Effect of aqueous extract of *Dicranopteris linearis* leaves against paracetamol and carbon tetrachloride-induced liver toxicity in rats

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Abstract: The present study aimed to determine the hepatoprotective activity of *Dicranopteris linearis* L. (family Gleicheniaceae) leaf aqueous extract (DLAE) using two models of liver injury in rats. Rats were divided into ten groups (n=6) and received dH_2O (negative control), 200 mg/kg silymarin (positive control) or DLAE (50, 250 and 500 mg/kg) orally once daily for 7 consecutive days and on the 8th day 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, only the highest dose of DLAE significantly (p<0.05) reduced the ALP, ALT and AST levels in CCl_4-and PCM-induced hepatotoxic rats while the other doses caused significant (p<0.05) reduction only in the levels of ALT and AST. The histological results obtained were in line with the biochemical analysis wherein reduction in the CCl_4- and PCM-induced tissue formation of necrosis, steatosis and inflammation occurred in a dose-dependent manner. In conclusion, the DLAE possesses hepatoprotective activity, which could be attributed to its free radicals scavenging and antioxidant activities, and high flavonoids content. Thus, in-depth studies regarding the hepatoprotective activity of DLAE are warranted.

Keywords: *Dicranopteris linearis*; Gleicheniaceae; *in vivo*; hepatoprotective activity; aqueous extract; leaves.

INTRODUCTION

Acute liver failure can results from toxic liver damage by drugs or poisons with oxidation process has been partly associated with the hepatic injury mediated by those agents (Adewusi and Afolayan, 2010). Despite extensive improvement in the field of modern medicine, it offers little benefit, particularly, towards the management of acute liver failure (Wagh *et al.,* 2010; Taub 2003). Furthermore, the incidence of relapse as well as side effects and development of tolerance upon uses of standard drugs on clinical evaluation make their efficacy arguable. This has been the basis for the development of new plant-based drugs, which include plant-based hepatoprotective agents (Mard *et al.,* 2008). One of the plants that have been reported to possess several pharmacological activities and is presently being studied in our laboratory for its hepatoprotective effect is *Dicranopteris linearis* L. Despite its limited usages within the Malay traditional medicine (Zakaria *et al.,* 2010), this plant, which is called '*pokok resam'* by the Malay and belongs to the family Gleicheniaceae, has been proven to possess several pharmacological properties (Zakaria *et al.,* 2010; Zakaria *et al.,* 2006; Zakaria *et al.,* 2008; Zakaria *et al.,* 2011a). Of those pharmacological activities, the mechanisms of anti-inflammation, antioxidation and anti-proliferation are known to be interrelated to each other as well as to the hepatoprotective mechanism (Dash *et al.,* 2007; Chattopadhyay 2003). Based on these facts, the potential of *D. linearis* leaf aqueous extract (DLAE) to exert hepatoprotective activity was investigated in the present study using various rat models.

MATERIALS AND METHODS

Plant material and preparation of the aqueous extract (DLAE)

The leaves of *D. linearis* were collected between July and August, 2010 from its natural habitat around Serdang, Selangor, Malaysia. A new voucher specimen, IR 0128/11, was deposited at the Herbarium of the Institute of Bioscience (IBS), Universiti Putra Malaysia. The preparation of DLAE was performed as previously described (Zakaria *et al*., 2008; Zakaria *et al*., 2011a). Eighty gram (80 g) of dried powdered leaves was soaked for 72 h in distilled water (dH_2O) in the ratio of 1:20 (w/v) at room temperature. After 72 h, the aqueous supernatant was collected and the residue was soaked again for another two times. The supernatant was pooled together and freeze-dried to yield approximately 15.7 g of dried DLAE (percentage yielded was ≈19.5%).

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Animals used

Adult male Sprague-Dawley rats (weighed 180-200 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) (Zakaria *et al*., 2008).

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 (Mohamed *et al*., 2011).

Hepatoprotective assay

DLAE, in the dose range in of 50, 250 and 500 mg/kg, were assayed against the paracetamol (PCM; 3 g/kg)- and carbon tetrachloride (CCl_4; 0.15 ml/kg)-induced liver toxicity models (Zakaria *et al*., 2011c). The hepatoprotective activity of extract was compared against dH_2O (vehicle group) and silymarin (200 mg/kg; standard drug).

