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Research Article

HEPATOPROTECTIVE ACTIVITY OF METHANOLIC EXTRACT OF *DIPTERACANTHUS PATULUS (JACQ) NEES:* POSSIBLE INVOLVEMENT OF ANTIOXIDANT AND MEMBRANE STABILIZATION PROPERTY[Δ]

SHRINIVAS BUMRELA[1*] AND SURESH R NAIK[1]

[1]Sinhgad Technical Education Society's, Sinhgad Institute of Pharmaceutical Science, Lonavala, Pune (MS), India 410401
Email: sbb2000@rediffmail.com

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ABSTRACT

The hepatoprotective activity as well as the possible underlying mechanism(s) of methanolic extract of *Dipteracanthus patulus (Jacq) nees* (MEDP) using carbon tetrachloride (CCl_4) and Paracetamol (PCM) rat models was evaluated. The rats were administered MEDP (250 and 500 mg/kg p.o.) and silymarin (100 mg/kg p.o.) daily for 5 days and received CCl_4 1ml/kg i.p. (1:1 v/v CCl_4:olive oil) on day 3 and 4, 30min after the silymarin and MEDP. The rats received PCM (2 gm/kg p.o.) on day 5, 30min after the silymarin and MEDP in PCM rat models. The hepatoprotective activity was assessed by determining serum biomarkers viz aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) along with total bilirubin (TB) and total proteins (TP). Furthermore, various antioxidant paradigms viz. malondialdehyde (MDA) formation, superoxide dismutase (SOD), glutathione peroxidise (GSHPx) and catalase (CAT) were determined in liver homogenates. In both CCl_4 and PCM rat models, MEDP treatment able to restore depleted antioxidant liver enzymes (SOD, GSHPx and CAT) and depleted the elevated serum biomarkers significantly respectively. In addition histoarchitecture of liver was also improved with the treatment of MEDP. The protective activity of MEDP (500 mg/kg p.o.) was comparable to that of silymarin (100 mg/kg p.o.). The hepatoprotective activity of MEDP seems to be related to its antioxidant activity possibly through free radicals scavenging mechanism by activating antioxidant enzymes. The phytoconstituents of MEDP may be responsible for antioxidant activity observed in the present study. [Δ] A part of the work forms SBB's Ph. D. research work.

Keywords: Hepatoprotective, *Dipteracanthus patulus,* Serum biomarkers, Carbon tetrachloride, Paracetamol, Enzyme antioxidants, Histopathology, β-carotene, β-sitosterol.

INTRODUCTION

The liver is a vital organ in which most of the drugs are metabolised. Some of the metabolites formed are either biologically inactive, and/or toxic. The drugs administered orally must pass through liver to reach systemic circulation. Liver therefore becomes a vulnerable target for injury from chemicals and drugs administered through oral route. Liver injury and altered liver functions manifest which is directly responsible to doses of drugs or chemicals. It is documented that both paracetamol (PCM) and carbon tetrachloride (CCl_4) elicit centrilobular necrosis largely due to formation of reactive toxic metabolites[1].

Despite of documents in modern medicine, there is not a single drug which can successfully cure the liver diseases or provide protection against known hepatotoxins. The recent herbal researches have indicated that number of plants e.g. *Plumbago zeylanica*[2], *Berberis asiatica*[3], *Amorphophallus campanulatus*[4] and polyherbal formulations are found to be useful as hepatoprotective agents.

Dipteracanthus, a genus of undershshrubs distributed in tropical and subtropical regions. Seven species occur in India[5]. *Dipteracanthus patulus (Jacq) Nees* (DP) is shrublet belonging to family Acantheaceae. In *tamil* it is known as *Kiranthinayagam* or *Kayappacchililai* and commonly distributed on wasteland in Tamil Nadu[6]. It grows in tropical Africa, Arabia, south west India, Srilanka and Pakistan[7]. Its flowering season is september to march.

In folklore medicine, it has been documented that DP is used for curing the eyesore by introducing the extract into eyelid. In addition, the leaves of various species of *Dipteracanthus* were used in various infectious diseases[8]. Phytochemical analysis of DP showed the presence of lyoniresinol-9'-O-β-D-glucoside, 5,5-dimethoxy-lariciresinol-9-O-β glucopyranoside, β-sitosterol, lupeol, α-ethyl galactose, apiginin-7-O-rutinoside, α-D-glucose, β-D-glucose and β-D-fructose[9]. Other phytochemical studies report the presence of ascorbic acid, phenolic compounds, tannin, lycopene, carotenoid and α-tocopherol in DP[10]. From our laboratory, we have quantitatively analysed compounds, β-carotene and β-sitosterol and also documented antioxidant activity (*in-vitro*)[11].

