
ORIGINAL PAPER

**CHEMOPROTECTIVE AND ANTIOXIDANT ACTIVITIES OF
METHANOLIC EXTRACT OF AMARANTHUS SPINOSUS LEAVES
ON PARACETAMOL INDUCED-LIVER DAMAGE IN RATS**

**Bagepalli Srinivasa
ASHOK KUMAR¹
Kuruba LAKSHMAN²
Koralkonta Narasimha
JAYAVEERA³
Devangam
SHESHADRI SHEKAR⁴
Rudrappa NANDEESH⁵
Chinnasamy VELMURUGAN⁴**

¹Department of Pharmacognosy, Sri K.V. College
of Pharmacy, Chickballapur, Karnataka

²Department of Pharmacognosy, PES College of
Pharmacy, Bangalore, Karnataka

³Department of Chemistry, Jawaharlal Nehru
Technological University of College of Engineer-
ing, Anantapur, Andhra Pradesh

⁴Department of Pharmacology, Sri K.V. College of
Pharmacy, Chickballapur, Karnataka

⁵Department of Pharmacognosy, Sree Sidhagan-
ga College of Pharmacy, Tumkur, Karnataka

India

Received: 30.04.2010

Accepted: 07.10.2010

Correspondence to:

Ashok Kumar, B.S.

Assistant Professor and Head,

Department of Pharmacognosy,

Sri K.V.College of Pharmacy,

Chickballapur-562101

Karnataka, India

e-mail: ashok4vani@gmail.com

ABSTRACT

Objective: *Amaranthus spinosus* Linn. (Amaranthaceae), the leaves were boiled without salt and consumed for 2–3 days to cure jaundice used by tribal of Kerala (India). Methanol extract of whole plant of *A. spinosus* Linn, (MEAS) was screened for hepatoprotective potency against paracetamol (PCM) (3 gm/kg. o.p.) induced-liver damage in Wistar rats at dose of 200 and 400 mg/kg respectively.

Materials and methods: *A. spinosus* was collected from surroundings of Chickballapur, Karnataka (India). Whole plant was extracted with methanol. Hepatoprotective activity of MEAS was evaluated in PCM induced hepatotoxicity in Wistar rats by measuring liver marker serum enzymatic levels of serum glutamate oxaloacetate transaminase (AST), serum glutamate pyruvate transaminase (ALT), albumin (ALB), total protein (TP), total bilirubin, direct bilirubin levels and the markers for oxidative defense namely malondialdehyde (MDA), reduced glutathione (GSH), catalase (CAT) and total thiols (TT). Histopathological studies of liver were done to assess the cellular damage.

Results: The results of our study showed significant ($p < 0.001$) protection against PCM induced hepatic damage in experimental animals following the administration of MEAS. Our results were compared with silymarin (100 mg/kg), a known hepatoprotective drug.

Conclusions: This presence of amino acids, flavonoids and phenolic compounds in the MEAS might be responsible for its marked chemoprotective and antioxidant activities in paracetamol induced-liver damage in Wistar rats.

Keywords: *Amaranthus spinosus* leaves, chemoprotective, paracetamol, antioxidant activity.

INTRODUCTION

Amaranthus spinosus Linn., (Amaranthaceae), commonly known as “Mulluharivesoppu” in Kannada, is an annual or perennial herb, native to tropical America and found throughout India as a weed in cultivated as well as fallow lands. ¹ The juice of *A. spinosus* is used by tribal of Kerala (India) to prevent swelling around stomach while the leaves are boiled without salt and consumed for 2–3 days to cure jaundice. ² Plant has one of the vegetable have high concentration of antioxidant components, ^{3–5} high nutritive values due to presence of fibre, proteins and high concentration

of essential amino acids, especially lysine. ⁶ In Indian traditional system of medicine (Ayurveda) the plant is used as antipyretic, laxative, diuretic, digestible, anti-diabetic, anti-snake venom, antileprotic, blood diseases, bronchitis, piles and anti-gonorrheal. ^{7–9} The Chinese use *A. spinosus* as traditional medicine to treat diabetes and seeds used as poultice for broken bones. Some tribes in India apply *A. spinosus* to induce abortion. ¹⁰

A. spinosus is also used as reported for its anti-inflammatory, ¹¹ anti-malarial, ¹² anti-androgenic, ¹³ immunomodulatory, ¹⁴ anti-diabetic, anti-hyperlipidemic and spermatogenic activities of stem. ¹⁵ Effect on he-

Table 1. Effect of methanolic extract of *A. spinosus* on paracetamol induced hepatotoxicity in rats

