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Evaluation of methanol extract of *Polygonum minus* Huds. leaves for its hepatoprotective activity

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ABSTRACT

Aims: Hepatotoxicity is a serious health risk and treatment options are inadequate. *Polygonum minus* Huds. (Family: Polygonaceae) is an antioxidant rich, commonly available plant in Malaysia and used in the Malay folk medicine. The leaves are also considered as one of the salad plants and flavouring agent for food delicacies. The present study evaluates the hepatoprotective activity of methanol extract of *P. minus* leaves on carbon tetrachloride (CCl_4) and paracetamol-induced hepatotoxicity in Sprague Dawley rats.
Methodology and results: Methanol extract of *P. minus* (MEPM) was prepared by maceration method. The standard drug and MEPM treated groups of rats were administered with silymarin (50 mg/kg) or MEPM (200 mg/kg or 400 mg/kg), respectively for 14 days in both experimental models. All the animals in the CCl_4-induced model were administered CCl_4 and paracetamol in the other model except to respective normal control group to induce liver toxicity. Estimation of body weight and liver weight, biochemical parameters including total protein, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and total bilirubin levels and histopathological studies were conducted. The MEPM was found to have significant hepatoprotective activity in rats with CCl_4 and paracetamol-induced liver damage as noted from the analysis of body weight, serum marker enzyme activity and histopathology.
Conclusion, significance and impact study: The MEPM possesses significant hepatoprotective activity while the activity is increased with dose in both experimental models. Inclusion of *P. minus* leaves in the food may be recommended as it may help to counteract different types of chemical-induced liver damage.

Keywords: Carbon tetrachloride, paracetamol, silymarin, *Polygonum minus*, hepatoprotective effect

INTRODUCTION

Liver is a major metabolizing organ in the human body. The extensive metabolic activities make the liver vulnerable to many disorders. Hepatotoxicity or injury to liver cells is caused by exposure to chemicals, drugs or non-infectious agents. Some drugs used for diseases and few chemicals could cause mild to severe liver injury. Liver disorders are one of the major health problems in the world. Liver disorders cause high morbidity, mortality and its occurrence is frequent. The liver disorders caused by drugs or chemicals are progressive in nature. The injury starts from scarring, then fibrosis and then non-functioning cirrhosis and then ultimately liver failure. The therapeutic option to prevent liver disease progression or cure is limited. None of the existing chemical drug options are well suited to prevent or treat this serious condition and they are having side effects as well. The need for an effective hepatoprotective agent with no or minimal side effects is essential to treat chronic liver diseases. Micronutrients and other plant-derived chemical compounds could be a suitable option to overcome the complications associated with these disorders (Adesanoye and Farombi, 2010).

Carbon tetrachloride (CCl_4)-induced hepatotoxicity is one of the established experimental methods to evaluate the potential hepatoprotective activity. Chemicals such as chloroform, ethanol and CCl_4 are known for their hepatotoxic action. The chemical, CCl_4 mainly acts by the generation of free radicals and increasing the lipid peroxidation that disturbs membrane integrity and function and thus inducing the liver damage (Kumar *et al*., 2013). Drugs such as paracetamol, isoniazid, trioglitazone and others are also found to have hepatic toxicity. Paracetamol-induced liver toxicity is also an established method to test the liver cell injuries. The mechanism of liver toxicity exhibited by both CCl_4 and paracetamol are almost similar. The activation of these chemicals through cytochrom P (CYP) 450 enzyme system leads to generation of free radicals. Free radicals are known to

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have the potential to damage the cells. The imbalance in free radical and endogenous antioxidants leads to rise in active free radicals and they start to destroy the cell membrane and there by induce liver damage (Kumar *et al.*, 2009; Adesanoye and Farombi, 2010).