In-vitro antioxidant[11], wound healing[6], antidiabetic10, cardiovascular[7] activities of DP have been reported. DP leaves are used to cure liver complaints by the peoples of Sholapur region (MS), India but this empirical knowledge has not been documented in any herbal literature. Furthermore, no studies on the hepatoprotective activity of DP have been reported. Keeping in mind, wide usage of DP in folklore medicine and therapeutically important phytochemical constituents present in this plant, it was felt necessary to evaluate hepatoprotective activity of methanolic extract of DP leaves (MEDP) against $CCl_4$and PCM induced liver damage in rats.

MATERIALS AND METHODS

Chemicals

Thiobarbituric acid (TBA) and malonaldehyde-bis-dimethyl acetyl (MDA), β-carotene, β-sitosterol, 5,5'-Dithiobis (2-nitrobenzoic acid) (DTNB) and epinephrine (Sigma Aldrich, St. Louis, MO, USA), Trichloro acetic acid (TCA), Carbon tetrachloride (Qualigens, Mumbai), gallic acid, Folin Ciocalteu and all other chemicals of research grade were purchased. The diagnostic kits for biochemical estimations of AST, ALT, ALP, LDH (Agappe Diagnostics Ltd, Kerala, India), TB (Merck specialities Ltd, India), TP (Medsource ozone Biomedicals Pvt. Ltd., India) were procured. Silymarin was obtained as gift sample from INDENA, s.p.a., Milan, Italy and Paracetamol from Serum Institute, Pune, India.

Plant material

The plant material was collected in the month of June 2010 from the nearby villages of Sholapur district (MS), India [It is located between 17.10 to 18.32 degrees to the north latitude while it is about 74.42 to 76.15 degrees to the east longitude]. The soil is black and of fertile quality and rain fall is scanty. The collected plant material was botanically authenticated (Certificate No. BSI/WC/Tech/2007/460 dated: 03/08/2007) by Botanical Survey of India (BSI), Pune (MS), India and the voucher specimen is deposited in BSI, Pune, MS (India).Shade dried leaves of DP (5gm) were extracted with

methanol (500ml) using soxhlet apparatus for 12hrs. MEDP was evaporated to dryness under reduced pressure using rotary vacuum evaporator. The percentage yield was calculated.

Determination of total phenolic content

The total phenolic content in MEDP was determined by the method of Slinkard and Singleton[12] using Folin Ciocalteu (FC) reagent. The standard curve was drawn using gallic acid (50-500µg/ml) in ethanol. Briefly, 1.0ml of extract containing 1.0gm extract was diluted to 46ml and 1.0ml of FC reagent was added and allowed to stand for 3min. Aliquot of 3ml of 2% (w/v) sodium carbonate was added and 2hr later the absorbance was measured at 760nm. The total phenolic content was expressed as microgram of gallic acid equivalent (GAE) per gram dry weight of the sample. The results mentioned were average of triplicate reading.

HPTLC fingerprinting of MEDP

HPTLC was performed on 2 separate precoated silica gel aluminium TLC plates $60F_{254}$ (E-Merk, Germany) for qualitative evaluation of β-carotene and β-sitosterol in MEDP. In brief, concentrated MEDP (10µL) and standard markers (5µL) were loaded on TLC plates with Camag Linomat 5 applicator with nitrogen supply. The mobile phase used for β-carotene and β-sitosterol was n-hexane: acetone (70:30 v/v) and toluene: ethyl acetate (80:20 v/v) respectively. The plates were developed to a distance of 80mm in a Camag twin-trough chamber previously equilibrated with mobile phase for 20min. After development of β-sitosterol plate, derivatization was carried out with 5% sulphuric acid in methanol and heated at 110°C on Camag TLC plate platform heater for 5min. Camag TLC visualizer-150503 was used for photodocumentation of β-sitosterol and β-carotene at 254nm, 366nm and White R. The β-carotene HPTLC chromatogram was obtained using Camag Scanner-170422 in conjunction with WinCATS-5 software. The Rf values and percentage of separated compounds were determined.

