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Protective potential of methanol extract of *Digera muricata* on acrylamide induced hepatotoxicity in rats

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This study was aimed to evaluate the probable protective effects of *Digera muricata* methanol extract (DME) against acrylamide (AA) induced hepatocellular injuries in female Sprague-Dawley rat. Phytochemical screening for the presence of different bioactive chemical groups was also carried out. The daily dose (6 mg/kg bw i.p.) injection of AA for 15 days caused significant increase in serum level of liver marker enzymes and metabolites: AST, ALT, ACP, ALP, LDH, BUN, creatinine, direct bilirubin and total bilirubin, while significant decrease in total protein and albumin. Hepatic level of antioxidant enzymes; CAT, POD, SOD, GSH-Px, GST and QR, and GSH contents were significantly decreased, while γ-GT and MDA was significantly increased. Treatment of DME (100, 150 and 200 mg/kg), dose dependently, ameliorated the toxicity of AA and the studied parameters were reversed towards the control level. Hepatic lesions induced with AA were reduced with DME treatment. Phytochemical screening indicates the presence of flavonoids, alkaloids, terpenoids, saponins, tannins, phlobatanin, coumarins, anthraquinones and cardiac glycosides. Total phenolic and flavonoids contents were 205±0.23 and 175.0±0.65 mg/g as equivalent to gallic acid and rutin, respectively in DME. In conclusion, the results suggest that the hepatoprotective effects of DME against AA-induced oxidative injuries could be attributed to the phenolics and flavonoids.

Key words: *Digera muricata*, acrylamide, alanine aminotransferase, antioxidant enzymes, TBARS, flavonoids.

INTRODUCTION

Acrylamide (AA) is a water-soluble vinyl monomer used in the production and synthesis of polyacrylamides (Dorman, 2000; Paulsson et al., 2001; Friedman, 2003; Nordin et al., 2003). These high molecular weight polymers can be modified to develop nonionic, anionic or cationic properties for specific uses. The principle end use of AA is in water-soluble polymers used as additives for water treatment, enhanced oil recovery, flocculants, paper making aids, thickeners, soil conditioning agents, sewage and waste treatment, ore processing and permanent-press fabrics (Paulsson et al., 2001; Friedman, 2003). On the other hand, AA is a component of tobacco smoke, which is formed by heating of biological material. Therefore, smoking could potentially be a source of AA in indoor air. Moreover, AA has been reported to be present in plant material like potatoes, carrots, radish, lettuce, Chinese cabbage, parsley, onions, spinach and rice paddy (Arikawa and Shiga, 1980), in sugar (Schultzova and Tekel, 1996) and olives (Friedman, 2003). It has been documented that AA is formed during the cooking of starchy foods at high temperature (Taubert et al., 2004). Furthermore, exposure to AA in foodstuffs has become a worldwide concern (Mitka, 2002) because of its generation in a variety of fried and oven-baked foods (Tareke et al., 2002) during cooking through Maillard reactions of sugars with asparagines residues (Mottram et al., 2002; Taubert et al., 2004).

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Abbreviations: DME, *Digera muricata* methanol extract; **AA,** acrylamide; **DM,** *Digera muricata*; **GSH-Px,** glutathione peroxidase; **QR,** quinone reductase; **GST,** glutathione-s-transferase; **GSR,** glutathione reductase; **γ-GT,** γ-glutamyl transpeptidase; **CAT,** catalase; **POD,** peroxidase; **SOD,** sodium dimutase; **GSH,** reduced glutathione reductase; **MDA,** malondialdehyde; **BUN,** blood urea nitrogen; **AST,** asparate transaminase; **ALT,** alanine transaminase; **ALP,** alkaline phosphatase; **ACP,** acid phosphatase; **LDH,** lactate degydrogenase.

