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ORIGINAL PAPER

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

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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, antidiabetic, anti-snake venum, 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±96.59c	416.2±34.14c	4.76±0.22c	2.85±0.10a	0.707±0.04c	0.225±0.02b	6.52±0.28a
PCM+ MEAS (400 mg/kg)	545.34±60.92c	259.81±60.92c	5.24±0.21c	3.38±0.24b	0.578±0.03c	0.187±0.01c	7.41±0.79b
PCM+ Silymarin (100 mg/kg)	229.23±36.67c	219.79±19.31c	5.24±0.36c	3.51±0.15c	0.442±0.04c	0.165±0.01c	7.46±0.60

Date represents Mean±SEM., n=6. [a]p<0.05; [b]p<0.01; [c]p<0.001 compared with PCM group and [#] p<0.001 compared with control.

matology [16] 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 stigmasterol 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).

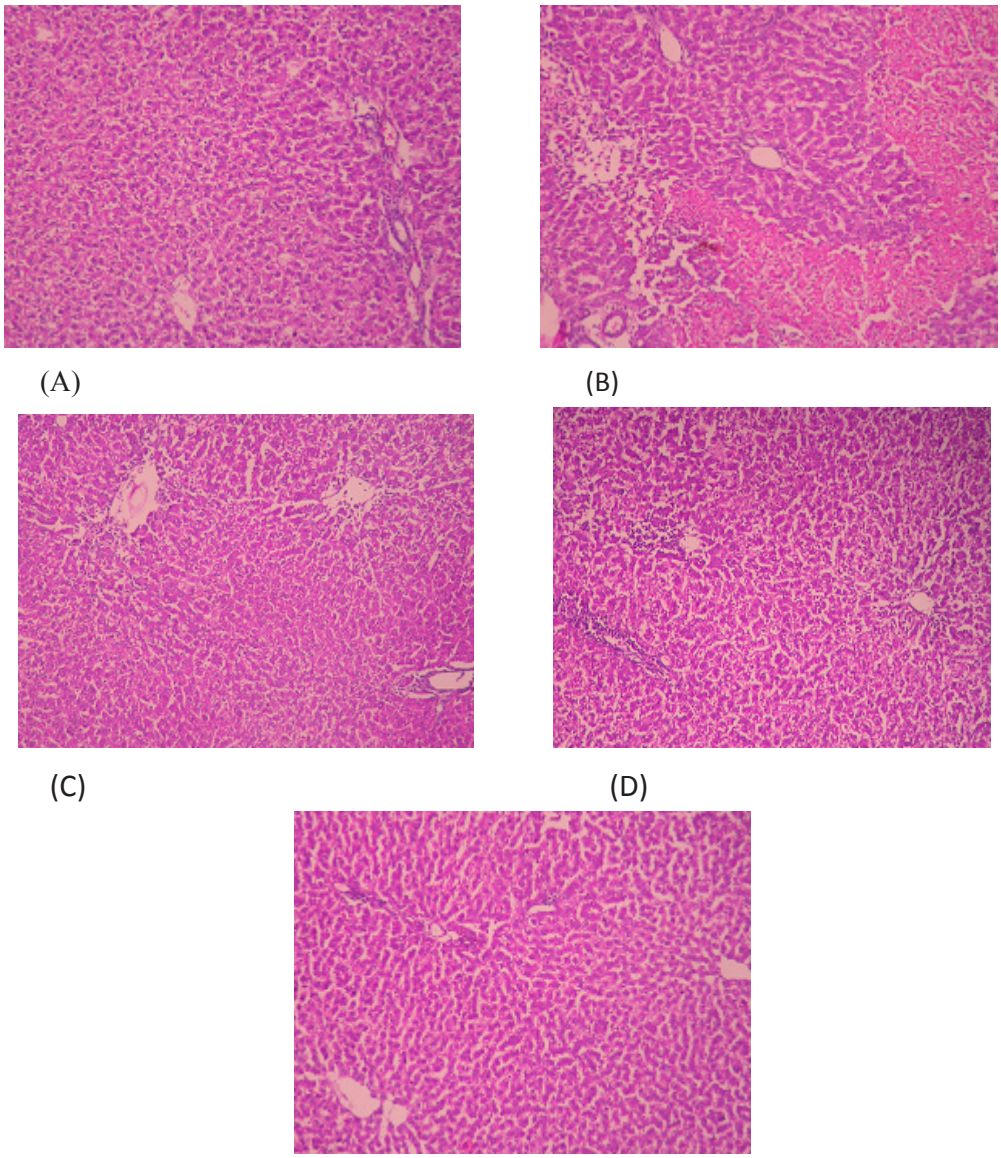

Figure 1. *Histopathology of liver tissues (A) liver section of normal rat. (B). PCM treated group showing: section shows patches of liver cell necrosis with inflammatory collections, (C). PCM+MEAS (200 mg/kg) treated group which showed central veins and sinusoids show reasonable congestion and focal 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.