Animals

Wistar rats of either sex (150-180 g) for hepatoprotective activity and Swiss albino mice (15-25 g) for acute toxicity study were used. The animals were purchased from National Toxicology Centre, Pune and National Biosciences, Pune respectively. Animals were housed in clean polypropylene cages under controlled environmental conditions (Temp 25±1°C, Relative humidity 60% and 12h light/ dark cycle). They received standard pellets of rat chaw (M/s. Hindustan Lever Ltd., Mumbai, India) and water *ad libitum* and acclimatized for 7 days in our animal house prior to experiments. The study protocols were approved by animal ethics committee and animal house has CPCSEA recognition. Animal welfare and experimental procedures were carried out strictly in accordance with the guide for the care and use of laboratory animals (National Research Council of USA, 1996)[13].

Acute toxicity study

Swiss albino mice were divided into 5 groups of 6 animals each. Mice were administered orally with graded doses (0.1, 0.5, 1, 2 and 5 g/kg b.w.) of MEDP as per OECD guidelines 420 and observed for clinical signs of toxic reactions and mortality for 6 days. One-tenth of this dose was selected as the maximum therapeutic dose for the evaluation[14].

Carbon tetrachloride induced hepatotoxicity

The study was carried out as per method described by Shanmugasundaram and Venkataraman[15]. The wistar albino rats were divided into 5 groups, each containing six animals. MEDP was administered as suspension in 1% carboxy methyl cellulose (CMC) in distilled water via oral gavages. Group I (Normal Control) received CMC orally for 5 days and olive oil (1 ml/kg, i.p.) on day 2 and 3, 30min after CMC administration. Group II (Toxicant) received CMC orally for 5 days and CCl_4: olive oil (1:1, 1 ml/kg, i.p.) on day 2 and 3, 30min after CMC administration. Group III (reference drug) received silymarin (100mg/kg p.o.), Group IV and V (Test) received MEDP (250 and 500 mg/kg p.o.) for 5 days. Group III to V received CCl_4: olive oil (1:1, 1 ml/kg, i.p.) on day 2 and 3, 30min after silymarin or MEDP administration. All the animals were sacrificed on 6thday (24hr after the last dose) by cervical dislocation.

Paracetamol induced hepatotoxicity

The experiment was designed based on published work of Chattopadhyay[16]. The wistar albino rats were divided into 5 groups, each containing 6 animals. Group I (Normal Control) and Group II (Toxicant) received 1% CMC for 7 days. Paracetamol was administered on day 5 as single dose (2 g/kg p.o.) to Group II to V, 30min after the silymarin or MEDP treatment. Group III (reference drug) received silymarin (100mg/kg p.o.) for seven days. Group IV and V (Test) received MEDP (250 and 500 mg/kg p.o.) for 7 days. MEDP was administered as suspension in CMC in distilled water via oral gavages. All the animals were sacrificed on 8th day (24hr after the last dose) by cervical dislocation.

Estimation of biochemical parameters in serum

Blood was collected 24h after the last dose from retro orbital plexus under mild anaesthesia and serum was separated by centrifugation at 3000rpm for 10min. Aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total bilirubin (TB) and total protein (TP) in serum were assayed using readymade reagent kits on biochemistry analyser (Merck Lx300, Germany).

Preparation of liver homogenate

Livers were quickly removed after sacrificing the animals, washed with ice cold normal saline solution and 10% (w/v) homogenate were prepared in saline phosphate buffer (pH 7.4) using teflon rotary homogenizer. The homogenate were centrifuged at 4000g for 10min to obtain supernatant fraction which was used for determination of superoxide dismutase (SOD), glutathione peroxidase GSHPx and catalase (CAT).

Antioxidant enzyme assay in liver homogenate

SOD activity was measured by the method of Misra and Fridovich[17]. Briefly, epinephrine undergoes auto-oxidation rapidly at pH 10.0 to produce adrenochrome, a pink coloured product that was detected at 480nm in kinetic mode using double beam UV-visible spectrophotometer-V520 (Jasco, Japan). The amount of enzyme required to produce 50% inhibition was defined as one unit of enzyme activity. The SOD activity was expressed as units/mg of protein.

GSHPx activity was carried out using the method of Rotruck *et al*[18]. GSHPx in the tissue homogenate oxidises glutathione and simultaneously, H_2O_2 is reduced to water. This reaction is arrested at 10min using TCA and remaining glutathione is reacted with DTNB solution to form a coloured product which is measured spectrophotometrically at 420nm. The GSHPx activity was expressed as units/mg of protein.

Catalase was assayed according to the method of Aebi[19]. An aliquot of liver homogenate supernatant was added into a quartz cuvette. The reaction was started by the addition freshly prepared H_2O_2 (30mM) in phosphate buffer (50mM, pH 7.0). The rate of H_2O_2 decomposition was measured spectrophotometrically at 240nm during 2min. The activity of catalase was expressed as µmol H_2O_2/mg protein per min. Protein in liver was estimated using the method of Lowry et al[20].