Monomeric AA has been shown to cause diverse toxic effects in experimental animals. Acrylamide is carcinogenic to laboratory rodents (Sadek and Abou-Gabal, 1999) and is described by the International Agency for Research of Cancer as a probable carcinogen to humans (IARC, 1994). In the human body, AA is oxidized to the epoxide glycidamide (2, 3-epoxypro-pionamide) via an enzymatic reaction involving cytochrome P450 2E1 (Sumner et al.1999; Mottram et al., 2002). AA undergoes biotransformation by conjugation with glutathione (Tong et al., 2004) and is probably being the major route of detoxification. Both AA and glycidamide can form hemoglobin adducts (Calleman et al., 1992; Bergmark et al., 1993), but only glycidamide has been shown to form adducts with amino groups of the DNA (Klaunig, 2008). It was shown by Park et al. (2002) that high levels of AA can cause mutations and cellular transformation. Free radicals are continuously produced *in vivo* and there are number of protective antioxidant enzymes (superoxide dismutase, catalase, glutathione-*S*-transferase, glutathione peroxidase and reduced glutathione) for dealing with these toxic substances (Tong et al., 2004). Oxidative stress describes the steady state level of oxidative damage in a cell, tissue or organ, caused by the reactive oxygen species. It is caused by an imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. The superoxide dismutase which is the product of highly conserved gene converts the free oxygen radical to H_2O_2. This molecule which is itself toxic for the cells is broken down to release hydroxyl radical ($\cdot OH$), a reactive species which is more toxic either $O\cdot^-_2$ and H_2O_2. The enzymes responsible for converting H_2O_2 to other harmless substances are catalase and glutathione peroxidase. Thus, this enzyme family may act in a sequential fashion to dismutate the toxic oxygen species to another which then can be rapidly broken down to non toxic byproducts (Gilani et al., 2005). Catalase function is to detoxify H_2O_2 to oxygen and water. Glutathione peroxidase (GSH-Px) is a cytosolic enzyme and also eliminates H_2O_2, but in comparison to catalase, (GSH-Px) has a wider range of substrate including lipid peroxides. The kinetics of this enzyme is very complex, although, it has a greater affinity for H_2O_2 when compared with catalase. Glutathione peroxidase primarily functions to detoxify low level of H_2O_2 in the cells (Halliwell and Whiteman, 2004). AA can also cause glutathione depletion, resulting in intracellular oxidative stress (Tong et al., 2004). It was reported by Tong et al. (2004) that glutathione is the principal thiol and redox buffer in mammalian cells, while serum albumin is the principal protein and thiol in the plasma fraction of blood. Reaction between these two thiols and the AA appear to account for most of AA's elimination from the body. Disturbing the fine balance between survival and death signals inside the cell by favoring the proapoptotic factors initiates a cascade of intracellular reactions leading to loss of viability or can lead to apoptosis or necrosis in liver. It impairs nervous system function in the short term, characterized by ataxia, skeletal muscle weakness and weight loss and is also responsible for reproductive toxicity affecting the male gonads in rodents. Since the generation of AA is inevitable in food, approaches to reducing its toxic effects should be established. Daily exposure to AA might present a risk factor for neurotoxicity and reproductive toxicity as well as carcinogenicity in humans (Svensson et al., 2003; Klaunig, 2008). There is a need to evaluate the various phytochemicals or extracts against the toxicity induced with AA in experimental animals (Ademiluyi and Oboh, 2008).

Digera muricata (DM) is grown in open fields and also in the maize fields in summer season. *D. muricata* is used ethnopharmacologically in renal disorders (Anjaria et al., 2002) aperient, refrigerant (Hocking, 1962). Antioxidant effects of DM was determined in kidneys (Khan et al., 2009) and testis (Khan and Ahmed, 2009) of rats against oxidative stress induced with carbon tetrachloride, but its protective potential against AA was not evaluated in liver of rat. On account of increasing importance of plant metabolites as alternative medicine, this study was designed to determine the phytochemical composition and to evaluate the protective effects of DME against liver toxicity induced by AA in rat model.

MATERIALS AND METHODS

Chemicals

Reduced glutathione (GSH), oxidized glutathione (GSSG), glutathione reductase, γ-glutamyl p-nitroanilide, glycylglycine, bovine serum albumin (BSA), 1,2-dithio-bis nitro benzoic acid (DTNB), 1-chloro-2,4-dinitrobenzene (CDNB), reduced nicotinamide adenine dinucleotide phosphate (NADPH), acrylamide (AA), flavine adenine dinucleotide (FAD), glucose-6-phosphate, Tween-20, 2,6-dichlorophenolindophenol, thiobarbituric acid (TBA), picric acid, sodium tungstate, sodium hydroxide, trichloroacetic acid (TCA) and perchloric acid (PCA) were purchased from Sigma Aldrich Chemicals Co. USA.

Plant collection and extract preparation

At maturity, the plants of *D. muricata* (L.) Mart. were collected, shade dried, chopped and ground of 1 mm mesh size. 750 g powder of *D. muricata* (L.) Mart. was extracted twice with absolute methanol for 72 h filtered and evaporated under reduced pressure at 4°C to obtain 12.0 g of DME.

Phytochemical studies

Qualitative studies of DME for the presence of flavonoids, alkaloids, terpenoids and saponins were carried out according to Harborne (1973), tannins (Sofowara, 1993), while coumarins, cardiac glycosides, anthraquinones and phlobatanins as described by Trease and Evans (1989). Percentage of flavonoids in DME were quantitatively determined according to Boham and Kocipai (1974), alkaloids (Harborne, 1973), tannins (Van-Buren and

Robinson, 1981) and saponins (Obadoni and Ochuko, 2001).

