20 mesh sieve. About 500 g of the leaf powder was extracted with 2.5 L of methanol: water (7:3) by maceration at room temperature for 4 h using a mechanical shaker. The hydromethanol extract (27% w/v) was partitioned separately into petroleum ether, chloroform and ethyl acetate fractions separately in the order of increasing polarity. The fractions were dried at 40°C under vacuum. The percentage yield of the fractions was: petroleum ether (2%), chloroform (1.2%), ethyl acetate (1.4%) and residual fractions (18%).

Experimental protocol

Animals were divided into 7 groups consisting of 6 animals each. Group I received 0.5% carboxymethyl cellulose (CMC) at a dose of 10 ml/kg b.w., orally and served as solvent control. Group II mice were similarly treated as group I. Groups III–VI received the petroleum ether (PEF), chloroform (CF), ethyl acetate (EAF), and residual fractions of *C. grandis* respectively at a dose of 200 mg/kg orally. Group VII received silymarin (25 mg/kg, p.o.) and served as the positive control (Muriel *et al.* 1992). All the fractions and the standard drug were suspended in 0.5% CMC and administered for 7 consecutive days.

On the 7th day, animals belonging to the groups II-VII were administered a single dose of paracetamol (2 g/kg orally) 1 h before final drug administration to induce acute hepatotoxicity (Hiroshini *et al.* 1987). After 24 h of final drug administration, blood was collected by retro orbital puncture under mild ether anesthesia. The serum was used for the assay of marker enzymes *viz.*, aspartate amino transferase (AST), alanine amino transferase (ALT), acid phosphatase (ACP), alkaline phosphatase (ALP) and uric acid and bilirubin. The enzyme levels were assayed using standard commercial kits. The results were expressed as units/liter.

Immediately after blood collection, animals were sacrificed by cervical dislocation and liver was dissected out and washed with ice-cold saline to remove as much blood as possible. Liver homogenate (5% w/v) were prepared in cold 50 mM phosphate buffer (pH 7.4) using a Remi homogenizer. The unbroken cells and cell debris were removed by centrifugation at 5000 g for 10 min and the resulting supernatant was used for the estimation of lipid peroxidation and enzymatic and non-enzymatic antioxidants.

The amount of total protein present in the tissue was estimated by the method of Lowry *et al*. (1951). Lipid peroxidation as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides (LH) were measured by the method of Nichans and Samuelson (1986). Superoxide dismutase (SOD) activity was determined by the inhibition of autocatalyzed adrenochrome formation in the presence of the homogenate at 480 nm (Kakkar *et al.* 1984). Catalase (CAT) activity was estimated by the catalysis of hydrogen peroxide (H_2O_2) to H_2O in an incubation mixture adjusted to pH 7.0 and recorded at 254 nm (Sinha 1972). Gutathione reductase (GSSH) activity was determined spectrophotometrically by the decrease in absorbance of NADPH at 340 nm (Racker 1955). Glutathione peroxidase (GPx) activity was measured by the procedure of Paglia and Valentine (1967). Peroxidase activity (Px) was measured spectrophotometrically by following the change in absorbance at 460 nm due to *o*-dianisidine oxidation in the presence of H_2O_2 and enzyme (Lobarzewski and Ginalska 1995). The estimation of reduced glutathione (GSH) was based on the reaction of reduced glutathione with dithionitrobenzoic acid (DTNB) to give a compound that absorbs at 412 nm (Ellmann 1959).

Histopathological studies

A portion of liver tissue in each group was fixed in 10% formalin, dehydrated in gradually increasing concentrations of ethanol (50-100%), cleared in xylene and embedded in paraffin for histopathological studies. Liver sections of 5 µm thickness were prepared. Heamotoxylin and eosin were used for staining and later the microscopic slides of liver cells were photographed at 100X magnification.

Statistical analysis

Statistical analysis was carried out using GraphPad software (GraphPad InStat) by one-way Analysis of Variance (ANOVA) followed by Dunnett's test. Results are expressed as mean ± SEM from six rats in each group. P values < 0.05 were considered significant.

