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Hepatoprotective effect of *Amaranthus hypochondriacus* seed extract on sodium arsenite-induced toxicity in male Wistar rats

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The hepatoprotective effect and antioxidant activity of ethanol extract of *Amaranthus hypochondriacus* seed (ESAH) were evaluated in sodium arsenite (SA) treated rats. Animals were divided into eight groups. The first group was used as control, group II was administered SA (2.5 mg/kg body weight). Groups III, IV and V were administered different doses of ESAH (100, 200 or 300 mg/kg b.w., respectively); groups VI, VII and VIII were administered ESAH at 100, 200, 300 mg/kg b.w. plus 2.5 mg SA/kg; respectively. Rats were orally administered different doses of ESAH daily for fourteen days. The SA was administered on the 7th and 14th day. Results showed that sodium arsenite-induced elevation of alanine aminotransferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) levels were substantially reduced by pre-treatment with 200 and 300 mg/kg b.w. of ESAH. Histopathological observations also showed hepatic protective activity of ESAH. Sodium arsenite increased the concentrations of malondialdehyde (MDA), hydrogen peroxide and reduced the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), and increased frequency of micronucleated polychromatic erythrocytes. Pre-treatment with 200 and 300 mg/kg of ESAH reduced the levels of MDA and H_2O_2; increased the activities of antioxidant enzymes (SOD, CAT, GPx) and reduced frequency of induction of micronucleated polychromatic erythrocytes (mPCEs) in bone marrow cells of the rats. Treatment with SA decreased body weight and increased relative liver weight compared to control and ESAH. Thus ESAH mitigates arsenic-induced hepatotoxicity and oxidative damage in rats.

Key words: *Amaranthus hypochondriacus*, antioxidant enzymes, hepatoprotective effect, sodium arsenite.

INTRODUCTION

Arsenic occupies number one position of hazardous substances in the Comprehensive Environmental Response Compensation and Liability Act (CERCLA), Agency for Toxic Substances and Disease Registry

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(ATSDR, 2007). Environmental and occupational exposure to arsenite has been linked with a range of harmful effects in both experimental animals and humans leading to various ailments and dysfunctions of vital organs such as liver, kidney and lung (Chowdhury et al., 2001; Odunola et al., 2011; Tseng et al., 2002; Waalkes et al., 2003). Exposure to arsenic and its compounds through underground waters has been associated with lesions of the skin, certain forms of cancers (Chiou et al., 2001; Jayanthika et al., 2001) and other health effects such as neurological disorders, damaged cognitive development in children (Parvez et al., 2006; Valentine, 1994) and immunotoxicity (Patrick, 2003). Sodium arsenite is widely used as a component of herbicides, fungicides, insecticides and algaecides, and in the manufacture of arsenical soap (Chen et al., 2006; Cullen, 2008). Incidence of arsenic contamination posing serious threat to public health has been reported in various countries like India, Poland, Argentina, Bangladesh, Chile, China, India, USA, and Taiwan where it occurs in drinking water above the permissible upper limit of 10 ppm (Chatterjee et al., 1995; Das et al., 1995; Frost et al., 1993; IARC, 2004). Several studies have demonstrated that liver is the most important target organ for arsenic toxicity (Guha, 2005; Hughes et al., 2003). The liver regulates several important metabolic functions and the hepatic injury is associated with distortion of these metabolic functions (Wolf, 1999).

Plants are one of the most important resources for human foods and medicines. There has been strong evidence that intake of plant dietary factors improve health, as well as prevent diseases (Jian, 2007). Consumption of whole grain has been associated with reduced risk of chronic diseases (Bonaccio et al., 2012; Jones and Engleson, 2010; Lillioja et al., 2013). Other studies have demonstrated the protective role of diets high in grain against cancer (Nicodemus et al., 2001) and diabetes (Meyer et al., 2000). Antioxidant activity, mediation of hormones and enhancement of the immune system are the plausible physiological mechanisms health benefits exerted by grain phytochemicals (Lupton and Meacher, 1998). Substantial amount of antioxidants have also been discovered in grain-based cereal products (Baublis et al., 2000; Emmons et al., 1999; Handelman et al., 1999).

It has been proposed that the antioxidants found in grain-based foods may actually contribute to health by lowering and/or alleviating the occurrence of aging-related diseases such as cardiovascular disease, neurodegenerative disease and some kinds of cancer (Miller et al., 2002).

Amaranth (*Amaranthus hypochondriacus,* family: Amaranthaceae) is an underutilized pseudo cereal that has promising potential as an excellent source of nutrients and bioactive compounds (Escudero et al., 1999; Muyonga et al., 2008). Amaranth contains significant amounts of bioactive components such as protease inhibitors, phytosterols, squalene, amino acids, flavonoids and polyphenols (Berghofer and Schoenlechner, 2002; Taylor and Parker, 2002). Amaranth contains lysine, methionine and tryptophan that help prevent cold sores, reduce disease infection and boost the body's immune system (Bressani, 1989). Hepatoprotective activity of whole plant extract of *Amaranthus spinosus* (Zeashan et al., 2008) and *Amaranthus viridis* (Kumar et al., 2011) in experimental animals has been reported. The present study was aimed at evaluating the potential protective effects of ethanol extract of *A. hypochondriacus* seed against sodium arsenite-induced toxicity in liver of male rats.

MATERIALS AND METHODS

Chemicals

Sodium arsenite ($NaAsO_2$) was purchased from Sigma Chemical Company, St. Louis MO, USA. Sodium arsenite was orally administered to rats at a dose of 2.5 mg/kg b.w. corresponding to 1/10th of the LD_{50} sodium arsenite (Preston et al., 1987). Kits for alkaline phosphatase, aspartate amino transferase (AST), alanine amino transferase (ALT), and gamma glutamyl transferase (GGT) were obtained from Randox Laboratories Ltd, Crumlin, UK. Colchicine, giemsa stain and all other chemicals were of analytical grade and purchased from Sigma Chemical or BDH Chemical Ltd, Poole, England.

Source of plant material and preparation of the extracts

A. hypochondriacus (accession number PI590991) seeds were obtained from United States Department of Agriculture, Research Station (USDA-ARS) North Central Regional Plant Introduction Station (NCRPIS) in Ames, USA and planted at the experimental field of National Horticultural Research Institute (NIHORT), Ibadan, Nigeria, in June, 2010. At maturity, seeds were harvested, dried, milled into flour and extracted in 80% ethanol using a Soxhlet apparatus. The extract was concentrated at reduced temperature (40°C) using a rotary evaporator (Buchi, USA). The resulting molten extract was further lyophilized using a freeze dryer (Freezone 4.5, Labconco, USA) at high vacuum (133×10^{-3} mBar) with a yield of 3.91% (w/w). The dried extract was stored in air tight amber bottle and kept at -20°C.

