Original Article

Ameliorative effect of Azima tetracantha extract on Cadmium induced Hepato-Renal oxidative stress: An in vivo approach

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Abstract

Cadmium (Cd), an environmental pollutant affects humans through contaminated foodstuffs and industrial processes. Cd accumulates in various organs upon exposure and the primary targets are kidney and liver. Its toxicity induces free radicals, creating oxidative stress, defects in antioxidant mechanism, damage to membrane proteins, DNA damage, necrosis, and apoptosis. In this study hepatoprotective and nephroprotective activity of Azima tetracantha towards Cd-induced toxicity was investigated by in vivo approach. Ultra Violet (UV) spectral and Fourier Transform Infra-Red (FTIR) analysis of methanol extract of the Azima tetracantha leaves revealed phenolic compounds. Cadmium Chloride (CdCl2) exposure in Wister Albino rats showed a marked increase in lipid profile, enzymatic antioxidants, and non-enzymatic antioxidants. Co-administration of a methanol extract of Azima tetracantha leaves (500 mg/kg/day) with CdCl2 resulted in the reversal of toxicity effects in the kidney and hepatic cells by maintaining the normal histological architecture. Further, reduced lipid peroxidation and increased antioxidant defense system activity were observed. Hence, the current study suggests that the nephroprotective and hepatoprotective efficacy of Azima tetracantha in Cd-induced toxicity might result from its antioxidant and metal chelating characteristics that might benefit in reaching the best remedy in Cd-induced damage.

Keywords: Cadmium, hepatotoxicity, nephrotoxicity, Azima tetracantha, heavy metal toxicity.

INTRODUCTION

Ingress in population growth and rapid industrialization results in environmental issues such as pollution. Heavy metals (HMs) are toxic, inorganic environmental pollutant released from soil, water, and air and enters the food chain (Almeer et al. 2018; Baskaran et al. 2018). Though heavy metals including copper, cobalt, iron, manganese, molybdenum, and zinc are essential for the normal metabolism of cells, certain heavy metals including arsenic, cadmium, lead, nickel, and mercury do not have useful biological functions and cause toxicity at low dose. (PB Tchounwou, CG Yedjou, AK Patlolla 2012). The characteristics of HMs are (1) high affinity towards thiol, histidine, and carboxyl groups and react indirectly with protein molecules. Hence, these metals can bind with the active site of enzymes, structural components of cells, and transport proteins (2) it’s potential to replace essential elements such as calcium in bones and iron in erythrocytes leading to structural changes and alterations in metabolic pathway (Jaishankar et al. 2014; Puerto-Parejo et al. 2017) (3) accelerate reactive forms of oxygen and modify the antioxidant system (Mao et al. 2018). Cadmium (Cd) is a non-essential HM and a multi-organ toxicant, released into the environment by both natural and anthropogenic operations. The non-biodegradable nature and maximum half-life (>20 years) are the important characteristics of this highly hazardous metal (Bhardwaj et al. 2021). Cd as cadmium chloride is absorbed by the intestine when ingested orally and cadmium oxide is inhaled and absorbed by cells through respiration. This HM enters the cells with ease and gets accumulated in the organs of the central nervous system (CNS), respiratory, excretory, cardiovascular, and reproductive systems (Galán et al. 2001; Kumar et al. 2018). Cd acts as a substitute for Zinc (Zn) and affects several physiological processes (Unsal et al. 2020). Acute or chronic exposure to Cd causes damage to the kidney and liver (Helal Ashour et al. 2014; El-Boshy et al. 2015; Shagirtha et al. 2017; Everson et al. 2018).

The generation of free radicals beyond the capacity of cells to counterbalance xenobiotics results in oxidative stress (Skipper et al. 2016). Further, Cd deposition decreases superoxide dismutase (SOD) and catalase (CAT) activity; impairs antioxidant and oxidant balance (Branca et al.; Gustin et al. 2018). Established theories on the mechanism of Cd-induced toxicity in kidney and liver cells suggest the possible role of oxidative stress, inflammatory responses, and apoptosis (Liu et al.; Gong et al.; Fouad and Jresat 2011; Dkhil et al. 2014). Due to the lack of possible remedies to abate Cd in foodstuffs, humans are continuously exposed to this heavy metal. Hence an alternate approach to dwindle Cd concentration in tissues becomes vital. Nutraceuticals can be effective in reducing the absorption of HMs by tissues, increasing their excretion, and enhancing the antioxidant defense mechanism to chelate HMs. Bioactive compounds of plants serve as effective nutraceuticals (Almeer et al. 2018; Mao et al. 2018) and dietary supplements of plant phenolic compounds with antioxidant activity alleviate the toxicity of HMs (Borowska et al. 2018).

Azima tetracantha, (Family: Salvadoracea) is traditionally used in Siddha and Ayurvedic medicine systems for treating several illnesses (Namik et al. 2016). The plant commonly known as "Mulsangu" or "Cankakiranam" in Tamil is used to treat microbial infections, cancer, ulcer, inflammatory diseases, and fever. The plant possesses antioxidant, analgesic, anti-venom, hepatoprotective and nephroprotective activities (Syed Ismail et al. 1997; Duraipandiyan et al. 2010; Ilyas et al. 2009; Sekar et al. 2010). A. tetracantha is rich in alkaloids, glycosides, terpenoids, saponins, volatile oils, and fatty acids (G Gayathri et al. 2012). Previous literature documented that ethanol extract of A. tetracantha leaves is effective against CCl_4 induced (Ilyas et al. 2009; Ekbote et al. 2010) and sulfate-induced toxicity in liver cells (Manikandaselvi et al. 2013). Similarly, the Cd-induced kidney cell damage was effectively reversed with A. tetracantha treatment (Sunil et al. 2013). Hence, in this background, the leaves of A. tetracantha have been analyzed for their phytochemical constituents and their role in hepatoprotective and nephroprotective activity utilizing in vivo model.

Materials and methods

Collection of Plant material

The leaves of A. tetracantha were collected from a single herb from the campus of Tamil University, Thanjavur, Tamil Nadu, India. The plant was authenticated at Rabinat Herbarium and Centre for Molecular Systematics, St. Joseph College, Tiruchirappalli, Tamilnadu, India, and a voucher specimen (EP001) was submitted.

Preparation of methanol extract of A. tetracantha leaves

The leaves of A. tetracantha were thoroughly rinsed with water multiple times to eliminate contaminants present on the leaf surface. The leaves were dried and finely pulverized. The powder was extracted for 48 hours with 70% methanol using the soxhlet apparatus. After completely evaporating alcohol at reduced pressure using a rotary evaporator, a semisolid extract was obtained. The extract was kept in a desiccator until further experiments.

UV and FTIR analysis

The methanol extract of A. tetracantha leaves was centrifuged for 10 minutes (3000 rpm) and filtered through Whatman No. 1 filter paper for UV and FTIR spectrophotometer analysis. After diluting the centrifuged sample in methanol to a concentration of 1:10, was scanned using a Perkin Elmer Spectrophotometer at wavelengths ranging between 190 and 900 nm. The absorption peaks were analyzed for the identification of functional molecules. The FTIR analysis was carried out in the range of 400-4000cm-1 (Perkin Elmer Spectrophotometer) to identify distinctive peaks that correspond to functional groups.

HPLC analysis

The fermented broth of A. tetracantha leaves (2 ml) was mixed with 50 ml of methanol (95%) and extraction of phytochemicals was carried out for 30 min twice at 45oC with an ultrasonic frequency of 80 kHz. The extract was filtered and concentrated at 50oC under reduced pressure using a rotary evaporator. The crude extract obtained was dissolved in 100 ml of mobile phase and filtered using filter paper and with Millipore membrane (0.45 mm). The sample was injected into HPLC (Paranthaman et al. 2012).