Treatment	SGPT (IU/L)	SGOT (IU/L)	Serum ALB	Serum TP	Bilirubin (mg/dl)		Liver weight
					Total	Direct	
Normal control	27.78±3.50	61.91±5.77	6.05±0.14	4.28±0.2	0.592±0.05	0.15±0.01	7.68±0.43
PCM alone (3 g/kg)	1379.74±147.45 [#]	1412.77±109.22 [#]	2.99±0.23 [#]	2.32±0.17 [#]	2.685±0.15 [#]	0.345±0.03 [#]	4.86±0.23 [#]
PCM+ MEAS (200 mg/kg)	784.58±96.59 ^c	416.2±34.14 ^c	4.76±0.22 ^c	2.85±0.10 ^a	0.707±0.04 ^c	0.225±0.02 ^b	6.52±0.28 ^a
PCM+ MEAS (400 mg/kg)	545.34±60.92 ^c	259.81±60.92 ^c	5.24±0.21 ^c	3.38±0.24 ^b	0.578±0.03 ^c	0.187±0.01 ^c	7.41±0.79 ^b
PCM+ Silymarin (100 mg/kg)	229.23±36.67 ^c	219.79±19.31 ^c	5.24±0.36 ^c	3.51±0.15 ^c	0.442±0.04 ^c	0.165±0.01 ^c	7.46±0.60

Date represents Mean±SEM., n=6. ^ap<0.05; ^bp<0.01; ^cp<0.001 compared with PCM group and [#]p<0.001 compared with control.

matology¹⁶ and biochemical changes in Epididymis.¹⁷ The betalains in stem bark of *A. spinosus* were identified as amaranthin, isoamaranthine, hydroxycinnamates, rutin, quercetin and kaempferol glycosides.¹⁸⁻²² It also contains amaranthoside, a lignan glycoside, amaricin, a coumaroyl adenosine along with stigmasteryl glycoside, betaine such as glycinebetaine and trigonelline.^{23,24} Betalains are well known for their antioxidant, anticancer, antiviral and antiparasitosis properties.²⁵⁻²⁷

A. spinosus is used for the treatment of liver diseases in traditional system of medicine. However, there is lack of scientific report regarding chemoprotective and antioxidant activities of *A. spinosus*. The present communication is an endeavour in the evaluation of chemoprotective and antioxidant activities of methanolic extract of *A. spinosus* leaves against paracetamol induced hepatic damage in experimental animals.

MATERIALS AND METHODS

Collection of Plant Material and Extraction

The fresh plant of *A. spinosus* was collected from Chickballapur, and was authenticated by Prof. B.K.Venkatesh, Department of Botany, Government First grade College, Chickballapur, Karnataka. A voucher specimen (SKVCP 11) was deposited in college herbarium. The whole plant was shade dried and coarsely powdered. The coarse powder was subjected to extraction with methanol by soxhlet apparatus and extract was concentrated to dryness in vacuum.

Preliminary Phytochemical screening

The methanol extract of *A. spinosus* was screened for the presence of various phytoconstituents like steroids, alkaloids, glycosides, flavonoids, carbohydrates, proteins and phenolic compounds.²⁸

Animals

Male Swiss Wister rats weighing 150-250 gm were acclimatized to the experimental room at temperature 23 ± 2 °C, controlled humidity conditions (50-55%) and 12 h light and 12 h dark cycle. They were caged with a maximum of two animals in polypropylene cage and were fed with standard food pellets (Kamadenu Enterprises, Bangalore) and water ad libitum. All the studies conducted were approved by the institutional animal ethical committee of Sri K.V.College of Pharmacy, Chickballapur, Karnataka, according to prescribed guidelines of CPCSEA, Government of India (Reg. No. 117/1998/CPCSEA).

