

PROTECTIVE ROLE OF *AERVA MONSONIAE* AND SELENIUM ON CADMIUM- INDUCED OXIDATIVE LIVER DAMAGE IN RATS

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ABSTRACT

Objective: The present study was aimed to investigate the protective effect of methanolic extract of the whole plant of *Aerva monsoniae* (MEAM) and selenium on cadmium (Cd)-induced oxidative liver damage in experimental rats.

Methods: In the present study, albino Wistar rats were treated with Cd (5 mg/kg), selenium (1 mg/kg), and MEAM (250 and 500 mg/kg) for 21 days. After 21 days of the treatment, the rats were sacrificed, and blood was collected for estimation of biochemical parameters and liver was used for histopathological studies.

Results: Oral administration of Cd significantly elevated the levels of hepatic markers such as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ -glutamyl transferase, cholesterol, total bilirubin, direct bilirubin, and decreased levels of total proteins and albumin. We also observed that elevated oxidative stress markers such as malondialdehyde reduced the enzymatic antioxidants such as superoxide dismutase, catalase, and non-enzymatic antioxidants such as reduced glutathione in the liver. Co-administration of MEAM and selenium in Cd-intoxicated rats, the altered biochemical parameters, and pathological changes were recovered significantly ($p > 0.01$) than the individual effects of MEAM and selenium.

Conclusion: From the above findings, it was concluded that combination of MEAM and selenium exhibited remarkable protective effects against Cd-induced oxidative liver damage in rats.

Keywords: Cadmium, Selenium, *Aerva monsoniae*, Oxidative damage, enzymatic antioxidants.

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INTRODUCTION

The occurrence of liver intoxication has increased in current years due to exposure to high levels of environmental toxicants such as pesticides, industrial chemicals, fertilizers, and heavy metals, which constitute a serious threat to human health [1]. Cadmium (Cd) is one of the most toxic and non-essential heavy metals. It is present in air, water, and soil. It is used in electrical industries in the manufacture of batteries, electroplating plastic, and also in the preparation of pigments and fertilizers. Humans get exposed to Cd primarily through the air pollution, smoking, and consumption of Cd-contaminated food and water [2,3]. Cd has a very long half-life in the body (10–30 years) and can build up over a long time [4]. Acute exposure to Cd causes dysuria, polyuria, chest pain, fatigue, and headache. Chronic intake of Cd produces organ dysfunction, resulting in cell death, pulmonary, hepatic, and renal tubular diseases. Liver is the most important target organ when considering Cd-induced toxicity because Cd primarily accumulates in the liver. Lipid peroxidation (LPO) is considered as the primary mechanism for Cd-induced toxicity as it is involved in the generation of free radicals [5]. Cd exerts its toxic effects through induction of oxidative stress by generating reactive oxygen species (ROS) and disturbing the antioxidant defense system [6].

By understanding the mechanism of oxidative stress on exposure to Cd, we consider that administration of antioxidants should be an important therapeutic approach in Cd intoxication. Selenium is a trace element that is essential in small amounts but can be toxic at the large amount. Humans and animals require selenium for the function of a number of selenium-dependent enzymes, also known as selenoproteins. Selenium maximizes the activity of antioxidant selenoenzymes such as selenium-containing glutathione peroxidases which are antioxidant enzymes that reduce potentially damaging ROS [7]. Selenium shows the beneficial effect of Cd-induced oxidative stress and hepatotoxicity [8].

Aerva monsoniae (L.f.) bennet (Amaranthaceae) is a perennial herb. It is found all over India. Traditionally, the whole plant of *A. monsoniae* is used in the management of diabetes, urinary tract infections, wounds, sore throat, and also used as diuretic and hepatoprotective. *A. monsoniae* has been reported for analgesic, anti-inflammatory, and antibacterial activities [9], an antidiabetic activity [10], and the methanolic extract of whole plant of *A. monsoniae* (MEAM) was reported to contain three known flavonoidal C-glycosides i.e., isoswertisin, 2-O- β -Dgalactosyl isoswertisin, and 2-O- β -D-xylosyl isoswertisin [11].