RESULTS

After 24 h of paracetamol treatment at a dose of 2 g/kg, the levels of hepatocellular marker enzymes like AST, ALT, ACP and ALP and in addition, serum uric acid and bilirubin levels were found to be significantly ($P<0.01$) elevated compared with the control group. Pre-treatment with the petroleum ether, chloroform, and ethyl acetate fractions of *C. grandis* significantly ($P<0.05$) reduced the serum marker enzymes and bilirubin level when compared to the paracetamol control. The reduction exhibited by the chloroform fraction is almost similar to the silymarin-treated group. However, the observed reduction in biochemical parameters in mice administered with the residual fraction did not produce any reduction ($P>0.05$) compared to paracetamol control (**Table 1**).

Our study showed that paracetamol significantly ($P<0.01$) reduced the amount of total protein and enhanced the end products of hepatic lipid peroxidation namely, MDA and LH in mice compared to the control. Pre-treatment with the petroleum ether, chloroform, and ethyl acetate fractions of *C. grandis* and silymarin significantly ($P<0.01$) increased the total protein and inhibited the lipid peroxidation. The reduction in MDA and LH levels produced by the residual fraction was found to be insignificant ($P>0.05$) compared to paracetamol control (**Table 2**).

The effect of the fractions of *C. grandis* on liver enzymatic and non-enzymatic antioxidants is presented in **Table 3**. Acute paracetamol administration produced a significant ($P<0.01$) decrease in the levels of tissue enzymatic and non-enzymatic antioxidants when compared to the solvent control. Pre-treatment with the petroleum ether, chloroform, and ethyl acetate fractions of *C. grandis* significantly increased the enzyme levels and brought them to near normalcy. The effect produced by the chloroform fraction was almost comparable to the standard drug silymarin. There was no significant ($P>0.01$) increase in the enzyme levels in

Table 1 Effect of the fractions of *Coccinia grandis* on serum transaminases, phosphatases, uric acid and bilirubin in control and experimental animals.

Group	Dose (p.o.)	AST (IU/L)	ALT (IU/L)	ACP (IU/L)	ALP (IU/L)	Uric acid (mg/dl)	Bilirubin (mg/dl)
Control (0.5% CMC)	10 ml/kg	605.3 ± 10.5	4693 ± 28.38	99.6 ± 19.37	88.9 ± 15.90	3.27 ± 0.04	2.85 ± 0.48
Paracetamol control	2 g/kg	1210 ± 11.26 a	7685 ± 31.54 a	242.3 ± 21.85 a	191.5 ± 10.50 a	6.98 ± 0.43 a	5.54 ± 0.57 a
PEF	200 mg/kg	720.5 ± 11.98 b	5519 ± 14.18 b	124.0 ± 8.78 b	108.6 ± 16.45 b	4.12 ± 0.29 b	3.00 ± 0.27 b
CF	200 mg/kg	700 ± 5.84 b	5470 ± 17.53 b	104.6 ± 11.64 b	99.7 ± 16.61 b	4.10 ± 0.32 b	3.04 ± 0.19 b
EAF	200 mg/kg	740 ± 16.62 b	5549 ± 12.33 b	138.1 ± 17.22 b	112.75 ± 18.42 b	4.02 ± 0.21 b	3.24 ± 0.42 b
RF	200 mg/kg	1087.5 ± 13.9 c	7315 ± 16.74 c	188.3 ± 21.85 c	180.16 ± 10.62c	6.19 ± 0.14 c	3.40 ± 1.16 c
Silymarin	25 mg/kg	652 ± 6.14 b	5079 ± 18.22 b	103.5 ± 11.75 b	93.0 ± 18.23 b	3.93 ± 0.30 b	3.15 ± 0.22 b

Values are expressed as mean ± SEM; n = 6 in each group. [a]P <0.01 when compared to control; [b]P <0.05 and [c]P>0.05 when compared to paracetamol control (one way ANOVA followed by Dunnett's test).

Table 2 Effect of fractions of *Coccinia grandis* on tissue protein, MDA and LH in control and experimental animals.

