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Hepatoprotective Activity of Methanolic Extract of *Amaranthus cruentus* in Paracetamol Intoxicated Rats

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KEYWORDS
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Antioxidant

ABSTRACT:

Objectives:This research assesses the hepatoprotective activity of the methanolic extract of *Amaranthus cruentus* (MEAC) against paracetamol paracetamol-intoxicated rat model.

Methods: Methanolic extract of leaves of *Amaranthus cruentus*was collected through the soxhlet apparatus. The extract is further utilized for physical examination,phytochemical screening, and GC-MS analysis. Albino Wistar rats (120-150 g) were used to assess the hepatoprotective activity of the extract. After 14 days of treatment of either distilled water (10 ml/kg/day, p.o.), silymarin (100 mg/kg/day, p.o.), or MEAC at doses of 200 mg/kg and 400 mg/kg body weight, p.o., respectively, animals were subjected to intoxicated with a single high dose of paracetamol (2 g/kg, o.p.) after overnight fasting. Liver function tests(ALP, ALT, AST), total bilirubin, total protein level, and oxidative stress markers(MDA, GSH, GPx, and SOD) were examined for examined hepatoprotective activity.

Results: Physical examination, Phytochemical screening, and GC-MS analysis revealed that *Amaranthus cruentus* has flavonoids and polyphenolic compounds. The liver profile of MEAC has significantly reduced ALP, AST, and ALT levels as compared to the paracetamol-treated groups. Moreover, MEAC significantly reduced the total bilirubin and increased the total protein contents, as compared to the paracetamol group. MEAC also significantly restored the oxidative stress biomarkers.

Conclusions: This study data revealed that *Amaranthus cruentus* has hepatoprotective potential against paracetamol-induced experimental animals due to its high phenolic compounds.

1. Introduction

The liver is the vital organ for detoxification, metabolism, and excretion of a variety of xenobiotics. Exposure to noxious chemicals and drugs like anticancer agents, high alcohol consumption high doses of paracetamol, etc. leads to serious liver toxicity and diseases[1,2,3]. In the world, jaundice, cirrhosis, and fatty liver are very common and increase the risk of a healthy life. But in modern medicine, there is a lack of effective liver protective medicine and still, we majorly depend on liver transplantation. The existing medications have substantial side effects with limited therapeutic benefits [4]. Paracetamol is the most popular antipyretic drug and it is easily available in the market without prescription in many countries[5]which may enhance the risk of paracetamol toxicity. It is well-known that paracetamol is the first-line drug for fever and is commonly used in children, adults, and pregnant women. But in chronic and high doses uses of paracetamol lead to hepatotoxicity as a side effect[6]. Paracetamol acts as a 'pro-poison,' exerting its hepatotoxic effect by producing harmful toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI),which is mostly generated by CYP2E1 and CYP3A4 cytochrome P450 enzymes[7,8]. In therapeutic doses, NAPQI is detoxified by conjugation with glutathione[9].This metabolic pathway via CYP2E1 produces reactive species (ROS)[10], that may be further responsible for hepatic cell injury in paracetamol overdose[11].The mitochondria are a key target for NAPQI adduct formation. The oxidative stress caused by excess production of ROS leads to activation of c-jun N-terminal kinase (JNK)[12-14]. The formation of drug-protein adducts between NAPQI and mitochondrial protein triggers Mitochondrial

dysfunction[15,16] that leads to cellular necrosis[17,18]. Severe liver injury results in the loss of hepatic synthetic function, coagulopathy, and hypoglycemia. Additionally, the loss of hepatic metabolic functions can cause encephalopathy and lactic acidosis.[19]

Amaranthus cruentus is a member of the Amaranthaceae family within the Caryophyllales order[20,21], which comprises 33 families, 692 genera, and 11,155 species[22]. Among these, only three Amaranthus species are known for grain production: *Amaranthus caudatus* L., *Amaranthus hypochondriacus* L., and *Amaranthus cruentus* L. [23].In amaranth, 65% of the proteins are concentrated in the embryo, whereas only 35% are found in the perisperm[21]. This plant is known for its balanced amino acid composition. The protein content of *A. cruentus* seeds is composed of albumins, globulins, prolamins, and glutelins, which constitute 48.9-65%, 13.7-18.1%, 1.0-3.2%, and 22.4-42.3% of the total protein content, respectively[24,25].