Analysis for flavonoids was carried out using an RP-HPLC technique (WeerasakSamee 2007), with an LC-10ATVp pump, a system controller (SCL 10A), a variable Shimadzu SPD-10ATVp UV-VIS detector, and a loop injector with a loop size of 20 l. The area of the peak was determined with the CLASSVP program. Reverse-phase chromatography was performed at 25oC using a C-18 reverse phase column (2504.6 mm i.d., particle size 5 m, Luna 5 C-18; Phenomenex, Torrance, CA, USA). The generation of solvent gradient was done with a dual pump system by altering the percentage of solvent A (water: acetic acid at 25:1 ratio) and solvent B (Methanol). The percentage of solvent B was increased to 50% (4 min) and 80% (10 min) at a 1 mL/min flow rate. The wavelength was detected at 280 nm.

Inhibition of protein denaturation

The inhibition of protein denaturation was determined using a slightly modified procedure of (Mizushima and Kobayashi 1968; S Sakat, AR Juvekar 2010). Fresh egg albumin (0.2 ml) was added to A. tetracantha leaf extract of different concentrations (100, 200, 300, 400, and 500 µg/ml) and standard diclofenac sodium (100, 200, 300, 400, and 500 µg/mL) and 2.8 mL phosphate-buffered saline (pH 6.4). Denaturation was accomplished by maintaining the reaction mixture at 70°C for 10 minutes in a water bath. Following cooling, the absorbance was read at 660 nm using double distilled water as a blank. Each experiment was repeated three times and the average was calculated. The following formula was used to determine the percentage inhibition of protein denaturation.

$$\%\text{ of Inhibition} = (At - Ac)/Ac\ x100$$

were, At=absorbance of test sample; Ac=absorbance of control

Animals

In this investigation, male Albino rats of the Wister strain weighing around 150-180 g were used. Healthy animals were procured from Bangalore-based, Venkateswar enterprises. The animals were maintained in large polypropylene cages that were lined with rice husk. The animal chamber was adequately ventilated and maintained at standard experimental settings (temperature 27±2oC and 12-hour light/dark cycle). All animals were fed a regular pellet diet and were provided access to water daily. They were exposed to the natural environment for one week before being used in the experiments. The criteria laid down by the Committee for Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India (Ethical No: MC/1416/a/13/CTCSEA) were followed.

Experimental design

The animals were randomly grouped into four with six animals in each group.

Group I: Control rats received only normal saline.

Group II: Rats were orally administered with cadmium (as CdCl2) in 0.5-ml sterile physiological saline at a dose of 5 mg/kg/day for 4 weeks (Renugadevi and Prabu 2010).

Group III: Rats were orally fed with A. tetracantha leaves extract (suspended in distilled water) alone at a dose of 500 mg/kg/day for 4 weeks.

Group IV: Rats received A. tetracantha leaves extract orally (suspended in distilled water) at a dose of 500 mg/kg/day and intra-peritoneal administration of Cd (5 mg/kg/day) after1 h of administration of A. tetracantha leaves extract for 4 weeks.

Biochemical investigations

After 4 weeks of treatment, the animals in each group were decapitated cervically. To separate serum, blood was centrifuged at 1000 g for 15 minutes. The kidney and liver were removed, weighed, and washed with a cold saline solution. The tissue was minced and homogenized (10% w/v) in phosphate buffer (pH 7.4), followed by centrifugation at 3000 g for 10 minutes. The supernatant obtained was utilized in a wide range of biological tests.

Determination of lipid profile

The lipid profile evaluated includes total cholesterol (Allain et al. 1974), TGL (Werner et al. 1981), HDL (Allain et al. 1974), LDL, and VLDL (Friedewald et al. 1972).

Determination of diagnostic markers

The diagnostic markers evaluated include total protein (Lowry et al. 1951), ALP (Kind et al. 1954), SGOT, SGPT (Reitman et al. 1957), LDH (Kind et al. 1954), γ-Glutamyl transferase (Ochei and Kolhatkar, 2000), and Creatinine (Chem and 1945). Urea was estimated using the Sigma Diagnostics kit.

Determination of Oxidative stress markers

The homogenized tissues of the kidney and liver were evaluated for oxidative stress markers. The enzymatic antioxidants, non-enzymatic antioxidants, superoxide dismutase (SOD) (Marklund and Marklund 1974), reduced glutathione (GSH) (Moron et al. 1979), glutathione peroxidase (GPx) (Rotruck et al. 1973), catalase (CAT) (Beers et al. 1952), Ascorbic acid (Vit C) (Omaye et al. 1979), α-tocopherol (Vit E) (Baker and Davies 1997) were determined using standard methods prescribed.

Histopathology

The kidney and liver tissues were fixed in 10% normal saline for 72 h and were sliced to a thickness of 2.1mm each. The slice of tissues was dehydrated using alcohol, treated with paraffin wax, and cast into blocks. The section of tissues was made with a microtome (5µm), attached to a slide, and dried. The slides were observed on a photographic microscope for the evaluation of histological changes.

Result and Discussion

Cadmium is a pervasive environmental pollutant that causes hepatotoxicity, and nephrotoxicity (Ezedom and Asagba 2016; Wang et al. 2017), induces carcinogenicity, mutagenicity, and cytotoxicity (Park et al. 2020). The cells of proximal convoluted tubules in the kidney are the most targeted site of Cd toxicity (Veljkovic et al. 2012). Cadmium complexes with metallothionein (Cd-MT complex) in the liver upon ingestion and are taken by the renal tubules when they are released into circulation. The influence of Cd on liver metabolic enzymes containing sulfhydryl groups and uncoupling of oxidative phosphorylation in mitochondria induces an increase in lipid peroxidation, oxidative stress, inflammatory responses, DNA damage, necrosis, and apoptosis. The acute exposure to Cd causes an imbalance in free radicals and antioxidants which results in oxidative stress. The antioxidant defense system protects against acute toxicity due to Cd exposure (Liu et al. 2016).

Cd is an exceptional HM that cannot generate free radicals. However, they are indirectly involved in free radical production (Galán et al. 2001) via Fenton chemistry. Copper and iron substituting property of Cd results in an increase in the concentration of these unbound ions which initiates oxidative stress through Fenton reactions (Casalino et al. 2002; Waisberg et al. 2003; Wätjen et al. 2004). An excessive amount of ROS and failure of antioxidant defense mechanism lead to cell damage (Namik et al. 2016; Zhang et al. 2019). Plants rich in phenolic compounds possess effective antioxidant activity and A. tetracantha being a rich source of phenolic compounds is being used in curing various ailments including kidney and liver disorders (Ognjanović et al. 2003).