STATISTICAL ANALYSIS

The results are presented as Mean ± Standard Error of Mean (S.E.M), and analyzes 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 DLAE (p.o) with normal behavior pattern observed.

Effects of DLAE on the blood liver enzymes level

The effects of DLAE on the levels of three rat's liver enzymes (ALP, AST and ALT) following induction with CCl_4 or PCM are shown in table 1. The two inducers caused significant ($p<0.05$) liver toxicity in the treated rats as indicated by remarkable increase in the level of these enzymes when compared to the normal rats (control). Pretreatment with 200 mg/kg silymarin (positive control) followed by CCl_4 or PCM resulted in significant ($p<0.05$) decrease in the level of ALP, ALT and AST. Interestingly, DLAE also exerted significant ($P<0.05$) hepatoprotective effect against CCl_4 and PCM as indicated by reduction in the levels of ALP, ALT and AST.

Histopathological study

Histopathological studies of the livers removed from CCl_4 (fig. A2) and PCM (fig. B2) -induced rats pretreated with dH_2O demonstrated severe damage to the architecture of the liver with necrosis of hepatocytes, infiltration of leukocyte and steatosis observed throughout the tissues when compared to the normal untreated tissues (fig. A1 and B1). The 200 mg/kg silymarin reversed the hepatotoxic effects of CCl_4 and PCM leading to the recovery of the liver towards its normal architecture (fig. A3 and B3). Interestingly, the DLAE, at the dose of 50-500 mg/kg, reversed the hepatotoxic effect of CCl_4 (fig. A4-6) and PCM (fig. B4-6) when compare to the respective toxic liver (fig. A2 and B2). The biochemical findings were, therefore, supported by the microscopical observations and the histopathological scoring (table 1).

DISCUSSION

D. linearis leaf exhibited antinociceptive, anti-inflammatory and antipyretic (Zakaria *et al.,* 2006;

Table 1: Effects of DLAE on the liver enzymes following CCl_4- and PCM-induced hepatotoxicity in rats

Hepatotoxicity models	Treatment	Dose (mg/kg)	ALP	ALT	AST	Histological Scoring
dH_2O	dH_2O	-	94.90±3.19	77.25±7.428	72.55± 2.95	-
CCl_4-induced	dH_2O	-	316.83±16.04[a]	991.77±26.57[b]	494.67±28.30[c]	7.2 ± 0.8
	Silymarin	200	254.08±35.28	720.32±20.70[d]	204.43±31.72[d]	4.0 ± 0.0[x]
	DLAE	50	292.67±17.76	328.02±16.26[d]	385.27±9.30[d]	6.8 ± 0.3
		250	242.33±9.94	144.33±12.89[d]	236.23±19.99[d]	6.5 ± 0.4
		500	244.50±14.92	168.32±15.97[d]	188.40±15.39[d]	4.7 ± 0.3[x]
PCM-induced	dH_2O	-	312.83±29.95[e]	861.33±27.48[f]	864.13±37.28[g]	8.3 ± 0.4
	Silymarin	200	161.00±10.95[h]	412.13±32.41[h]	612.13±17.28[h]	4.5 ± 0.5[y]
	DLAE	50	307.17±17.98	561.40±18.29[h]	301.47±14.95[h]	7.3 ± 0.2
		250	330.00±18.063	1063.87±13.45[h]	411.62±28.25[h]	7.7 ± 0.2
		500	215.67±32.23[h]	485.35±16.73[h]	272.47±42.06[h]	4.0 ± 0.8[y]

Values are expressed as means ± S.E.M; n=6

[a,b,c] Data differed significantly ($p<0.05$) when compared to the respective column within the normal group

[d]Data differed significantly ($p<0.05$) when compared to the respective column within the CCl_4-induced group pretreated with dH_2O

[e,f,g] Data differed significantly ($p<0.05$) when compared to the respective column within the normal group

[h]Data differed significantly ($p<0.05$) when compared to the respective column within the PCM-induced group pre-treated with dH_2O

[x,y]Data differed significantly ($p<0.05$) when compared to the dH_2O-treated group induced with CCl_4 or PCM

Zakaria *et al.,* 2008), and cytotoxic and antioxidant (Zakaria *et al*., 2011a) activities. In an attempt to further establish the pharmacological profile of *D. linearis* leaf, we study the hepatoprotective effect of DLAE against the CCl_4- and PCM-induced liver toxicity models. In the present study, we demonstrate the potential of DLAE as hepatoprotective agent against CCl_4- and PCM-induced liver toxicity.