Herbal drugs are widely prescribed due to their effectiveness, fewer side effects and relatively low cost even though their chemical constituents are not identified or standardized (Levy *et al.*, 2004). The use of herbal medicines for liver disorders has a long history. Many herbs have been used in liver diseases and associated ailments in different traditional systems of medicine. The hepatoprotective activity of plants is mainly related with their antioxidant, anti-inflammatory and anti-fibrotic potentials (Thyagarajan *et al.*, 2002). The hepatoprotective effects of plants via antioxidant mechanism is studied and confirmed for plants such as *Silybum marianum*, *Glycyrrhiza glabra*, *Phyllanthus* species (*amarus, niruri, emblica*), and *Picrorhiza kurroa* (Asadi-Samani *et al.*, 2015).

Polygonum minus, locally known as kesum in Malay, is a common aromatic plant in Southeast Asia. In many Southeast Asian countries, this plant is used as a traditional medicine for different ailments and also used in food delicacies as a flavouring agent. It is one of the salad leaves (ulam-people eat raw leaves with other food) in Malaysia (Christapher *et al.*, 2015). Cure for digestive disorders (decoction), warm up the body after child birth, improved eye sight, reduction of body pain and sprains (paste form with rice) and as a shampoo to clean dandruff are the traditional uses of *P. minus*. This plant belongs to the list of essential oil producing crop in the Malaysian Herbal product blue print. Many of the chemical constituents of the plant including myricetin, quercetin, methyl flavonol, flavone, decanal, dodecanal were identified. It contains phenolic compounds and secondary metabolites such as aldehydes, flavonoids, terpenoids, gerniol and geranial (Ismail *et al.*, 2011; Shukor *et al.*, 2013). Several researchers have conducted pharmacological, toxicological and clinical studies and reported that *P. minus* has high antioxidant, anti-inflammatory, antiulcer, antimicrobial, immunomodulatory and cognitive enhancing properties (Qader *et al.*, 2012; Christapher *et al.*, 2015). This plant extract is found to be non-toxic even at doses up to 5000 mg/kg (Wasman *et al.*, 2010). Clinical studies suggested that *P. minus* is effective in improving the sexual well-being of 45-55 years of aged adult males (Udani *et al.*, 2014), cognitive and psychosocial parameters according to mood in middle-aged (35-55) women (Shahar *et al.*, 2015) and concentration and mood of 35-65 years healthy adults (Udani, 2013).

The present study evaluates the hepatoprotective potential of methanol extract of leaves of *P. minus* in CCl_4 and paracetamol-induced liver damage in Sprague Dawley (SD) rats.

MATERIALS AND METHODS

Plant material

Fresh leaves of cultivated variety of *P. minus* were collected in September 2014 from the sub-urban region of Penang, Malaysia. The plant was identified and authenticated by a taxonomist in School of Biological Sciences, Universiti Sains Malaysia (USM), Penang, Malaysia and a voucher specimen has been deposited at USM herbarium (Voucher no: USM Herbarium. 11542). The collected leaves were washed to remove dirt and shade dried. The leaves were separated and dried in a hot air oven (50 °C) for 3 days. The dried leaves were blended to get coarse powder. The methanol extract of *P. minus* (MEPM) is prepared by the following maceration method: the powdered leaves was weighed and mixed with methanol at a ratio of 1: 10 w/v and kept for shaking in an orbital shaker. Each day the solution was decanted and replenished with equal volume of solvent, the decanted solution was kept in refrigerator. The procedure was repeated for 5 days, and then the solutions decanted in each day were pooled and filtered through muslin cloth. The filtrate was then evaporated a rotary evaporator (Yamato Rotary Evaporator RE300, Yamato Scientific Co., Ltd. Tokyo, Japan) to get a concentrated liquid, and then that was freeze-dried (Thermo Scientific, Massachusetts, USA) to obtain dry extract. The yield obtained for the methanol extraction was 15.64% w/v. The dry extract was stored at 0-4 °C until further use. The extract was suspended in 0.3% carboxymethyl cellulose (CMC) when required for the experiments. The extract was administered by oral feeding to the experimental animals.