Estimation of lipid peroxidation

Estimation of lipid peroxidation in liver homogenate was carried out by the method of Ohkawa and Yagi[21]. In this method, the malondialdehyde (MDA) was quantified by the reaction with thiobarbituric acid (TBA) and used as an index of lipid peroxidation. The results were expressed as nmol of MDA per mg of protein.

Histoarchitecture studies

The liver samples fixed in 10% formalin were embedded in paraffin wax and cut into 4-6 µm thick sections on microtome (Leica RM2125, Germany). The sections were stained with haematoxylin and eosin and observed under microscope for cell necrosis, fatty

changes, hyaline degeneration, ballooning and infiltration of kupffer cells and lymphocytes. The photodocumentation of histomicrographs were carried out using digital binocular microscope (Labomed, USA).

Statistical analysis

The results are expressed as mean ± SEM (n=6). Results were analysed statistically by one way ANOVA followed by Tukey's multiple comparison test. The difference was considered significant if $p<0.05$.

RESULTS

Priliminary phytochemical analysis showed the presence of many biologically active constituents' viz. glucosides, iridoid glycosides, steroids, phenolic compounds, tannin, carotenoid in MEDP. The total phenolic content in MEDP was found to be 300.08 ± 2.06 µg GAE/g. HPTLC analysis of MEDP confirmed the presence of β-carotene and β-sitosterol at Rf values 0.77 and 0.40 respectively using standard β-carotene **(Fig. 1)** and β-sitosterol **(Fig. 2)**. In HPTLC densitometric chromatogram of MEDP, the β-carotene and β-sitosterol found was 0.08±0.004 and 0.19±0.03 gm% of sample respectively. The percentage of β-sitosterol is more in DP plant as compared to β-carotene.

MEDP showed no symptoms or toxic reactions and devoid of adverse effects. MEDP did not showed mortality at 5gm/kg, p.o. in mice. The dose selected of MEDP for the present study was 250 and 500 mg/kg, p.o. ($1/10^{th}$ or less of maximum dose used in acute toxicity study).

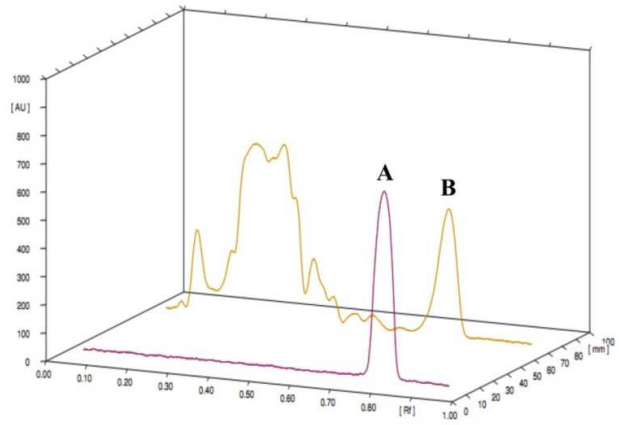

Fig. 1: HPTLC fingerprinting of MEDP (A) Standard β-carotene (B) β-carotene in MEDP.

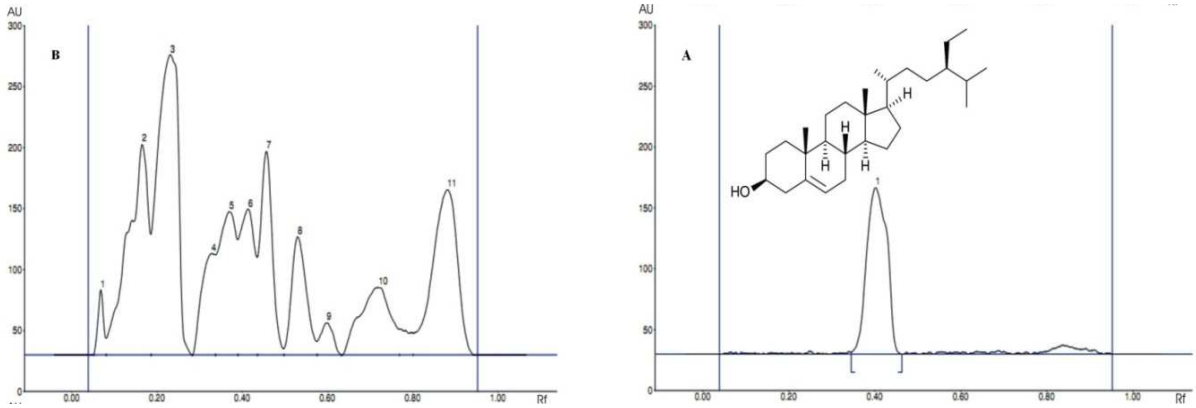

Fig. 2: HPTLC fingerprinting of MEDP (A) Standard β-sitosterol (B) β- sitosterol in MEDP-Peak No. 6.