Fig.1: UV- visible spectum of of Azima tetracantha leaves extract

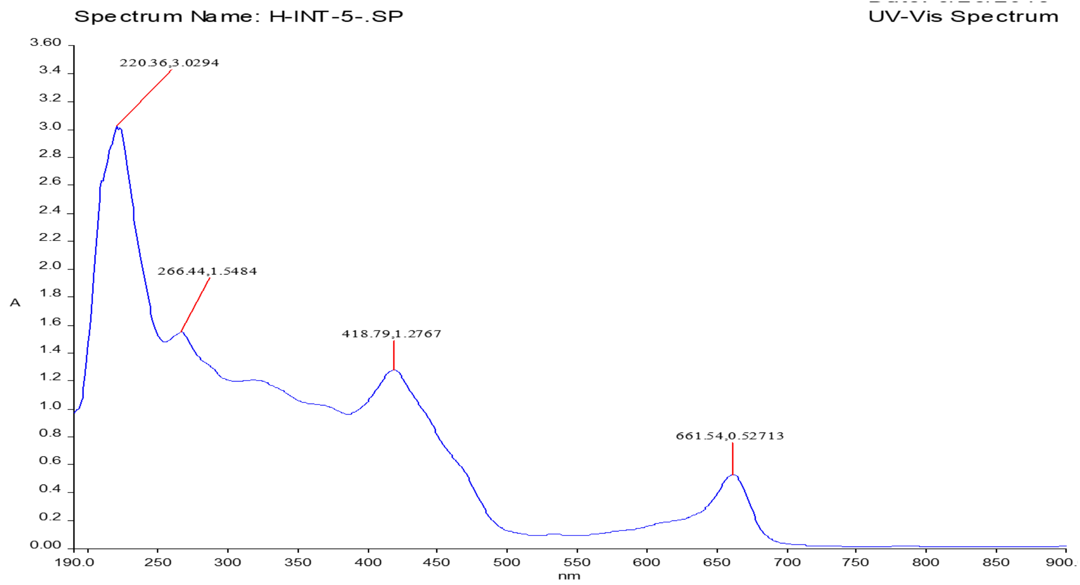


Fig.2: FTIR spectum of of Azima tetracantha leaves extract

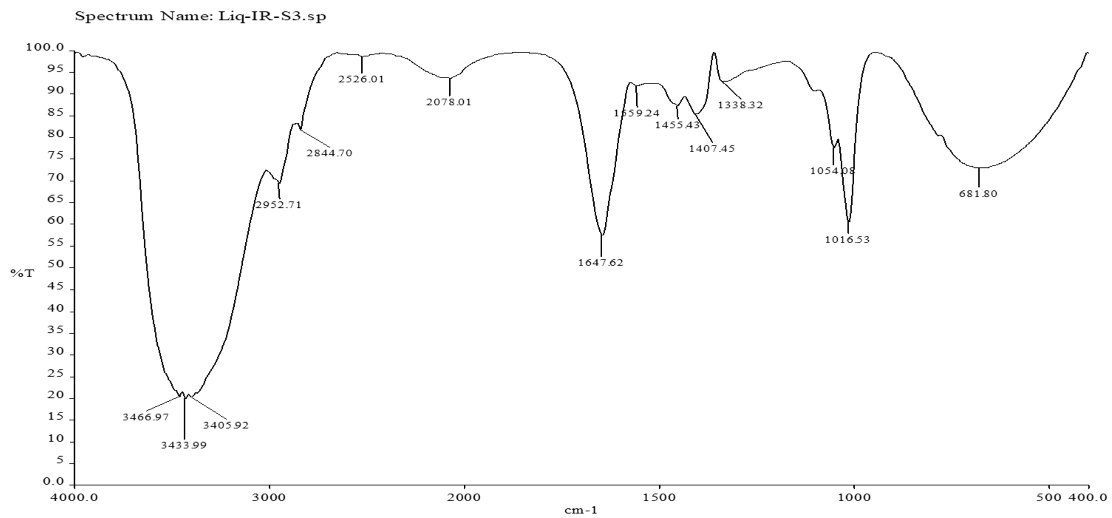
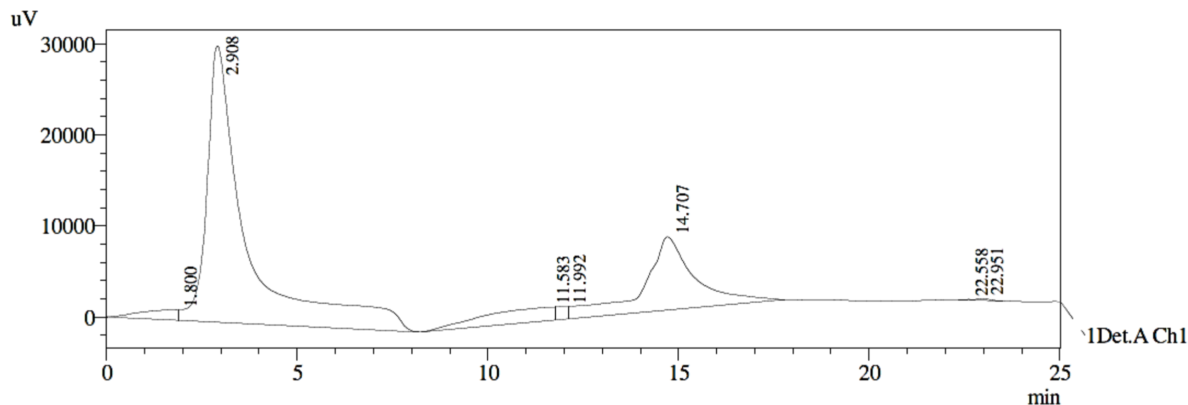


Fig.3: HPLC analysis of Azima tetracantha leaf extract



Characterization of phytochemicals of A. tetracantha leaves

UV- visible spectroscopy study of methanol extract of A. tetracantha exhibited four distinct peaks at wavelength measuring 220, 266, 418, and 661 nm with the absorption of 3.0294,1.5484, 1.2767, and 0.52713 respectively (Fig.1). The absorption peaks in the range between 400-420 and 650-660 nm show the presence of quinones and chlorophyll respectively. The peaks between 200-280 nm (220 and 266 nm) correspond to phenolic acids and their derivatives such as quinones, phenylpropones, flavones, and flavonols. The peak at 418 indicates phenolic acids (flavonoids). This interpretation of UV spectra was based on previous literature on the characterization of aromatic plants (Suica-Bunghez et al.). From the UV spectral analysis, it could be concluded that A. tetracantha contains phenolic compounds and flavonoids.

The functional groups of phytochemicals in A. tetracantha methanol leaf extract were identified with FTIR analysis (Fig.2). The narrow band at 3466.97, 3433.99, and 3405 cm-1 represents the N-H stretching of primary amines. The peaks at 2952.71 and 1455.43 cm-1 represent the methyl C-H stretch and bend respectively. Methoxy, methyl ether O-CH3 and C-H stretch are represented by the peak at 2744.70 cm-1. The carbonate group was shown by the peak at 2526.01 cm-1. The transition metal carbonyl was represented by a peak at 2078.01 cm-1. N-H stretch of secondary amines was indicated with the peak at 1647.62 cm-1. The aliphatic nitro and Bromo compounds were represented by peaks at 1559.24 and 681.80 cm-1 respectively. The peaks at 1054.08 and 1.01.53 cm-1 indicate aliphatic fluoro compounds (Coates, 2000). The results of FTIR analysis confirm phenolic compounds in A. tetracantha leaf extract.