Experimental animals

Male SD rats were used in the present study. The rats were obtained from the AIMST University Central Animal house. Rats were kept for an acclimatization period of 7 days before the commencement of experiments. The animals were housed in polypropylene cages (five/cage), maintained at room temperature and appropriate humidity and a normal day and night cycle. Free access to normal diet (commercial rat chow) and water was allowed during the acclimatization period. All the experiments in this study were in accordance with the guidelines of institutional animal experiment guidelines and the same was approved by the AIMST University Human and Animal Ethics Committee, (AUHAEC 3/FOP/2015).

Hepatoprotective activity

Carbon tetrachloride- induced hepatotoxicity model

The animals were divided into 5 groups each consist of 5 rats and the experiment was conducted according to the following protocol;

GROUP I: Normal control [n=5, the animals were given 1 mL CMC orally; once daily]

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GROUP II: CCl_4 control [n=5, the animals were administered with carbon tetrachloride (2 mg/kg of body weight; in olive oil (1:1 v/v) by intraperitoneally on day 6 , 9 and 12]

GROUP III: Standard drug [n=5, the animals were administered with carbon tetrachloride (2 mg/kg of body weight) in olive oil (1:1 v/v) by intraperitoneally on day 6 , 9 and 12; treated with silymarin (50 mg/kg body weight; orally; once daily for 14 days]

GROUP IV: MEPM I [n=5, the animals were administered with carbon tetrachloride (2 mg/kg of body weight) in olive oil (1:1 v/v) by intraperitoneally on day 6 , 9 and 12; treated with MEPM (200 mg/kg body weight) orally; once daily for 14 days]

GROUP V: MEPM II [n=5, the animals were administered with carbon tetrachloride (2 mg/kg of body weight) in olive oil (1:1 v/v) by intraperitoneally on day 6 , 9 and 12; treated with MEPM (400 mg/kg body weight) orally; once daily for 14 days]

The study was conducted for 14 days and all the animals were sacrificed at the end of the study.

Paracetamol-induced hepatotoxicity model

The animals were divided into 5 groups each consist of 5 animals and the experiment was conducted according to the following protocol;

GROUP I: Normal control [n=5, the animals were given 1 mL CMC orally; once daily]

GROUP II: Paracetamol control [n=5, the animals were administered with paracetamol (750 mg/kg of body weight; in CMC by orally on every 72 h for 14 days]

GROUP III: Standard drug [n=5, the animals were administered with paracetamol (750 mg/kg of body weight) in CMC by orally on every 72 h for 14 days; treated with silymarin (50 mg/kg body weight) orally; once daily for 14 days]

GROUP IV: MEPM I [n=5, the animals were administered with paracetamol (750 mg/kg of body weight) in CMC by orally on every 72 h for 14 days; treated with MEPM (200 mg/kg body weight) orally; once daily for 14 days]

GROUP V: MEPM II [n=5, the animals were administered with paracetamol (750 mg/kg of body weight) in CMC by orally on every 72 h for 14 days; treated with MEPM (400 mg/kg body weight) orally; once daily for 14 days]

The study was conducted for 14 days and all the animals were sacrificed at the end of the study.

Body weight analysis

Body weight of all animals was taken in regular interval and recorded. The variation in body weight of silymarin and MEPM treated groups were compared with the normal control group.

Biochemical estimations

At the end of the experiments each animal was anesthetized by diethyl ether, retro-orbital plexus in the eye was punctured and about 1 mL blood was drawn to sodium EDTA tubes. The biomarkers including aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total protein and bilirubin levels were estimated using standard clinical laboratory methods.

Liver weight analysis

After the blood collection, the animals were sacrificed by cervical dislocation and the abdominal cavity was cut and opened to harvest the liver. The harvested liver was washed with ice cold saline and blotted to dry. The weight of the liver was measured to calculate the absolute and relative liver weight.

Histopathology

A part of the liver was excised and kept in 10% formalin for histopathology studies. The paraffin blocks were prepared and sections were prepared, stained with haemotaxylin and eosin (H&E) and mounted in neutral DPX medium. The slides were observed under light microscope (400×).

Statistical analysis

Data are expressed as mean ± SEM. Significant differences between groups were analyzed using one-way ANOVA followed by Tukey's multiple comparison tests. The statistical analysis was done using IBM-SPSS (version 20). The $P < 0.05$ values were considered to be statistically significant.