Determination of total phenolic contents

The amount of total phenolics in extract was determined according to the Folin-Ciocalteu procedure (Singleton et al., 1999). Samples (200 µl, three replicates) were introduced into test tubes; 1.0 ml of Folin-Ciocalteu's reagent and 0.8 ml of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured. The total phenolic content was expressed as gallic acid equivalents (GAE) mg/g of dry weight of DME.

Determination of total flavonoid contents

Flavonoids (extracted with 5% $NaNO_2$, 10% $AlCl_3$ $6H_2O$ and 1 M NaOH) were measured at 510 nm with a known rutin concentration as a standard. The results were expressed as mg of rutin equivalents (RTE)/g dry weight of DME (Singleton et al., 1999).

Animals and treatment

The study protocol was approved by Ethical committee of Quaid-i-Azam University Islamabad for laboratory animal feed and care. This study was conducted (with 42 mature Sprague-Dawley female rats) in the laboratory of the department of biochemistry, Quaid-i-Azam University, Islamabad, Pakistan. The animals weighing 155 to 175 g, were divided equally into 7 groups with six rat in each group and maintained under standard laboratory conditions (12 h light/darkness; at 25±3°C). The animals have free access to standard animal diet and water *ad libitum*. The experimental protocols were as follows:

Estimation of DME dose

Male Sprague-Dawley rats (3; six week old) were kept fasting for overnight providing only water, after which the extract was administered intragastrically at the dose of 300 mg/kg bw and rats were remained under observation for 14 days to observe the mortality. Toxicity was not observed and the procedure was repeated for further higher doses, that is, 600, 1000, 1500 and 2000 mg/kg bw. One-tenth (200 mg/kg bw) of the maximum dose of the extract tested (2000 mg/kg bw) did not indicate mortality was selected for evaluation of hepatoprotective activity (Handa and Anupama, 1990).

Group I, animals remained untreated (control); Group II was given saline 1 ml/kg (0.9%) intragastrically once a day for 15 days; Group III was treated with aqueous AA (6 mg/kg bw; i.p.) aqueous solution once a day for 15 days; Group IV was given acrylamide along with DME (100 mg/kg bw) intragastrically once a day for 15 days; Group V was given acrylamide along with DME (150 mg/kg bw) intragastrically once a day for 15 days; Group VI was given acrylamide along with DME (200 mg/kg bw) intragastrically once a day for 15 days; Group VII rats were treated with DME (200 mg/kg bw; orally) once a day for 15 days as a control group.

At the end of the experiment, animals were weighed, abdominal scision was made and blood was collected from atrium in routine biochemical test tubes having EDTA and centrifuged at 2000 × g for 20 min at 4°C to get the serum. Liver was removed immediately after euthanasia and weighted. A part of the liver was fixed, while other dried in liquid nitrogen and stored at -70°C for various biochemical and enzymatic analysis. Histopathological studies of liver were carried out in 4 to 5 µm thin sections.

Assessment of biochemical markers

Serum analysis of various liver marker enzymes such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), acidic phosphatase (ACP), lactate dehydrogenase (LDH), total protein, albumin, direct bilirubin, total bilirubin, blood urea nitrogen (BUN) and creatinine were estimated by using standard AMP (Autoryzowany Przedstawiciel w Poice) diagnostic kits (Stattogger Strasse 31b 8045 Graz, Austria).

Assessment of antioxidant enzymes

Liver tissue was homogenized in 10 volume of 100 mmol KH_2PO_4 buffer containing 1 mmol EDTA (pH 7.4) and centrifuged at 12,000 × g for 30 min at 4°C. The supernatant was collected and used for the following measurements as described further. Protein concentration of the supernatant of liver tissue was determined by the method of Lowry et al. (1951) using crystalline BSA as standard.

Catalase (CAT) and peroxidase (POD) activities were determined by the method of Chance and Maehly (1955). CAT activity was determined by adding H_2O_2 at 240 nm, while POD activity was measured by using guaiacol as substrate at 470 nm. One unit of CAT and POD activity was defined as an absorbance change of 0.01 as units/min. Superoxide dismutase (SOD) activity was estimated using phenazine methosulphate and sodium pyro-phosphate buffer according to Kakkar et al. (1984). Enzyme reaction was initiated by adding NADH (780 µmol) and stopped after 1 min by adding glacial acetic acid and color intensity at 560 nm was recorded. Results are expressed in units/mg tissue protein.