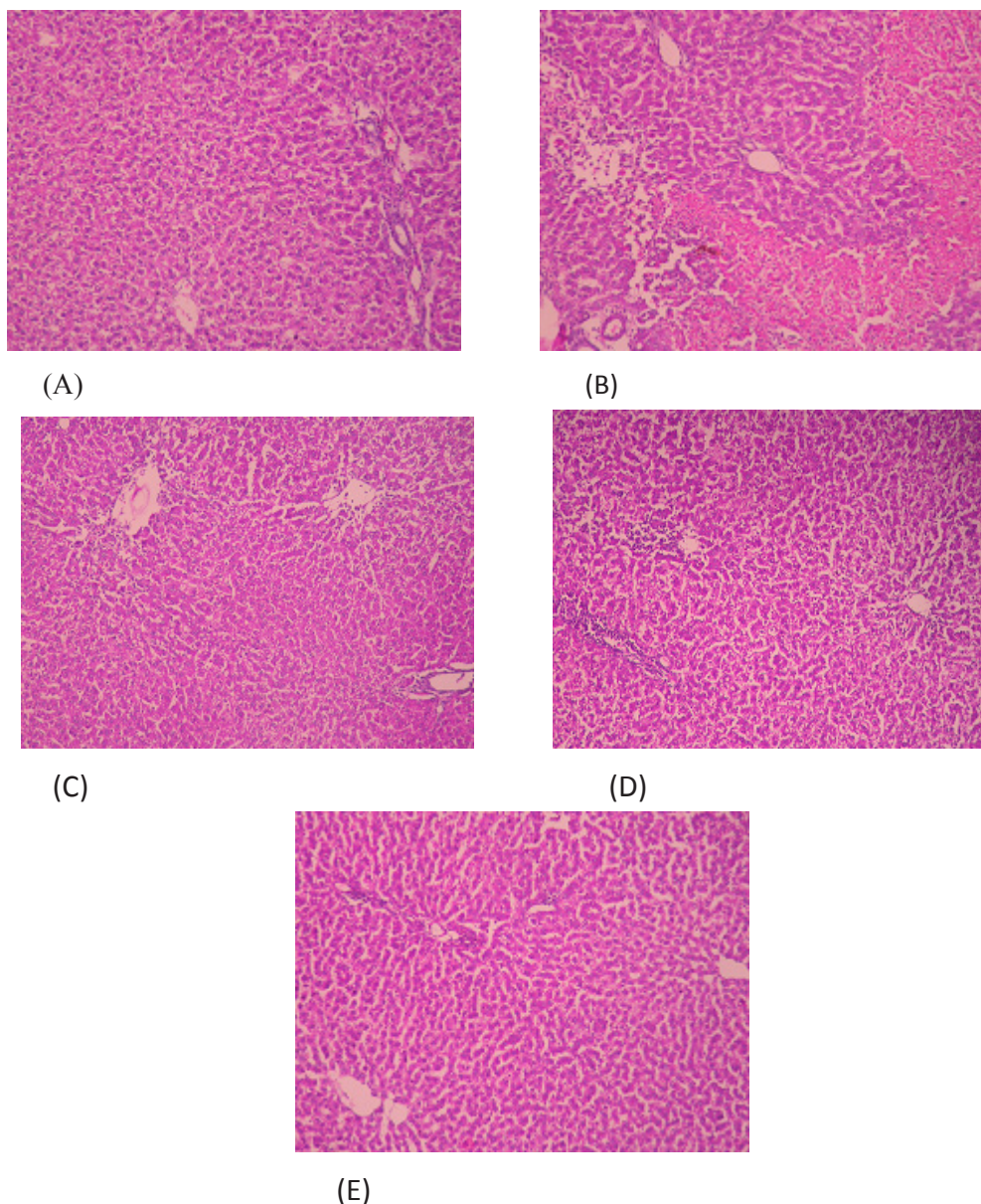


Figure 1. Histopathology of liver tissues (A) liver section of normal rat. (B). PCM treated group showing: section shows patches of liver cell necrosis with inflammatory collections, (C). PCM+MEAS (200 mg/kg) treated group which showed central veins and sinusoids show reasonable congestion and focal haemorrhage or extensive centrilobular degeneration and diffuse inflammatory process. (D) & (E) showed central veins and sinusoids show less congestion and focal haemorrhage or small centrilobular degeneration with slight degree of inflammatory process; less portal triaditis and spotty necrosis in PCM+ MEAS (400 mg/kg) and PCM+ Silymarin (100 mg/kg) groups.

Acute toxicity studies

Methanol extract of *A. spinosus* was studied for acute oral toxicity as per revised OECD (Organization for Economic Cooperation and development) guidelines No. 423. The extract was devoid of any toxicity in rats when given in dose up to 2000 mg/kg by oral route. Hence, for further studies 200- 400 mg/kg doses of extract were used.²⁹

Assessment of Chemoprotective activity

Rats were divided into five groups (n=6):

Group I- received a single daily dose of sodium carboxy methyl cellulose (1 ml of 1%, w/v, p.o. body weight) for 14 days.

Group II- animals received single dose of 3 gm/kg paracetamol (PCM) orally on 14th day,

Group III and IV- animals received orally 200, 400 mg/kg body weight of MEAS once daily for 14 days and along with paracetamol single dose (3 gm/kg).

Group V - received Silymarin (100 mg/kg once daily p.o) respectively for 14 days along with paracetamol single dose (3 gm/kg) as in group second.