Animals

Forty eight male albino rats weighing 120 to 150 g were used in this experiment. They were kept in departmental animal house in well cross ventilated room at 27 ± 2°C and 12 h light/12 h dark cycle. Animals were given commercial pellets (Ladokun Livestock Feeds, Ibadan, Nigeria) and water *ad libitum*. After two weeks of acclimatization, animals were randomly divided into eight treatment groups of six animals each. Group I received only distilled water and was used as control. Group II received 2.5 mg/kg b.w. of sodium arsenite (SA). Groups III, IV and V were administered ethanol extract of *A. hypochondriacus* seed (ESAH) at 100, 200 and 300 mg/kg body weight, respectively. Groups VI, VII and VIII were administered ESAH at 100, 200, 300 mg/kg body weight, respectively; plus SA at 2.5 mg/kg b.w. Rats were orally administered their respective doses of ESAH daily for 14 days, while SA was administered on the 7th and 14th day. At the end of

the experiment, body weights of rats were recorded.

Collection of samples

Animals were sacrificed by cervical dislocation 24 h after administration of the last dose of sodium arsenite following standard rules laid down by the University of Ibadan Ethics Committee on the treatment of experimental animals. Two hours prior to the sacrifice, the animals were intraperitoneally injected with 0.04% colchicine. Blood samples were collected by cardiac puncture and liver samples were harvested, rinsed in ice cold 1.15% KCl solution and weighed. The blood and liver samples were used for biochemical analyses and histopathological examination. Femoral bone marrow from each animal was collected and used for micronucleus assay.

Sample preparation for the assays

The blood was allowed to clot; serum was separated by centrifugation at 3000 × g for 10 min and used for the estimation of serum enzymes. Alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) activities were assayed using standard diagnostic kits from Randox Laboratories Ltd., UK. Femoral bone marrow cells were prepared according to the method of Salamone et al. (1980). The fixed cells on the mounted slides were scored under light microscope to detect the presence of micronucleated polychromatic erythrocytes (mPCEs), using tally counter to make scoring easier. Livers were weighed, minced and homogenized in four volume 0.01 M phosphate buffer (pH 7.4) using a Potter-Elvegin homogenizer. The homogenate was centrifuged at 20,000 × g for 20 min at 4°C using an ultracentrifuge (HERMLE LABNET Z 323K). The resulting supernatant was used for the assay of antioxidant parameters and protein content.

Evaluation of antioxidant parameters

Assessment of lipid peroxidation

Extent of lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) as described by Varshney and Kale (1990).

Determination of superoxide dismutase (SOD) activity

Superoxide dismutase activity was determined by the method previously described by Misra and Fridovich (1972). The ability of superoxide dismutase to inhibit the auto-oxidation of adrenaline (epinephrine) in an alkaline medium (pH 10.2) to adrenochrome makes this reaction a basis for a simple assay for this dismutase. Epinephrine was added to the assay mixture containing tissue supernatant and the change in extinction coefficient was followed at 480 nm in a spectrophotometer.

Determination of catalase (CAT) activity

Catalase activity was determined according to the method of Aebi (1984). The method involves spectrophotometric monitoring the rate at which H_2O_2 is decomposed by the enzyme catalase at 240 nm.

Determination of glutathione peroxidase (GPX) activity

Hepatic GPx activity was determined spectrophotometrically according to the method of Rotruck et al. (1973). The absorbance was read at 412 nm against a blank.

Determination of hydrogen peroxide (H_2O_2) level

The level of hydrogen peroxide generated was determined as described by the method of Wolf (1994). This is based on the principle of ferrous oxidation with xylenol orange reagent. The colour development was measured spectrophotometrically at 560 nm.

Protein estimation

The protein content of the liver homogenate was determined according to the method Lowry et al (1951) using bovine serum albumin as a standard.

Histopathological studies

Liver tissues were fixed in 10% neutral buffered formalin. These tissues were processed for histopathological examination using a routine paraffin-wax embedding method. Sections of about 5 µm thickness were stained with haematoxylin-eosin dye. Photomicrographs of the stained tissue sections prepared on slides were taken with a camera attached to the compound light Nikon microscope at × 400 magnification.

Statistical analysis

Data were analysed by statistical program for the social sciences (SPSS) version 17 (SPSS, Chicago, IL, USA) and presented as mean ± standard deviation (S.D). Duncan's multiple range tests was used to compare the significance of differences at $p<0.05$ significance level.

RESULTS

Effect of treatment on body weight and relative liver weight

Results indicated that treatment with the three different doses of *A. hypochondriacus* seed extracts (groups III, IV and V) did not cause any significant changes in body and relative liver weights (Table 1). However, a significant ($p<0.05$) decrease in the percentage body weight and increase in relative liver weight was observed in rats treated with SA compared to control group. Pre-treatment with ESAH at 100, 200 and 300 mg/kg b.w. before administration of SA (groups VI, VII and VIII) did not cause any significant changes in body weight and relative liver weight when compared with control.

Effect of ethanol extract of *A. hypochondriacus* seed on sodium arsenite-induced micronucleated polychromatic erythrocytes (mPCEs)

Treatment with sodium arsenite induced significantly ($p<0.05$) formation of micronuclei in the polychromatic

Table 1. Percentage change in body weight of experimental animals administered ethanol extract of *A. hypochondriacus* seed (ESAH), mean liver weight and percentage mean relative liver weight.