Hepatic glutathione-*S*-transferase (GST) activity was determined according to Habig et al. (1974), using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. Glutathione reductase (GSR) activity determined according to Carlberg and Mannervik (1975), was measured at 340 nm by using reduced nicotinamide adenine dinucleotide phosphate (NADPH) as a substrate. Glutathione peroxidase (GSH-Px) activity was measured by using reduced nicotinamide adenine dinucleotide phosphate (NADPH) as a substrate (Mohandas et al., 1984). γ-Glutamyl transpeptidase (γ-GT) activity was determined by the method of Orlowaski and Meister (1973) using glutamyl *p*-nitroanilide as substrate. The activity of quinone reductase (QR) was determined according to Benson et al. (1980). The reduction of dichlorophenolindophenol (DCPIP) was recorded at 600 nm.

Reduced glutathione assay (GSH)

Reduced glutathione was estimated by the method of Jollow et al. (1974) by using 1,2-dithio-bis nitro benzoic acid (DTNB) as substrate. The yellow color was read immediately at 412 nm and expressed as µM GSH/g tissue.

Estimation of lipid peroxidation assay (MDA)

Malondialdehyde (MDA) of liver were measured at 535 nm by using 2-thiobarbituric acid (2,6-dihydroxypyrimidine-2-thiol; TBA). An extinction coefficient of 156,000 M^{-1} cm^{-1} was used for calculation according to Iqbal et al. (1996).

Histopathalogical determination

For microscopic evaluation, liver were fixed in a fixative (absolute ethanol 60%, formaldehyde 30% and glacial acetic acid 10%) and embedded in paraffin, sectioned at 4 to 5 µm and subsequently

Table 1. Effect of DME on body weight, absolute liver and relative liver weight in rat.

Group	Treatment	Initial body weight (g)	Percent increase in body weight	Absolute liver weight (g)	Relative liver weight as (%) body weight
I	Control	164.5±7.2	21.0±2.6^b	5.20±0.55^b	2.62±0.25^b
II	Saline (1 ml/kg)	164.5±5.6	20.4±2.1^b	4.95±0.25^b	2.50±0.15^b
III	AA (6 mg/kg)	163.3±7.2	12.8±2.0^C	7.96±0.24^C	4.33±0.29^C
IV	AA (6 mg/kg)+DME (100 mg/kg)	162.8±3.7	14.6±2.0^C	7.21±0.65^{Ca}	3.86±0.37^{Ca}
V	AA (6 mg/kg)+DME (150 mg/kg)	164.2±7.0	17.8±1.5^{Aa}	6.93±0.52^{Cb}	3.59±0.31^{Cb}
VI	AA (6 mg/kg)+DME (200 mg/kg)	167.3±4.4	20.0±3.7^b	5.96±0.40^{Bb}	2.97±0.25^{Ab}
VII	DME (200 mg/kg)	160.5±3.7	21.8±1.6^b	4.81±0.30^b	2.46±0.11^b

Mean ±SD (n= 06). A= $P < 0.05$; B= $P < 0.0$; C= 0.001 significance from control group. a=$P < 0.01$; b= $P < 0.001$ significance from AA group.

Table 2. Effect of DME on liver marker enzymes in rat.

Group	Treatment	AST (U/dl)	ALT (U/dl)	ALP (U/dl)	ACP (U/dl)	LDH (U/dl)
I	Control	0.61±0.04^b	0.49±0.06^b	2.70±0.26^b	7.96±0.10^b	0.43±0.02^b
II	Saline (1 ml/kg)	0.59±0.05^b	0.49±0.05^b	2.66±0.28^b	7.51±0.70^b	0.43±0.04^b
III	AA (6 mg/kg)	2.36±0.07^C	1.74±0.07^C	4.66±0.60^C	13.50±1.33^C	0.91±0.05^C
IV	AA (6 mg/kg)+DME (100 mg/kg)	1.84±0.40^{Cb}	1.51±0.06^{Cb}	4.05±0.46^{Ca}	11.21±1.02^{Cb}	0.82±0.04^{Ca}
V	AA (6 mg/kg)+DME (150 mg/kg)	1.11±0.11^{Cb}	1.05±0.11^{Cb}	3.88±0.40^{Ca}	9.43±0.74^{Ab}	0.67±0.08^{Cb}
VI	AA (6 mg/kg)+DME (200 mg/kg)	0.79±0.10^b	0.65±0.08^{Bb}	2.98±0.21^b	8.03±0.90^b	0.57±0.05^{Cb}
VII	DME (200 mg/kg)	0.64±0.04^b	0.51±0.03^b	2.68±0.20^b	7.20±0.62^b	0.42±0.04^b

Mean ±SD (n= 06). A= $P < 0.05$; B= $P < 0.01$; C= $P < 0.001$ significance from control group. a= $P< 0.01$; b= $P < 0.001$ significance from AA group. AST, Asparate transaminase; ALT, alanine transaminase; ALP, alkaline phosphatase; ACP, acid phosphatase; LDH, lactate degydrogenase.

stained with hematoxylin/eosin. Sections were studied under light microscope (DIALUX 20 EB) at 40 and 100 magnifications. Slides of all the treated groups were studied and photographed.