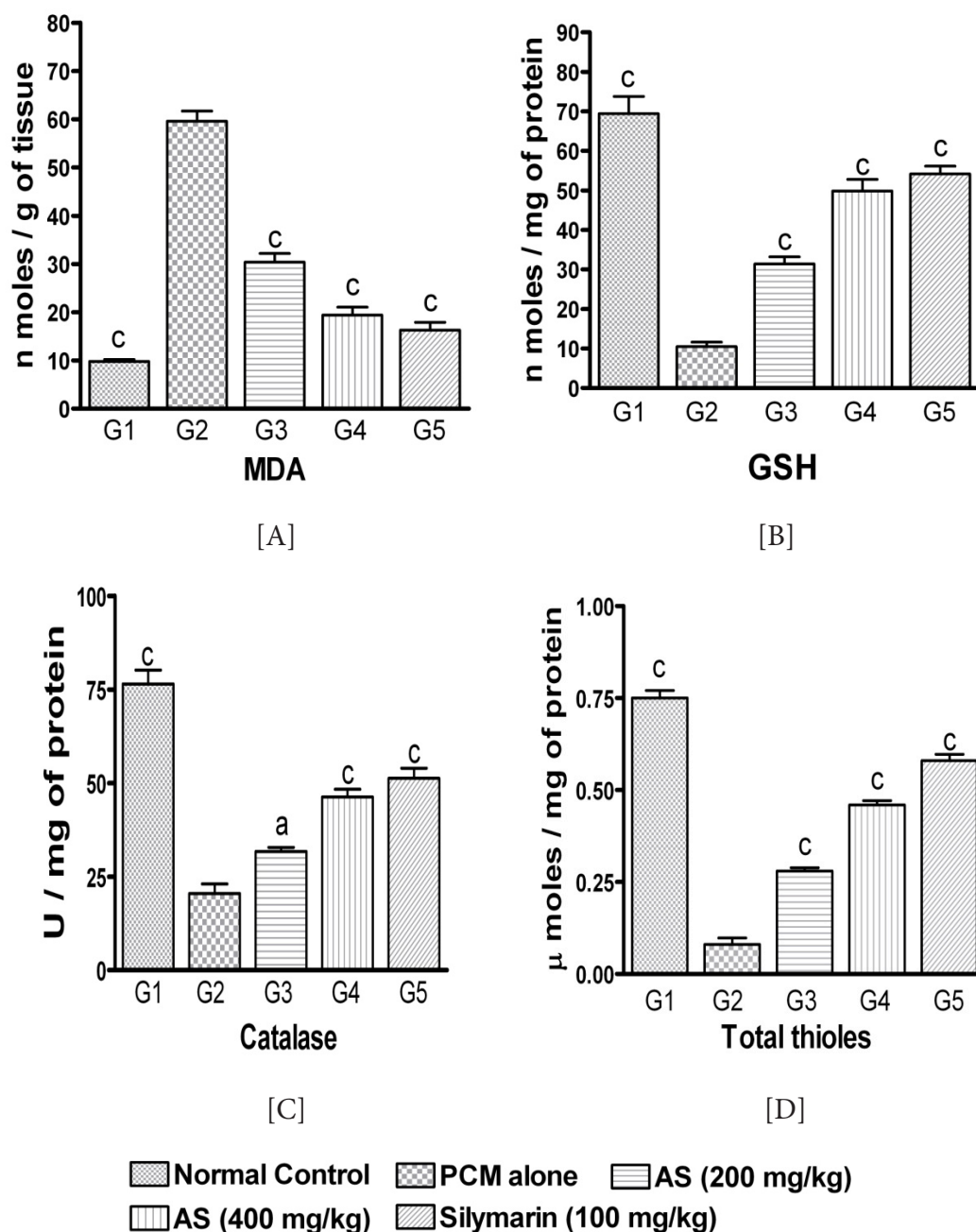


Figure 2. *In vivo* antioxidant methanolic extract of *A. spinosus* [A] MDA, [B] GSH, [C] CAT and [D] total thiols, in the liver homogenates of PCM induced liver damage. Each bar represent Mean \pm S.E.M., $n=6$. ^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$ compared with PCM alone group. One-way ANOVA followed by Tukey's post-test.

Animals were sacrificed 48 h after the paracetamol administration; blood and liver were collected.³⁰ The blood collected and allowed to clot and serum was separated at 3000 rpm for 10 min to obtain serum, which was later used for the estimation of serum enzymes. Serum glutamate oxaloacetate transaminase (AST, U/L), serum glutamate pyruvate transaminase (ALT U/L),³¹ Albumin (ALB), total bilirubin and direct bilirubin³² were assayed using autoanalyser (Maygun MS 500) using kits (ERBA, Transasia Biomedicals Ltd. India).

Assessment of antioxidant potential

Collected Liver was homogenized in ice-cold saline-EDTA using Teflon-glass homogenizer (Remi, pvt ltd., Mumbai). The required quantity of liver homogenates was used for the estimation of lipid peroxidation as malonaldehyde (MDA).³³ The homogenate was centrifuged at 10,000 rpm for 10 min and the pellet was discarded. The supernatant was again centrifuged at 20,000 rpm for 1 hour at 40C and the supernatant obtained was used for the estimation of total protein,³⁴ Catalase,³⁵ GSH and total thiols.³⁶

Histopathological studies

The livers were removed from the animals and the tissues were fixed in 10% formalin for at least 24 h dehydrated with alcohol and embedded in paraffin. Thin section (5µm) were cut and stained with haematoxylin-eosin dye (H & E) for photo microscopic assessment including cell necrosis, fatty changes ballooning degeneration, focal haemorrhage and centrilobular degeneration and lymphocyte infiltration.