Group	Treatment	Mean body weight change (%)	Mean liver weight (g)	Mean relative liver weight (%)
I	Distilled water alone	36.00[a]	6.61±1.45	3.68±0.84[b]
II	[#]SA alone	20.00[b]	10.51±2.21	6.42±2.06[a]
III	100 mg/kg ESAH	26.67[a]	7.46±0.42	3.93±0.29[b]
IV	200 mg/kg ESAH	30.00[a]	6.99±2.52	3.65±1.60[b]
V	300 mg/kg ESAH	26.67[a]	8.51±3.27	4.57±2.03[b]
VI	100 mg/kg ESAH + SA	30.00[a]	7.22±2.28	3.69±1.09[b]
VII	200 mg/kg ESAH + SA	30.00[a]	7.16±0.83	3.58±0.41[b]
VIII	300 mg/kg ESAH + SA	28.33[b]	6.46±1.03	3.32±0.92[b]

[#]SA = sodium arsenite. Values are expressed as means ± standard deviation; n = 6 for each treatment. Mean values within a column with same superscript letter are not significantly different, while different superscripts denote significant difference ($p<0.05$).

erythrocytes of the rat bone marrow cells and the degree of induction was about 2.3-fold compared to control (Table 2). Pre-treatment with ESAH at the dose of 300 mg/kg b.w. before administration of SA resulted in a significant ($p<0.05$) decrease in the induction of mPCEs to the value close to control group.

Effect of ethanol extract of *A. hypochondriacus* seed on sodium arsenite-induced changes in serum enzyme activities

Hepatic injury induced by SA caused significant changes of marker enzymes as ALT and AST activities increased significantly ($p<0.05$) compared to control group (Table 3). The changes in marker enzymes of pre-treated groups at 100, 200, 300 mg/kg doses of ESAH before administration of SA (groups VI, VII and VIII) as ALT 14.12 ($p<0.05$), 12.62 ($p<0.05$, 9.18 ($p<0.05$), respectively and AST 26.90 ($p<0.05$), 24.30 ($p<0.05$), 23.30 ($p<0.05$), respectively, are significantly different compared to SA treated group ALT (16.74) and AST (54.61). Maximum protection was observed in marker enzyme at the dose of 300 mg/kg b.w. of ESAH close to the value of control - ALT (9.39) and AST (38.17). In the case of ALP there was no significant difference observed between tested groups. The increased activity of GGT (3.47) induced by SA (group II) was significantly different from the level observed when rats were administered 300 mg/kg b.w. ESAH (group V).

Effect of ethanol extract of *A. hypochondriacus* seed on sodium arsenite-induced changes in antioxidant enzyme activity in liver

Treatment with SA caused a significant ($p<0.05$) decrease in the activities of SOD, CAT and GPx in liver tissue compared to control (Table 4). Treatment with ESAH alone at the dose of 200 and 300 mg/kg (groups IV and V) caused significant ($p<0.05$) increase in the activities of SOD and CAT compared to SA-treated rats. The GPx activity significantly ($p<0.05$) increased in treatment with 300 mg/kg ESAH alone (group V), when compared with SA-treated group. The CAT and GPx activities had significantly increased in ESAH pre-treated groups at the dose of 200 and 300 mg/kg (groups VII and VIII). The activity of GPx also increased significantly ($p<0.05$) at the dose of 100 mg/kg (group VI) when compared to SA group. The significant change as CAT 99.20 ($p<0.05$), 117.63 ($p<0.05$) and GPx 473.08 ($p<0.05$), 483.80 ($p<0.05$) ameliorated the effect of SA-induced toxicity as CAT 71.38 ($p<0.05$) and GPx 398.45 ($p<0.05$) close to control CAT (123.83), GPx (539.75). In the different dose levels of ESAH, 300 mg/kg showed maximum protection.

Effect of seed extracts of *A. hypochondriacus* on sodium arsenite-induced changes in hepatic lipid peroxidation and hydrogen peroxides

The results showed significant ($p<0.05$) elevation in the levels of MDA (11.15) and hydrogen peroxides (41.42) in SA intoxicated rats when compared to control, MDA (4.71) and H_2O_2 (29.87); respectively (Table 4). Treatment with ESAH alone at doses of 100, 200 and 300 mg/kg (groups III, IV and V) caused significant ($p<0.05$) decrease in the levels of MDA and H_2O_2 compared to control. Pre-treatment with ESAH at doses of 100 and 200 mg/kg before administration of SA (groups VI and VII) did not cause any significant reduction in the levels of MDA and H_2O_2 when compared with control. Pre-treatment with 300 mg/kg ESAH before administration of SA (Group VIII) as MDA (4.40), H_2O_2

Table 2. Induction of micronucleated polychromatic erythrocytes (mPCEs) in rat bone marrow cells after exposure to ethanol extract of *A. hypochrondriacus* seed (ESAH) and/or sodium arsenite.

Groups	Number of mPCE/1000
Control	4.43±0.32^{d}
#SA only	10.24±0.43^{a}
100 mg/kg ESAH	5.74±0.70^{c}
200 mg/kg ESAH	4.93±0.36^{cd}
300 mg/kg ESAH	4.22±0.44^{d}
100 mg/kg ESAH + SA	6.84±0.39^{b}
200 mg/kg ESAH + SA	5.53±0.45^{c}
300 mg/kg ESAH + SA	4.96±0.20^{cd}

#SA = sodium arsenite. Values are expressed as means ± standard deviation; n = 6 for each treatment. Mean values within a column with same superscript letters are not significantly different, while different superscripts denote significant difference ($p<0.05$).

(31.66) significantly ($p<0.05$) reduced the elevation of SA-induced lipid peroxidation to a level close to control as MDA (4.71) and H_2O_2 (29.87).

Histopathological observations

Results of the histopathological examination (Figure 1) of liver sections in control animals (group I) showed central vein surrounded by hepatic cord of cells, while SA treated rats liver section (group II) showed severe hepatic necrosis, with periportal cellular infiltration by mononuclear cells. Treatment with the three different doses of ESAH alone (groups III, IV and V) animals showed normal liver architecture similar to that observed in control group. Groups VI, VII and VIII animals pre-treated with different doses of the seed extracts before SA administration showed absence of necrosis and mild inflammatory collections around central vein. Normal liver architecture was observed in animals pre-treated with ESAH at 300 mg/kg b.w. (group VIII). This demonstrated hepatoprotective potential of the seed extract at a higher dose of 300 mg/kg b.w. compared to treatment with 100 and 200 mg ESAH/kg body weight.

DISCUSSION

Arsenite exerts its cellular toxicity by binding to sulfhydryl groups which results in enzyme inhibition (El-Demerdash et al., 2009). Arsenic toxicity involves oxidative damage in organs (Izquierdo-Vega et al., 2006) mainly by interaction of arsenic with protein thiols that are central components of redox-sensitive proteins in redox signalling and control pathways (Hansen et al., 2006). Sodium arsenite has been shown to decrease glutathione levels and increase lipid peroxidation in liver, kidney and heart (Ramos et al., 1995). In addition elevation in the level of serum enzymes (ALT, ALP, AST and GGT) has emerged as an index of a liver lesion (Zeashan et al., 2008).