According to the literature survey, the MEAM has not been evaluated for its protective effect against Cd-induced oxidative damage in rats, which drive us to take up the present study.

METHODS

Chemicals

Cd (CdCl_2), selenium (Na_2SeO_3), and thiobarbituric acid were purchased from HiMedia, Mumbai, India. The drugs and chemicals were purchased from various companies and the details are as follows: Cd (CdCl_2), selenium (Na_2SeO_3), and thiobarbituric acid - HiMedia, Mumbai, India; Silymarin, reduced glutathione (GSH) -Sigma-Aldrich, Spruce Street, St. Louis, China; Biochemical analytical kits and trichloroacetic acid - merck specialities Pvt., Ltd., Mumbai, India; 1,1,3,3-tetraethoxypropane Deoxyribose, Griess reagent, H_2O_2 , nitro blue tetrazolium (NBT), phenazine methosulphate (PMS), nicotina-mide adenine dinucleotide (NADH), phosphate buffer saline (PBS), ethylene amine adenine dinucleotide phosphate (EDTA) - Sigma, Germany; all other chemicals and solvents used were of analytical grade.

Collection of plant material and authentication

Whole plant of *A. monsoniae* was collected in and around Kakatiya University Campus, Warangal, Andhra Pradesh, in the month of

November 2010. The plant material was authenticated by Dr. V.S. Raju (taxonomist), Department of Botany, Kakatiya University, Warangal. The voucher specimen (KU/UCPSC/27/2010) of this plant material has been retained in the Department of Pharmacognosy and Phytochemistry for future reference.

Preparation of plant extract

The whole plant was collected, washed under running water, dried under shade, and then ground into coarse powder for the maceration process with methanol at room temperature. After exhaustive extraction, the methanolic extract was concentrated under reduced pressure to yield brownish green-colored mass. It was coded as MEAM.

Animals

Male Wistar albino rats weighing between 150 and 200 g were purchased from Sainath Agencies, Hyderabad, India, with a prior permission from our Institutional Animal Ethical Committee (IAEC 32 UCPSC 2016) and used for the studies. The animals were housed in standard polypropylene cages and maintained under standard laboratory conditions (12:12 h light and dark cycle; at an ambient temperature of 25±50°C; 35–60% of relative humidity). The animals were fed with standard rat pellet diet and water *ad libitum*.

Acute oral toxicity studies

Acute oral toxicity study was conducted according to OECD guidelines for the testing of chemicals, test No. 423 (OECD 2001; acute oral toxicity-acute toxic class method). Wistar albino rats (n=6) were used for the acute toxicity study. The animals were kept overnight with access to water but not food, after which the whole plant of *A. monsoniae* methanolic extract was administered orally at dose levels 5, 50, 300, and 2000 mg/kg body weight and the animals were observed for 24 h. Further, they were observed continuously for the first 2 h for morbidity and up to 24 h for mortality.

Cadmium induced hepatotoxicity

Male Wistar albino rats were randomly divided into nine groups (n=6 in each group). A control group of animals received normal water and remaining eight experimental groups received Cd (5 mg/L) in their drinking water for 21 days. The details of the treatment given to these groups are as follows:

- Group I: Served as control group, received only 1 ml/kg b.w. of 2% gum acacia in water daily for 21 days.
- Group II: Served as a toxic group, received Cd-polluted water for 21 days.
- Group III (standard group): Treated with silymarin (100 mg/kg b.w.) + selenium (Se as Na₂SeO₃, 1 mg/kg b.w.) for 21 days followed by cadmium.
- Group IV: Treated with silymarin (100 mg/kg b.w.) for 21 days followed by cadmium.
- Group V: Treated with selenium (1 mg/kg b.w.) for 21 days followed by cadmium.
- Group VI (MEAM-250): Treated with MEAM (250 mg/kg b.w.) for 21 days followed by cadmium.
- Group VII (MEAM 500): Treated with MEAM (500 mg/kg b.w.) for 21 days followed by cadmium.
- Group VIII (MEAM-250+Se): Treated with MEAM (250 mg/kg b.w.) + selenium (1 mg/kg b.w.) for 21 days followed by cadmium.
- Group IX (MEAM 500+Se): Treated with MEAM (500 mg/kg b.w.) + selenium (1 mg/kg b.w.) for 21 days followed by cadmium.