Statistical analysis

All the values were expressed as mean±SEM. the results were analyzed statistically by one-way ANOVA followed by Tukey's post-test, $P < 0.05$ were considered significant.

RESULTS

Preliminary phytochemical screening

Methanolic extract of *A. spinosus* showed the presence of flavonoids, saponins, glycosides, terpenoids aminoacids, alkaloids, carbohydrates, phenolic compounds and proteins.

Acute toxicity studies

Methanolic extract of *A. spinosus* did not show any behavioral changes or mortality up to a dose of 2000 mg/kg.

Effect of MEAS on SGPT, SGOT, ALB, bilirubin (Total and Direct bilirubin) and liver weight

PCM alone treated animals showed increase in serum marker enzymes (SGPT and SGOT), bilirubin and decrease in ALB levels. Oral administration of MEAS at a doses of 200 and 400 mg/kg significantly ($P < 0.001$) normalized the PCM-induced biochemical changes. Silymarin significantly reversed the PCM hepatotoxicity. The liver weight of rats treated with PCM only decreased significantly ($p < 0.05$), which was blunted by MEAS and Silymarin (Table 1).

Estimation of MDA, GSH, CAT, TT and TP

PCM showed significant ($p < 0.001$) increase in lipid peroxidation (MDA) 508.16% and decrease GSH 84.82%, CAT 71.35% and Total thiols 89.33% levels in group II compared to control group. MEAS and silymarin (200, 400 and 100 mg/kg) significantly ($p < 0.001$) decrease in MDA level (209.8, 97.7, 66.22%), while increase in GSH (54.76, 28.13, 21.97%), CAT (58.51, 39.52, 32.92%), and total thiols (62.66, 38.66, 22.66%)

levels respectively (Fig.1).

Histopathological observations

Histopathological examination of the liver sections, control groups (Group I) showed normal hepatic cell structure (A). PCM treated group (Group II) shows marked hemorrhagic areas, centrolobular degeneration and multiple foci of hepatocellular necrosis with dilation of sinus congestion and diffuse inflammatory process (B). The histological architecture of liver sections of the rats treated with MEAS at 200 mg/kg showed moderate changes in degeneration of hepatocytes, central vein and sinus congestion, centrolobular necrosis was completely prevented (C). Both MEAS at 400 mg/kg and silymarin (100 mg/kg) showed mild congestion of central vein and sinus, no centrolobular degeneration and necrosis was observed (D & E).

DISCUSSION

The liver is the largest organ in the vertebrate body, and is the major site of xenobiotic metabolism and excretion. Liver injury can be caused by drugs, toxic chemicals, and virus infiltration from ingestion or infection. The toxins absorbed from the intestinal tract gain access first to the liver resulting in a variety of liver ailments. Thus liver disease remains one of the serious health problems ³⁷.

Paracetamol, a widely used analgesic and antipyretic, is extremely safe at therapeutic dose ³⁸. However, overdose or chronic use of high-dose PCM has been the major cause of hospital admissions for emergency liver transplantation for acute liver failure (ALF) in UK, over the last 30 years ³⁹. It also currently accounts for approximately 40% of cases of ALF in the USA ⁴⁰. PCM is mainly metabolized in liver to extractable glucuronide and sulphate conjugates ⁴¹. However, these metabolites eventually converted into a reactive (toxic) metabolite known as N-acetyl-p-benzo-quinoneimine by the liver cytochrome P-450 enzyme system. The major finding observed in PCM intoxication in humans and animals is acute centrolobular necrosis. In the present study, the haemorrhagic and necrotic findings observed in the histopathological examination of the liver of rats which were administered PCM; demonstrate PCM-induced damage in the liver (Fig. 1 B). A sharp increase in the serum AST and ALT levels is considered to be a significant indicator of PCM-induced acute liver damage ⁴². In our study, when compared to the control group, the sharp increase in SGPT and SGOT levels were observed in PCM administered group (Table 1), indicating PCM induced hepatic injury ⁴³. A significant prevention of a change in the enzymatic levels (AST and ALT) of the PCM treated rats following adminis-

tration of MEAS and evidenced an effective prevention of the PCM toxicity on the liver.

Total protein measurement is used in the diagnosis of a variety of diseases involving the liver or kidney disorders. A decrease in albumin level has been attributed to several causes, such as massive necrosis of the liver, deterioration of liver function, hepatic resistance to insulin and glycogen impairment of oxidative phosphorylation⁴⁴. MEAS normalizes the protein and albumin levels when compared to PCM.