The observed reduction in the mean body weight of experimental rats treated with sodium arsenite when compared with control group in this study is in agreement with result of El-Demerdash et al. (2009) and Bashir et al. (2006). The weight loss in rats exposed to arsenic has been associated with the induction of diabetes mellitus by the arsenite (Rahman et al., 1998). Kaltreider et al. (2001) demonstrated that exposure to low levels of arsenic altered hormonal function in the glucocorticoid system. The glucocorticoid hormones play an important role in glucose regulation as well as carbohydrate, lipid and protein metabolism. Dysfunction in the glucocorticoid system has been linked to weight loss/gain; this may explain the loss in weight observed in this study. The increase in relative liver weight in the rats treated with sodium arsenite alone when compared with control group is in agreement with previous findings in rats and mice (Gbadegesin and Odunola, 2010; Jana et al., 2006; Odunola et al., 2011; Yousef et al., 2008). Sodium arsenite intoxication has been shown to compromise the integrity of the liver in mouse, rat, fish and goat (Sharma et al., 2009; Yousef et al., 2008). The results of this study showed that sodium arsenite significantly ($p<0.05$) induced the formation of micronuclei in the polychromatic erythrocytes of the rat bone marrow cells. The induction by sodium arsenite led to about 2.3 times the mean number of mPCEs formation in the bone marrow cells of control group. Pre-treatment with ESAH at 100, 200 or 300 mg/kg before SA administration (Groups VI, VII and

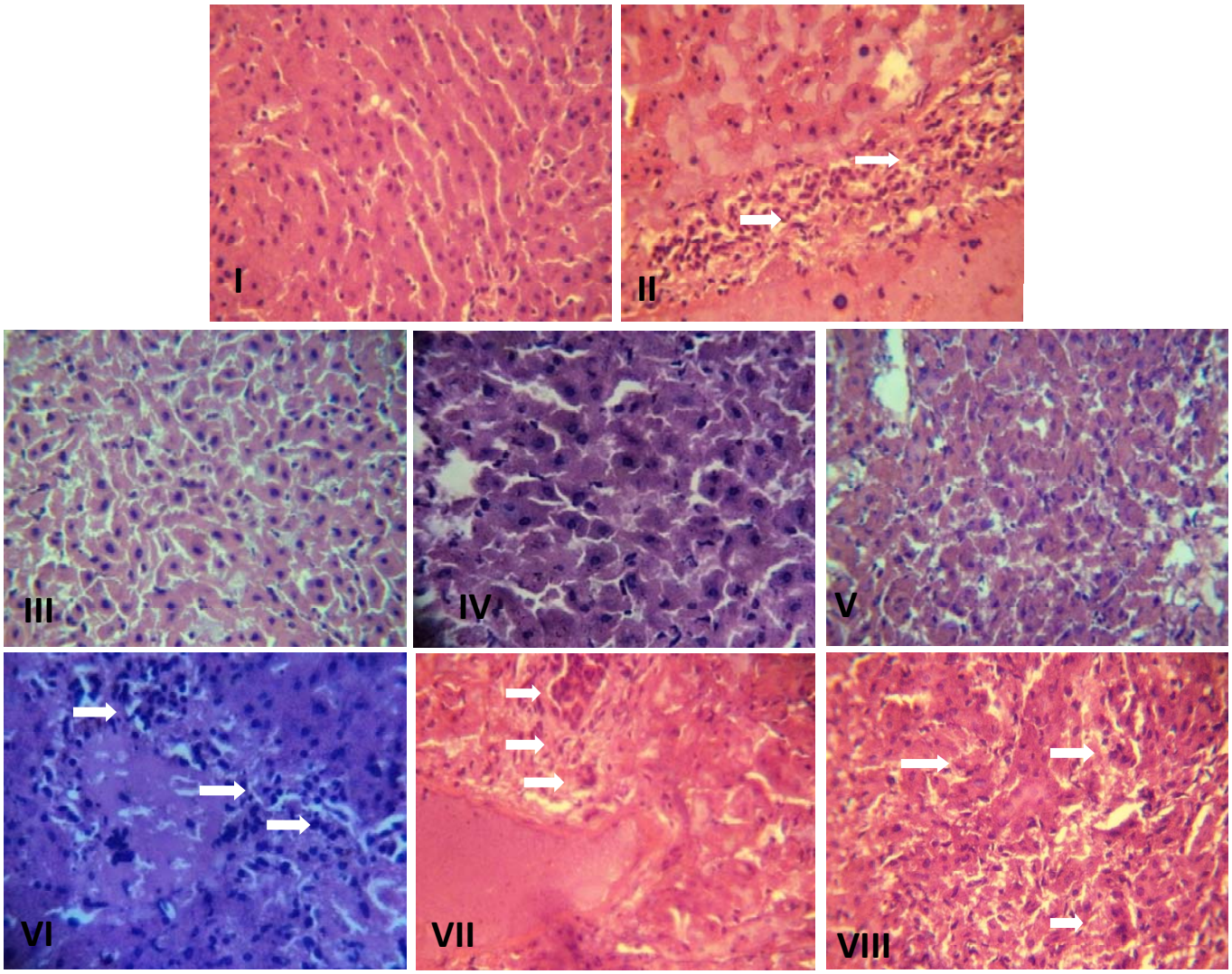

Figure 1. Results of the histopathological examination of liver sections. (I) Liver section of control rats showing normal architecture (× 400); (II) Liver section of rats treated with sodium arsenite (2.5 mg/kg b.w.) showing severe hepatic necrosis, periportal cellular infiltration by mononuclear cells (× 400); (III) Liver section of rat treated with 100mg/kg b.w. of *A. hypochondriacus* seed extract showing no visible lesions (× 400); (IV and V) Liver section of rat treated with 200 and 300 mg/kg *A. hypochondriacus* seed extract respectively, showing no visible lesions, cytoplasm and nucleus are intact and well differentiated (× 400); (VI) Liver section of rat treated with 100mg/kg *A. hypochondriacus* seed extract and 2.5 mg/kg b.w. sodium arsenite. Inflammation changes were observed, with mild hepatic degeneration (× 400); (VII) Liver section of rat treated with 200 mg/kg *A. hypochondriacus* seed extract and 2.5 mg/kg b.w. sodium arsenite. Cellular infiltration around the portal area is mild (× 400); (VIII) Liver section of rat treated with 300 mg/kg *A. hypochondriacus* seed extract and 2.5 mg/kg b.w. sodium arsenite showing very mild hepatic degeneration (×400).