RESULTS

Body weight analysis

The effects of CCl_4 and paracetamol administration on body weight of the rats were studied and the results were obtained as shown in Table 1. The effects of CCl_4 and paracetamol administration on body weight of the rats were studied and the results were obtained as shown in Table 1. The terminal body weight of the animals was compared with the initial (0-day) body weight. Statistically significant difference was noted in normal control ($^{***}P < 0.001$) in CCl_4-induced model and paracetamol control ($^{*}P < 0.05$) in paracetamol-induced model. The variation

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Table 1: Effect of methanol extract of *P. minus* leaves on body weight (in g) of rats.

CCl_4 model			PCM model		
Group	0 day	Terminal	Group	0 day	Terminal
Normal Control	188.40 ± 3.94	220.0 ± 4.36***	Normal Control	196.40 ± 2.60	209.00 ± 3.99
CCl_4 Control	189.00 ± 2.29	187.8 ± 9.71$^{\#}$	PCM Control	203.40 ± 3.24	194.80 ± 0.80$^{*\#}$
CCl_4 + silymarin	183.60 ± 11.31	181.6 ± 11.90*	PCM + silymarin	202.80 ± 4.04	207.00 ± 4.92
CCl_4 + MEPM 200 mg/kg	184.40 ± 3.73	185.8 ± 5.94	PCM + MEPM 200 mg/kg	199.60 ± 4.40	207.20 ± 4.72
CCl_4 + MEPM 400 mg/kg	188.20 ± 3.71	194.0 ± 6.77	PCM + MEPM 400 mg/kg	198.60 ± 5.98	209.40 ± 3.69

CCl_4, Carbon tetrachloride; PCM, Paracetamol. Values are expressed as mean ± SEM (n=5).
*, P < 0.05; ***, P < 0.001 compared with 0-day values (paired t-test). $^{\#}$, P < 0.05 compared with normal control (One-way ANOVA followed by Tukey's multiple comparison *post hoc* test).

Table 2: Effect of methanol extract of *P. minus* leaves on absolute and relative liver weight of rats.

Liver weight (in g)-CCl_4 model			Liver weight (in g)-PCM model		
Group	Absolute	Relative	Group	Absolute	Relative
Normal Control	7.73 ± 0.27	3.51 ± 0.11	Normal Control	8.57 ± 0.30	4.17±0.11
CCl_4 Control	8.50 ± 0.30	4.57 ± 0.24	PCM Control	9.46 ± 0.29	4.54±0.18
CCl_4 + silymarin	6.55 ± 0.35	3.65 ± 0.20	PCM + silymarin	7.41 ± 034*	3.54±0.25*
CCl_4 + MEPM 200 mg/kg	7.16 ± 0.58	3.88 ± 0.34	PCM + MEPM 200 mg/kg	8.12 ± 0.36	3.95±0.22
CCl_4 + MEPM 400 mg/kg	7.31 ± 0.86	3.76 ± 0.38	PCM + MEPM 400 mg/kg	8.07 ± 0.70	4.08±0.26

CCl_4, Carbon tetrachloride; PCM, Paracetamol. Values are expressed as mean ± SEM (n=5). No significant changes in liver weights in CCl_4 model. *P < 0.05 compared with PCM control (One-way ANOVA followed by Tukey's multiple comparison *post-hoc* test)

Table 3: Effect of methanol extract of leaves of *P. minus* on biochemical parameters in CCl_4 model.