After 21 days of the treatment, blood was collected by puncturing the retro-orbital plexus of the rats of all groups under ether anesthesia and serum was separated by centrifugation. Further serum was analyzed for various biochemical parameters. The liver was dissected out used for histopathological studies and analysis of antioxidant parameters.

Assessment of hepatoprotective activity

The collected blood samples were allowed to stand for 30 min at room temperature and then centrifuged (Remi, model: R8-C, India)

at 3000 rpm for 30 min to separate the serum. The serum was used for estimation of various biochemical parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ -glutamyl transferase (GGT), cholesterol (CHOL), total bilirubin (TB), direct bilirubin (DB), total proteins (TP) and albumin (ALB) by using test kits.

Assessment of antioxidant parameters

The liver tissue was dissected out, weighed, and washed using chilled saline solution. Tissue was minced and homogenized (10%, w/v) in 1.15% KCl and centrifuged (4000 rpm for 5 min). The resulting clear supernatant was used for estimation of LPO [12], reduced GSH [13], superoxide dismutase (SOD) [14], and activity of catalase (CAT) [15].

Histopathological studies

The animals were then dissected and the livers were carefully removed and washed with 0.9% saline solution. The liver tissue was kept in 10% formalin and embedded in paraffin wax. 5 μ m slices were stained with hematoxylin and eosin (H and E) and photographs were taken.

Statistical analysis

All the values were expressed as a mean \pm standard deviation. The data were statistically evaluated using one-way analysis of variance (ANOVA) followed by Dunnett's t-multiple comparison test using GraphPad Prism 3 computer software. p=0.05 or less was considered to be significant.

RESULTS

Acute oral toxicity studies

The MEAM did not cause any adverse effects and mortality up to a dose level of 2000 mg/kg b.w.p.o. and were considered as safe. Hence, two doses of MEAM (i.e., 250 and 500 mg/kg b.w.) were selected for the present study.

Effect of MEAM and selenium on serum hepatic marker enzymes

Table 1 shows the serum AST, ALT, ALP, and GGT levels of control and experimental animals. Toxic group (Cd-administered group) showed significant (p<0.01) increases in the levels of AST, ALT, ALP, and GGT compared to normal Group 1. Upon supplementation with MEAM alone and combination with selenium reduce the elevated levels of serum hepatic marker enzymes. Among all the test groups, co-administration of MEAM at 250 mg/kg and selenium significantly (p<0.01) prevented the elevated levels of serum hepatic marker enzymes.

Effect of MEAM and selenium on serum CHOL, bilirubin, TP and ALB

Table 2 shows the levels of serum CHOL, TB, DB, TP, and ALB. Cd-polluted water-administered rats showed significant (p<0.01) increase in serum CHOL, TB, and DB levels and decrease in TP and ALB levels compared to normal (Group 1). Upon supplementation with MEAM alone and combination with selenium reduce the elevated levels of CHOL, TB, and DB and increases the decreased levels of TP and ALB. Among all the test groups, co-administration of MEAM at 250 mg/kg and selenium significantly (p<0.01) prevented the elevated levels CHOL, TB, and DB and decreased levels of TP and ALB.

Effect of MEAM and selenium on serum oxidative stress parameters

Oral administration of cadmium caused increased levels of malondialdehyde (MDA) and decreased levels of GSH, SOD, and CAT which are reversed upon administration of either selenium or MEAM or both, the better reversal effects were observed in co-administration of selenium (1 mg/kg b.w.), and MEAM at the dose of 250 mg/kg which was comparable with combination of silymarin and selenium. The results were presented in Table 3.