VIII) significantly ($p<0.05$) reduced the frequency of mPCEs formation in bone marrow cells when compared with group treated with SA alone. Das et al. (1993) reported a similar decrease in the degree of mPCEs formation in mice pre-treated with garlic extracts before sodium arsenite challenge.

The increased activities of serum marker enzymes (AST, ALT, ALP and GGT) are conventional indicators of liver injury (Achliya et al., 2004; Thabrew et al., 1987). Yadav and Dixit (2003) reported the ability of a drug to reduce the injurious effects or to restore the normal hepatic physiological mechanisms that have been disturbed by a hepatotoxin, as the index of its protective effects. The present study revealed a significant ($p<0.05$) increase in the level of ALT and AST on administration of sodium arsenite, this may be due to the increased permeability of the plasma membrane indicating considerable hepatocellular damage. Similar results of elevated serum enzymes were earlier reported (Mallick et al., 2003; Odunola et al., 2011). The serum marker enzymes are mainly intracellular ones which mean that any damage to the hepatocytes could lead to their

Table 3. Effect of ethanol extract of *A. hypochondriacus* seed (ESAH) on serum alanine amino transaminase (ALT), aspartate amino transaminase (AST), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) in rats treated with sodium arsenite.

Group	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
Treatment	Distilled water	#SA alone	100 mg ESAH	200 mg ESAH	300 mg ESAH	100 mg ESAH +SA	200 mg ESAH+ SA	300 mg ESAH+ SA
ALP(U/L)	66.93±10.42^{a}	71.76±19.52^{a}	62.10±5.28^{a}	50.37±15.51^{a}	43.47±16.77^{a}	69.69±4.14^{a}	53.82±17.24^{a}	49.68±9.82^{a}
ALT (U/L)	9.39±1.32^{d}	16.74±1.04^{a}	10.35±0.94^{cd}	10.58±2.26^{cd}	8.76±2.21^{d}	14.12±0.80^{b}	12.62±0.33^{bc}	9.18±0.23^{d}
AST (U/L)	38.17±3.61^{b}	54.61±11.94^{a}	38.96±3.60^{b}	33.13±1.45^{bc}	32.44±1.23^{bc}	26.90±2.89^{c}	24.30±2.18^{c}	23.30±2.35^{c}
GGT (U/L)	1.74±0.67^{ab}	3.47±0.95^{a}	2.32±1.64^{ab}	1.74±0.67^{ab}	1.16±0.00^{b}	3.18±0.58^{ab}	2.61±1.11^{ab}	1.74±0.67^{ab}

#SA = sodium arsenite; Values are expressed as means ± standard deviation; n = 6 for each treatment; Mean values within a row with same superscript letter are not significantly different, while different superscript denote significant difference ($p<0.05$).

Table 4. Changes in the activities of SOD, CAT, GPx, MDA, H_2O_2 and protein in liver of male rats treated with ethanol extract of *A. hypochondriacus* seed (ESAH) and/or sodium arsenite.

Treatment	Distilled water	#SA alone	100 mg/kg ESAH	200 mg/kg ESAH	300 mg/kg ESAH	100 mg/kg ESAH+SA	200 mg/kg ESAH+SA	300 mg/kg ESAH+SA
	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
SOD	4.22±0.40^{a}	2.82±0.33^{dc}	3.32±0.47^{bc}	3.52±0.35^{b}	3.55±0.26^{b}	2.39±0.11^{d}	3.12±0.21^{bc}	3.31±0.47^{bc}
CAT	123.83±20.50^{a}	71.38±8.33^{e}	71.95±4.81^{de}	86.05±6.99^{cd}	110.05±5.34^{ab}	80.99±11.69^{e}	99.20±1.08^{bc}	117.63±7.55^{a}
GPX	539.75±55.70^{a}	398.45±31.25^{cd}	361.10±44.15^{d}	447.05±56.90^{bc}	482.29±55.61^{ab}	451.89±15.23^{bc}	473.08±24.02^{b}	483.80±24.99^{ab}
MDA	4.71±1.48^{c}	11.15±0.85^{a}	4.52±0.55^{c}	4.38±0.62^{c}	4.15±0.75^{c}	6.54±0.46^{b}	5.86±0.53^{b}	4.40±0.40^{c}
H_2O_2	29.87±6.70^{c}	41.42±5.89^{a}	21.85±2.57^{d}	27.33±2.36^{c}	29.39±4.80^{c}	36.38±1.33^{b}	34.19±2.04^{b}	31.66±1.37^{c}
Protein	0.66±0.06^{a}	0.60±0.05^{ab}	0.50±0.02^{cd}	0.53±0.04^{bc}	0.54±0.05^{bc}	0.44±0.05^{e}	0.49±0.02^{cde}	0.43±0.04^{e}

#SA = sodium arsenite; Values are expressed as means ± standard deviation; n = 6 for each treatment. Mean values within a column with same superscript letter are not significantly different, while different superscripts denote significant difference ($p<0.05$). Superoxide dismutase (SOD) activity is expressed as units/mg protein; catalase (CAT) activity as µmol H_2O_2 consumed/mg protein; glutathione peroxidase (GPx) as units/mg protein; malondiadehyde (MDA) as units/g tissue × 10^{-6}; Hydrogen peroxide (H_2O_2) as µmol/L.

release into the plasma leading to the observed increase in plasma activities. Hepatocellular necrosis leads to elevation of the serum marker enzymes, which are released from liver cells into blood (Ashok et al., 2001). In addition, the increased GGT activity of rats exposed to SA observed in this study is indicative of oxidative stress and cytogenetic damage (Lee et al., 2006). Pre-treatment with ESAH at the different dose levels of 100, 200 and 300 mg/kg (Groups VI, VII and VIII) attenuated the increased levels of the serum enzymes produced by SA and caused a subsequent recovery towards normalization.

