academicJournals

Vol. 7(19), pp. 1305-1309, 17 May, 2013
DOI: 10.5897/JMPR12.630
ISSN 1996-0875 ©2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Effect of methanol extract of *Dicranopteris linearis* leaves against paracetamol- and carbon tetrachloride (CCl_4)-induced liver toxicity in rats

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Accepted 8 May, 2012

The present study aimed to determine the hepatoprotective activity of methanol extract of *Dicranopteris linearis* leaves (MEDL) using two models of liver injury in rats. Rats (n = 6) received 10% DMSO (negative control), 200 mg/kg silymarin (positive control) or MEDL (50, 250, and 500 mg/kg) orally once daily for 7 days and 3 hours after the last administration of the test solutions, they were subjected to the hepatotoxic induction either using carbon tetrachloride (CCl_4) or paracetamol (PCM). The bloods and livers were collected and subjected to biochemical and microscopical analysis. From the data obtained, all doses of MEDL significantly ($P < 0.05$) reduced the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in CCl_4-induced hepatotoxic rats while only the 500 mg/kg MEDL caused significant ($P < 0.05$) reduction in the level of both enzymes in the PCM-induced liver toxicity model. The histological results obtained were in line with the biochemical analysis. In conclusion, the MEDL-induced hepatoprotective activity is attributed partly to its free radicals scavenging and antioxidant activities and high flavonoids content.

Key words: *Dicranopteris linearis*, Gleicheniaceae, *in vivo*, hepatoprotective activity, methanol extract, leaves.

INTRODUCTION

Acute liver failure is caused by a variety of insults, including toxic liver damage by poisons or drugs, and oxidative damage has been known to play a prominent role in hepatic injury mediated by these agents (Adewusi and Afolayan, 2010). Management of liver disease is still a challenge to the modern medicine, which has little to offer alleviation of hepatic ailments (Wagh et al., 2010; Taub, 2003). Therefore, interest and effort have been shifted towards medicinal plants as new sources of hepatoprotective agents. *Dicranopteris linearis* L. (Family Gleicheniaceae) is locally known to the Malay as '*Resam*' but has limited usages within the Malay traditional

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medicine, wherein it is used to treat cold and fever. However, this plant has been used in various countries to treat external wound, ulcers, boils, asthma and intestinal worm infection (Zakaria et al., 2006). The leaves of *D. linearis* have been scientifically proven to possess antinociceptive, anti-inflammatory, antiproliferative and antioxidant activities (Zakaria et al., 2006, 2008, 2011a).

The link between antioxidant and hepatoprotective activities has been established elsewhere (Dash et al., 2007; Chattopadhyay, 2003), and has triggered the present study. Therefore, the present study aimed to determine the hepatoprotective activity of methanol extract of *D. linearis* leaves (MEDL) using various animal models.

MATERIALS AND METHODS

Plant and preparation of the extract

The leaves of *D. linearis* were collected from its natural habitat around Serdang, Selangor, Malaysia between July and August, 2010, and a new voucher specimen, IR 0128/11, was deposited at the Herbarium of the Institute of Bioscience (IBS), Universiti Putra Malaysia. The preparation of MEDL was carried out according to previously described method (Zakaria et al., 2011a). Forty gram of coarse powdered dried leaves was soaked three times, each for 72 h, in methanol (1:20, w/v) at room temperature. The methanol supernatant collected was evaporated to yield approximately 12.3 g of dried MEDL (percentage yielded was ≈ 31%).

Animals used

Adult male Sprague-Dawley rats (weighed 180 to 200 g) were adopted in the present study and the animal ethics approval was obtained from the Animal Ethics Committee, UPM (reference no: UPM/FPSK/PADS/BR-UUH/00383). The animals were cared and handled according to the methods described by Zakaria et al. (2012). Animals were fasted for 48 h prior to all assays, and standard drug (200 mg/kg silymarin) and extract were administered orally (by gavage) with 10% dimethyl sulfoxide (DMSO; 10 ml/kg) as the vehicle.

Pharmacological study

Acute toxicity study in rats

The extract was subjected to the acute toxicity study using a single dose administration of 5000 mg/kg (p.o.) prior to the hepatoprotective study. The effects of a single oral dose of MEDL were monitored over a 14 days period and no symptoms or signs of toxicity were observed in any of the animals treated (Mohamed et al., 2011; Zakaria et al., 2012).