Significant increase in serum enzymes viz AST, ALT, ALP, LDH and total bilirubin while total protein content was lowered in PCM and CCl_4 groups as compared to normal group. The groups treated with silymarin or MEDP markedly reduced enzyme levels compared to that of toxicant treated groups (Table 1 and 2).

The enzymes viz. SOD, GSHPx and CAT in liver homogenate were found to be depleted in toxicant treated groups as compared to normal control group. In the present study, it was observed that pretreatment with MEDP significantly ($p<0.05$) restored the activity of antioxidant enzymes as compared to toxicant group (Table 3 and 4).The levels of MDA (an index of lipid peroxidation) in liver homogenate were significantly elevated in toxicant treated groups ($p<0.05$) as compared to normal control group. Silymarin (100 mg/kg p.o.) and MEDP (500mg/kg p.o.) treated groups significantly reduced the MDA levels as compared to toxicant groups.

Histoarchitecture of liver tissues in normal control rats in carbon tetrachloride model showed central vein surrounded by hepatic cord of cells and distinct sinusoidal spaces (normal architecture). In CCl_4treated rats, massive fatty infiltrations, necrosis, ballooning degeneration, and excess infiltration of the lymphocytes whereas in PCM treated rats, centrilobular necrosis surrounded by fatty infiltration and granular degeneration were observed. Silymarin or MEDP treatment ameliorated such histopathological changes in both the models (Fig. 3 and 4).

Table 1: Effect of MEDP on serum enzyme (AST, ALT, ALP, LDH), total bilirubin (TB) and total protein (TP) in CCl_4 intoxicated rats.

Group		AST U/l	ALT U/l	ALP U/l	LDH U/l	TB mg/dl	TP gm/dl
I	Normal Control	65 ± 5.177	85 ± 7.662	150.5 ± 9.219	161.3 ± 10.89	0.49 ± 0.028	7.89 ± 0.21
II	CCl_4	158 ± 4.676#	341.3 ± 15.31#	401.3 ± 36.53#	287.8 ± 3.909#	0.98 ± 0.028#	5.80 ± 0.28#
III	Silymarin	66.67 ± 1.994a	124 ± 7.476a	195.3 ± 4.178a	211.1 ± 2.87a	0.61 ± 0.016a	7.79 ± 0.11a
IV	CCl_4 + MEDP 250 mg/kg	93.28 ± 3.798a	200.5 ± 2.456a	264.1 ± 10.65a	219.9 ± 3.3a	0.69 ± 0.021a	6.72 ± 0.25a
V	CCl_4 + MEDP 500 mg/kg	84.2 ± 2.230a	168.4 ± 6.676a	205.2 ± 4.75a	194.7 ± 5.75a	0.50 ± 0.022a	7.10 ± 0.09a

Values are mean±SEM; N=6 in each group. #*p<0.05* when group II compared with group I, a*p<0.05* when group III, IV and V compared with group II.

Table 2: Effect of MEDP on serum enzyme (AST, ALT, ALP, LDH), total bilirubin (TB) and total protein (TP) in Paracetamol intoxicated rats.

Group		AST U/l	ALT U/l	ALP U/l	LDH U/l	TB mg/dl	TP gm/dl
I	Normal Control	106.7 ± 3.503	88.17 ± 8.92	255.7 ± 16.76	211 ± 3.47	0.389 ± 0.018	8.133 ± 0.21
II	PCM	244.6 ± 6.409#	406.9 ± 5.98#	560.1 ± 28.89#	506.9 ± 10.45#	0.808 ± 0.042#	6.452 ± 0.13#
III	Silymarin	142.7 ± 4.624a	180.3 ± 6.00a	272.6 ± 11.66a	264.4 ± 11.17a	0.452 ± 0.024a	7.578 ± 0.23a
IV	PCM + MEDP 250 mg/kg	207.8 ± 3.134a	212.6 ± 5.91a	380.9 ± 26.94a	332.4 ± 12.4a	0.548 ± 0.021a	6.917 ± 0.12a
V	PCM + MEDP 500 mg/kg	127.1 ± 5.166a	176.4 ± 8.42a	303.9 ± 6.45a	274 ± 11.6a	0.425 ± 0.022a	7.463 ± 0.18a

Values are mean±SEM; N=6 in each group. #*p<0.05* when group II compared with group I, a*p<0.05* when group III, IV and V compared with group II.