It has been reported that one of the principal causes of SA induced liver injury is formation of lipid peroxides by free radical derivatives of SA (Manna et al., 2007). The body has an effective defence mechanism to prevent and neutralize the free radical induced damage. This is proficient by a set of endogenous antioxidant enzymes such as SOD, CAT, GST and GPx. These enzymes constitute the first line of cellular antioxidant defence and provide a mutually supportive team of defence against ROS (Amresh et al., 2007a). The reduced activities of SOD, CAT and GPx observed in this study points out hepatic damage in the rats administered with SA, and is probably due to exhaustion of these enzymes to scavenge excessively-generated superoxide and hydrogen peroxides, respectively; in tumour cells

(Manoharan et al., 2006). The reduced activity of SOD, CAT and GPx of rats treated with SA is in agreement with previous results (Bashir et al., 2006; Gopalkrishnan and Rao, 2006; Wu et al., 2001). Reduced activities of SOD, CAT and GPx in erythrocytes have been reported in cancer (Balasenthil et al., 2000). Reduced activity of CAT after exposure to sodium arsenite in this study could be correlated to increased generation of hydrogen peroxide. Similar observations were reported by El- Demerdash et al. (2009), during treatment of experimental rats with sodium arsenite. The decreased SOD activity in the liver suggests that accumulation of superoxide anion radical might be responsible for increased lipid peroxidation following arsenic treatment. Free radicals are produced by arsenic treatment (Yamanaka et al., 1990), by the reaction of molecular oxygen with dimethylarsine, a metabolite of dimethyl arsenic acid. Pre-treatment with 100, 200 and 300 mg/kg b.w. of *A. hypochondriacus* seed extract increased the activities of SOD, CAT and GPx in a dose dependent manner. This observation is similar to report of El-Demerdash et al., (2009) which showed that treatment with *Curcumin* extract improved the levels of liver SOD and CAT activity in rats.

Free radical-induced lipid peroxidation is regarded as one of the basic mechanism of cellular damage and therefore, the extent of tissue damage can be monitored by measuring the concentration of plasma or serum lipid peroxides (Selvendiran and Sakthisekaran, 2004). In this study, elevation of lipid peroxidation and hydrogen peroxides in the liver of rats treated with SA suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defence mechanisms to prevent the formation of excessive free radicals (Amresh et al., 2007b). Increase in plasma lipid peroxides in DMBA-painted animals has also been observed and was due to overproduction and diffusion from the damaged erythrocyte membranes and some other host tissues such as the liver (Manoharan et al., 2006). Increased plasma lipid peroxidation has been reported in several types of cancer patients (Ozdemirler et al., 1998). Pre-treatment with 100, 200 and 300 mg/kg ESAH significantly reduced the elevated levels of MDA and H_2O_2 caused by SA; restoring towards normalization. Similar observation was reported in CCl_4-induced toxicity in rats and administration of different doses of ethanol extracts of *Amaranthus spinosus* significantly prevented the heave in levels of MDA and H_2O_2 (Zeashan et al., 2008). It is likely that the mechanism of hepatoprotection of extract of *A. hypochondriacus seed* may be due to its antioxidant activity.

Conclusion

From this study, *A. hypochondriacus* seed extract seems to exhibit hepatoprotective effects and mitigates sodium arsenite-induced oxidative damage in rats. The ESAH has shown dose dependent activity as 300 mg/kg b.w. has greater activity which is comparable with the control group.

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Conflict of interest statement

The authors declare that there is no conflict of interests.

REFERENCES

Achliya GS, Wadodkar SG, Dorle AK (2004). Evaluation of hepatoprotective effect of *Amalkadi Ghrita* against carbon tetrachloride induced hepatic damage in rats. J. Ethnopharmacol. 90:229-232.

Aebi H (1984). Catalase *in vitro*. Methods Enzymol. 105:121-126.

Amresh G, Kant R, Zeashan H, Gupta RJ, Rao Ch V, Singh PN (2007a). Gastroprotective effects of ethanolic extract from *Cissampelos pareira* in experimental animals. J. Nat. Med. 61:323-328.

Amresh G, Rao Ch V, Singh PN (2007b). Antioxidant activity of *Cissampelos pareira* on benzo(a)pyrene-induced mucosal injury in mice. Nutr. Res. 27:625-632.

Ashok Shenoy K, Somayaji SN, Bairy KL (2001). Hepatoprotective effects of Ginkgo biloba against carbon tetrachloride induced hepatic injury in rats. Indian J. Pharmacol. 33:260-266.

Agency for Toxic Substances and Disease Registry (ATSDR) (2007). CERCLA Priority List of Hazardous Substances. Agency for Toxic Substances and Disease Registry.

Balasenthil S, Arivazhagan S, Nagini S (2000). Garlic enhances circulatory antioxidants during 7,12 dimethylbenz(a)anthracene induced hamster bucchal pouch carcinogenesis. J. Ethnopharmacol. 72(3):429-433.

Bashir S, Sharma Y, Irshad M, Gupta SD, Dogra TD (2006). Arsenic-induced cell death in liver and brain of experimental rats. Basic Clin. Pharmacol. Toxicol. 98:38-43.

Baublis AJ, Decker EA, Clydesdale FM (2000). Antioxidant effect of aqueous extracts from wheat based ready-to-eat breakfast cereals. Food Chem. 68:1-6.

Berghofer E, Schoenlechner R (2002). In Grain amaranth pseudocereals and less common cereals: grain properties and utilization potential. Belton PS, Taylor JRN (Eds.). Berlin: Springer-Verlag. Heidelberg, Germany pp. 219-260.

Bonaccio M, Iacoviello L, de Gaetano G, Moli-Sani I (2012). The Mediterranean diet: the reasons for a success. Thromb. Res. 129(3):401-404.

Bressani R (1989). The proteins of grain amaranth. Food Rev. Int. 5:13-38.

Chatterjee A, Das D, Mandal BK, Chowdhury TR, Samanta G, Chakraborty D (1995). Arsenic in ground water in six districts of West bengal, India: the biggest arsenic calamity in the world. Part 2. Arsenic concentration in drinking water, hair, nails, urine, skin-scale and liver tissue (biopsy) of the affected people. Analyst 120(3):643-656.

Chen Z, Cai Y, Solo-Gabriele H, Snyder GH, Cisar JL (2006). Interactions of arsenic and the dissolved substances derived from turf soils. Environ. Sci. Tech. 40(15):4659-4665.