To speculate on the possible mechanisms of action responsible for DLAE hepatoprotective activity, it is necessary to understand the different mechanism of liver toxicity triggered CCl_4 and PCM. The CCl_4 toxicity could be associated with the binding of its active metabolite, trichloromethyl radicals, with polyunsaturated fatty acid (PUFA) to form alkoxy and peroxy radicals, which resulted in the induction of lipid peroxidation (Weber *et al.,* 2003; Feroz Khan *et al.,* 2009). Lipid peroxidation is a process associated with damage in cell membrane and disruption of normal enzymes' activity by free radicals that finally induce hepatic injury or necrosis (Popovic *et al.,* 2006). On the contrary, the mechanism of toxicity induced by PCM could be attributed to the oxidative action of its toxic metabolite, N-acetyl-p-benzoquinone-imine (NAPQI). The NAPQ1 binds to DNA, proteins, and cellular proteins to produce protein adducts (Somchit *et*

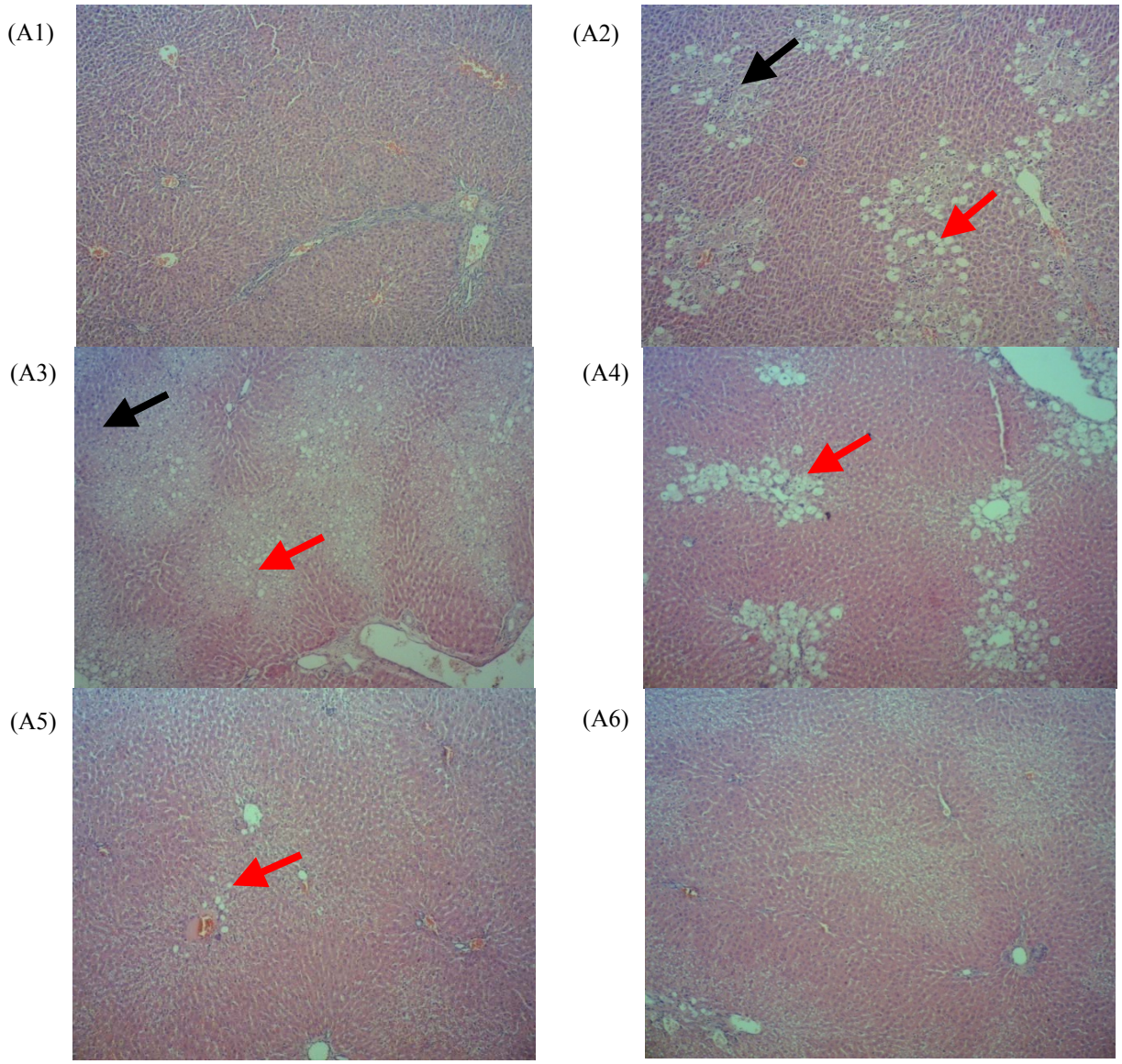

Fig. 1: Liver sections of CCl_4-induced rats after pretreatment with test solutions compared to the normal untreated rats. The liver sections were stained with haematoxylin and eosin (H & E) and magnified (x40) for the evaluation of general morphology. (A1) dH_2O+dH_2O (normal), (A2) dH_2O+CCl_4 (negative control), (A3) Silymarin+CCl_4 (positive control), (A4) 50 mg/kg DLAE+CCl_4, (A5) 250 mg/kg DLAE+CCl_4, (A6) 500 mg/kg DLAE+CCl_4. The red solid arrow represents steatosis, and the black solid arrow represents inflammation.