The effects of the MEAS on liver weight of rats are shown in Table 1. The PCM treated rats showed significant loss in liver weight. The administration of extract, however, significantly prevented the weight loss due to PCM challenge, which was comparable with that of silymarin.

Elevation of lipid peroxidation is measure of membrane damage and alterations in structure and function of cellular membrane. We observed a significantly increased in MDA an index of lipid peroxidation, in the liver of rats treated with PCM. Oral administration of MEAS significantly reversed MDA level, suggesting that MEAS might have antioxidant principles to produce such response.

It is well known fact that the deficiency of GSH an intrinsic antioxidant enzyme, in a living organisms leads to tissue injury. The liver injury including one caused by alcohol abuse or by drugs like acetaminophen; lung injury caused by smoking and muscle injury by intense physical activity etc.,⁴⁵ have been correlated with low tissue levels of GSH. Administration of PCM alone (group II) showed a significant reduction GSH, CAT and TT suggesting high oxidative stress. MEAS (200 and 400 mg/kg) in the groups III and IV prevented the loss of antioxidant enzymes GSH and CAT and also the TT and thus exhibits its antioxidant potential. The reduction in the oxidative damage caused by PCM and the stimulation of GSH synthesis has been indicated in protection of liver against PCM challenge in rats treated with MEAS and silymarin⁴⁶.

A. spinosus contains amino acids namely, lysine, arginine, histidine, cystine, phenylalanine, leucine, isoleucine, valine, threonine, methionine, tyrosine and tryptophan⁴⁷.

De Toranzo et al., have reported that methionine and tyrosine exhibit protective effect against carbon tetrachloride-induced acute liver damage⁴⁸. Pisarenko has demonstrated increased intracellular leucine, valine and isoleucine concentrations in myocardial cells to stimulate the synthesis of acetyl coenzyme A and succinyl coenzyme A, and to deteriorate the oxidative

metabolism, and methionine and cysteine to may reduce myocardial damage caused by oxygen radicals⁴⁹. Lysine, which is a nucleophilic amino acid, is reported to facilitate the exchange of electrophiles derived from lipid peroxidation⁵⁰. The present study showed the hepatoprotective and antioxidant effects of MEAS in PCM induced hepatotoxicity, may be due to the presence of amino acids, flavonoids⁵¹, terpenoids⁵², saponins⁵³, glycosides⁵⁴, and phenolic compounds⁵⁵.

REFERENCES

1. Anonymous. The Wealth of India. A Dictionary of Indian Raw Materials and Industrial Products. Vol. I. CSIR, New Delhi. 1988. 219.
2. Hema ES, Sivadasan M, Anil KN. Studies on edible species of Amaranthaceae and Araceae used by Kuruma and Paniya tribes in Wayanad district, Kerala, India. *Ethnobotany* 2006; 18:122-126.
3. Odhavo B, Beekrum S, Akula US, Baijnath H. Preliminary assessment of nutritional value of traditional leafy vegetables in Kwazulu-Natal, South Africa. *J Food Composition and Analysis* 2007; 20:430-435. doi:10.1016/j.jfca.2006.04.015
4. Cao C, Sofic E, Prior RL. Antioxidant capacity of tea and common vegetables. *J. Agri. Food Chem* 1996; 44: 3426-3431. doi:10.1021/jf9602535
5. Gil MI, Ferreres F, Thomas-Arberan FA. Effect of post harvest storage and processing on the antioxidant constituents (flavonoids and Vitamin C) of fresh cut spinach. *J. Agri. Food Chem* 1999; 47:2213-2217. doi:10.1021/jf981200I PMID:10794612
6. Vinson JA, Hap Y, Su X, Zubik L. Phenolic antioxidant quantity and quality in foods, vegetables. *J Agri Food Chem* 1998; 46:3630-3634. doi:10.1021/jf980295o
7. Teutonico RA, Knorr D. Amaranth- composition, properties and applications of a rediscovered food crop. *Food Technology* 1985; 39:49-60.
8. Vaidyaratnam PS, Varier S. *Indian Medicinal Plants*, 1996.
9. William d'ymock. *Pharmacographia Indica*, Part III, 1976; 138-139.
10. Kirtikar KR, Basu BD. *Indian Medicinal Plants*. International book distributors, Dehra Dun, 1987. Grubben GJH, Denton OA. *Plant Resources of Tropical Africa 2. Vegetables* 2004.
11. Olumayokun A, Olajid, Babatunde R, Ogunleya, Temitope O, Erinle. Anti-inflammatory properties of *Amaranthus spinosus*. *Pharm Biol* 2004;521-525.
12. Hilou A, Nacoulma OG, Guiguemde TR. In vivo antimalarial activities of extract from *Amaranthus spinosus* L., and *Boerhaavia erecta* L., in mice. *J Ethnopharmacol* 2006;103: 236-240. doi:10.1016/j.jep.2005.08.006 PMID:16171960
13. Murgan K, Vanithakumari G, Sampathraj R. Effects of combined extracts of *Dolichos biflorus* seeds and *Amaranthus spinosus* roots on the accessory sex organs of male rats. *Ancient Science of Life* 1993; 13: 351-357.
14. Tatiya AU, Surana SJ, Khope SD, Gokhale SB, Sutar MP. Phytochemical investigation and immunomodulatory activity of *Amaranthus spinosus* Linn. *Indian J Pharm Educ Res* 2007; 44(4):337-341.
15. Sangameswaran B, Jayakar B. Anti-diabetic, anti-hyperlipidemic and spermatogenic effects of *Amaranthus spinosus* Linn. On streptozotocin-induced diabetic rats. *J Nat Med* 2008; 62:79-82. doi:10.1007/s11418-007-0189-9 PMID:18404348
16. Olufemi BE, Assiak IE, Ayoade GO, Onigemo MA. Studies on the effect of *Amaranthus spinosus* leaf extract on the Hematology of growing pigs. *Afri J Biomed Res* 2003; 6:149-150.
17. Murgan K, Vanithakumari G, Sampathraj R. Biochemical Changes in epididymis following treatment with combined extracts of *Amaranthus spinosus* roots and *Dolichos biflorus* seeds. *Ancient Science of*