GROUP	TP (g/L)	AST (U/L)	ALT (U/L)	ALP (U/L)	TB (µmol/L)
Normal Control	71.67 ± 1.05	171.50 ± 6.04	112.50 ± 6.3	158.83 ± 13.48	1.33 ± 0.21
CCl_4 Control	71.67 ± 2.40	1968.50 ± 91.03***	1092.17 ± 32.31***	481.50 ± 63.25***	6.67 ± 2.42***
CCl_4 + silymarin	73.00 ± 1.51	213.67 ± 13.45$^{\#\#\#}$	126.67 ± 15.18$^{\#\#\#}$	181.17 ± 25.28$^{\#\#\#}$	2.33 ± 0.21$^{\#\#\#}$
CCl_4 + MEPM 200 mg/kg	70.17 ± 2.09	262.83 ± 44.48$^{\#\#\#}$	156.17 ± 16.28$^{\#\#\#}$	240.83 ± 11.93$^{\#\#\#}$	2.00 ± 0.26$^{\#\#\#}$
CCl_4 + MEPM 400 mg/kg	72.17 ± 2.01	196.00 ± 25.36$^{\#\#\#}$	145.33 ± 7.60$^{\#\#\#}$	151.00 ± 15.80$^{\#\#\#}$	1.67 ± 0.33$^{\#\#\#}$

CCl_4, Carbon tetrachloride; TP, Total Protein; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase; TB, Total Bilirubin. Values are expressed as mean ± SEM (n=5). ***, P < 0.001 compared with normal control; $^{\#\#\#}$, P < 0.001 compared with CCl_4 control (One-way ANOVA followed by Tukey's multiple comparison *post-hoc* test).

between body weight of animals groups in each model was compared and noted that the body weight of CCl_4 and paracetamol control groups were significantly different ($^{\#}$P < 0.05) with the respective normal control group.

Absolute and relative liver weight

The influence of challenging CCl_4 and paracetamol on liver weight was estimated by the measurement of absolute and calculation of relative weight of liver. The values are tabulated on Table 2. It was estimated that none of the animal groups in CCl_4-induced hepatotoxicity showed significant variation in the absolute and relative organ weight when compared with the normal control. In paracetamol-induced toxicity model, silymarin treated group alone showed a significant variation (P < 0.05) in the absolute and relative liver weight.

Biochemical analysis

The biochemical parameters such as total protein, AST, ALT, ALP and bilirubin level have been studied. In the CCl_4-induced hepatotoxicity study (Table 3), there was no significant difference in the protein level in the CCl_4 control group when compared with normal control. The protein contents in standard drug (silymarin; 50 mg/kg) treated and different doses of extract (200 and 400 mg/kg) treated groups were not significantly different when compared with CCl_4 control group. In the case of paracetamol-induced hepatotoxicity (Table 4), a significant (P < 0.001) decrease in the protein level was observed in the paracetamol control group. The protein contents in standard drug treated group and higher dose (400 mg/kg) of MEPM treated groups were noted to be significantly increased (P < 0.001) when compared with paracetamol control group.

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The effect of MEPM on AST level in both the models has been studied. In the CCl_4-induced liver injury model (Table 3), a significant ($P < 0.001$) increase in AST level was observed in the CCl_4 control group as compared to normal control group. The AST levels in standard drug treated group and different doses of MEPM treated groups were significantly decreased ($P < 0.001$) when compared with CCl_4 control group. In the normal control group in paracetamol-induced hepatotoxicity model (Table 4), a significant ($P < 0.001$) increase in AST level was observed for the paracetamol control group when compared with normal control group. The AST levels in standard drug and MEPM at 400 mg/kg treated groups were noted to be significantly decreased ($P < 0.001$) when compared with paracetamol control group.

In CCl_4-induced hepatotoxicity model (Table 3), there was a significant ($P < 0.001$) increase in the ALT level of CCl_4 control group when compared with normal control. The ALT levels in silymarin treated group and different doses of MEPM treated groups were significantly decreased ($P < 0.001$) when compared with CCl_4 control group. In the case of other experimental model (Table 4), a significant ($P < 0.001$) increase in AST level was observed in the paracetamol control group as compared to normal control group. The ALT level in standard and higher dose of MEPM (400 mg/kg) treated groups were found to be significantly decreased ($P < 0.001$) when compared with paracetamol control group.