Table 1: Bioactive compounds identified in A.tetracantha leaf extract through HPLC analysis

Peak#	R. Time	Area	Height	Area %	Height %	Compounds identified
1	1.800	75553	1143	2.298	2.679	Kaempferol
2	2.908	2194324	30343	66.735	71.140	Ascorbic Acid
3	11.583	214585	1476	6.526	3.459	Unidentified
4	11.992	29155	1385	0.887	3.247	Ellagic acid
5	14.707	768770	8035	23.380	18.838	Delphinidin -3-O-retinoside
6	22.558	1120	59	0.034	0.139	Unidentified
7	22.951	4627	212	0.141	0.497	Unidentified
Total		**3288135**	**42653**	**100.000**	**100.000**	

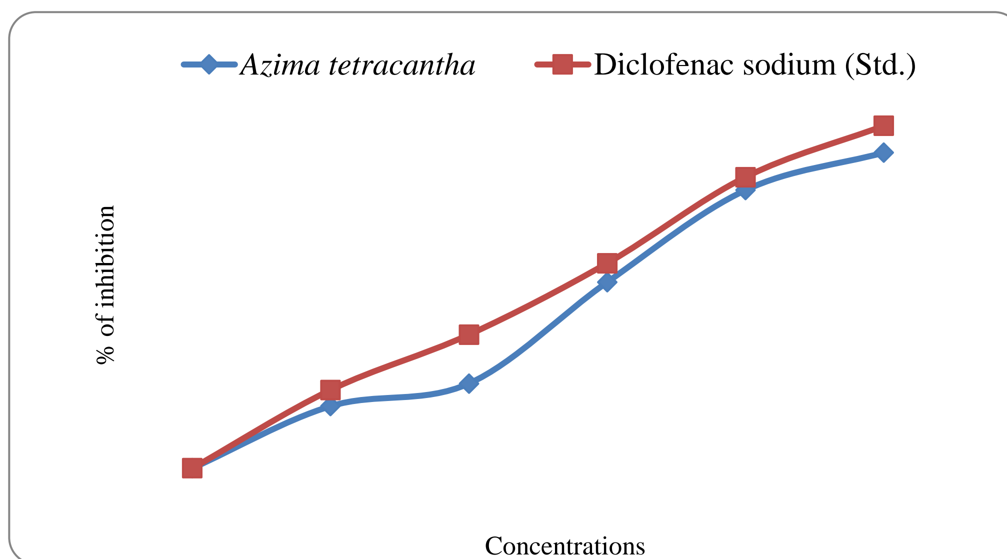
The compounds identified in A. tetracantha leaves by HPLC analysis were presented in Table 1. The HPLC spectrum (Fig.3) showed 7 distinct bands indicating kaempferol, ellagic acid, delphinidin-3-O-retinoid, ascorbic acid, and other unidentified compounds. The HPLC data was in concordance with the results of UV spectral and FTIR analysis which confirmed phenolic compounds in the leaf extract. The phenolic compounds such as gentistic acid, gallic acid, benzoic acids, salicylic acid, and vanillin were reported with methanol extract of A. tetracantha leaves (Gayathri et al. 2014).

in vitro anti-inflammatory activity

The in vitro assessment of anti-inflammatory activity of A. tetracantha methanol leaf extract was examined with protein (fresh egg albumin) denaturation bioassay. The stabilization of proteins is observed with an increase in absorbance of the sample compared to control. 86.12% inhibition of protein denaturation was observed with a 500 µg/ml concentration of A. tetracantha leaf extract compared to 93.45% inhibition exhibited by diclofenac sodium. Dose-dependent inhibition of protein denaturation observed in the present study (Table 2) was in agreement with the inhibition of protein denaturation by coffee extract (Chandra et al. 2012).

Table 2: Effect of Azima tetracantha on protein denaturation (Fresh egg albumin)

Groups	**Concentrations**	**% of inhibition**	
		A. tetracantha	**Diclofenac sodium**
Group I	100µg/ml	16.95±1.18	21.37±1.98
Group II	200µg/ml	23.12±1.61	36.45±2.37
Group III	300µg/ml	50.78±3.55	55.94±3.47
Group IV	400µg/ml	75.89±5.31	79.45±4.65
Group V (Std)	500µg/ml	86.12±6.02	93.45±6.84



Effect of Azima Tetracantha on plasma diagnostic markers

Cd toxicity affects the total protein concentration in serum. The decrease in protein concentration might have resulted due to alterations in amino acid and protein metabolism because of damaged liver cells. Thus, defects in protein synthesis or degradation of synthesized proteins result in low total protein (Ranatunge et al. 2012). The total protein in control (7.07±0.02 gm/dl) was reduced (5.53±0.06gm/dl) in Cd-administered animals (Group II). But it was restored to normal (7.33±0.06gm/dl) with the administration of A. tetracantha along with Cd (Table 3). Konda et al. (2016) reported the nephroprotective role of A. tetracantha leaf extract with an increase in the concentration of total protein at a dose of 500 mg/body weight. An increase in urea and creatinine due to Cd-toxicity was reported by (Renugadevi and Prabu 2010). Cd toxicity significantly increased blood

urea and decreased creatinine clearance in serum compared to control. The administration of Cd (Group II) increased the serum creatinine (1.38±0.09mg/dl) compared to the control group (0.92±0.96mg/dl). Treatment with A. tetracantha leaf extract along with Cd administration significantly restored these renal functional markers. Increased level of serum creatinine with nephrotoxicity due to glycerol was effectively managed with an A. tetracantha leaf extract dose of 500mg/bodyweight (Konda et al. 2016).

Table 3: Effect of Azima tetracantha on plasma oxidative stress markers and antioxidants in control and experimental rats

Parameters	Group I	Group II	Group III	Group IV
MDA (nmol/l)	12.33±0.86[a]	20.26±1.41 [b]	13.65±0.95 [a]	14.53±1.01 [a]
GSH (mg/dl)	9.09±1.03 [a]	6.11±0.80 [b]	9.87±1.12 [a]	8.56±1.08 [a]
SOD (U/ml)	4.38±0.30 [a]	3.44±0.31 [b]	4.72±0.19 [a]	3.93±0.27 [a]
CAT (U/ml)	7.15±0.50 [a]	6.50±0.45 [b]	7.85±0.40 [a]	7.08±0.54 [a]
GPX (U/ml)	8.4±0.58 [a]	6.8±0.47 [b]	8.6±0.53 [a]	8.01±0.56 [a]
Vit C (mg/dl)	1.80±0.12 [a]	1.36±0.09 [b]	1.86±0.16 [a]	1.63±0.11 [a]
Vit E (mg/dl)	3.21±0.038 [a]	2.47±0.033 [b]	3.52±0.036 [a]	3.13±0.035 [a]

Effect of Azima tetracantha on plasma oxidative stress markers and antioxidants in control and experimental rats

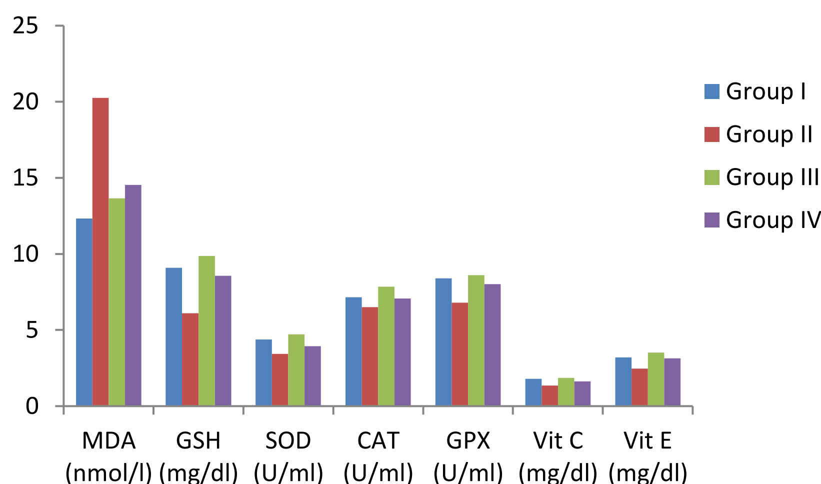
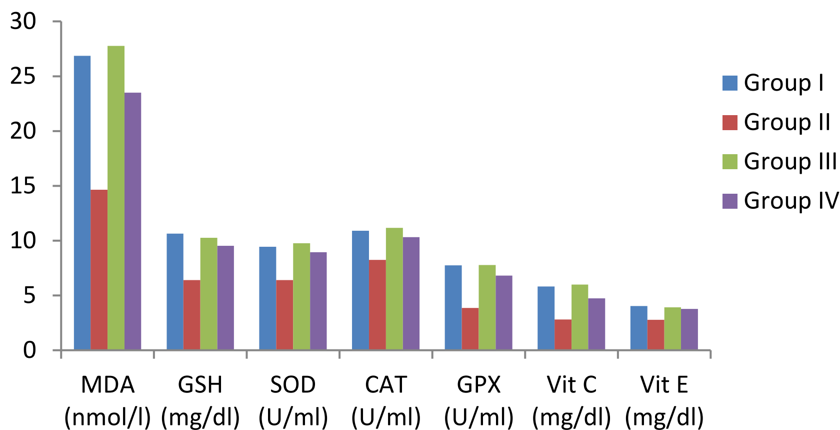


Table 4: Effect of Azima tetracantha on liver oxidative stress markers and antioxidants in control and experimental rats.