Group	Dose (p.o.)	Protein (mmoles/min/mg wet tissue)	MDA (nmoles/min/mg protein)	LH (nmoles/min/mg protein)
Control (0.5% CMC)	10 ml/kg	94.98 ± 3.60	0.152 ± 0.01	0.264 ± 0.01
Paracetamol control	2 g/kg	71.50 ± 0.88 a	0.393 ± 0.07 a	0.604 ± 0.01 a
PEF	200 mg/kg	86.55 ± 5.85 b	0.205 ± 0.03 b	0.310 ± 0.03 c
CF	200 mg/kg	88.47 ± 3.51 c	0.185 ± 0.01 c	0.305 ± 0.01 c
EAF	200 mg/kg	85.26 ± 4.10 c	0.216 ± 0.02 c	0.395 ± 0.03 c
RF	200 mg/kg	75.66 ± 1.17 d	0.329 ± 0.08 d	0.534 ± 0.01 d
Silymarin	25 mg/kg	89.53 ± 2.08 c	0.179 ± 0.01 c	0.290 ± 0.02 c

Values are expressed as mean ± SEM; n = 6 in each group. [a]P <0.01 when compared to control; [b]P <0.05, [c]P<0.01 and [d]P>0.05 when compared to paracetamol control (one way ANOVA followed by Dunnett's test).

Table 3 Effect of fractions of *Coccinia grandis* on tissue enzymatic and non-enzymatic antioxidants in experimental animals.

Group	Dose (p.o.)	Catalase (µmoles/min/ mg protein)	GPx (nmoles/min/ mg protein)	SOD (nmoles/min/ mg protein)	GSSH (nmoles/min/ mg protein)	Peroxidase (nmoles/min/ mg protein)	GSH (nmoles/min/ mg protein)
Control (0.5% CMC)	10 ml/kg	1.59 ± 0.13	3.26 ± 0.07	3.50 ± 0.22	2.26 ± 0.25	1.273 ± 0.06	2.92 ± 0.23
Paracetamol control	2 g/kg	0.70 ± 0.01 a	1.97 ± 0.04 a	0.69 ± 0.03 a	0.43 ± 0.01 a	0.279 ± 0.03 a	1.11 ± 0.14 a
PEF	200 mg/kg	0.92 ± 0.09 b	2.72 ± 0.18 b	1.43 ± 0.20 b	1.64 ± 0.35 b	0.870 ± 0.08 b	2.45 ± 0.21 b
CF	200 mg/kg	1.44 ± 0.12 b	2.83 ± 0.11 b	1.56 ± 0.17 c	1.98 ± 0.30 b	0.918 ± 0.04 b	2.59 ± 0.16 b
EAF	200 mg/kg	0.95 ± 0.04 c	2.54 ± 0.17 b	1.42 ± 0.10 b	1.47 ± 0.09 b	0.720 ± 0.09 b	2.26 ± 0.11 b
RF	200 mg/kg	0.70 ± 0.01 d	2.16 ± 0.11 d	0.81 ± 0.02 d	0.52 ± 0.09 d	0.413 ± 0.06 d	1.28 ± 0.11 d
Silymarin	25 mg/kg	1.43 ± 0.03 b	2.81 ± 0.04 b	1.64 ± 0.30 b	2.17 ± 0.02 b	0.980 ± 0.08 b	2.65 ± 0.13 b

Values are expressed as mean ± SEM; n = 6 in each group. [a]P <0.01 when compared to control; [b]P <0.05, [c]P<0.01 and [d]P>0.05 when compared to paracetamaol control (one way ANOVA followed by Dunnett's test).

residual fraction treated group compared to paracetamol control.

The results of the histopathological studies of control and experimental animals are presented in **Fig. 1A-G**. Section of control mouse liver shows normal hepatic parenchyma, central vein, and sinusoids (**Fig. 1A**). Section of mouse liver treated with paracetamol shows foci of extensive haemorrhagic necrosis, more around the central vein (**Fig. 1B**). Liver section of mice treated with the pet-ether fraction shows few hepatocytes with microvesicular steatosis and congestion of sinusoids (**Fig. 1C**). Liver section of mice treated with the chloroform fraction shows hepatic parenchyma with congestion of central vein and microvesicular steatosis (**Fig. 1D**). Liver section of mice treated with the ethyl acetate fraction shows normal liver parenchyma with the periphery showing haemorrhagic necrosis (**Fig. 1E**). Liver section of mice treated with the residual fraction shows few hepatocytes with microvesicular steatosis and the periportal area shows focal inflammatory cell collection (**Fig. 1F**). Liver section of mice treated with the standard drug silymarin shows normal hepatic parenchyma with mild congestion of sinusoids (**Fig. 1G**).