Histopathology

Histopathological investigations showed that the administration of Cd in rats produced severe hepatic damage including dilatation in sinusoidal spaces, degeneration and deformation of hepatocytes, centrilobular

Table 1: Effect of MEAM and selenium on serum hepatic marker enzymes in Cd-induced hepatotoxicity

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	GGT (IU/L)
I	46.517±3.974	65.467±3.974	455.12±26.16	5.567±0.233
II	186.8±3.9**	226.02±4.510**	1227.9±5.17**	46.900±1.88**
III	74.7±3.33** (80.07%)	76.117±2.666** (93.36%)	556.82±27.24** (86.8%)	12.33±0.507** (83%)
IV	80.1±7.054** (76.09%)	80.950±3.321** (90%)	589.0±15.52** (82.6%)	13.417±0.231** (81%)
V	95.6±5.66** (65.1%)	92.83±1.821** (82.9%)	568.63±22.90** (69%)	16.6±0.116** (73%)
VI	100.33±3.143** (61.6%)	98.556±2.94** (79.3%)	736.72±41.01** (63.5%)	16.95±0.187** (72%)
VII	110.35±4.456** (54.5%)	104.42±8.533** (75.7%)	833.83±24.31** (50.9%)	17.400±0.141** (71%)
VIII	83.886±3.023** (73.3%)	82.650±3.011** (89.4%)	620±26.00** (78.6%)	14.533±0.163** (78%)
IX	90.10±2.836** (68.97%)	88.57±9.001** (85.66%)	747.62±29.01** (71.2%)	15.36±0.160** (76.2%)

n=6, data expressed as mean±SD, values in parenthesis indicate percentage recovery. P value- control versus other groups, **p<0.01, *p<0.05. MEAM: Methanolic extract of whole plant of *Aevra monsoniae*, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, GGT: γ-Glutamyl transferase, SD: Standard deviation

Table 2: Effect of MEAM and selenium on serum CHOL, bilirubin, TP, and ALB in Cd-induced hepatotoxicity in rats

Groups	CHOL (mg/dl)	TB (mg/dl)	DB (mg/dl)	ALB (g/dl)	TP (g/dl)
I	42.05±6.8	0.0600±0.008	0.0216±0.007	4.59±0.087	8.517±0.17
II	95.53±8.1**	2.90±0.209**	2.1±0.187**	2.53±0.23**	5.233±0.17**
III	52.42±1.3** (80%)	0.51±0.040** (84%)	0.27±0.018** (88%)	4.183±0.085** (80%)	8.033±0.19** (85%)
IV	58.16±2.4** (69%)	0.58±0.024** (81%)	0.29±0.023** (87%)	3.98±0.089** (70%)	7.667±0.12** (75%)
V	78.35±2.6** (32.1%)	0.89±0.034** (70%)	0.41±0.016** (81%)	3.382±0.072** (58%)	6.76±0.24** (46%)
VI	79.16±8.6** (30.6%)	0.92±0.251** (69%)	0.43±0.012** (80%)	3.223±0.096** (33%)	6.43±0.27** (36%)
VII	82.31±8.6** (24.7%)	1.23±0.234** (58%)	0.48±0.02** (77%)	3.132±0.083** (29%)	6.233±0.10** (30%)
VIII	62.31±1.1** (61%)	0.68±0.014** (78%)	0.33±0.018** (85%)	3.887±0.2753** (65%)	7.485±0.11** (68%)
IX	72.36±1.5** (43.3%)	0.72±0.231** (76%)	0.38±0.014** (82%)	3.532±0.833** (63%)	7.31±0.17** (63%)

n=6, data expressed as mean±SD, values in parenthesis indicate percentage recovery. P value-control versus other groups, **p<0.01, *p<0.05. DB: Direct bilirubin, CHOL: Cholesterol, ALB: Albumin, TP: Total protein, TB: Total bilirubin, MEAM: Methanolic extract of whole plant of *Aevra monsoniae*, SD: Standard deviation