Parameters	Group I	Group II	Group III	Group IV
MDA (nmol/l)	26.86±1.88[a]	14.64±1.02 [b]	27.75±0.54 [a]	23.51±0.66 [a]
GSH (mg/dl)	10.63±7.25 [a]	6.40±0.44 [b]	10.27±0.71 [a]	9.54±0.87 [a]
SOD (U/ml)	9.45±0.66 [a]	6.41±0.44 [b]	9.76±0.64 [a]	8.95±0.62 [a]
CAT (U/ml)	10.91±0.69 [a]	8.25±0.42 [b]	11.17±0.72 [a]	10.31±0.51 [a]
GPX (U/ml)	7.73±0.57 [a]	3.86±0.27 [b]	7.76±0.49 [a]	6.8±0.47 [a]
Vit C (mg/dl)	5.81±0.40 [a]	2.81±0.19 [b]	5.98±0.35 [a]	4.72±0.33 [a]
Vit E (mg/dl)	4.02±0.28 [a]	2.77±0.19 [b]	3.92±0.26 [a]	3.76±0.27 [a]

Effect of Azima tetracantha on liver oxidative stress markers and antioxidants in control and experimental rats.



Effect of A. tetracantha on lipid profile

The effect of Cd-induced toxicity on plasma lipids indicates an increase in the concentration of total cholesterol (150.33±0.36 mg/dl), triglycerides (177.27±0.04 mg/dl), HDL (16.76±0.15), LDL (131.44±0.15 mg/dl) and VLDL (35.61±0.12 mg/dl). But the treatment with methanol extract of A. tetracantha leaves reduced the levels of total cholesterol (69.63±0.40), TGL (98.74±0.06), HDL (15.81±0.07), LDL (69.30±0.21), and VLDL (19.69±0.04) (Table 4). A similar reduction in HDL, LDL, and VLDL in FeSO4-induced hepatotoxic rats was reported earlier (Manikandaselvi et al. 2013).

Table 5: Effect of Azima tetracantha on diagnostic markers in control and experimental rats.

Parameters	Group I	Group II	Group III	Group IV
Protein (gm/dl)	7.07±0.49 [a]	5.53±0.32 [b]	7.69±0.57 [a]	7.38±0.51 [a]
SGOT (IU/L)	38.82±4.11 [a]	57.53±4.72 [b]	42.28±3.65 [a]	37.23±4.27 [a]
SGPT (IU/L)	31.11±2.17 [a]	52.88±3.70 [b]	32.41±2.94 [a]	34.88±2.72 [a]
ALP (IU/L)	73.47±5.11 [a]	96.56±6.37 [b]	72.27±5.25 [a]	78.52±4.9 [a]
LDH(IU/L)	117.77±12.44 [a]	261.11±18.27 [b]	156.55±14.38 [a]	120.78±12.35 [a]
GGT(IU/L)	22.39±1.56 [a]	32.16±2.04 [b]	22.91±1.60 [a]	24.64±2.00 [a]
Urea(mg/dl)	35.71±2.49 [a]	52.85±3.69 [b]	35.32±2.8 [a]	38.57±2.69 [a]
Creatinine (mg/dl)	0.92±0.06 [a]	1.38±0.09 [b]	0.94±0.08 [a]	1.05±0.08 [a]

Effect of A. tetracantha on Plasma Oxidative markers

Liver tissues are a rich source of transaminase and acute damage to liver tissues profoundly increases the level of transaminase. An increase in SGOT, SGPT, ALP, and ACP was reported in CCl4-induced hepatotoxic rats (Ilyas et al. 2009). An increase in serum enzymes such as SGOT (58.36±0.06 IU/L), SGPT (53.41±0.41 IU/L), ALP (95.56±1.40 IU/L), and LDH (261.41±0.21 IU/L) in animals of Group II compared to Group I was observed (Table 5). But oral administration of A. tetracantha combined with intra-peritoneal Cd in Group IV animals, reduced SGOT (38.15±0.65 IU/L), SGPT (34.88±0.01 IU/L), ALP (78.52±0.33 IU/L) and LDH (120.78±0.05 IU/L). The elevation of hepatic marker enzymes is an indication of liver damage and destruction of liver membranes. The antioxidant defense mechanism restricts ROS and maintains the structural integrity of the membranes.

Inflammatory responses and oxidative stress cause acute kidney damage. Kidney damage due to Cd intoxication can be predicted by the increase in urea which suggests the modification of glomeruli and tubular capacity (Wang et al. 2017). A. tetracantha exerts protective activity by altering the renal biochemical parameters. The phytochemicals of A. tetracantha

decreased urea and creatinine concentrations while increasing the total protein content in plasma. The antioxidant and anti-inflammatory mechanism of A. tetracantha might be attributed to phytochemicals such as flavonoids, alkaloids, tannins, terpenoids, and saponins (Olagunju et al. 2009). Flavonoids are responsible for anti-inflammatory activity (Syed Ismail et al. 1997).

Table 6: Effect of Azima tetracantha on Plasma lipid profile in control and experimental rats.

Parameters	Group I	Group II	Group III	Group IV
TC (mg/dl)	53.33±3.73 [a]	150±10.5 [b]	63.33±4.43 [a]	70±4.9 [a]
TG (mg/dl)	78.48±5.49 [a]	177.21±12.40 [b]	86.07±6.02 [a]	98.73±6.91 [a]
HDL (mg/dl)	22.91±1.60 [a]	16.66±1.16 [b]	14.58±1.02 [a]	18.75±1.31 [a]
LDL (mg/dl)	46.11±3.22 [a]	131.22±9.18 [b]	60.64±4.24 [a]	69.01±4.83 [a]
VLDL (mg/dl)	15.69±1.09 [a]	35.44±2.48 [b]	17.21±1.20 [a]	19.74±1.38 [a]

Table 7: Effect of Azima tetracantha on kidney oxidative stress markers and antioxidants in control and experimental rats.