Table 3: Effect of MEDP on antioxidant enzymes (SOD, GSHPx, CAT) and MDA levels in CCl_4 intoxicated rats.

Group		SOD U/mg protein	GSHPx U/mg protein	CAT U/mg protein	nmol MDA/mg protein
I	Normal Control	4.53 ± 0.19	4.13 ± 0.30	3.67 ± 0.21	2.74 ± 0.21
II	CCl_4	1.68 ± 0.12#	1.33 ± 0.16#	1.64 ± 0.15#	9.90 ± 0.35#
III	Silymarin	3.75 ± 0.13a	2.41 ± 0.14a	3.62 ± 0.17a	4.04 ± 0.23a
IV	CCl_4 + MEDP 250 mg/kg	2.99 ± 0.12a	2.16 ± 0.17a	2.04 ± 0.09	6.35 ± 0.22a
V	CCl_4 + MEDP 500 mg/kg	3.84 ± 0.10a	2.77 ± 0.13a	3.33 ± 0.16a	4.85 ± 0.34a

Values are mean ± SEM; N=6 in each group. #*p<0.05* when group II compared with group I, a*p<0.05* when group III, IV and V compared with group II. 1 U of SOD = 50% inhibition of epinephrine to adrenochrome transition by the enzyme, 1 U of CAT = µmol of H_2O_2 consumed/min/mg protein, 1U of GSHPx = µg GSH utilised/min/mg protein.

Table 4: Effect of MEDP on antioxidant enzymes (SOD, GSHPx, CAT) and MDA levels in paracetamol intoxicated rats.

Group		SOD U/mg protein	GSHPx U/mg protein	CAT U/mg protein	nmol MDA/mg protein
I	Normal Control	4.288 ± 0.234	2.641 ± 0.149	4.822 ± 0.339	1.785 ± 0.104
II	PCM	1.712 ± 0.144#	0.773 ± 0.117#	1.257 ± 0.167#	10.33 ± 0.238#
III	Silymarin	3.642 ± 0.167a	2.158 ± 0.126a	4.043 ± 0.386a	3.815 ± 0.185a
IV	PCM + MEDP 250 mg/kg	2.742 ± 0.137a	1.427 ± 0.159	2.505 ± 0.179a	8.13 ± 0.238 a
V	PCM + MEDP 500 mg/kg	3.868 ± 0.131a	2.235 ± 0.263a	3.891 ± 0.169a	5.934 ± 0.131 a

Values are mean±SEM; N=6 in each group. #*p<0.05* when group II compared with group I, a*p<0.05* when group III, IV and V compared with group II. 1 U of SOD = 50% inhibition of epinephrine to adrenochrome transition by the enzyme, 1 U of CAT = µmol of H_2O_2 consumed/min/mg protein, 1U of GSHPx = µg GSH utilised/min/mg protein.