Table 3: Effect of MEAM and selenium on oxidative stress parameter

Groups	GSH (nM/mg)	MDA (mM/mg)	CAT (U/mg)	SOD (U/mg)
I	5.59±0.5	1.64±0.1	12.48±1.2	14.28±1.2
II	1.51±0.7**	4.21±0.2**	2.64±1.3**	3.13±1.4**
III	4.99±0.9** (85%)	1.88±0.15** (90%)	10.4±1.1** (78%)	12.32±2.1** (82%)
IV	4.65±0.8** (76%)	2.05±0.13** (84%)	9.3±1.4** (67%)	10.43±0.9** (65%)
V	3.6±0.6** (52%)	2.71±0.4** (58%)	6.34±1.5** (37%)	7.83±1.1** (42%)
VI	3.46±0.9** (47%)	2.88±0.3** (51%)	5.83±1.2** (32%)	6.52±1.5** (30%)
VII	3.21±0.8** (41%)	3.01±0.6** (46%)	4.82±1.6** (22%)	5.21±1.6** (18%)
VIII	4.49±0.4** (75%)	2.30±0.1** (74%)	8.37±1.4** (58%)	9.81±1.3** (59%)
IX	4.34±0.5** (69%)	2.40±0.3** (70%)	7.86±1.3** (53%)	8.14±0.8** (44%)

n=6, data expressed as mean±SD, values in parenthesis indicate percentage recovery. P value- control versus other groups, **p<0.01, *p<0.05. GSH: Glutathione, MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, MEAM: Methanolic extract of whole plant of *Aevra monsoniae*, SD: Standard deviation

necrosis, and inflammatory infiltration of hepatocytes (Fig. 1b) when compared with control rats (Fig. 1a). The above changes were reduced

in all treated groups. Among all the groups, the histological pattern was almost normal in rats treated with MEAM at 250 mg/kg with selenium

(Fig. 1h) and it was well comparable with standard drug silymarin with selenium.

DISCUSSION

Environmental contamination by cadmium is recognized as a global problem. Cd is a toxic metal, promotes an early oxidative stress, and contributes to the development of serious pathological conditions because of its long retention in some tissues. In this context, the present study also confirmed that the administration of *A. monsoniae* (250 mg/kg) significantly preserved the hepatic functions against the toxic effects exerted by cadmium.

Liver dysfunction was accompanied by elevated levels of serum hepatic marker enzymes (AST, ALT, ALP, and GGT), CHOL, TB, and DB and decreased levels of ALB and TP. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver. High levels of AST and ALT are the crucial parameters to detect the liver damage [16]. The membrane-bound enzyme ALP and bilirubin levels are related to the status and function of hepatic cells. Increased serum ALP is due to increased synthesis in the presence of increasing biliary pressure [17]. GGT is a microsomal brush border enzyme found notably in the liver. It is involved in the transfer of amino acids across the cellular membrane. It is also involved in GSH metabolism by transferring the glutamyl moiety to a variety of acceptors leaving the cysteine product to preserve the intracellular homeostasis of oxidative stress [18]. Serum GGT level is considered as a better index and to be highly sensitive to biliary tract damage [19]. HMG CoA reductase is the rate-limiting enzyme in CHOL biosynthesis. The activity of this enzyme might have been stimulated by cadmium, leading to the increased CHOL levels observed in serum [20]. The serum levels of TB and DB in the present study have also been found to be increased in cadmium-intoxicated animals. TB and DB levels in serum are a clear marker of hepatic dysfunction due to obstructions in bile ducts. The decreased level of ALB and TP in serum is due to decreased synthetic capacity of the liver [21].

Administration of *A. monsoniae* (250 mg/kg) alone and in combination with selenium (1 mg/kg)-attenuated cadmium-induced hepatotoxicity as shown by the decreased levels of the serum AST, ALT, ALP, GGT, TBL, DBL, and CHOL and increased levels of ALB and TP. The above effects clearly indicate that MEAM may offer protection by stabilizing the cell membrane in hepatic damage induced by cadmium. Furthermore, it