This plant is cultivated as a minor crop in the region of Asia-Pacific including India, China, and Nepal in India, it is cultivated in both the hills and plains states like Himachal Pradesh, Uttarakhand, Sikkim, Assam, Jammu and Kashmir, Chhattisgarh, Maharashtra, Gujarat, and Karnataka[26].Red amaranth (Amaranthus cruentus) seed extract is notable for the high biological significance of its flavonoids and phenolic acids[27,28]. The seeds and sprouts of this plant contain a variety of phytoconstituents. The research shows that this plant contains a variety of phytoconstituents mainly gallic acid. Moreover, seeds contain p-coumaric acid, caffeic acid, p-hydroxybenzoic acid, vanillic acid, and cinnamic acid, while p-coumaric acid, syringic acid, and ferulic acid are found in the sprouts. The main flavonoid identified in the sprouts is rutin.[29–31]. Considering these characteristic features, we evaluated of hepatoprotective activity of methanolic extract of *Amaranthus cruentus* (ACMC) against paracetamol intoxicated rat model.

2. Methods

Drugs and chemicals

Paracetamol was obtained from Shreya Chemicals in Bilaspur, whereas Silymarin (Micro Labs Limited) was found as a gift sample. All the other analytical grade chemicals and reagents were procured from the departmental central store.

Diagnostic kits

For biochemical assessment,diagnostic kits like aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin, total protein were procured from Beacon Diagnostics Pvt. Ltd., Gujrat, India.

Collection and authentication of plant material

The plant material was collected from the region of Bilaspur, Chhatisgarh, India in May 2023. The plant materials were identified and authenticated by a botanist, Professor A.K. Dixit, Department of Botany, Guru Ghasidas Vishwavidyalaya, Koni, Bilaspur (C.G.), 495009. The specimen voucher, with the identification number (Bot/GGV/2023/74 dated 21/04/2023), was submitted to the department for verification.

Preparation of plant extract

Air-dried and coarsely powdered leaves of the plant (1kg) were subjected to a hot extraction (at 80°C) process by using a soxhlet apparatus. Methanol (90 % v/v) was used as a solvent for extraction. The extraction continued until the initial volume was reduced to one-fourth. The extract was then concentrated for further studies using by rotatory vacuumed evaporator (at 50 °C) and the dried extract was stored at 8 °C in an ambered colored bottle.

Laboratory Animals

Wistar albino rats of either sex weighing between 120-150g were purchased from Chakraborty Enterprises, 3D Girish Vidya Ratna Lane, Narkeldanga, Kolkata-700011, and were kept in the department animal house for experimental purposes. Approval from the Institutional Animal Ethical Committee (IAEC) of the Department of Pharmacy, Guru Ghasidas Vishwavidyalaya, Bilaspur (Chhattisgarh) was obtained before the experiments (Reference No. 09/IAEC/Pharmacy/2023 dated 28/03/2023). All protocols and experiments were conducted in strict compliance with the guidelines provided by the IAEC and Committee for Control and Supervision of Experiments on Animals (CCSEA) (Approval No. 994/GO/Re/S/06/CCSEA).

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Experimental design

Wistar strain albino rats of either sex, weighing 100-160g, were randomly assigned to five groups, each comprising six animals. Group I, the normal control group, received distilled water (10 ml/kg/day, p.o.) for 14 days. Group II, the negative control (toxic) group, received distilled water (1 ml/kg/day, p.o.) for 14 days. Group III, the standard control group, received silymarin (100 mg/kg/day, p.o.) for 14 days. Groups IV and V, treated as Test I and II, and received methanolic extract Amaranthus cruentus (MEAC) at doses of 200 mg/kg and 400 mg/kg /day, p.o., respectively for 14 days. Food was withdrawn 12 hours before paracetamol administration to enhance acute liver toxicity. On the 14th day, Groups II, III, IV, and V were treated with paracetamol (2 g/kg, p.o.) diluted with distilled water, administered 1 hour after silymarin and MEAC administration, respectively, and were sacrificed 4 hours after paracetamol administration.