The effect of MEPM on ALP level in both models has been studied. In CCl_4-induced liver injury model (Table 3), a significant ($P < 0.001$) increase in ALP level was observed in the CCl_4 control group as compared to normal control group. The ALP levels in standard drug (silymarin at 50 mg/kg) treated group and different doses of MEPM (200 and 400 mg/kg) treated groups were significantly decreased ($P < 0.001$) when compared with CCl_4 control group. In paracetamol-induced hepatotoxicity (Table 4), a significant ($P < 0.001$) increase in ALP level was observed in the paracetamol control group as compared to normal control group. The ALP level in standard drug ($P < 0.01$) and MEPM ($P < 0.01$) at a dose of 400 mg/kg treated groups were found to be significantly decreased when compared with paracetamol control group.

In the CCl_4-induced hepatic injury model (Table 3), a significant ($P < 0.001$) increase in bilirubin level was observed in the CCl_4 control group as compared to normal control group. The bilirubin levels in standard drug treated group and different doses of extract treated groups were noted to be significantly decreased ($P < 0.001$) when compared with CCl_4 control group. In paracetamol-induced hepatotoxicity (Table 4), a significant ($P < 0.001$) increase in bilirubin level was observed in the paracetamol control group as compared to normal control group. The bilirubin level in standard drug treated and higher dose of MEPM treated groups were found to be significantly decreased ($P < 0.05$) when compared with paracetamol control group.

Table 4: Effect of methanol extract of leaves of *P. minus* on biochemical parameters in PCM model.

GROUP	TP (g/L)	AST (U/L)	ALT (U/L)	ALP (U/L)	TB (µmol/L)
Normal Control	74.40 ± 1.33	253.00 ± 10.01	127.80 ± 4.69	147.80 ± 3.81	1.60 ± 0.07
PCM Control	60.40 ± 1.37***	319.20 ± 12.32***	173.80 ± 4.40***	317.60 ± 24.40***	1.95 ± 0.01***
PCM + silymarin	71.20 ± 0.97$^{\#\#\#}$	232.00 ± 5.32$^{\#\#\#}$	120.80 ± 1.97$^{\#\#\#}$	206.20 ± 24.03$^{\#\#}$	1.73 ± 0.05$^{\#}$
PCM + MEPM 200 mg/kg	60.80 ± 0.79	290.60 ± 2.99	163.20 ± 3.91	261.20 ± 20.81	1.88 ± 0.03
PCM + MEPM 400 mg/kg	69.20 ± 1.40$^{\#\#\#}$	245.80 ± 2.24$^{\#\#\#}$	131.00 ± 3.65$^{\#\#\#}$	200.00 ± 11.90$^{\#\#}$	1.77 ± 0.02$^{\#}$

PCM, Paracetamol; TP, Total Protein; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase; TB, Total Bilirubin. Values are expressed as mean ± SEM (n=5). ***, $P < 0.001$ compared with normal control; $^{\#}$, $P < 0.05$; $^{\#\#}$, $P < 0.01$; $^{\#\#\#}$, $P < 0.001$ compared with paracetamol control. One-way ANOVA followed by Tukey's multiple comparison *post-hoc* tests.

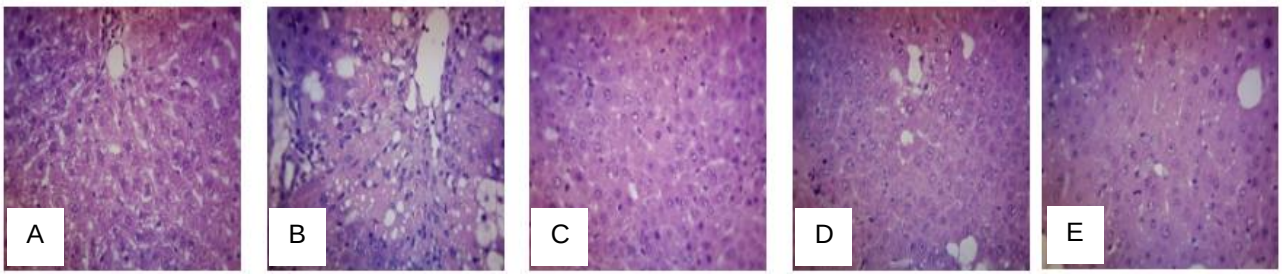

Figure 1: Photomicrograph of liver sections of different groups of CCl_4-induced hepatotoxicity. A, Normal control (Carboxymethyl cellulose); B, CCl_4 control (2 mg/kg in 1:1 olive oil); C, Silymarin (50 mg/kg) treated; D, Methanol extract of *P. minus* (200 mg/kg) treated; E, Methanol extract of *P. minus* (400 mg/kg) treated. The slides were stained with H&E and viewed at 400× magnification.