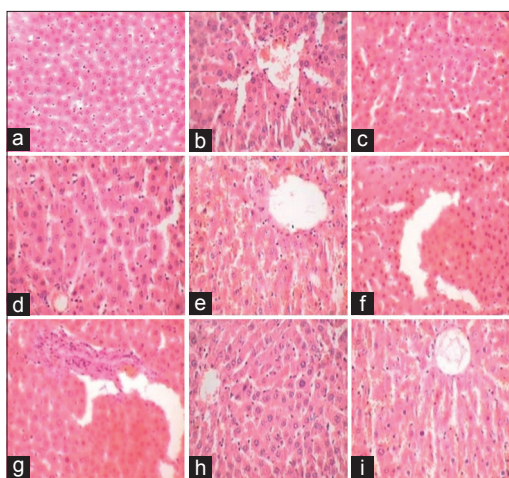


Fig. 1: (a-i) Photomicrographs of liver sections. (a) Control group. (b) Toxic group treated with Cd. (c) Standard group treated with Se and silymarin. (d) Treated with silymarin only. (e) Treated with Se only. (f) Treated with methanolic extract of the whole plant of *Aevra monsoniae* (MEAM) 250 mg/kg (g) Treated with MEAM 500 mg/kg. (h) Treated with a combination of Se and MEAM 250 mg/kg. (i) Treated with combination of Se and MEAM 500 mg/kg

has been observed that co-administration of MEAM and selenium (1 mg/kg) showed profound significant hepatoprotective activity against cadmium-induced liver toxicity compared with MEAM alone.

Cd has been reported to interact with critical subcellular sites such as the cytosol, mitochondria, and peroxisomes, resulting in the excessive generation of free radicals [22]. The free radicals, in turn, to induce oxidative stress through the oxidative damage of biomolecules such as lipids, proteins, and DNA, which may contribute to various pathological conditions [23]. LPO is one of the main manifestations of Cd-induced oxidative damage and has been found to play an important role in the toxicity of cadmium [24]. A significant increase in the level of hepatic MDA in Cd-intoxicated rats could be possibly due to excessive formation of free radicals which leads to the deterioration of biological macromolecules [6].

GSH is a tripeptide and a cysteine-rich protein participates in the maintenance of cytoplasmic and membrane thiol status. It is the most potent intracellular antioxidant and a powerful nucleophile, critical for cellular protection such as detoxification of ROS. In the present investigation, the reduced level of hepatic GSH may be due to increased utilization of GSH by the hepatic cells act as scavengers of free radicals produced by cadmium [25,26].

SOD and CAT were enzymatic antioxidants. SOD is a metalloenzyme that catalyzes the dismutation of superoxide radicals [27]. CAT is a hemeoprotein which catalyzes the reduction of H_2O_2 to water and oxygen and thus protects the cells from the oxidative damage of H_2O_2 and OH^- [28]. In the present investigation, Cd-intoxicated rats showed a significant decrease in the activity of these antioxidant enzymes in liver tissue might be due to the overproduction of ROS, the primary mechanism of Cd toxicity [29,30].

The decreased levels of LPO and elevated levels of GSH, SOD, and CAT were observed after treatment with MEAM at 250 mg/kg and 500 mg/kg, co-administration of selenium and MEAM which may be attributed to the antioxidant activity.

Our biochemical findings were corroborated with the histopathological findings of the liver. Histopathology studies of liver photomicrographs of different groups were showed to mitigate the damage and structural abnormalities observed in Cd-intoxicated rats. Among all groups, combination of MEAM and selenium showed the significant protective effect and it was well comparable with standard drug silymarin with selenium. These findings were substantiated with traditional claim of *A. monsoniae* used in the liver damage.

CONCLUSION

Conclusively, our results suggest that Cd is a potent liver toxicant capable of damaging the hepatocytes and also caused marked oxidative stress which is evident from the elevated biochemical parameters and decreased antioxidant parameters, increased LPO, respectively, in Cd-treated rats. Treatment with MEAM in combination with selenium at two test doses significantly attenuated the Cd-induced oxidative damage in rats. To conclude, among the two test doses, a combination of MEAM (250 mg/kg) and selenium (1 mg/kg) has shown synergistic protective action against cadmium-induced oxidative damage in rat.

CONFLICTS OF INTERESTS

All authors have none to declare.

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