Collection of blood and tissue

Blood samples were collected from sacrificed animals via cardiac puncture. Blood samples were centrifuged (3000 rpm, 15 min, 4°C) to separate the serum. The liver was isolated and homogenized (10% w/v) with potassium phosphate buffer (0.1M, pH 7.4) and centrifuged (8000 rpm, 30 min, 4°C) to collect the supernatant. Serum and supernatant of liver tissue were stored at -20°C for further biochemical analysis. A liver of each group was stored in a formalin solution for histological studies.

Biochemical Analysis

Hepatic biomarkers like AST, ALT, AST, bilirubin, and total protein content were estimated by using diagnostic kits, and oxidative stress biomarkers like superoxide dismutase (SOD), glutathione reduced (GSH),and malonaldehyde (MDA) were estimated by spectrophotometric methods described in Choudhary et al. 2016 [32].

Histopathological studies

Isolated liver tissue was subjected to histopathological study. 10 % buffered formalin was used to store the tissue until the histopathological section. Paraffin-embedded tissue blocks were sectioned (4-5 microns) and stained with hematoxylin and eosin for gross histopathological observations [33].

Statistical analysis

The results were displayed as mean ± SEM and statistically analyzed by one-way analysis of variance (ANOVA) by using GraphPad Prism software, version 5.03

3. Results

Phytochemical analysis

The results showed that a highly viscous blackish color of MEAC (18% w/w extractive yield) contains a variety of phytochemicals (Table 1) including alkaloids, glycosides, and flavonoids, which have a variety of pharmacological activity. The GC-MS chemical profiling and spectra of MEAC are presented the Figure 1 and Figure 2.

Table 1. Phytochemical analysis of MEAC

SN.	Phytochemical	Name of test	Result
1.	Alkaloid	Brontrager test	+
2.	Glycoside	Modified Brontrager's test	-
		Legal's test	+
3.	Tannins	Ferric chloride test	+
4.	Flavonoids	Alkaline test	+
		Lead acetate test	+
5.	Carbohydrates	Molisch test	+
		Felhling test	-
6.	Steroids	Salkowaski test	-
		Liebermann Burchard's Test	+
7.	Protein	Biuret test	+
		Ninhydrin test	+

(+) indicates presence and (-) sign indicates absence of compound

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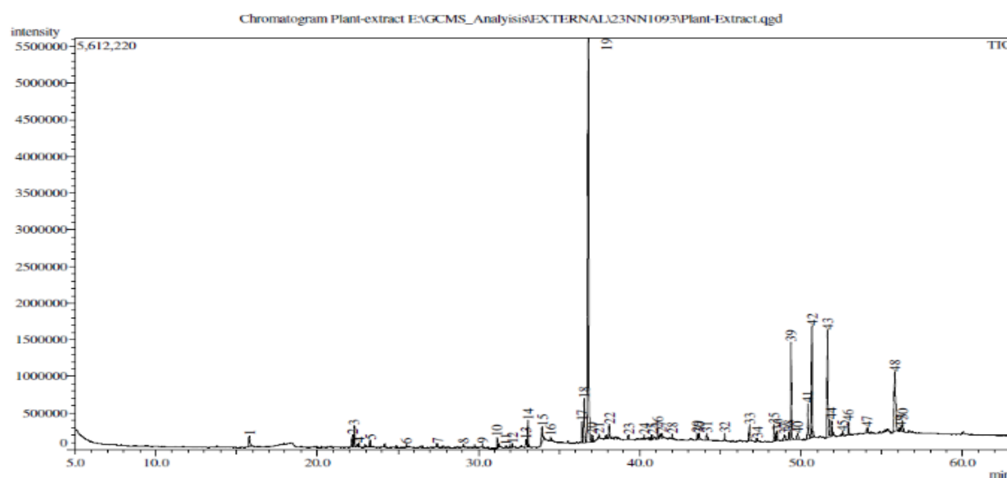