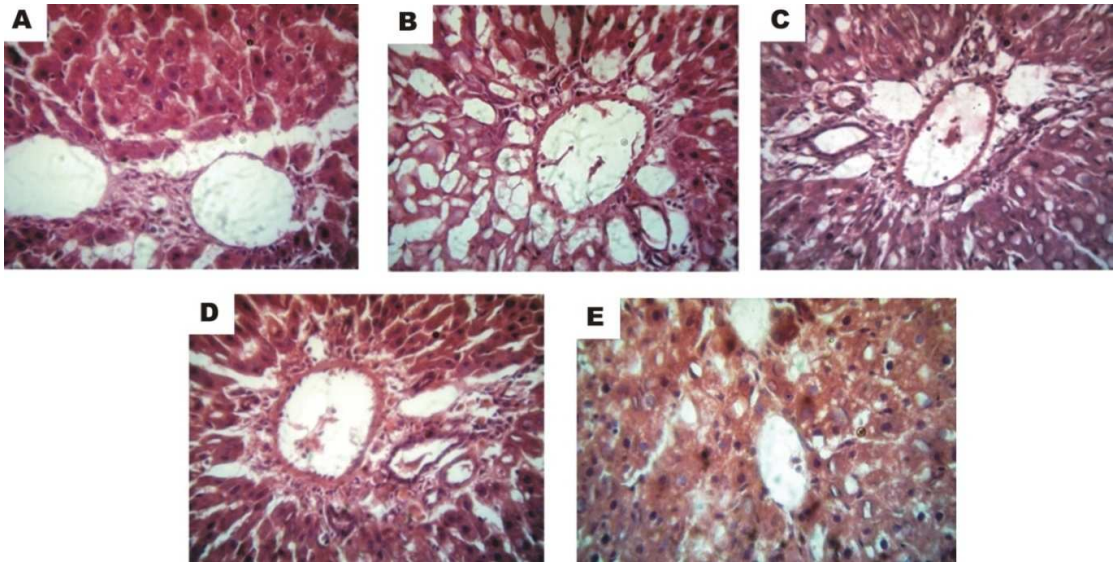

Fig. 3: The photomicrographs [x (40 x 10) = x 400] of rat liver section stained with Haematoxylin-Eosin. (A) H-E staining of liver tissues of normal rats, (B) H-E staining of liver tissues of CCl_4 treated rats, (C) H-E staining of liver tissues of rats treated with CCl_4 + Silymarin (100 mg/kg p.o.), (D) H-E staining of liver tissues of rats treated with CCl_4 + MEDP (250 mg/kg p.o.) and (E) H-E staining of liver tissues of rats treated with CCl_4 + MEDP (500 mg/kg p.o.)

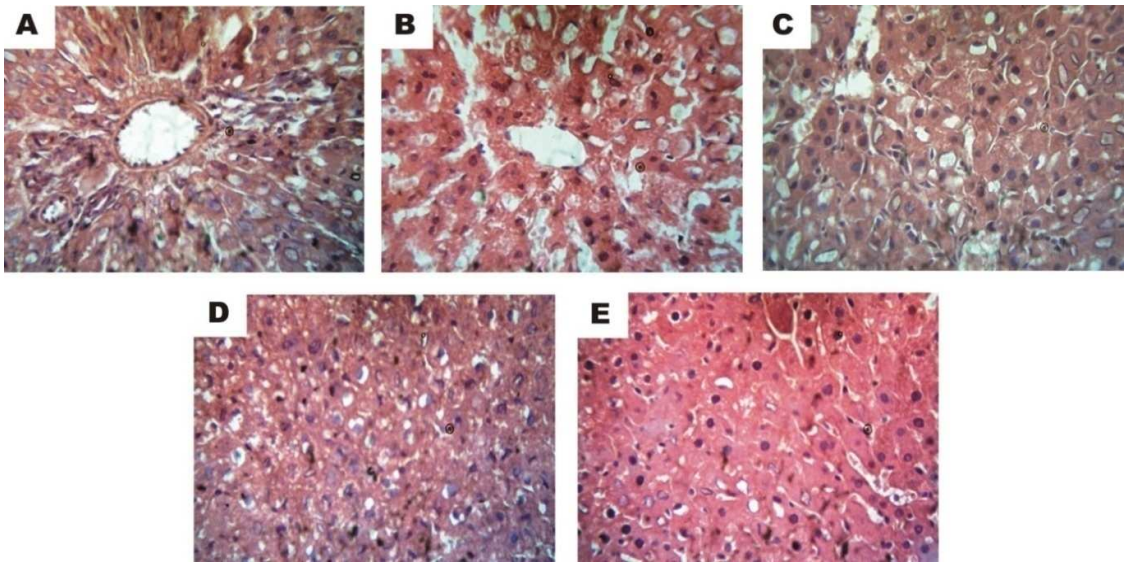

Fig. 4: The photomicrographs [x (40 x 10) = x 400] of rat liver section stained with Haematoxylin-Eosin. (A) H-E staining of liver tissues of normal rats, (B) H-E staining of liver tissues of PCM treated rats, (C) H-E staining of liver tissues of rats treated with PCM + Silymarin (100 mg/kg p.o.), (D) H-E staining of liver tissues of rats treated with PCM + MEDP (250 mg/kg p.o.) and (E) H-E staining of liver tissues of rats treated with PCM + MEDP (500 mg/kg p.o.)

DISCUSSION

The liver cells contain abundant enzyme biomarkers which are diagnostically important. In liver injury, damage to the membranes of hepatocytes and cell organelles allows intracellular enzymes to leak into the blood that helps to predict liver damage and/or injury[22]. AST, ALT and LDH are the predominant biomarker enzymes of hepatocellular necrosis, whereas ALP largely represents chloestasis. The other paradigms selected for the assessment of liver disorders include bilirubin and protein[23]. The elevated serum marker enzymes and bilirubin, and decreased total protein in PCM and CCl_4 intoxicated rats clearly indicate severe damage of plasma membrane of hepatocytes and cholestasis. The elevated serum biomarker enzymes were significantly ($p<0.05$) restored nearby to normal levels in MEDP and silymarin treated groups suggesting their promising hepatoprotective activity. The restoration of biomarker enzymes might be achieved through plasma membrane stabilisation of hepatocytes or alternatively regeneration of hepatic cells via enhanced protein synthesis.