Parameters	Group I	Group II	Group III	Group IV
MAD (nmole/mg protein)	13.78±1.66[a]	19.20±2.74[b]	13.66±1.72[a]	14.87±1.88[a]
GSH (µg/mg protein)	10.60±0.70[a]	7.20±0.64[b]	10.87±0.85[a]	9.93±0.83[a]
SOD (U/mg protein)	8.95±0.62[a]	6.15±0.43[b]	8.44±0.59[a]	8.57±0.59[a]
CAT (U/mg protein)	10.21±0.71[a]	6.94±0.48[b]	9.35±0.56[a]	9.70±0.65[a]
GPx (U/mg protein)	6.93±0.48[a]	3.6±0.25[b]	5.73±0.40[a]	6.8±0.47[a]
Vit C (µg/mg protein)	4.90±0.34[a]	2.36±0.16[b]	5±0.35[a]	4.81±0.33[a]
Vit E (µg/mg protein)	3.52±0.24[a]	2.57±0.18[b]	3.32±0.23[a]	3.27±0.22[a]

Effect of A. tetracantha on oxidative stress markers and non-enzymatic antioxidants

An increase in free radicals leads to auto-oxidation of fatty acids present in phospholipids of the cell membrane. This auto-oxidation results in altering the function and morphology of the cell membrane (Pandit et al. 2004). Cells produce antioxidants to counteract the auto-oxidation of fatty acids caused by free radicals. However, under oxidative stress, the antioxidant defense mechanism becomes ineffective (Halliwell and Gutteridge 2015) resulting in increased intracellular GSH and reduced activity of CAT and SOD (Yamamoto et al. 1999). The acute exposure to Cd and the subsequent toxicity leads to increased activity of copper/zinc-containing enzymes in the antioxidant system (Ognjanović et al. 2003). A decrease in enzymatic antioxidants in Cd toxicity is due to the overconsumption of enzymes to cleanse ROS (Amin et al. 2006). Oxidative stress markers of the kidney (Table 6) and liver (Table 7) were decreased due to Cd-induced toxicity. But with the administration of A. tetracantha leaf extract, the markers enzymes were restored to normal levels. Oral administration of A. tetracantha ethanol root extract significantly increased SOD, CAT, GSH, GR, and GPx activity in animals with renal cell damage (Manikandaselvi et al. 2013; Konda et al. 2016). Manikandaselvi et al. (2013) reported a decrease in vitamin E due to increased production of ROS was consistent with the present findings, as a decrease in vitamin E (2.57±0.18 mg/dl) compared to control (3.52±0.24 mg/dl) was observed in kidney cells (Table 6). In liver cells, vitamin E in control animals (4.02±0.28 mg/dl) was reduced (2.77±0.19) in Cd-treated animals (Table 7 and Fig.9). GSH is a strong endogenous antioxidant and a tripeptide that is rich in cysteine residues. The enzyme maintains the cytoplasmic membrane and detoxifies ROS. The cells produce excessive GSH during Cd toxicity. The epidemiologic and experimental studies indicate that Cd exposure results in oxidative stress, inflammation, fibrosis, and functional impairment in liver cells (Fan et al. 2018; Zhang et al. 2019).

The degradation process of polyunsaturated lipids by reactive oxygen species generates Malondialdehyde (MDA). The researchers used this aldehyde product as a biomarker to assess the level of oxidative stress. Cd toxicity has previously been shown to cause lipid peroxidation in a variety of organs, including the ovaries, uterus, kidneys, and liver. Increased levels of liver MDA due to CdCl2 toxicity was restored with a decrease in MDA by 43.65% (Yang et al. 2021). A similar observation in CCl4-induced hepatotoxicity was reported by (Prakash et al. 2015). The decreased oxidative stress markers of the kidney (Table 6) were ameliorated with the treatment of A. tetracantha leaf extract. Similarly, the oxidative stress markers of the liver, observed in Cd-treated animal groups were increased with A. tetracantha treatment (Table 7).

Fig.4: Effect of Azima tetracantha extract on Cd – induced histological changes in liver cells

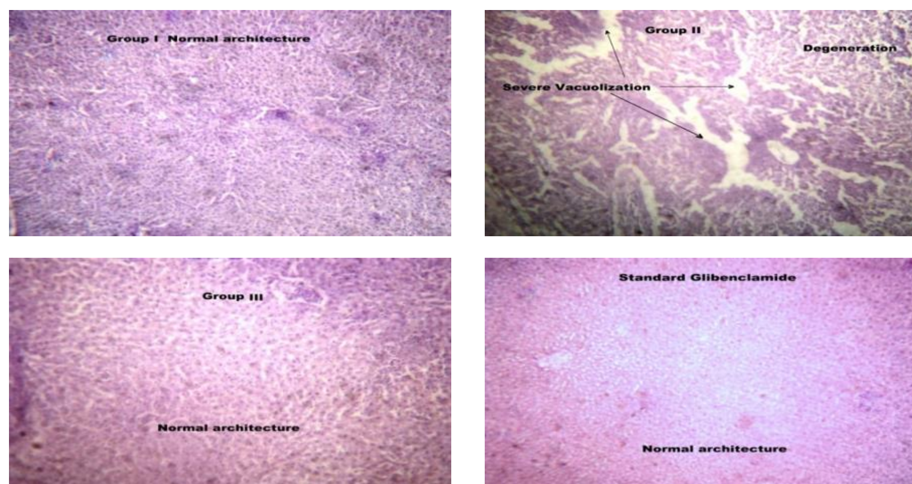
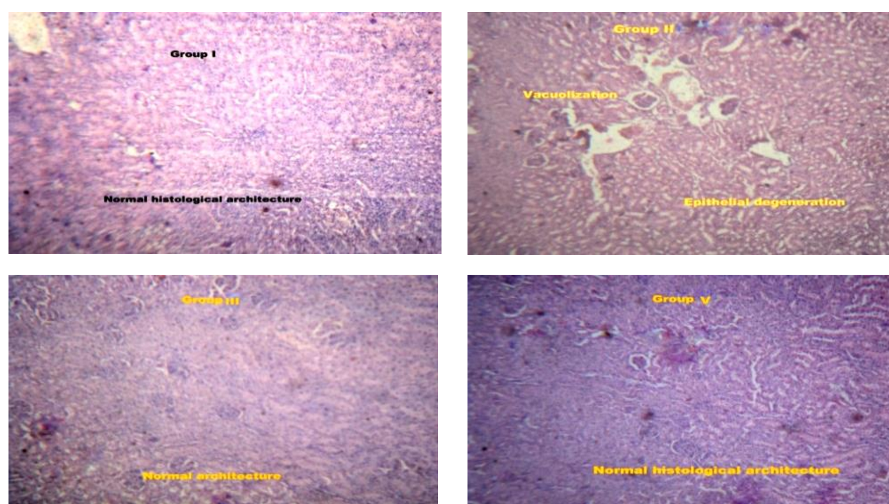


Fig.5: Effect of Azima tetracantha extract on Cd – induced histological changes in kidney cells



Histopathology

The control groups showed normal glomeruli with intact Bowman's capsule and proximal and distal convoluted tubules. No signs of hemorrhage or capillary congestion were observed. But Cd exposed cells showed distortion in glomeruli, convoluted tubules, apical blebbing, and capillary congestion (Fig.4). Treatment with methanol extract of A. tetracantha leaves reversed the abnormalities significantly with reduced congestion of capillaries, tubular damage, and distortion of capillaries (Fig.5). The influence of Cd-induced toxicity clearly showed morphological alterations in the hepatic cells with significant nicks on the central venous wall. Degeneration of cells with necrosis and inflammatory infiltrations of the cells were observed. The histopathological examination of Cd-induced hepatic cells reported by (Wang et al. 2017) was in support of the findings of the present study.

Conclusion

The methanol extract of A. tetracantha was observed to possess hepatic and nephroprotective effects in cadmium-induced toxicity. Effective protective activity of A. tetracantha leaf extract was achieved with a concentration of 500 mg/kg. The protective effect of the plant may be attributed to the presence of phenolic compounds. Hence the study recommends the use of A. tetracantha in treating acute liver and kidney disorders and also suggests further clinical evaluation for the use of A. tetracantha phytochemicals as a drug.