Hepatoprotective assay

In the hepatoprotective study, the 50, 250, and 500 mg/kg MEDL were assayed against paracetamol (PCM; 3 g/kg)- and carbon tetrachloride ($CC1_4$; 1.5 ml/kg)-induced liver toxicity models (Zakaria et al., 2011c). The 10% DMSO was used as the vehicle group, while silymarin (200 mg/kg) was used as the standard drug.

The test solutions were administered for 7 consecutive days and followed 3 h after the last test solutions administration by a single oral administration of PCM or intraperitoneal administration of CCl_4. After the administration of the respective inducer, the rats were fasted for 48 h and later anaesthetized. The blood were collected for biochemical parameters analyses and then the rats were sacrificed and their livers were excised immediately, and the liver were fix in 10% formalin for histopathology studies.

Biochemical studies of blood collected after treatment with hepatotoxicants

The blood (3 ml) was collected by cardiac puncture using sterile disposable syringe and was kept in plain tube. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and was utilized for the estimation of two biochemical parameters, namely, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Zakaria et al., 2011c). After collection of blood samples, the rats in different groups were sacrificed and their livers were excised immediately and were fix in 10% formalin for histopathology studies.

Histopathology studies of liver collected after treatment with hepatotoxicants

Small pieces of liver tissues in each group were collected in 10% formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Sections of 5 µm in thickness were cut and stained with hematoxylin and eosin (H&E). These sections were examined under light microscope for histological changes.

Statistical analysis

The results are presented as mean ± standard error of mean (SEM), and are analyzed using the one-way analysis of variance (ANOVA) test with Dunnet post-hoc test with $P < 0.05$ as the limit of significance.

RESULTS

Acute toxicity study

No signs and symptoms of toxicity and mortality were detected in rats receiving 5000 mg/kg MEDL (p.o.) with normal behavior pattern observed.

Effects of MEDL on the blood liver enzymes level

The effects of MEDL on the levels of two liver enzymes (AST and ALT) in rats induced with CCl_4 or PCM are shown in Table 1. The ability of CCl_4 and PCM to induce liver toxicity was confirmed by the increase in the level of liver enzymes (that is, AST and ALT) when compared with the control group. Interestingly, all doses of MEDL significantly ($P < 0.05$) attenuate the levels of AST and ALT in CCl_4-induced hepatotoxic rats, while only the 500 mg/kg MEDL significantly ($P < 0.05$) reduced the liver enzymes' level in PCM-induced hepatotoxic rats when compared with the respective control group (10% DMSO-treated).

Table 1. Effects of MEDL on liver enzymes of CCl_4- and PCM-induced hepatotoxicity in rats.

Hepatotoxicity model	Treatment	Dose (mg/kg)	ALT	AST	Histological scoring
CCl4-induced	Control	-	42.8 ± 3.6	107.5 ± 8.3	-
	DMSO	-	803.7±70.1[a]	1051.0 ± 155.4[a]	7.2 ± 0.8
	Silymarin	200	66.0 ± 19.2[b]	414.4 ± 72.1[ab]	4.0 ± 0.0[x]
	MEDL	50	583.0 ± 138.6[ab]	745.5 ± 110.5[a]	6.8 ± 0.3
		250	390.1 ± 131.8[ab]	669.4 ± 125.9[ab]	6.5 ± 0.4
		500	161.4 ± 50.9[ab]	540.5 ± 82.5[ab]	5.7 ± 0.8
PCM-induced	DMSO	-	589.8 ± 87.9[a]	1396.0 ± 199.0[a]	8.3 ± 0.4
	Silymarin	200	359.1 ± 75.8[a]	612.1± 155.4[ac]	4.5 ± 0.5[y]
	MEDL	50	750.2 ± 58.4	2238.0 ± 363.2[ab]	7.3 ± 0.2
		250	703.5 ± 115.7	2205.0 ± 297.0[ab]	7.7 ± 0.2
		500	46.04 ±7.229*	135.7 ± 15.9*	4.0 ± 0.8[y]

Values are expressed as means ± SEM; n = 6. [a]Data differed significantly ($P < 0.05$) when compared with the normal group. [b]Data differed significantly ($P < 0.05$) when compared with the DMSO-treated group induced with CCl_4. [c]Data differed significantly ($P < 0.05$) when compared with the DMSO-treated group induced with PCM. [x]Data differed significantly ($P < 0.05$) when compared with the DMSO-treated group induced with CCl_4. [y]Data differed significantly ($P < 0.05$) when compared with the DMSO-reated group induced with PCM.