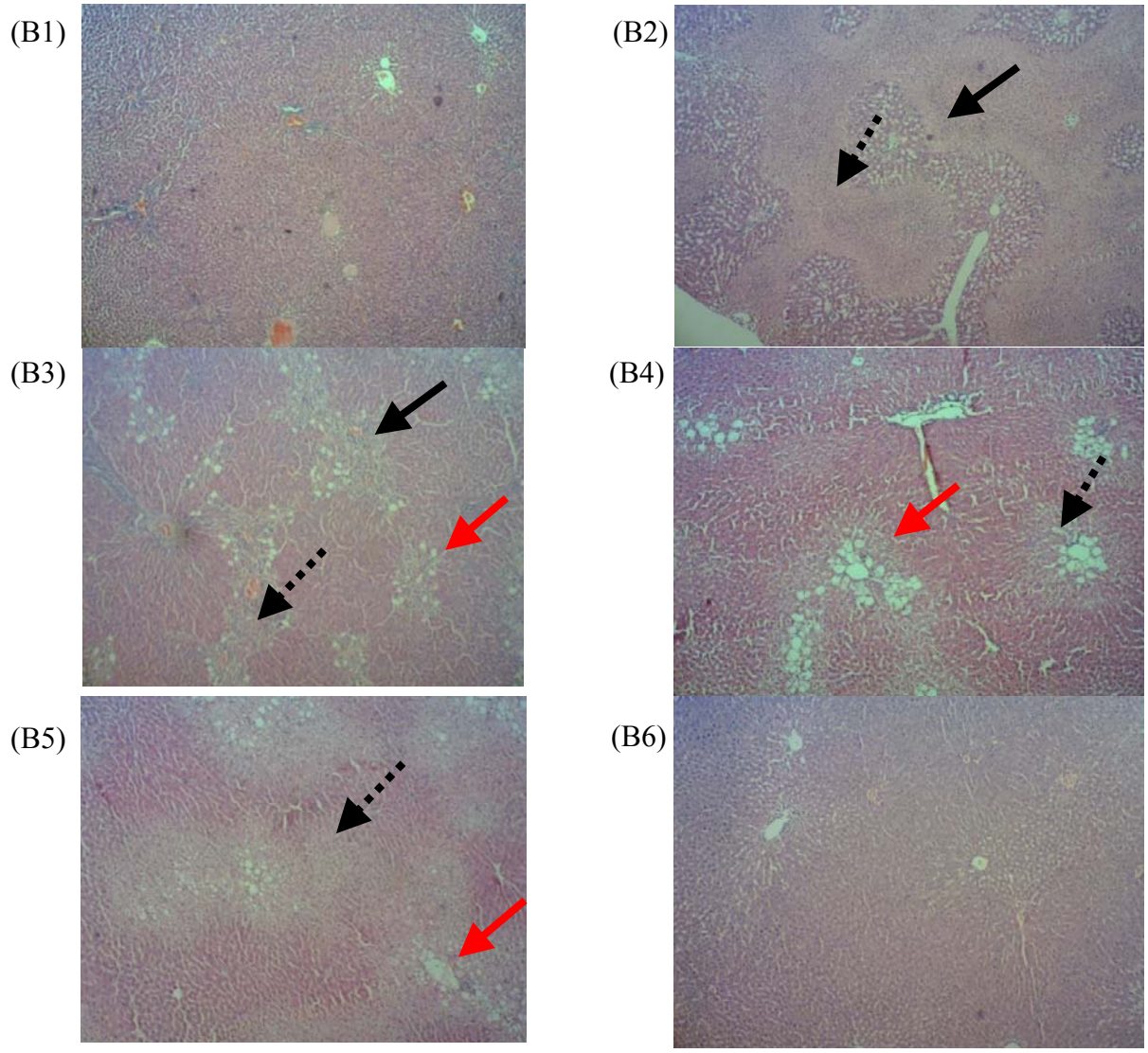

Fig. 2: Liver sections of PCM-induced rats after pretreatment with test solutions compared to the normal untreated rats. The liver sections were stained with haematoxylin and eosin (H & E) and magnified (x40) for the evaluation of general morphology. (B1) dH_2O+dH_2O (normal), (B2) dH_2O+PCM (negative control), (B3) Silymarin+PCM (positive control), (B4) 50 mg/kg DLAE+PCM, (B5) 250 mg/kg DLAE+PCM, (B6) 500 mg/kg DLAE+PCM. The red solid arrow represents steatosis, the black solid arrow represents inflammation and the dashed arrow represents necrosis.

al., 2005) that contributes to the dysfunction and death of hepatocytes, and, finally, leading to liver necrosis (Zakaria *et al.,* 2011b). Based on the above facts, the free radicals and oxidative processes are two of the possible factors that contribute towards the development of hepatotoxicity. Therefore, it is postulated that any extracts/compounds bearing free radical scavenging and antioxidant activities may also exert hepatoprotective agents. Interestingly, DLAE has been proven to possess free radical scavenging and antioxidant activities (Zakaria *et al.,* 2011a). These activities might work synergistically with the extract's anti-inflammatory and antiproliferative activities to produce the observed hepatoprotective activity. Furthermore, the extract also contained high total phenolic content (TPC), which has been reported to contribute to the extracts antioxidant and hepatoprotective activities (Weber *et al*., 2003; Feroz Khan *et al*., 2009; Desmarchelier *et al*., 1998; Hort *et al*., 2008). The aqueous extract of *D. linearis* leaf has been reported to contain flavonoids, tannins and saponins (Zakaria *et al.,* 2011a). These classes of compounds have been reported to exert hepatoprotective activity and, thus, suggested to contribute to the observed hepatoprotection of DLAE (Popovic *et al*., 2006; Somchit *et al*., 2005; Zakaria *et al*., 2011b; Desmarchelier *et al*., 1998; Hort *et al*., 2008).

CONCLUSION

The present study demonstrated that the leaf of *D. linearis* possesses hepatoprotective activity against PCM and

CCl4-induced liver toxicity, which could be attributed, partly, to the extract's free radical scavenging and antioxidant activities and high phenolics and flavonoids contents. Thus, further extensive studies are warranted to determine the responsible bioactive compound (s) with hepatoprotective activity.

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