Figure 1. GC–MS spectrum of Methanolic extract of *Amaranthus cruentus*

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	15.803	15.725	15.883	598367	0.95	140274	0.84	4.27		1-Dimethyl(isopropyl)silyloxypropane
2	22.171	22.108	22.225	499330	0.79	155529	0.94	3.21		(3E,5E)-2,6-Dimethylocta-3,5,7-trien-
3	22.293	22.225	22.383	995020	1.58	287607	1.73	3.46	V	3-Buten-2-one, 4-(3-hydroxy-6,6-dim
4	22.585	22.533	22.642	168047	0.27	57855	0.35	2.90		4-(2,6,6-Trimethylcyclohexa-1,3-dien
5	23.270	23.217	23.333	286041	0.46	95044	0.57	3.01		Ethanone, 1-(1,3-dimethyl-3-cyclohex
6	25.524	25.475	25.575	121540	0.19	44311	0.27	2.74		3-Octadecene, (E)-
7	27.432	27.383	27.508	168154	0.27	47332	0.28	3.55		8-Pentadecanone
8	29.066	29.017	29.142	151621	0.24	44189	0.27	3.43		6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-t
9	30.227	30.183	30.292	148494	0.24	53857	0.32	2.76		1-Tricosene
10	31.173	31.117	31.233	413947	0.66	141057	0.85	2.93		Neophytadiene
11	31.692	31.667	31.933	50439	0.08	22403	0.13	2.25		9-Eicosyne
12	32.096	32.050	32.150	142599	0.23	50367	0.30	2.83		3,7,11,15-Tetramethyl-2-hexadecen-1
13	32.934	32.875	32.975	359755	0.57	109354	0.66	3.29		7-Hexadecenoic acid, methyl ester, (Z
14	33.051	32.975	33.117	1301528	2.07	377793	2.27	3.45	V	Hexadecanoic acid, methyl ester
15	33.953	33.792	34.075	1476164	2.35	217684	1.31	6.78		n-Hexadecanoic acid
16	34.481	34.433	34.558	156494	0.25	51186	0.31	3.06		1-Octadecanol
17	36.424	36.358	36.475	854115	1.36	284641	1.71	3.00		9,12-Octadecadienoic acid (Z,Z)-, me
18	36.541	36.475	36.633	2096843	3.34	601756	3.62	3.48	V	9,12,15-Octadecatrienoic acid, methy
19	36.803	36.633	36.942	20562908	32.72	5506956	33.11	3.73	V	Phytol
20	37.066	36.942	37.125	361287	0.57	90820	0.55	3.98	V	Methyl stearate
21	37.463	37.417	37.550	155985	0.25	47966	0.29	3.25		Ethyl 2-(2-(2-butoxyethoxy)ethoxy)ac
22	38.113	37.942	38.183	560496	0.89	185690	1.12	3.02	V	Hexadecanamide
23	39.289	39.242	39.350	168384	0.27	58699	0.35	2.87		Tributyl acetylcitrate
24	40.275	40.225	40.325	165318	0.26	61119	0.37	2.70		Octacosane
25	40.740	40.683	40.783	195422	0.31	56076	0.34	3.48		13,16-Octadecadiynoic acid, methyl e
26	41.127	41.075	41.183	412857	0.66	139947	0.84	2.95		4,8,12,16-Tetramethylheptadecan-4-o
27	41.356	41.300	41.475	347333	0.55	67259	0.40	5.16	V	8-Methyl-6-nonenamide
28	42.007	41.958	42.067	168507	0.27	62738	0.38	2.69		Heneicosane
29	43.564	43.500	43.625	358200	0.57	88928	0.53	4.03		Behenic alcohol
30	43.672	43.625	43.725	254238	0.40	85353	0.51	2.98	V	Heneicosane
31	44.175	44.083	44.258	426405	0.68	94700	0.57	4.50		Bis(2-ethylhexyl) phthalate
32	45.273	45.225	45.325	245487	0.39	89570	0.54	2.74		Nonacosane
33	46.787	46.717	46.908	954504	1.52	223538	1.34	4.27		1-Heptacosanol
34	47.265	47.217	47.325	130089	0.21	42013	0.25	3.10		Heptacosanoic acid, methyl ester
35	48.331	48.258	48.425	909284	1.45	230179	1.38	3.95		10-Hydroxy-1,6-dimethyl-9-(propan-2
36	48.510	48.425	48.575	459045	0.73	134010	0.81	3.43	V	Squalene
37	48.960	48.892	49.058	383574	0.61	72186	0.43	5.31		8-Hexadecene, 8,9-diheptyl-
38	49.253	49.058	49.300	527735	0.84	99370	0.60	5.31	V	1-Triacontanol
39	49.378	49.300	49.517	4700038	7.48	1331222	8.00	3.53	V	.gamma.-Tocopherol
40	49.770	49.708	49.850	308505	0.49	79110	0.48	3.90		Tetracosyl pentafluoropropionate
41	50.416	50.325	50.567	2072822	3.30	482777	2.90	4.29		.delta.-Tocopherol
42	50.654	50.567	50.725	5261047	8.37	1527147	9.18	3.45	V	dl-.alpha.-Tocopherol
43	51.643	51.550	51.800	5252677	8.36	1470999	8.84	3.57		(R)-6-Methoxy-2,5,7,8-tetramethyl-2-
44	51.878	51.800	51.950	952245	1.52	219938	1.32	4.33	V	.gamma.-Tocopherol
45	52.567	52.517	52.642	144826	0.23	47158	0.28	3.07		Androstane-17-carboxylic acid, 3,11-l
46	52.932	52.867	53.042	633150	1.01	175331	1.05	3.61		Vitamin E
47	54.105	54.017	54.225	400373	0.64	64154	0.39	6.24		Ergosterol
48	55.807	55.692	55.967	4414875	7.02	813311	4.89	5.43		Chondrillasterol
49	56.152	56.092	56.208	227213	0.36	53736	0.32	4.23		.beta.-Amyrone
50	56.286	56.208	56.408	750511	1.19	148985	0.90	5.04	V	7,22-Ergostadienone
				62853838	100.00	16633224	100.00			