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Conflict of Interest

The authors declare that they have no conflict of interest in the publication.

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Author Contributions

All authors contributed to the study's conception and design. Material preparation and data collection were performed by Dr.S. Vasthi Gnana Rani and analysis were performed by Dr. K. Hema, and Dr. S. Ananth. The first draft of the manuscript was written by Dr. S.Vasthi Gnana Rani and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

REFERENCES

1. Allain CC, Poon LS, Chan CSG, et al (1974) Enzymatic determination of total serum cholesterol. academic.oup.com 20:470
2. Almeer RS, Alarifi S, Alkahtani S, et al (2018) The potential hepatoprotective effect of royal jelly against cadmium chloride-induced hepatotoxicity in mice is mediated by suppression of oxidative stress and upregulation of Nrf2 expression. Biomedicine & Pharmacotherapy 106:1490–1498. https://doi.org/10.1016/J.BIOPHA.2018.07.089
3. Amin A, Hamza A, Daoud S, et al (2006) Spirulina protects against cadmium-induced hepatotoxicity in rats. Citeseer 1:21–25
4. Baker RTM, Davies SJ (1997) Modulation of tissue α-tocopherol in African catfish, Clarias gariepinus (Burchell), fed oxidized oils, and the compensatory effect of supplemental dietary vitamin E. Aquaculture Nutrition 3:91–97. https://doi.org/10.1046/J.1365-2095.1997.00078.X
5. Baskaran R, Priya LB, Sathish Kumar V, Padma VV (2018) Tinospora cordifolia extract prevents cadmium-induced oxidative stress and hepatotoxicity in experimental rats. Journal of Ayurveda and Integrative Medicine 9:252–257. https://doi.org/10.1016/J.JAIM.2017.07.005
6. Beers R, chem IS-JB, 1952 undefined A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. researchgate.net
7. Bhardwaj JK, Paliwal A, Saraf P (2021) Effects of heavy metals on reproduction owing to infertility. Journal of Biochemical and Molecular Toxicology 35:. https://doi.org/10.1002/JBT.22823
8. Borowska S, Brzoska MM, Tomczyk M (2018) Complexation of Bioelements and Toxic Metals by Polyphenolic Compounds - Implications for Health. Current Drug Targets 19:1612–1638. https://doi.org/10.2174/1389450119666180403101555
9. Branca J, Morucci G, research AP-N regeneration, 2018 undefined Cadmium-induced neurotoxicity: still much ado. ncbi.nlm.nih.gov
10. Casalino E, Calzaretti G, Sblano C, Landriscina C (2002) Molecular inhibitory mechanisms of antioxidant enzymes in rat liver and kidney by cadmium. Toxicology 179:37–50. https://doi.org/10.1016/S0300-483X(02)00245-7
11. Chandra S, Chatterjee P, … PD-APJ of, 2012 undefined Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein. Elsevier
12. Chem RB-JB, 1945 undefined On the colorimetric determination of creatinine by Jaffe reaction. ci.nii.ac.jp
13. Coates J Interpretation of Infrared Spectra, A Practical Approach. 10815–10837
14. Dkhil MA, Al-Quraishy S, Diab MMS, et al (2014) The potential protective role of Physalis peruviana L. fruit in cadmium-induced hepatotoxicity and nephrotoxicity. Food and Chemical Toxicology 74:98–106. https://doi.org/10.1016/J.FCT.2014.09.013
15. Duraipandiyan V, Gnanasekar M, Ignacimuthu S (2010) Antifungal activity of triterpenoid isolated from Azima tetracantha leaves. Folia Histochemica et Cytobiologica 48:311–313. https://doi.org/10.2478/V10042-010-0005-3
16. Ekbote MT, Ramesh CK, Mahmood R, et al (2010) Hepatoprotective and antioxidant effect of Azima tetracantha Lam. leaves extracts against CCl4-induced liver injury in rats. Indian Journal of Natural Products and Resources 1:493–499
17. El-Boshy ME, Risha EF, Abdelhamid FM, et al (2015) Protective effects of selenium against cadmium induced hematological disturbances, immunosuppressive, oxidative stress and hepatorenal damage in rats. Journal of Trace Elements in Medicine and Biology 29:104–110. https://doi.org/10.1016/J.JTEMB.2014.05.009
18. Environment AT-S of the T, 2019 undefined (2019) Cadmium pigments in consumer products and their health risks. Elsevier. https://doi.org/10.1016/j.scitotenv.2018.12.096
19. Everson TM, Punshon T, Jackson BP, et al (2018) Cadmium-associated differential methylation throughout the placental genome: epigenome-wide association study of two US birth cohorts. ehp.niehs.nih.gov 126:. https://doi.org/10.1289/EHP2192
20. Ezedom T, Asagba SO (2016) Effect of a controlled food-chain mediated exposure to cadmium and arsenic on oxidative enzymes in the tissues of rats. Toxicology Reports 3:708–715. https://doi.org/10.1016/J.TOXREP.2016.07.002
21. Fan R, Hu P, Wang Y, et al Betulinic acid protects mice from cadmium chloride-induced toxicity by inhibiting cadmium-induced apoptosis in kidney and liver. Elsevier
22. Fouad AA, Jresat I (2011) Protective effect of telmisartan against cadmium-induced nephrotoxicity in mice. Life Sciences 89:29–35. https://doi.org/10.1016/J.LFS.2011.04.019