Statistical analysis

The values were expressed as the mean ± SEM for the 06 rats in each group. Differences between groups were assessed by one-way analysis of variance (ANOVA) using the statistical package for social sciences (SPSS) software package for Windows (version 13.0). Post hoc testing was performed for intergroup comparisons using the least significant difference (LSD) test. A value corresponding to $P < 0.05$ was considered statistically significant.

RESULTS

Biochemical composition of DME

Analysis of various fractions of the *D. muricata* indicated the presence of flavonoids, alkaloids, terpenoids, saponins, coumarins, tannins, cardiac glycosides and anthraquinones. Flavonoids percentage (5.26±0.09), saponins (3.13±0.11), alkaloids (0.64±0.01) and tannins (0.35±0.14) had been determined in DME. Total phenolic contents as equivalent to gallic acid were 205±0.23 mg/g of the extract, while the total flavonoid contents were 175.0±0.65 mg/g extract as equivalent to rutin in DME.

General toxicity

Treatment of AA significantly decreased the percent increase in body weight of rat compared with the control group (Table 1). Treatments with DME consistently increased the percent of body weight. However, this increase was more pronounced at the higher dose of DME (200 mg/kg bw) and it was statistically similar to the control group of rat. Contrary to body weight, the absolute and relative liver weight was increased with the AA treatment when compared with the control group. Treatment of rats with the DME along with AA treatment ameliorates the effects of AA and weight of body, absolute and relative liver weight reversed towards the control group in dose dependent way. The earlier mentioned parameters did not statistically change ($P > 0.05$) with saline and DME (200 mg/kg bw) alone as against the control group.

Effects of DME on liver marker enzymes

Effects of AA and various doses of DME on liver marker enzymes are shown in Table 2. It was depicted from the results that the AA treatment of rats for 15 days caused hepatotoxicity and an increase in the serum level of AST, ALT, ALP and ACP ($P < 0.001$), while LDH ($P < 0.05$)

Table 3. Effect of DME on liver biochemical markers in rat.

Group	Treatment	Total protein (mg/dl)	Albumin (mg/dl)	Direct bilirubin (mg/dl)	Total bilirubin (mg/dl)	BUN (mg/dl)	Creatinine (mg/dl)
I	Control	45.0 ± 1.8^c	21.0 ± 2.3^c	0.75 ± 0.02^c	1.32 ± 0.05^c	28.26 ± 1.23^c	0.66 ± 0.028^c
II	Saline (1 ml/kg)	45.0 ± 2.4^c	21.2 ± 1.9^c	0.75 ± 0.04^c	1.31 ± 0.07^c	29.80 ± 1.36^c	0.67 ± 0.027^c
III	AA (6 mg/kg)	32.2 ± 3.0^C	10.2 ± 1.9^C	1.23 ± 0.06^C	2.00 ± 0.10^C	55.58 ± 2.0^C	0.90 ± 0.025^C
IV	AA (6 mg/kg)+DME (100 mg/kg)	35.5 ± 1.9^{Ca}	13.5 ± 1.0^{Cb}	1.08 ± 0.06^{Cc}	1.63 ± 0.08^{Cc}	48.88 ± 2.44^{Cc}	0.85 ± 0.008^{Cc}
V	AA (6 mg/kg)+DME (150 mg/kg)	39.3 ± 2.7^{Cc}	16.3 ± 1.4^{Cc}	0.93 ± 0.06^{Cc}	1.44 ± 0.08^{Ac}	37.96 ± 2.02^{Cc}	0.77 ± 0.023^{Cc}
VI	AA (6 mg/kg)+DME (200 mg/kg)	41.5 ± 2.4^{Ac}	17.8 ± 1.5^{Bc}	0.80 ± 0.07^c	1.34 ± 0.06^c	30.96 ± 2.09^{Ac}	0.71 ± 0.016^{Bc}
VII	DME (200 mg/kg)	43.5 ± 2.4^c	22.1 ± 2.2^c	0.76 ± 0.05^c	1.31 ± 0.06^c	28.76 ± 1.34^c	0.64 ± 0.021^c

Mean ±SD (n= 06). A= $P < 0.05$; B= $P < 0.01$; C= $P < 0.001$ significance from control group. a= $P < 0.05$; b = $P < 0.01$; c= $P < 0.001$ significance from AA group. BUN, Blood urea nitrogen.