Life 1993; 13:154-159.

18. Ibewuik JC, Ogundaini AO, Bohlin L, Ogungbamila FO. Anti-inflammatory activity of selected Nigerian medicinal plants. *Nigerian Journal of Natural Products and Medicine* 1997;1:10-14.

19. Rastogi RP, Mehrotra BN. *Compendium of Indian Medicinal Plants*. Lucknow, 1999.

20. Stintzing FC, Kammerer D, Schieber A, Hilou A, Nacoulma O, Carle R. Betacyanins and phenolic compounds from *Amaranthus spinosus* L., and *Boerhaavia erecta*. *Zeitschrift fur Naturforschung* 2004; 59:1-8.

21. Ashok Kumar BS, Lakshman K, Chandrasekhar KB, Saleemul-lakhan, Narayana Swamy VB. Estimation of rutin and quercetin in *Amaranthus spinosus* L. *Asian J Chem* 2008; 20(2):1633-1635.

22. Blunden G, Yang M, Janicsak MI, Carabot-Cuervo A. Betaine distribution in the *Amaranthaceae*. *Biochem Syst Ecology* 1999; 27:87-92.

23. doi:10.1016/S0305-1978(98)00072-6

24. Azhar-UI-Haq M, Afza N, Khan SB, Muhammad P. Coumaroyl adenosine and lignan glycoside from *Amaranthus spinosus* Linn. *Polish J Chem* 2006; 80:259-263.

25. Kapadia G, Balasubramanian V, Tokuda H, Iwashima A, Nishino H. Inhibition of 12-O-tetradecanoylphorbol-13-acetate induced Epstein-Barr virus early antigen activation by natural colorant. *Cancer Letters* 1995; 115:173-178. doi:10.1016/S0304-3835(97)04726-5

26. Kapadia G, Tokuda H, Harukuni K, Takao M, Nishino H. Chemoprevention of lung and skin cancer by *Beta vulgaris* (beet) root extract. *Cancer Letter* 1996; 100:211-214. doi:10.1016/0304-3835(95)04087-0

27. Patkai G, Barta J, Varsanyi I. Decomposition of anticarcinogen factors of the beet root during juice and nectar production. *Cancer Letters* 1997; 114:105-106. doi:10.1016/S0304-3835(97)04636-3

28. Kokate CK. Preliminary phytochemical analysis. In: Kokate CK (eds.) *Practical Pharmacognosy*, New Delhi 1986.

29. OECD, Guidelines for testing chemicals. Guidelines 423, acute oral toxicity. *Acute Toxic Class Methods*, Paris 2000.

30. Rao KS, Mishra SH. Hepatoprotective activities of whole plant of *Fumaria indica*. *Indian Drugs* 1997; 59:165.

31. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamate oxaloacetate transaminase. *American J Clin Pathology* 1957; 28:53-56.

32. Malloy HT, Evelyn KA. The determination of bilirubin with the photometric colorimeter. *J Biol Chem* 1937; 119:481-490.

33. Gelvan D, Saltman P. Different cellular targets of Cu- and Fe-catalyzed oxidation observed using a Cu-compatible thiobarbiturate acid assay. *Biochimica Biophysica Acta* 1990; 1035:353-360. PMID:2119808

34. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Bio Chem* 1951; 193:265-75.