Figure 2. GC–MS spectral analysis of Methanolic extract of *Amaranthus cruentus*

Effect of MEAC on ALT, AST, and ALP level

The effects of MEAC on the level of ALT, AST, and ALP are presented in Table 2. The serum hepatic enzymes are well-known biomarkers to identify hepatocellular damage. The overdose of paracetamol in the rats of the paracetamol control group led to considerable hepatocellular damage,

which was indicated by the elevated serum hepatic enzymes (ALT, AST, and ALP). Group-II rats showed increase in blood serum ALT (256 ± 5.37 IU/L, $P < 0.05$), AST (265.3 ± 24.00 IU/L, $P < 0.05$) and ALP (119 ± 1.38 IU/L, $P < 0.05$) compared to group-I animals of ALT 32.5 ± 5.92 IU/L, AST 39.50 ± 5.42 IU/L and ALP 56.8 ± 2.15 IU/L in that order. The treatments of MEAC in group IV and group V significantly reduced the levels of ALT to (69.2 ± 3.70 IU/L, $P < 0.05$ and 55.2 ± 4.14 IU/L, $P < 0.05$), AST to (59.8 ± 4.71 IU/L, $P < 0.05$ and 55.5 ± 4.14 IU/L, $P < 0.05$), and ALP to (72.8 ± 4.16 IU/L, $P < 0.5$ and 71.3 ± 9.27 IU/L, $P < 0.05$), compared to group-II animals. Silymarin treatments in groupIII rats significantly ($P < 0.05$) decreased the ALT, AST, and ALP levels to 41.70

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± 4.68 IU/L, 48.33 ± 3.72 IU/L, and 59.00 ± 2.10 IU/L in that order compared to rats in the groupII.