Table 4. Effect of DME on antioxidant enzymes, GSH and MDA in rat.

Group	Treatment	CAT (U/min)	POD (U/min)	SOD (U/mg protein)	GSH (µg/g tissue)	MDA (nmol/mg protein)
I	Control	4.3 ± 0.14^c	3.25 ± 0.23^c	6.20 ± 0.32^c	0.76 ± 0.05^c	40.7 ± 4.3^c
II	Saline (1 ml/kg)	4.3 ± 0.19^c	3.25 ± 0.36^c	6.23 ± 0.32^c	0.76 ± 0.07^c	41.6 ± 2.8^c
III	AA (6 mg/kg)	2.9 ± 0.33^B	1.71 ± 0.35^B	1.71 ± 0.46^B	0.35 ± 0.06^B	75.2 ± 8.0^B
IV	AA (6 mg/kg)+DME (100 mg/kg)	3.1 ± 0.30^B	1.95 ± 0.33^B	2.33 ± 0.28^{Ba}	0.44 ± 0.06^{Ba}	63.8 ± 4.8^{Bc}
V	AA (6 mg/kg)+DME (150 mg/kg)	3.5 ± 0.23^{Bc}	2.61 ± 0.18^{Ac}	3.00 ± 0.31^{Bc}	0.59 ± 0.04^{Bc}	56.4 ± 4.0^{Bc}
VI	AA (6 mg/kg)+DME (200 mg/kg)	3.8 ± 0.37^{Ac}	3.20 ± 0.18^c	4.43 ± 0.67^{Bc}	0.72 ± 0.07^c	51.8 ± 5.2^{Ac}
VII	DME (200 mg/kg)	4.4 ± 0.30^c	3.23 ± 0.32^c	6.21 ± 0.33^c	0.75 ± 0.06^c	40.0 ± 4.6^c

Mean ±SD (n= 06); A= $P < 0.01$; B= $P < 0.001$ significance from control group. a= $P < 0.05$; b = $P < 0.01$; c = $P < 0.001$ significance from AA group. CAT, Catalase; POD, peroxidase; SOD, sodium dimutase; GSH, reduced glutathione reductase; MDA, malondialdehyde

was significantly increased to that of the control group. Treatment of DME for 15 days ameliorated the toxicity of AA and the serum level of these enzymes was reduced ($P < 0.01$ or $P < 0.001$) in a concentration dependent manner. Serum level of AST, ALP, ACP and LDH at 200 mg/kg bw dose was reversed towards the control level and it was statistically similar to the control group. However, serum level of ALT was significantly decreased ($P < 0.001$) with only DME (200 mg/kg bw).

Effects of DME on biochemical markers of liver

Mean values for total protein and albumin of serum was significantly decreased ($P < 0.001$), while direct bilirubin, total bilirubin, BUN and creatinine level was significantly increased with the treatment of AA for 15 days (Table 3). Toxicity of AA was ameliorated with the administration of DME and the level of earlier mentioned biochemical markers in serum of rats was restored as against the AA

group in a dose dependent manner.

Effects of DME on antioxidant enzymes

As shown in Table 4, the treatment of female rats with AA for 15 days significantly ($P < 0.001$) suppressed the activity of CAT, POD, SOD, GST, GSH-Px, GSR and QR in liver tissue when compared with the control group. In the DME along with AA treated groups, the activity of CAT, POD, SOD, GST, GSH-Px, GSR and QR was increased as against the AA group in a dose dependent fashion. However, at the lowest dose of DME (100 mg/kg bw), CAT and POD enzyme activity were statistically not different from the AA-treated group. By contrast, CAT, POD, SOD, GST, GSH-Px, GSR and QR were restored significantly ($P < 0.001$) at higher doses of DME when compared with the AA-treated group. Mean values of the earlier mentioned enzymes did not change ($P > 0.05$) in the DME treatment alone compared with the control

Table 5. Effect of DME on hepatic antioxidant enzymes in rat.