DISCUSSION

In the assessment of liver damage by paracetamol, the determination of enzyme levels is largely used. Administration of a large dose of paracetamol (2 g/kg, p.o.) to fasted mice produces hepatic lesions within 24 h and serum aminotransferases *viz.* AST and ALT were significantly elevated (Drotman and Lawhorn 1978). These enzymes are cytoplasmic in origin, and necrosis or membrane damage releases the enzymes into circulation and therefore can be measured in the serum (Dixon *et al.* 1975). Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver. In addition, destruction of hepatic cells causes an elevation in the serum levels of ACP, ALP, and bilirubin. ACP is localized almost exclusively in the particles and its release parallels that of lysosomal hydrolases (Tanaka and Iizuka 1968). Increase in the serum level of ALP is due to increased synthesis, in the presence of increasing biliary pressure. Studies have demonstrated that administration of paracetamol promotes the conversion of the enzyme xanthine dehydrogenase to the oxidase form (Tirmenstein and Nelson 1990). This conversion in conjunction with the degradation of the adenine nucleotide pool may lead to the production of reactive oxygen species and increased uric acid production. In our study, there was a significant increase in the levels of serum aminotransferases, phosphatases, bilirubin, and uric acid, which evidenced paracetamol-induced hepatocellular damage. Pre-treatment with the fractions of *C. grandis* (except the residual fraction) reversed the increased enzyme activities produced by paracetamol. The subsequent recovery towards normalization of these enzymes strongly suggests the possibility of the fractions being capable of conditioning the hepatocytes so as to cause accelerated regeneration of parenchymal cells (**Table 1**).

Lipid peroxidation (LPO) is an autocatalytic process, which is a common consequence of cell death. It has been postulated as a destructive process in liver injury due to paracetamol administration. The formation of LPO indicates cellular injury mediated by reactive oxygen species with resultant destruction of membrane lipids and production of lipid peroxides leading to failure of antioxidant defense mechanisms (Amimoto *et al.* 1995). Treatment with paracetamol has produced a significant elevation in MDA and LH levels in liver suggesting enhanced lipid peroxidation. A significant decrease in the levels of MDA and LH in mice pre-treated with the PEF, CF, and EAF of *C. grandis* suggest that the fractions may have the ability to protect the liver from free radical injury induced by paracetamol. A