Chiou HY, Chiou ST, Hsu YH, Chou YL, Tseng CH, Wei ML, Chen CJ (2001). Incidence of transitional cell carcinoma and arsenic in drinking water: a follow-up study of 8, 102 residents in an arseniasis-endemic area in North-eastern Taiwan. Am. J. Epidemiol. 135(5):411

-418.
Chowdhury UK, Rahman MM, Mondal B, Paul K, Lodh D, Biswas BK (2001). Ground water arsenic contamination and human suffering in West Bengal, India and Bangladesh. Environ. Sci. 8:393-415.
Cullen WR (2008). Is Arsenic an Aphrodisiac? The Sociochemistry of an element. Royal Society of Chemistry, Cambridge, UK.
Das D, Chatterjee A, Mandal BK, Samanta G, Chakroborty D, Chanda B (1995). Arsenic in groundwater in six districts of West Bengal, India: the biggest arsenic calamity in the world. Part 2. Arsenic concentration in drinking water, hair, nails, urine, skin-scales and liver tissues (biopsy) of the affected people. Analyst 120:917-924.
Das T, Roychoudhury A, Sharma A, Talukder G (1993). Modification of Clastogenicity of Three known Clastogens by Garlic extract in Mice *in vivo*. Environ. Mol. Mutagen. 21(4):383-388.
El-Demerdash FM, Yousef MI, Radwan FME (2009). Ameliorating effect of curcumin on sodium aresenite-induced oxidative damage and lipid peroxidation in different rat organs. Food Chem. Toxicol. 47:249-254.
Emmons CL, Peterson DM, Paul GL (1999). Antioxidant capacity of oat (*Avena sativa* L.) extracts. In vitro antioxidant activity and contents of phenolic and tocol antioxidants. J. Agric. Food Chem. 47(12):4894-4898.
Escudero NL, Albarracin G, Fernández S, De Arellano LM, Mucciarelli, S (1999). Nutrient and antinutrient composition of *Amaranthus muricatus*. Plant Foods Hum. Nutr. 54:327-336.
Frost F, Frank D, Pierson K, Woodru L, Raasina B, Davis R, Davies J (1993). A seasonal study of arsenic in groundwater, Snohomish County, Washington, USA. Environ. Geochem. Health 15:209-213.
Gbadegesin MA, Odunola OA (2010). Aqueous and ethanolic leaf extracts of Ocimum basilicum (sweet basil) protect against sodium arsenite-induced hepatotoxicity in Wistar rats. Niger. J. Physiol. Sci. 25:29-36.
Gopalkrishnan A, Rao MV (2006). Amelioration by vitamin A upon arsenic induced metabolic and neurotoxic effects. J. Health Sci. 52:568-577.
Guha MDN (2005). Effect of chronic intake of arsenic-contaminated water on liver. Toxicol. Appl. Pharmacol. 206:169-175.
Handelman GJ, Cao G, Walter MF, Nightingale ZD, Paul GL, Prior RL, Blumberg JB (1999). Antioxidant capacity of oat (*Avena sativa* L.) extracts. 1. Inhibition of low-density lipoprotein oxidation and oxygen radical absorbance capacity. J. Agric. Food Chem. 47(12):4888-4893.
Hansen JM, Zhang H, Jones DP (2006). Differential oxidation of thioredoxin-1, thioredoxin-2 and glutathione by metal ions. Free Radic. Biol. Med. 40:138-145.
Hughes MF, Kenyon EM, Edwards BC, Mitchell CT, Del Razo LM, Thomas DJ (2003). Accumulation and metabolism of arsenic in mice after repeated oral administration of arsenate. Toxicol. Appl. Pharmacol. 191(3):202-210.
International Agency for Research on Cancer (IARC) (2004). International Agency for Research on Cancer Betal Quid and Areca Nut chewing and some Areca Nut derived Nitrosamines. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol 85. Lyon, France: IARC.
Izquierdo-Vega JA, Soto CA, Sanchez-Peña LC, De Vizcaya- Ruiz A, Del Razo LM (2006). Diabetogenic effects and pancreatic oxidative damage in rats subchronically exposed to arsenite. Toxicol. Lett. 160(2):135-142.
Jana K, Jana S, Samanta PK (2006). Effects of chronic exposure to sodium arsenite on hypothalamo-pituitary-testicular activities in adult rats: possible an estrogenic mode of action. Reprod. Biol. Endocrinol. 4:1-13.
Jayanthika B, Wijeweera A, Gandolfi J, Parrish A, Clark Lantz R (2001). Sodium arsenite enhances AP-1 and NF_kB DNA binding and induces stress protein expression in precission-cut Rat-Lung slices. Toxicol. Sci. 61(2):283-294.
Jian Z (2007). Nutraceuticals, Nutritional Therapy, Phytonutrients, and Phytotherapy for Improvement of Human Health: A Perspective on Plant Biotechnology Application. Recent Pat. Biotechnol. 1:75-97.
Jones JM, Engleson J (2010). Whole grains: benefits and challenges. Ann. Rev. Food Sci. Technol. 1:19-40.
Kaltreider RC, Davis AM, Lariviere JP, Hamilton JW (2001). Arsenic alters the function of the glucocorticoid receptor as a transcription factor. Environ. Health Perspect. 109(3):245-251.
Kumar ABS, Lakshman K, Swamy NVB, Kumar APA, Shekar SD, Manoj B, Vishwantha, GL (2011). Hepatoprotective and Antioxidant Activities of *Amaranthus viridis* Linn. Macedonian J. Med. Sci. 4(2):125-130.
Lee DH, Lim JS, Song K, Boo Y, Jacobs DR (2006). Grade Association of Blood Lead and Urinary Cadmium Concentrations with Oxidative Stress-Related Markers in the Population. Results from the Third National Health and Nutrition Examination Survey. Environ. Health Perspect. 114(3):354.
Lillioja S, Neal AL, Tapsell L, Jacobs DR, Jr (2013). Whole grains, type 2 diabetes, coronary heart disease, and hypertension: links to the aleurone preferred over indigestible fiber. Biofactors 39(3):242-258.
Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the Folin phenol Reagent. J. Biol. Chem. 193:269-275.
Lupton JR, Meacher MM (1998). Radiographic analysis of the effect of dietary fibers on rat colonic transit time. Am. J. Physiol. 255(5):633-639.
Mallick P, Mallick JC, Guha B, Khuda-Bukhsh AR (2003). Ameliorating effect of microdoses of a potentized homeopathic drug, Arsenicum Album, on arsenic-induced toxicity in mice. BMC Complement. Altern. Med. 22:3-7.
Manoharan S, Kavitha K, Senthil N, Renju GL (2006). Evaluation of anticarcinogenic effects of Clerodendron inerme on 7,12-dimethylbenz(a) anthracene-induced hamster bucchal pouch carcinogenesis. Singapore Med. J. 47(12):1038-1043.
Manna P, Sinha M, Pal P, Sil PC (2007). Arjunolic acid, a triterpenoid saponin, ameliorates arsenic-induced cyto-toxicity in hepatocytes. Chem Biol. Interact. 170(3):187-200.
Meyer KA, Kushi LH, Jacob DR Jr, Slavin J, Sellers TA, Folsom AR (2000). Carbohydrates dietary fiber, incident type 2 diabetes mellitus in older women. Am. J. Clin. Nutr. 71(4):921-930.
Miller BL, Boone K, Cummings JL, Read SL, Mishkin F (2000). Functional correlates of musical and visual ability in frontotemporal dementia. Br. J. Psychiatry 176:458-463.
Misra HP, Fridovich I (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247(10):3170-3175.
Muyonga JH, Nabakabya D, Nakimbugwe, DN, Masinde D (2008). Efforts to promote amaranth production and consumption in Uganda to fight malnutrition. Chapter 8. Using Food Science and Technology to Improve Nutrition and Promote National Development, (Eds: Robertson, G.L. and Lupien, J.R. (Eds), International Union of Food Science and Technology.
Nicodemus KK, Jacobs DR Jr, Folsom AR (2001). Whole and refined grain intake and risk of incident postmenopausal breast cancer. Cancer Causes Control. 12(10):917-925.
Odunola OA, Akinwumi KA, Ibegbu DM (2011).The Influence of Garlic and Spondias mombin on Sodium Arsenite induced Clastogenicity and Hepatotoxicity in Rats. Pac. J. Sci. Technol. 12(2):401-409.
Ozdemirler G, Pabuccuoglu H, Bulut T (1998). Increased lipid peroxide levels and antioxidant system in colorectal cancer. J. Cancer Res. Clin. Oncol. 128:555-559.
Parvez F, Yu C, Maria A, Hussain IAZM, Hassina M, Ratan D, Alexander G, Joseph HG, Habibul A (2006). Prevalence of Arsenic Exposure from Drinking Water and Awareness of Its Health Risks in a Bangladeshi Population: Results from a Large Population-Based Study. Environ. Health Perspect. 114(3):355-359.
Patrick L (2003). Toxic metals and antioxidants. Part II: The role of antioxidants in arsenic and cadmium toxicity. Altern. Med. Rev. 8:106-128.
Preston RJ, Dean BJ, Galloway S, Holden H, McFee AF, Shelby M (1987). Mammalian *in vivo* cytogenic assays: analysis of chromosome aberrations in bone marrow cells. Mutat. Res. 189:157-165.
Rahman M, Tondel M, Ahmad SA (1998). Diabetes mellitus associated with arsenic exposure in Bangladesh. Am. J. Epidemiol. 148(2):198-203.
Ramos O, Carrizales L, Yarez L, Barriga F (1995). Arsenic increased lipid peroxidation in rat's tissues by a mechanism independent of glutathione levels. Environ. Health Perspect. 103(1):85-88.

Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hekstra WG (1973). Selenium, biochemical role as a component of glutathione peroxidase purification and assay. Science 179:588-590.

Salamone M, Heddle J, Stuart E, Katz M (1980). Towards an improved micronucleus test: studies on 3 model agents, mitomycin C., cyclophosphamide and dimethylbenzanthracene, Mutat. Res. 74(5):347-56.

Selvendiran K, Sakthisekaran D (2004). Chemopreventive effect of piperine on modulating lipid peroxidation and membrane bound enzymes in benzo(a)pyrene induced lung carcinogensis. Biomed. Pharmacother. 58:264-267.

Taylor JRN, Parker ML (2002). Quinoa. In: Pseudocereals and less common cereals: grain properties and utilization: (Eds: Belton P.S. and Taylor J.R.N.). Berlin: Springer-Verlag pp. 93-122.

Thabrew MI, Joice PDTM, Rajatissa WA (1987). Comparative study of efficacy of Paetta indica and Osbeckia octandra in the treatment of liver dysfunction. Planta Med. 53:239-241.

Tseng CH, Tseng CP, Chiou HY, Hsueh YM, Chong CK, Chen CJ (2002). Epidemiologic evidence of diabetogenic effect of arsenic. Toxicol. Lett. 133:69-76.

Valentine JL (1994). Review of health assessments for US/Canada populations exposed to arsenic in drinking water. In: Arsenic Exposure and Health (a special issue of Environmental Geochemistry and Health, Vol.16) (Chappell, W.R., Abernathy, C.O., Cothern, C.R., eds). Surrey, UK: Laws and Stimson Associates pp. 139-152.

Varshney R, Kale RK (1990). Effects of calmodulin anatagonists on radiation induced lipid peroxidation in microsomes. Int. J. Radiat. Biol. 58:733-743.

Waalkes MP, Ward JM, Liu J, Diwan BA (2003). Transplacental carcinogenicity of inorganic arsenic in the drinking water: induction of hepatic, ovarian, pulmonary, and adrenal tumors in mice. Toxicol. Appl. Pharmacol. 186:7-17.

Wolf PL (1999). Biochemical diagnosis of liver diseases. Indian J. Clin. Biochem. 14:59-90.

Wolf SP (1994). Ferrous ion oxidation in presence of ferric ion indicator xylenol orange for measure- ment of hydroperoxides. Meth. Enzymol. 233:182-189.

Wu MM, Chiou HY, Wang TW, Hsueh YM, Wang IH, Chen CJ, Lee TC (2001). Association of blood arsenic levels with increased reactive oxidants and decreased antioxidant capacity in a human population of north-eastern Taiwan. Environ. Health Perspect. 109(10):1011-1017.

Yadav NP, Dixit VK (2003). Hepatoprotective activity of leaves of *Kalanchoe pinnata* Pers. J. Ethnopharmacol. 86:197-202.

Yamanaka K, Hoshino M, Okamoto M, Sawamura R, Hasegawa A, Okada S (1990). Induction of DNA damage by dimethylarsine, a metabolite of inorganic arsenics, is for the major part likely due to its peroxyl radical. Biochem. Biophys. Res. Commun. 168:58.

Yousef M, El-Demerdash F, Radwan F (2008). Sodium Arsenite Induced Biochemical Perturbations in Rats: Ameliorating Effect of Curcumin. Food Chem. Toxicol. 46:3506-3511.

Zeashan H, Amresh G, Singh S, Rao CV (2008). Hepatoprotective activity of *Amaranthus spinosus* in experimental animals. Food Chem. Toxicol. 46:3417-3421.