Group	Treatment	GSH-Px (nmol/mg protein)	QR (nmol/mg protein)	GST (nmol/mg protein)	GSR (nmol/mg protein)	γ-GT (nmol/ mg protein)
I	Control	114.7±4.9[b]	144.2±8.9[b]	153.8±6.3[b]	207.3±4.0[b]	52.8±6.0[b]
II	Saline (1 ml/kg)	115.5±5.0[b]	144.7±5.6[b]	154.0±8.6[b]	205.2±9.0[b]	52.8±6.8[b]
III	AA (6 mg/kg)	65.0±8.7[B]	83.8±5.8[B]	99.0±7.2[B]	152.0±10.3[B]	94.5±7.4[B]
IV	AA (6 mg/kg)+DME (100 mg/kg)	76.7±6.8[Ba]	104.2±7.3[Bb]	110.8±5.0[Ba]	171.7±11.8[Ba]	78.8±6.7[Bb]
V	AA (6 mg/kg)+DME (150 mg/kg)	86.3±6.8[Bb]	111.7±7.5[Bb]	122.8±7.4[Bb]	176.5±12.3[Bb]	72.3±4.0[Bb]
VI	AA (6 mg/kg)+DME (200 mg/kg)	94.7±9.0[Bb]	127.3±6.0[Bb]	136.8±7.0[Bb]	185.8±9.4[Bb]	61.7±6.3[Ab]
VII	DME (200 mg/kg)	116.5±6.0[b]	145.0±3.2[b]	150.0±6.7[b]	205.0±7.2[b]	54.0±3.6[b]

Mean ±SD (n= 06). A= $P < 0.05$; B= $P < 0.001$ significance from control group. a= $P < 0.01$; b= $P < 0.001$ significance from AA group. GSH-Px, Glutathione peroxidase; QR, quinone reductase; GST, glutathione-s-transferase; GSR, glutathione reductase; γ-GT, γ-glutamyl transpeptidase

group.

Effects of DME on hepatic GSH and MDA

Hepatic level of GSH contents significantly decreased, while MDA contents increased by the AA administration compared to that of the control group. In the DME-treated groups along with AA, hepatic level of GSH contents increased while MDA contents decreased in a dose dependent manner (Table 5). Administration of DME alone did not change the mentioned parameters ($P > 0.5$) compared with that in the control group.

Histopathology of liver

As regards to the histopathology of liver, the control animals revealed well determined hepatic lobules, separated by interlobular septa, traversed by portal veins, hepatic artery and bile ducts (hepatic triads). In the middle of each hepatic lobule is a central or intralobular vein with a few Kupffer cells around it. Within each lobule, plates of hepatic cells radiate from the central vein towards the periphery, while between the laminae, hepatic sinusoids prevail. Most of the cells are polyhedral and mononucleated (Figure 1a). Animals treated with AA showed drastic alterations in the internal structure of their livers. The main features were a disrupted pattern of hepatic cords, lymphocytes infiltration, inflammation, centrilobular vacuolization of hepatocytes and the incidence of necrosis, congestion and disruption of the central canal wall (Figure 1b, c). Rats treated with AA followed by DME, showed much less damage to liver structure. Hepatocytes were polyhedral and hepatic cords were well defined. The hepatic cells in the middle zone were normal and necrosis which were present, were restricted to the centrilobular region (Figure 1d).

DISCUSSION

Low level of enzyme activity of catalase, peroxidase, superoxide dismutase, glutathione peroxidase, glutathione-S-transferase and glutathione reductase was recorded in the liver tissues suggesting acute injuries caused by AA. Likewise, the activity of quinine reductase and glutathione reductase was decreased, while γ-GT activity was enhanced with AA. The antioxidant enzymes protect the major molecules such as lipids, proteins and DNA from oxidative damage by inactivating the oxidants. Increase in ROS with AA in liver HepG2 cells has been reported (Yousef et al., 2006; Zhang et al., 2008). The antioxidant enzymes may act in a coordinate manner to protect living tissues from oxidative damage. Damaging effects of the AA in liver were reduced with the DME administration thereby reversed the level of CAT, POD, SOD, GST, GSH-Px, GSR and QR towards the normal level in a dose dependent way. The ameliorating effects of the DME were more pronounced at the higher dose of 200 mg/kg bw.