Histopathological study

Histopathological studies of the livers removed from CCl_4 (Figure 1B1) and PCM (Figure 1C1)-induced rats pretreated with 10% DMSO demonstrated severe damage to the architecture of the liver with necrosis of hepatocytes, infiltration of leukocyte, and steatosis was observed throughout the tissues when compared with thenormal untreated tissues (Figure 1A). In addition, hemorrhages were also seen in tissues of liver treated with PCM (Figure 1C1). The 200 mg/kg silymarin also reversed the hepatotoxic effects of CCl_4 and PCM leading to the recovery of the liver toward its normal architecture (Figure 1B2 and C2). Interestingly, the MEDL, at the doses of 50 to 500 mg/kg, reversed the hepatotoxic effect of CCl_4 (Figure 1B3), while only the 500 mg/kg MEDL attenuated the hepatotoxic effect of PCM (Figure 1C3). Thus, these biochemical findings were supported by the microscopical observations and histopathological scoring (Table 1).

DISCUSSION

We have previously reported the potential of various extracts of *D. linearis* leaf to exert antinociceptive, anti-inflammatory and antipyretic (Zakaria et al., 2006; 2008), and cytotoxic and antioxidant (Zakaria et al., 2011) activities.

In our attempt to establish the pharmacological profile for *D. linearis* leaf, we study the hepatoprotective effect of MEDL against the CCl_4- and PCM-induced liver toxicity models.

The present study demonstrated the ability of MEDL to protect the liver of albino rats from injury induced by CCl_4 and PCM. The mechanism of liver toxicity triggered by CCl_4 and PCM are different altogether. CCl_4 toxicity was attributed to the binding of its active metabolite trichloromethyl radicals with polyunsaturated fatty acid (PUFA) to form alkoxy and peroxy radicals. These radicals induce generation of lipid peroxidation (Weber et al., 2003; Feroz Khan et al., 2009), a process associated with damage in cell membrane and disruption of normal enzymes' activity that finally induce hepatic injury or necrosis (Popovic et al., 2006). In contrast to CCl_4-induced toxic mechanism, PCM toxic effect is attributed to the oxidative action of its toxic metabolite, N-acetyl-p-benzoquinone-imine (NAPQI), binding to DNA, proteins, and cellular proteins to produce protein adducts (Somchit et al., 2005) resulting in the dysfunction and death of hepatocytes that lead to liver necrosis (Zakaria et al., 2011b). From the mechanisms of hepatoprotection described earlier, free radicals and oxidative processes are two of the important factors that contribute towards the development of hepatotoxicity.

Thus, it is suggested that any extracts/compounds that exhibit free radical scavenging and antioxidant activities may also possess hepatoprotective agents. As mentioned earlier, the MEDL did possess free radical scavenging and antioxidant activities (Zakaria et al., 2011a). This might, in part, justify our present findings on the extract ability to exert hepatoprotective activity. All these activities may be attributed to the extract's high total phenolic and flavonoids content (Zakaria et al., 2011a) as reported by the others (Desmarchelier et al., 1998; Hort et al., 2008).