Table 2: Effect of MEAC on ALT, AST, and ALP level

Group No	Groups	Liver Function Markers		
		ALT(IU/L)	AST(IU/L)	ALP(IU/L)
G-I	Normal Control	32.5 ± 5.92	39.50 ±5.42	56.8 ± 2.15
G-II	Paracetamol Control	256 ± 5.37#	265 ±24.00#	119 ± 1.38#
G-III	Silymarin	41.7 ± 4.68*	48.33 ±3.72*	59.0 ± 2.10*
G-IV	MEAC-200	69.2 ± 3.7*	59.8 ± 4.71#	72.8 ± 4.16*
G-V	MEAC-400	55.3 ± 4.14*#	54.7 ± 4.32*	71.3 ± 9.26*

Each value is expressed as the mean ± SEM (n=6) in each group and was estimated using one-way ANOVA followed by post hoc Tukey multiple comparison test.

* Significantly different from group I at $p<0.05$.

Significantly different from group II at $p<0.05$

Effect of MEAC on total bilirubin and total protein level

The effects of MEAC on the level of total bilirubin and total protein are presented in Table 3.Group-II rats displayed a significant ($P < 0.05$) increase in serum total bilirubin to 1.18 ± 0.06 mg/dl while, a decrease in total protein to 6.95 ± 0.18 g/dl compared to group-I rats of total bilirubin 0.367 ± 0.03 mg/dl and total protein 9.14 ± 0.18 g/dl. The treatments of MEAC in the groups IV and group V rats significantly decreased the levels of total bilirubin (0.450 ± 0.04 mg/dl, $P < 0.05$ and 0.333 ± 0.04 mg/dl, $P < 0.05$) while, increasing total protein level (7.67 ± 0.4 g/dl, $P < 0.05$ and 7.43 ± 0.25 g/dl, $P < 0.05$) respectively as compared to Group-II animals. Silymarin treatment in group-III rats significantly ($P < 0.05$) decreased the total bilirubin levels to 0.483 ± 0.03 mg/dl while, increasing the total protein level to 7.67 ± 0.33 g/dl compared to rats in groupII.

Table 3.Effect of MEAC on TB and TP level

Group No	Groups	Liver Function Markers	
		Total Bilirubin (mg/dl)	Total Protein (g/dl)
G-I	Normal Control	0.367 ± 0.03	9.14 ± 0.185
G-II	Paracetamol Control	1.18 ± 0.06 01#	6.95 ± 0.18 #
G-III	Silymarin	0.483 ± 0.03 #*	7.67 ± 0.33#*
G-IV	MEAC-200	0.450 ± 0.04 #*	7.67 ± 0.4#*
G-V	MEAC-400	0.333 ± 0.04 #*	7.43 ± 0.25#*

Each value is expressed as the mean ± SEM (n=6) in each group and was estimated using one-way ANOVA followed by post hoc Tukey multiple comparison test.

* Significantly different from group I at $p < 0.05$.

Significantly different from group II at $p < 0.05$

Effects of MEAC in SOD, GSH, GPx, and MDA level

The effects of MEAC on the level of SOD, GSH, and MDA are presented in Table4. Group-II rats showed a decrease in SOD (197 ± 6.82 U/mg, $P < 0.056$) and GSH (0.571 ± 0.018 g/mg, $P < 0.05$) compared to group-I animals of SOD 309 ± 5.64 U/mg and GSH 0.897 ± 0.022 g/mg in that order. The treatments of MEAC in groups IV and group V significantly increased the levels of SOD (246 ± 6.43 U/mg, $P < 0.05$ and 251 ± 14.5 U/mg, $P < 0.05$) and GSH (0.732 ± 0.031 g/mg, $P < 0.05$ and 0.691 ± 0.032 g/mg, $P < 0.05$) respectively compared to group-II animals. Silymarin

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treatments in group III significantly ($P < 0.05$) increased the SOD and GSH levels to 277 ± 9.01 U/mg and 0.858 ± 0.028 g/mg respectively compared to rats in groupII. There are no significant changes in the level of GPx among groups.