All forms of life maintain a reducing environment within their cells through a constant input of metabolic energy which is preserved by enzymes and the antioxidant GSH (Sridevi et al. 1998). The delicate balance between the production and catabolism of oxidants is critical for maintenance of the biological function (Sridevi et al., 1998). In this study, the liver tissues contents of GSH were decreased, while the MDA were increased with AA treatment. Induction in the levels of MDA; the marker of extent of lipid peroxidation, in liver tissues is in agreement with the finding of Tong et al. (2004) who suggested that enhancement of lipid peroxidation is a consequence of glutathione depletion resulting in oxidative stress. AA is oxidized to glycidamide, a reactive epoxide and undergoes conjugation with glutathione (Mottram et al., 2002) which is the most plausible reason for the decrease of GSH. Decrease in GSH with AA in
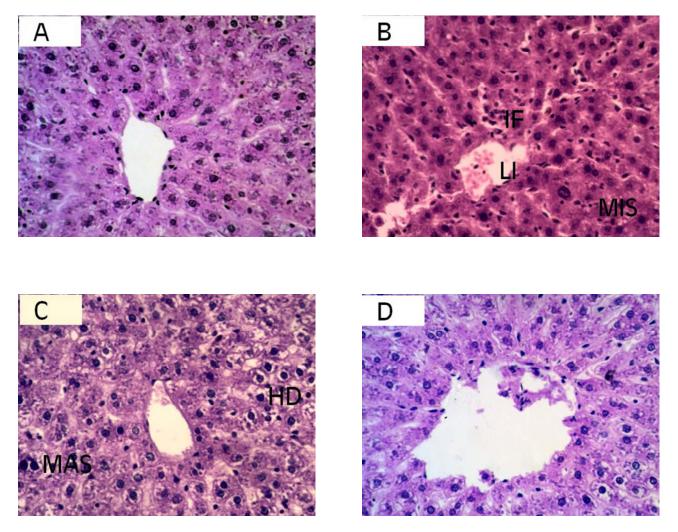
Figure 1. H and E stain. Representative sections of liver. Histopathology of liver with normal triad, hepatocytes and centrilobular region (A). Acrylamide (6 mg/kg) treated rats with lymphocytes infiltration (LI), inflammatory cells (IF), microvesicular steatosis (MIS) and necrosis in liver histopathology; (B) hydropic degeneration (HD), macrovesicular steatosis (MAS) and disruption of triad architecture in liver of acrylamide treated rats; (C) recovery of hepatic disruption with DME against the acrylamide, injuries are restricted to the centrilobular (D).

liver HepG2 cells has been determined (Zhang et al., 2008). Similar results have been obtained in this study. Post-treatment of DME exerts its protective effects against the oxidative stress induced by AA, reversing the GSH and MDA level towards the normal control. These ameliorating effects of DME possibly seems to be due to the presence of flavonoids and other constituents in this plant and are found similar to the previous studies (Khan and Ahmed, 2009: Khan et al., 2009) where *D. muricata* extracts ameliorate the oxidative stress induced with carbon tetrachloride in rat. Chemical constituents present in DME may be responsible for the observed protective effects against the AA.

Biochemical parameters are sensitive index to changes due to xenobiotics and can constitute important diagnostic tool in toxicological studies. Activities of transaminases (AST, ALT), acid and alkaline phosphatases (ACP

and ALP) and lactate dehydrogenase (LDH) were increaseed in the serum with the AA treatment indicating acute injuries to hepatocytes. Phosphatases are important and critical enzymes in biological processes, they are responsible for detoxification, metabolism and biosynthesis of energetic molecules for different essential functions. Any interference in these enzymes leads to biochemical impairment and lesions of the tissue and cellular function (Khan et al., 2001). Oxidative stress implicated with AA injured the membranous system of hepatocytes with the increased level of transaminases in the blood. Results obtained in this study were comparable to other studies where significant increase in transaminases and LDH was obtained with AA treatment and level of liver marker enzymes reversed towards the normal level with ginseng extract (Manna et al., 2006), as the ginseng possesses antioxidant and anti-inflammatory

properties are obtained with DM. Decrease in total protein and albumin while increase in direct and total bilirubin indicated the liver dysfunction obtained in this study. These results are in agreement with the histo-pathological studies of the liver tissues. AA treatment most prominently causes the centrilobular necrosis and inflammation was significantly recovered by DME. Enhanced liver weight was also reduced with the DME extract, possibly through the elimination of fibrosis and reduction in the invasion of inflammatory cells. This study suggested that tested extract of *D. muricata* possesses significant protective effect against hepatotoxicity induced by AA which may be attributed to the individuals or combined action of phyto-constituents present in it (Ademiluyi and Oboh, 2008; Huda-Faujan et al., 2009). These protective effects of DME may be attributed through the antioxidant or scavenging effects of the chemicals such as flavonoids, terpenoids, tannins and other chemicals alone or combined action of phyto-constituents present in it.

Conclusion

The results obtained in this study suggested the pro-tective potential of DME that consequently ameliorated the oxidative stress induced with AA. Disturbances in the normal redox state by AA can cause toxic effects through the production of cell damaging peroxides and free radicals. It is suggested that mechanism of action of DME is probably through scaven-ging of free radicals. The results herein are a good start for deep research with medical and pharmaceutical po-tential of DME.

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