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Histopathology

Histopathological examination of liver sections for the normal control groups in both models showed a normal liver parenchymal architecture (Figures 1A and 2A). There were no lesions observed in both groups. In the case CCl_4 (Figure 1B) and paracetamol (Figure 2B) control groups, hepatic cell damage was evident. Rupture in cell structure, invasion of fat globules and vein congestion was observed. It was found that the standard (Figures 1C and 2C) and MEPM treated (400 mg/kg) groups effectively prevented (Figures 1E and 2E) the extent of liver damage as they reduced the cell damage, fat globule invasion and vein congestion. Liver cell protective effect of both doses of MEPM (200 and 400 mg/kg) were observed and the protective effect is more evident in the higher dose (Figures 1D and 2D).

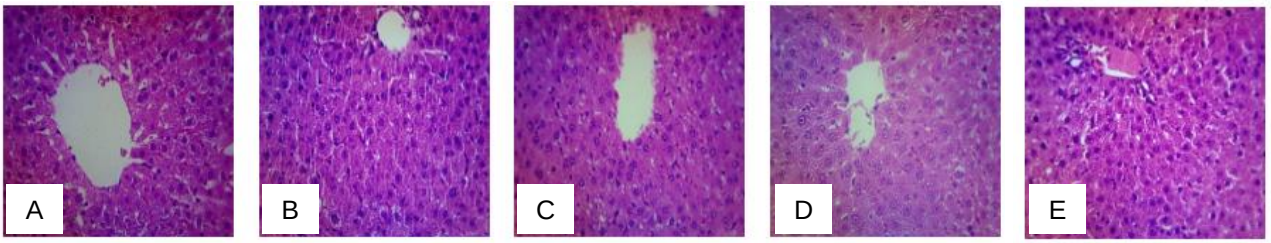

Figure 2: Photomicrograph of liver sections of different groups of paracetamol-induced hepatotoxicity. A, Normal control (Carboxymethyl cellulose); B, Paracetamol control (750 mg/kg); C, Silymarin (50 mg/kg) treated; D, Methanol extract of *P. minus* (200 mg/kg) treated; E, Methanol extract of *P. minus* (400 mg/kg) treated. The slides were stained with H&E and viewed at 400× magnification.

DISCUSSION

Carbon tetrachloride and paracetamol-induced hepatotoxicity on rats are established experimental models to study the potential hepatoprotective activity of herbal extracts and plant derived products. CCl_4 is a chemical, which manifests its toxicity as hepatic injury in animals. This is due to the generation of reactive metabolites (CCl_3• and CCl_3OO•) *via* the metabolism of CCl_4 by CYP-450. These free radicals bind with cellular components such as proteins, lipids and nucleic acids. There irreversible covalent bonding leads to stimulate series of intracellular reactions such as lipid peroxidation. Ultimately these reaction causes damage to the biologically important membranes such as mitochondrial, endoplasmic reticulum and plasma membranes. The permeability alteration in these membranes leads to loss of calcium sequestration and leads to cell destruction (Boll *et al.*, 2001; Halliwell *et al.*, 2004; Adesanoye and Farombi, 2010). Paracetamol is a well known antipyretic drug which causes hepatic injury to human and animals in high dose. The mechanism of liver damage is based on the generation of free radicals. The highly reactive metabolites are generated due to the altered metabolic pathway. The difference in kinetics due to high dose shifts the normal sulfation and glucuronidation to oxidation by CYP-450 enzymes and leads to the generation of highly reactive N-acetyl-p-benzoquinone imine. The conversion of N-acetyl-p-benzoquinone imine is reduced to form semiquinone radicals. These free radicals covalently bind with cellular components. The destruction of cell due to oxidative stress will occur due to the above discussed mechanism (Remirez *et al.*, 1995; Maheswari *et al.*, 2008).