Hepatotoxicity of CCl_4 has been attributed to its major metabolite, trichloromethyl radical ($CCl_3\cdot$) which reacts with oxygen to form the trichloromethyl peroxy radicals ($CCl_3OO\cdot$). These oxygen reactive species are known to cause hepatotoxicity, renal toxicity, liver cancer and other cell damage mainly by a) covalent bonding to cellular macromolecules and b) peroxidative degradation of membrane lipids and endoplasmic reticulum rich in polyunsaturated fatty acids[24].

Paracetamol is sulphated or glucoronated and excreted. After a large dose, the drug is converted into N-acetylbenzo-p-quinoneimine (NAPQI), a highly reactive species that can be conjugated with glutathione to produce an inert conjugate. In cases of severe overdose, hepatic glutathione reserve fall and NAPQI is free to react with hepatic protein and cause centrilobular necrosis, and results in acute non-infectious hepatitis[25]. Such cellular events lead to lipid peroxidation and formation of MDA formation. The MDA is responsible for the loss of integrity of cell membranes and subsequently manifest in hepatic damage. In the present findings significant reversal of MDA formation by MEDP (500 mg/kg p.o.) treatment clearly point out its hepatoprotective potential which is almost that of silymarin.

Reactive oxygen species (ROS) viz superoxide anions (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl free radicals ($OH\bullet$) cause damage to all major classes of macromolecules in cells. The cells have developed multiple ways to remove ROS, and thus, able to protect themselves against their oxidative damage. Mammals have 3 different isozymes of superoxide dismutase (SOD) that catalyse the conversion of superoxide to hydrogen peroxide. The cytosolic form of superoxide dismutase contains Cu/Zn at its active site (as does the extracellular enzyme) whereas mitochondrial enzymes contain Mn at its active site. Hydrogen peroxide is removed by catalase (CAT), a heme containing enzyme present in high concentration in peroxisomes, and to a lesser extent in mitochondria and cytosol. CAT is distributed in all animal tissues and the highest activity is observed in red blood cells and liver. A selenium containing enzyme, GSHPx catalyzes reduction of both hydrogen peroxide and lipid peroxides using the sulfhydryl group of glutathione (as a hydrogen donar with formation of oxidized or disulfide form of glutathione. All these antioxidant enzymes act in conjunction with each other hence able to remove/ scavenge/ neutralise excess free radicals (H_2O_2, OH and O_2) and able to ameliorate or offer protection of liver damage due to CCl4 and PCM. Such protection effect against ROS has been demonstrated by Vitamin C, Vitamin E and β-carotene[26].

Phytochemical analysis of DP showed presence of β-carotene[11] and moderate levels of β-sitosterol, phenolic compounds, ascorbic acid, tannin, lycopene, α-tocopherol[10] and iridoid glycosides[9]. The presence of such phytoconstituents might be responsible for hepatoprotective activity and which also supports this *in-vivo* hepatoprotective activity[27,28]. Furthermore, it has been suggested that iridoid glycoside in certain plants is a critical compound in hepatoprotective activity[29]. The hepatoprotective activity of MEDP (500 mg/kg p.o.) was further confirmed by liver histo-architecture studies.

Natural antioxidants, vitamin C and vitamin E have been implicated in liver as well as cardiac damage protection. Additionally, it has been determined that the antioxidant effect of plant products is mainly due to phenolic compounds, such as flavonoids, phenolic acids, tannins and phenolic diterpenes[30,31]. Therefore antioxidants have intrinsic capability to induce hepatoprotective activity largely through free radical scavenging and enhancing antioxidant enzyme defence systems[32,33].

The findings of present study reveal hepatoprotective action of MEDP against CCl_4 intoxication in rats. It is likely that the presence of principally bioactive phytochemical constituents in DP may be

responsible for hepatoprotective activity but further research is needed. This is only a footstep towards new hepatoprotective agent (*Dipteracanthus patulus Jacq nees*) from natural origin.

CONCLUSION

It can be concluded that MEDP provide significant protection against CCl_4 and paracetamol intoxication in rats. It may be stated that MEDP exerts its hepatoprotective action by dual mechanism – by enzymatic and non-enzymatic pathways. Controlled clinical trials on healthy volunteers can be warranted to prove the therapeutic benefit in human beings against such hepatotoxins.

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