Group-II rats displayed a significant ($P < 0.05$) increase in MDA to 3.47 ± 0.267 nmol/mg proteins compared to group-I rats of MDA 0.668 ± 0.246 nmol/mg protein. The treatments of MEAC in Groups IV and Group V significantly decreased the levels of MDA to (2.50 ± 0.419 nmol/mg protein, $P < 0.05$ and 2.07 ± 0.497 nmol/mg protein, $P < 0.05$) respectively as compared to Group-II animals. Silymarin treatments in group III rats significantly ($P < 0.05$) decreased the MDA levels to 0.801 ± 0.207 nmol/mg proteins compared to rats in group II.

Table 4. Effect of MEAC on SOD, GSH, and GPx level

		Oxidative Stress Biomarkers		
Group No	**Groups**	**SOD (µg /mg protein)**	**GSH (µg/mg protein)**	**MDA (nmol/mg)**
G-I	Normal Control	309 ± 5.64	0.897± 0.022	0.668 ± 0.246
G-II	Paracetamol Control	197 ± 6.82$^{\#}$	0.571 ± 0.018$^{\#}$	3.47 ± 0.267$^{\#}$
G-III	Silymarin	277 ± 9.01$^{\#*}$	0.858 ± 0.028*	0.801 ±0.207*
G-IV	MEAC-200	246 ± 6.53$^{\#*}$	0.732± 0.031$^{\#*}$	2.50 ± 0.419$^{\#*}$
G-V	MEAC-400	251 ± 14.5$^{\#*}$	0.691 ± 0.032$^{\#*}$	2.07 ± 0.497$^{\#*}$

Each value is expressed as the mean ± SEM (n=6) in each group and was estimated using one-way ANOVA followed by post hoc Tukey multiple comparison test.

* Significantly different from group I at $p < 0.05$.

$^{\#}$ Significantly different from group II at $p < 0.05$

Histopathology of Liver

Histopathological observations (Figure 3) support the evidence of the result of biochemical analysis. The normal control group showed a well-organized cellular structure into hexagonal lobules, with portal triads at the vertices and a central vein in the middle. In the tissue of the normal group, there was no signs of inflammation, necrosis fibrosis, or toxic changes were observed. The paracetamol control group displayed hepatocyte vacuolization, minor sinusoidal dilation, mild portal tract inflammation, and narcosis. The silymarin, MEAC-200, and MEAC-400 mg/kg groups showed considerable recovery of the hepatocyte from narcosis, indicating the pretreatment with extract protects the cellular integrity from paracetamol-induced toxicity.

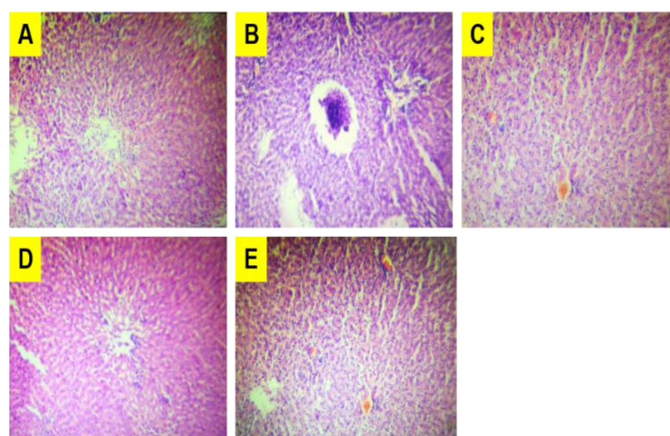

Figure 3. Histopathology of the liver (A) Normal Control, (B) Paracetamol control, (C) Silymarin, (D) MEAC-200, and (E) MEAC-400.