Body weight maintenance is one of the main evidences of hepato-protection. In the present study, it was noted that both low and high dose of MEPM extract effectively revert the changes in body weight. The extent of activity is comparable with the standard drug in both experimental models. AST, ALT and ALP are the major enzyme markers for the liver function. Increased AST is an indicator of hepatic injury. ALT elevation is an indicator of necrosis and increase in ALP level indicates obstructive liver injury (Kaplan, 1986; Zimmerman, 1999; Navarro and Senior, 2006). In the present study, the level of all these biomarkers was noted to be elevated after the administration of CCl_4. Administration of standard drug silymarin (50 mg/kg) and MEPM extracts (200 and 400 mg/kg) was helped to attenuate the toxic effects of CCl_4 in treated rats. Elevation of enzyme biomarkers such as AST, ALT and ALP was observed in the paracetamol-induced hepatotoxicity model also. The enzymes levels were noted to be markedly increased in the paracetamol control group when compared with normal control that indicates the liver toxicity. The elevation of enzymes level was effectively reverted by the administration of silymarin and MEPM. The preventive effect of MEPM at 200 mg/kg is not statistically significant whereas the effect was statistically significant at 400 mg/kg. Elevated serum bilirubin is an indication of bilary occlusion and liver damage. Reduction in total protein level suggests the lack of normal synthetic functions that indicates liver cell damage (Solomon *et al.*, 1993). In the present study, the bilirubin level has significantly increased in both experimental models. The elevation is more prominent in the CCl_4-induced model. The administration of standard and MEPM, except the low dose (200 mg/kg) treated group in paracetamol-induced model, noted to be significantly reduced the bilirubin levels in both the experiments. Alteration in total protein level was not observed in the CCl_4-induced toxicity whereas significant reduction was observed in higher dose (400 mg/kg)

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treated group in paracetamol-induced toxicity. The standard and higher dose of MEPM was found to be effective in preventing the functional damage of liver.

Histopathological examinations are essential in studying the cellular changes occurred in hepatotoxicity experiment (Pradeep *et al.*, 2009). The damage of liver cells caused by CCl_4 or paracetamol, prevention or cure of damage caused by the toxicant by standard drug silymarin and different doses of MEPM extract in both toxicity models are supported by the histopathological study observations.

Free radicals are mainly responsible for the oxidative stress that lead to alteration in various cell functions, and ultimately serious degenerative diseases including atherosclerosis, cancer, inflammatory joint disease, asthma, diabetes, senile dementia and degenerative eye diseases (Florence, 1995). Endogenous antioxidants in the body are the first line defense to counteract the free radicals. The imbalance between antioxidants and free radicals outpace antioxidant levels and cause cell damage. Supplementation of dietary antioxidants is one of the possible methods to overcome the oxidative stress. Consumption of herbs that are having promising antioxidant capacity is an option to supplement antioxidants. The positive correlation between high content of flavonoids and polypheonls and antioxidant capacity is well established (Pietta, 2000; Wojcik *et al.*, 2010).

Polygonum minus is a commonly available plant with high antioxidant capacity due to high content of flavonoids and phenolics such as rutin, myricetin, quercetin, quercitrin and others (Azlim Almey *et al.*, 2010; Sumazian *et al.*, 2010). In this study, the methanol extract of leaves of *P. minus* helped to prevent the liver damages induced by chemical and drug toxicants. The mechanism of hepato-protection is may be due to free radical scavenging, induction or supplementation of endogenous antioxidant system followed by reduction in oxidative stress which is the general mechanism of action of antioxidants.

CONCLUSION

The results of this study confirm the role of *P. minus* in preventing the chemical and drug-induced hepatotoxicity. Detailed studies on the *in vivo* antioxidant modulation and role on free radical scavenging are required to establish its hepatoprotective effect.

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