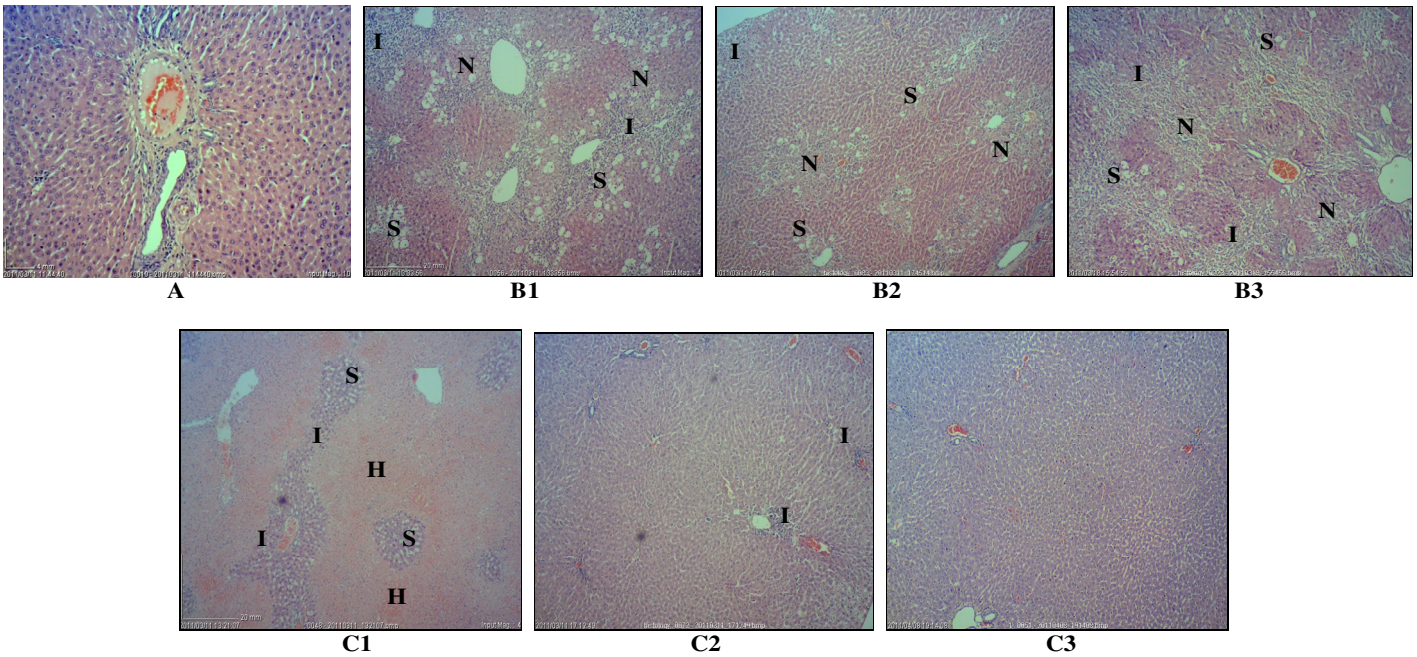

Figure 1. Photomicrograph (H&E staining, 100× magnification) showed (A) normal liver architecture showing portal vein (PV), hepatic artery (HA), and bile duct (BD), along with and normal hepatic cells with well preserved cytoplasm and well defined nucleus around the perivenular area. The sinusoids line is well defined; (B1) Hepatotoxic liver after treatment with CCl_4 showing severe necrosis (N) of hepatocytes in parenchyma region, infiltration of leukocytes (I) and diffuse steatosis (S); (B2) 200 mg/kg silymarin attenuated CCl_4-induced hepatotoxic effect indicated by the presence of normal hepatocytes architecture (NH) between areas filled with mild necrotic hepatocytes (N) and infiltration of leukocytes (IL), and reduction in the number of steatosis (S); (B3) 500 mg/kg MEDL reversed CCl_4-induced hepatotoxic effect indicated by the presence of normal hepatocytes architecture (NH) between areas filled with mild infiltration of leukocytes (I) and steatosis (S); (C1) Hepatotoxic liver after treatment with PCM showing severe necrosis (N), steatosis (S), infiltration of leucocytes (I) and hemorrhage (H) within the parenchyma region of the liver; (C2) 200 mg/kg silymarin attenuated PCM-induced hepatotoxic effect with normal hepatocytes architecture (NH) is seen between areas wherein focal infiltration of leukocytes (IL) is seen in the perivenular area. No signs of steatosis (S) and necrosis (MN) were observed; and (C3) 500 mg/kg MEDL reversed PCM-induced hepatotoxic effect with normal architecture of hepatocytes (NH) observed throughout the slides. No sign of necrosis N), steatosis (S) and infiltration of leukocytes (I) were observed.

Conclusion

The present study demonstrated that the leaf of *D. linearis* possesses hepatoprotective activity against PCM- and CCl_4-induced liver toxicity, which could be attributed, partly, to the free radical scavenging and antioxidant activities, and high phenolics and flavonoids contents. Thus, further extensive studies are warranted.

ACKNOWLEDGEMENTS

The authors thank the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia for providing the facilities to carry out this study. This research was supported by a Research University Grant Schemes 2010 (04/02/10/0925RU) and 2011 (04/02/11/1395RU) from the Universiti Putra Malaysia.

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