35. Claiborne A. In *Handbook of methods for oxygen radical research*. London 1985.

36. Moran A, Depierre JW, Mannervick B. Levels of glutathione, glutathione reductase, glutathione-S-transferase activities in rat liver. *Biochimica Biophysica Acta* 1979; 582: 67-68.

37. Chatterjee, T.K. Medicinal plants with hepatoprotective properties in herbal opinions, vol. III. Books and Allied (P) Ltd., Calcutta. 2000; 135.

38. Savides MC, Oehme FW. Acetaminophen and its toxicity. *Journal of Applied Toxicology*. 1983;3:96-111. doi:10.1002/jat.2550030209 PMID:6886301

39. Bernal, W. Changing patterns of causation and the use of transplantation in the United Kingdom. *Seminars in Liver Disease* 2003;23:27-37.

40. Lee WM: Acute liver failure in the United States. *Seminars in Liver Disease* 2003; 23: 217-226. doi:10.1055/s-2003-42641 PMID:14523675

41. Jollow DJ, Thorgeirsson SS, Potter WZ, Hashimoto M, Mitchell JR. Acetaminophen-induced hepatic necrosis in metabolic disposition of toxic and toxic doses of acetaminophen. *Pharmacol.* 1974; 12: 251-271. doi:10.1159/000136547 PMID:4449889

42. Navarro VJ, Senior JR. Drug related hepatotoxicity. *New England J Med.* 2006; 354: 731-739. doi:10.1056/NEJMra052270 PMID:16481640

43. Kupeli E, Orhan DD, Yesilada E. Effect of *Cistus laurifolius* L. leaf extracts and flavonoids on acetaminophen-induced hepatotoxicity in mice. *J Ethnopharmacol.* 2006; 103: 455-460. doi:10.1016/j.jep.2005.08.038 PMID:16216454

44. Rao RH. Fasting glucose homeostasis in the adaptation to chronic nutritional deprivation in rats. *American J Physiol.* 1995; 268: 873-879.

45. Leeuwenburgh C, Ji LL. Glutathione depletion in rested and exercised mice; biochemical consequence and adaptation. *Arch Biochem Biophys.* 1995; 316: 941-949. doi:10.1006/abbi.1995.1125 PMID:7864653

46. Potter DW, Hinson JA. Mechanisms of acetaminophen oxidation to N-acetyl p-benzoquinoneimine by horse radish peroxidase and cytochrome P-450. *J Bio Chem.* 1987; 262: 966-973.

47. Tee LBG, Davies DS, Seddon CE, Boobis AR. Species differences in the hepatotoxicity of paracetamol are due to differences in the rate of conversion to its cytotoxic metabolite. *Biochem Pharmacol.* 1987; 36: 1041-1052. doi:10.1016/0006-2952(87)90412-6

48. *Wealth of India-Raw materials*, Vol VI, New Delhi, Publication and Information

49. Directorate, Council of Scientific and Industrial Research, 1988; 215-216.

50. Pisarenko OI. Mechanisms of myocardial protection by amino acids: facts and hypotheses. *Clin Exp Pharmacol Physiol.* 1996; 23: 627-633. doi:10.1111/j.1440-1681.1996.tb01748.x PMID:8886480

51. Jamall IS, Mignano JE, Lynch VD, Bidanset JH, Lau Cam C, Greening M. Protective effects of zinc sulfate and L-lysine on acute ethanol toxicity in mice. *Environmental Res.* 1979; 191: 112-20. doi:10.1016/0013-9351(79)90039-2

52. De Feudis FV, Papadopoulos V, Drieu K. Ginkgo biloba extracts and cancer: a research area in its infancy. *Fund Clin Pharmacol.* 2003; 17: 405-417. doi:10.1046/j.1472-8206.2003.00156.x PMID:12914542

53. Takeoka GR, Dao LT. Antioxidant constituent of almond *Prunus dulcis* (Mill.) D.A. Webb. *Hulls. J Agri Food Chem.* 2003; 51: 496-501. doi:10.1021/jf020660i PMID:12517116

54. Hu J, Lee SO, Hendrich S, Murphy PA. Quantification of group B soya saponins by high-performance liquid chromatography. *J Agri Food Chem.* 2002; 50: 2587-2594. doi:10.1021/jf0114740 PMID:11958627

55. Quyang MA, He ZD, Wu CL. Antioxidative activity of glycosides from *Lingustrum sinense*. *Nat Prod Res.* 2003; 13: 381.

56. Di Carlo G, Mascolo N, Izzo AA, Capasso F. Flavonoids: old and new aspects of a class of natural therapeutic drugs. *Life Sci.* 1999; 65: 337. doi:10.1016/S0024-3205(99)00120-4