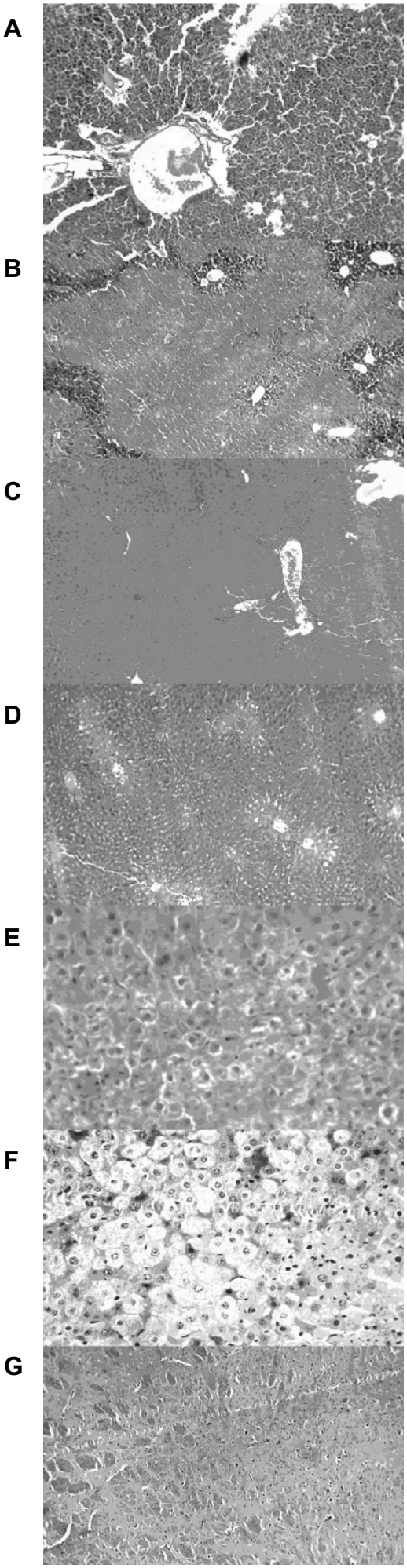

Fig. 1 (**A**) Section of control mouse liver shows normal liver parenchyma, central vein and sinusoids. (**B**) Section of mouse liver treated with paracetamol shows foci of extensive haemorrhagic necrosis, more around the central vein. (**C**) Liver section of mice treated with the pet-ether fraction shows few hepatocytes with microvesicular steatosis and congestion of sinusoids. (**D**) Liver section of mice treated with the chloroform fraction shows hepatic parenchyma with congestion of central vein and microvesicular steatosis. (**E**) Liver section of mice treated with the ethyl acetate fraction shows normal liver parenchyma with the periphery showing haemorrhagic necrosis. (**F**) Liver section of mice treated with the residual fraction shows few hepatocytes with microvesicular steatosis and the periportal area shows focal inflammatory cell collection. (**G**) Liver section of mice treated with the standard drug silymarin shows normal hepatic parenchyma with mild congestion of sinusoids.

decrease in total liver protein observed in the paracetamol treated mice may be associated with the decrease in the number of hepatocytes which in turn may result in decreased hepatic capacity to synthesize protein. But, when mice were pretreated with the PEF, CF, and EAF of *C. grandis*, a significant increase in liver protein was observed indicating the hepatoprotective activity of the fractions through the hepatic cell regeneration (**Table 2**).

It has been postulated that oxidative stress is an important mechanism in the development of paracetamol-induced hepatotoxicity. During toxic paracetamol overdoses, NAPQI formed from cytochrome P450 reacts very rapidly with GSH and hence the concentrations of GSH become very low in centrilobular cells (Reid *et al.* 2005). GSH is a very important tripeptide, which can remove the free radical species such as superoxide anion and alkoxy radicals and hydrogen peroxide. It maintains the membrane protein thiols and acts as a substrate for GPx and glutathione transferase. Under conditions of GSH depletion, GPx, the major peroxide detoxification enzyme is also inhibited (Davis *et al.* 1974). In our study, we observed a significant increase in GSH activity suggesting the protective mechanism of the fractions in response to free radicals. The activity produced by the chloroform fraction was found to be the highest among the fractions tested and is almost similar to the silymarin treated group. Silymarin, by its stabilizing action on the plasma membrane, has been shown to normalize paracetamol-induced elevated biochemical parameters in the serum and liver. It has protective effect on paracetamol-induced LPO and GSH depletion (Campos *et al.* 1989).

In addition, studies have demonstrated that superoxide anion and its dismutation product H_2O_2 have been generated during the formation of NAPQI by cytochrome P450, leading to increased superoxide toxicity (Mitchell *et al.* 1973). SOD is an enzymatic antioxidant that scavenges the superoxide anion and converts into H_2O_2, hence diminishing the toxic effect caused by this radical. The decomposition of H_2O_2 to H_2O is catalysed by CAT in association with GP_X. Thus, a decrease in the activities of both CAT and GP_X leads to an accumulation of H_2O_2. Also, a decrease in the activity of GSSH occurs due to the depletion of thiol leading to related pathologies (James *et al.* 2003). Treatment of mice with the fractions of *C. grandis* increased the activities of enzymatic antioxidants and prevented the accumulation of excessive free radicals from paracetamol intoxication (**Table 3**).

A number of scientific reports indicated certain flavonoids, triterpenoids and steroids have protective effect on liver due to its antioxidant properties (Raokurma and Mishra 1998; Gupta *et al.* 2004; Murugesh *et al.* 2005). We have previously reported the *in vitro* antioxidant activity of the fractions of the leaves of *C. grandis* and the presence of high phenolic and flavonoid content in the fractions has contributed directly to the antioxidant activity by neutralising the free radicals (Umamaheswari and Chatterjee 2008a). This antioxidant property is actually an added advantage for this species towards hepatoprotective activity.

CONCLUSION

The results of this study clearly demonstrated that the leaves of *C. grandis* exhibited potent hepatoprotective activity against paracetamol-induced hepatic damage in mice. This may be due to their antioxidant and free radical scavenging properties. Further studies are in progress to isolate and purify the active principles involved in the hepatoprotective efficacy of the leaves of *C. grandis*.

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