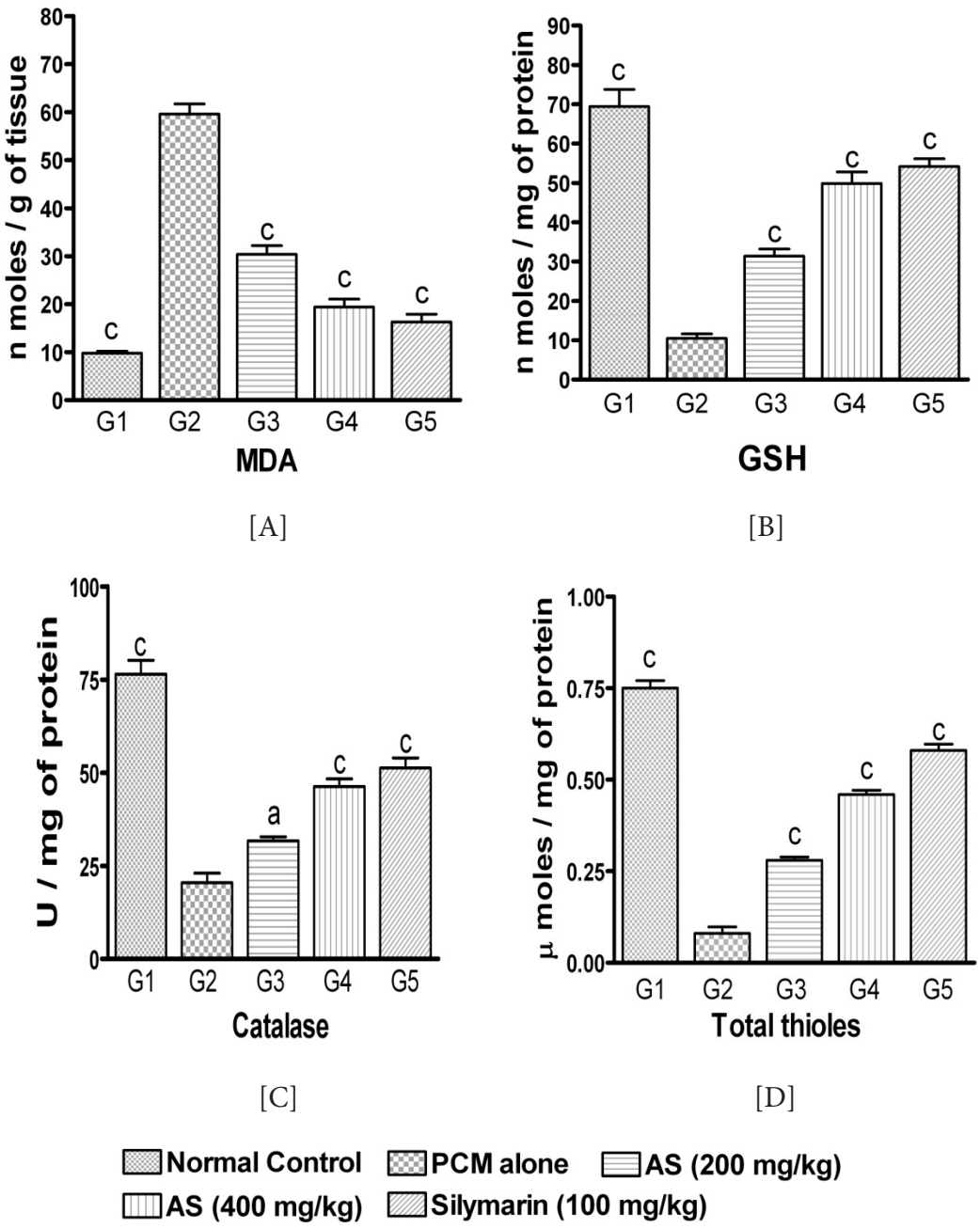

Figure 2. *In vivo antioxidant methanolic extract of A. spinosus [A] MDA, [B] GSH, [C] CAT and [D] total thiols, in the liver homogenates of PCM induced liver damage. Each bar represent Mean ± S.E.M., n=6. [a] p < 0.05; [b] p < 0.01; [c] p < 0.001 compared with PCM alone group. One-way ANOVA followed 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 bilirubin [32] 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 phosphorylation [44]. 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 it 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 silymarin [46].

A. spinosus contains amino acids namely, lysine, arginine, histidine, cystine, phenylalanine, leucine, isoleucine, valine, threonine, methionine, tyrosine and tryptophan [47].

De Toranzo et al., have reported that methionine and tyrosine exhibit protective effect against carbon tetrachloride-induced acute liver damage [48]. 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 radicals [49]. Lysine, which is a nucleophilic amino acid, is reported to facilitate the exchange of electrophiles derived from lipid peroxidation [50]. The present study showed the hepatoprotective and antioxidant effects of MEAS in PCM induced hepatotoxicity, may be due to the presence of amino acids, flavonoids [51], terpenoids [52], saponins [53], glycosides [54], and phenolic compounds [55].

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