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Discussion

Plant medicines play a crucial role in treating various diseases through their diverse formulations. Some have been analyzed and scientifically validated for their potential. In this study, we designed experiments to investigate the hepatoprotective activity of methanolic extracts from *Amaranthus cruentus* leaves, aiming to develop them into safe natural drug candidates. Paracetamol is a commonly utilized antipyretic and pain relieving sedate for treating fever, migraines, and other torments, and is accessible over the counter[34]. But in toxic quantities, it turns into a strong hepatotoxin that causes fulminant hepatic and renal tubular necrosis, which can kill both experimental animals and people. Paracetamol toxic metabolite (NAPQI) leads to oxidative stress and cellular damage in the liver, this happens due to the accumulation of ROS, which damages hepatocyte membranes, induces lipid peroxidation, and causes liver necrosis[35]. Serum hepatic biomarkers like ALT and AST are commonly used as indicators of hepatotoxicity and cellular damage. These enzymes play an important role in the reductive transfer of amino group form from alanine or aspartate to alpha-ketoglutarate, resulting in pyruvate or oxaloacetate[36]. Damaged hepatic tissues release these enzymes into extracellular space. Which can be observed as biomarkers. ALT is also present in other tissues including the heart, brain, and muscles, but it appears in higher amounts in the liver than in the other organs. Besides this, AST is almost similar to other organs so AST has lower specificity for the liver[37]. ALP is another important enzyme, it is a hydrolysable enzyme and also elevated in hepatobiliary damage. As displayed in the paracetamol control group, hepatotoxicity can result in biliary congestion, which impairs the body's ability to excrete ALP and elevated levels were observed[35]. The serum bilirubin concentration are well-known indices for determining the liver's secretary and synthetic functions and can be used to identify types of liver damage[38]. The reduction in total protein levels can serve as a useful indicator of cell dysfunction severity in chronic liver disease. Stimulating protein synthesis is considered a liver-protective mechanism that promotes hepatic cell formation. Excessive production of ROS leads to damage of cellular proteins, lipids, and nucleic acid, resulting in elevation in lipid peroxidation (MDA level) and depletion of antioxidant enzymes (SOD, GSH, GPx), resulting in oxidative stress.

The results showed, that MEAC significantly reduced AST, ALT, ALP, and total bilirubin levels and increased total protein levels when compared to the paracetamol control group. The restoration of serum hepato-biomarkers indicates its potential hepatoprotective effects and increase in total protein levels suggesting that MEAC stimulates biosynthesis and accelerated hepatocellular generation[39]. Moreover, as expected, the result shows that the paracetamol group showed significant induction of oxidative stress, which is indicated by a decrease in hepatic GSH and SOD levels and an increase in MDA level when compared to the normal group. However, pretreatment with MEAC before paracetamol administration significantly enhanced hepatic GSH and SOD levels and decreased MDA levels compared to the paracetamol control group, indicating its potential antioxidant activity[40]. The results also showed that hepatoprotective activity of MEAC at 200 mg/kg was similar to standard silymarin. This suggests that the inhibition of increased liver injury and normalization of liver function test (LFT) markers might contribute to the protective effect of MEAC against paracetamol-induced liver damage. Flavonoids and phenolics, known for their ability to scavenge free radicals and reduce their formation, are reported bioactive compounds. The hepatoprotective effect of MEAC might be due to the antioxidant activity of its phyto-constituents, which reduced oxidative stress induced by paracetamol and other similar analgesic and anti-inflammatory agents, thereby preventing inflammatory liver damage.

Conclusion

The plant for this study was selected based on a literature review collected from Koni, Bilaspur, Chhattisgarh. The leaves were ground into a powder and extracted using methanol. Phytochemical analysis of the methanolic extract of *Amaranthus cruentus* (MEAC) revealed alkaloids, carbohydrates, flavonoids, and phenolic compounds. Doses of MEAC were chosen based on previous research.To evaluate the hepatoprotective effects of MEAC against paracetamol-induced hepatotoxicity in rats, AVME and silymarin were administered orally for 14 days. Blood and liver samples were collected on the fourteenth day, and liver

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function and oxidative stress markers were measured. Statistical analysis showed significant hepatoprotective effects, with MEAC restoring liver function and oxidative stress markers. Histological analysis confirmed the attenuation of liver damage. Further studies are needed to elucidate the exact mechanisms and establish MEAC's therapeutic role as a hepatoprotective agent.

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