23. Friedewald W, Levy R, chemistry DF-C, 1972 undefined (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. academic.oup.com 18:499
24. G Gayathri, R Nair Bindu, V Babu (2012) GC-MS Analysis of Azima tetracantha. J Pharm Res,
25. Galán A, García-Bermejo L, Troyano A, et al (2001) The role of intracellular oxidation in death induction (apoptosis and necrosis) in human promonocytic cells treated with stress inducers (cadmium, heat, X-rays). European Journal of Cell Biology 80:312–320. https://doi.org/10.1078/0171-9335-00159
26. Gayathri G, Bindu N, Sci VB-IJPP, 2014 undefined (2014) Scavenging of free radicals and total phenols of Methanol extract of Azima tetracantha Lam. researchgate.net
27. Gong Z, Wang X, Wang J, et al Trehalose prevents cadmium-induced hepatotoxicity by blocking Nrf2 pathway, restoring autophagy and inhibiting apoptosis. Elsevier
28. Gustin K, Tofail F, Vahter M, Kippler M (2018) Cadmium exposure and cognitive abilities and behavior at 10 years of age: A prospective cohort study. Environment International 113:259–268. https://doi.org/10.1016/J.ENVINT.2018.02.020
29. Halliwell B, Gutteridge J (2015) Free radicals in biology and medicine
30. Helal Ashour T, Del Principe D, Rosado JA, Wachowicz B (2014) Preventative effects of caffeic acid phenyl ester on cadmium intoxication induced hematological and blood coagulation disturbances and hepatorenal damage. downloads.hindawi.com. https://doi.org/10.1155/2014/764754
31. Ilyas M, Jamal MH, College M, et al (2009) Effects of ethanolic leaf extract of Azim tetracantha Lam. on Ehrlich ascites carcinoma tumour bearing mice. researchgate.net 4:351–354
32. Jaishankar M, Tseten T, Anbalagan N, et al (2014) Toxicity, mechanism and health effects of some heavy metals. Interdisciplinary Toxicology 7:60. https://doi.org/10.2478/INTOX-2014-0009
33. Kind P, Pathology EK-J of clinical, 1954 undefined (1954) Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. ncbi.nlm.nih.gov 7:322
34. Konda VR, Arunachalam R, Eerike M, et al (2016) Nephroprotective effect of ethanolic extract of Azima tetracantha root in glycerol induced acute renal failure in Wistar albino rats. Journal of Traditional and Complementary Medicine 6:347–354. https://doi.org/10.1016/J.JTCME.2015.05.001
35. Kumar A, Singh N, Pandey R, et al (2018) Biochemical and molecular targets of heavy metals and their actions. Biomedical Applications of Metals 297–319. https://doi.org/10.1007/978-3-319-74814-6_14
36. Liu W, Gu J, Liu X, et al CaMKII plays a central role in alteration of calcium homeostasis and MAPKs involved in osteoblast apoptosis induced by cadmium. infona.pl
37. Lowry OC, Rosebrough N, … AF-J biol, 1951 undefined Protein measurement with the Folin phenol reagent. garfield.library.upenn.edu
38. Manikandaselvi S, Ravikumar R, Thinagarbabu R, et al (2013) Hepatoprotective potential of Azima tetracantha and Tribulus terrestris on ferrous sulfate-induced toxicity in rat. Bangladesh Journal of Pharmacology 8:357–360. https://doi.org/10.3329/BJP.V8I3.15579
39. Mao T, Han C, Wei B, et al (2018) Protective Effects of Quercetin Against Cadmium Chloride-Induced Oxidative Injury in Goat Sperm and Zygotes. Biological Trace Element Research 185:344–355. https://doi.org/10.1007/S12011-018-1255-8
40. Marklund S, Marklund G (1974) Involvement of the Superoxide Anion Radical in the Autoxidation of Pyrogallol and a Convenient Assay for Superoxide Dismutase. European Journal of Biochemistry 47:469–474. https://doi.org/10.1111/J.1432-1033.1974.TB03714.X
41. Mizushima Y, Kobayashi M (1968) Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. Journal of Pharmacy and Pharmacology 20:169–173. https://doi.org/10.1111/J.2042-7158.1968.TB09718.X
42. Moron M, Depierre J, (BBA BM-B et biophysica acta, 1979 undefined Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Elsevier
43. Namık ME, Üniversitesi K, Fidanol Z, et al (2016) Thymoquinone ameliorates cadmium-induced nephrotoxicity, apoptosis, and oxidative stress in rats is based on its anti-apoptotic and anti-oxidant properties. Springer 170:165–172. https://doi.org/10.1007/s12011-015-0453-x
44. Ognjanović BI, Pavlović SZ, Maletić SD, et al (2003) Protective influence of vitamin E on antioxidant defense system in the blood of rats treated with cadmium. biomed.cas.cz 52:563–570
45. Olagunju JA, Adeneye AA, Fagbohunka BS, et al (2009) Nephroprotective activities of the aqueous seed extract of Carica papaya Linn. in carbon tetrachloride induced renal injured Wistar rats: a dose-and time-dependent study. Biology and Medicine 1:11–19
46. Omaye S, Turnbull J, enzymology HS-M in, 1979 undefined Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. Elsevier
47. Pandit S, Sur T, Jana U, et al Prevention of carbon tetrachloride-induced hepatotoxicity in rats by Adhatoda vasica leaves. ijp-online.com
48. Paranthaman R, … AS-A journal of, 2012 undefined (2012) Determination of pesticide residues in banana by using high performance liquid chromatography and gas chromatography-mass spectrometry. researchgate.net. https://doi.org/10.3844/ajbbsp.2012.1.6
49. Park JH, Lee BM, Kim HS (2020) Potential protective roles of curcumin against cadmium-induced toxicity and oxidative stress. https://doi.org/101080/1093740420201860842 24:95–118. https://doi.org/10.1080/10937404.2020.1860842
50. PB Tchounwou, CG Yedjou, AK Patlolla DS (2012) Heavy metal toxicity and the environment in Molecular, clinical and environmental toxicology, vol 101 (ed. Luch, A.) 133–164. Clinical and Environmental Toxicology 133–164
51. Prakash E, Jeyadoss T, Velavan S (2015) In vitro hepatoprotective activity of Azima tetracantha leaf extract and silver nanoparticle in hepatocytes. Scholars Research Library Der Pharma Chemica 7:381–390
52. Puerto-Parejo LM, Aliaga I, Canal-Macias ML, et al (2017) Evaluation of the dietary intake of cadmium, lead and mercury and its relationship with bone health among postmenopausal women in Spain. mdpi.com. https://doi.org/10.3390/ijerph14060564
53. Ranatunge RAAR, Wijesinghe MR, Ratnasooriya WD, et al (2012) Cadmium-Induced Toxicity on Larvae of the Common Asian Toad Duttaphrynus Melanostictus (Schneider 1799): Evidence from Empirical Trials. Bulletin of Environmental Contamination and Toxicology 2012 89:1 89:143–146. https://doi.org/10.1007/S00128-012-0635-6
54. Reitman S, pathology SF-A journal of clinical, 1957 undefined A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. academic.oup.com
55. Renugadevi J, Prabu SM (2010) Cadmium-induced hepatotoxicity in rats and the protective effect of naringenin. Experimental and Toxicologic Pathology 62:171–181. https://doi.org/10.1016/J.ETP.2009.03.010
56. Rotruck JT, Pope AL, Ganther HE, et al (1973) Selenium: Biochemical role as a component of glatathione peroxidase. Science (1979) 179:588–590. https://doi.org/10.1126/SCIENCE.179.4073.588
57. S Sakat, AR Juvekar MG (2010) No Title. Int J Pharm Pharm Sci 33:454–468
58. Sekar S, Chidambaram SB, Thendral Hepsibha B, et al (2010) In vitro studies on antioxidant and free radical scavenging activities of Azima tetracantha. Lam leaf extracts. Article in Indian Journal of Science and Technology 3:. https://doi.org/10.17485/ijst/2010/v3i5/29757
59. Shagirtha K, Bashir N, Miltonprabu S (2017) Neuroprotective efficacy of hesperetin against cadmium induced oxidative stress in the brain of rats. Toxicology and Industrial Health 33:454–468. https://doi.org/10.1177/0748233716665301
60. Skipper A, Sims JN, Yedjou CG, Tchounwou PB (2016) Cadmium Chloride Induces DNA Damage and Apoptosis of Human Liver Carcinoma Cells via

Oxidative Stress. International Journal of Environmental Research and Public Health 2016, Vol 13, Page 88 13:88. https://doi.org/10.3390/IJERPH13010088
61. Suica-Bunghez I, … ST-IC, 2016 undefined Antioxidant activity and phytochemical compounds of snake fruit (Salacca Zalacca). iopscience.iop.org. https://doi.org/10.1088/1757-899X/133/1/012051
62. Sunil C, Duraipandiyan V, Ignacimuthu S, Al-Dhabi NA (2013) Antioxidant, free radical scavenging and liver protective effects of friedelin isolated from Azima tetracantha Lam. leaves. Food Chemistry 139:860–865. https://doi.org/10.1016/j.foodchem.2012.12.041
63. Syed Ismail T, Gopalakrishnan S, Hazeena Begum V, Elango V (1997) Anti-inflammatory activity of Salacia oblonga Wall. and Azima tetracantha Lam. Journal of Ethnopharmacology 56:145–152. https://doi.org/10.1016/S0378-8741(96)01523-1
64. Unsal V, Dalkiran T, Çiçek M, Kölükçü E (2020) The role of natural antioxidants against reactive oxygen species produced by cadmium toxicity: A review. Advanced Pharmaceutical Bulletin 10:184–202. https://doi.org/10.34172/APB.2020.023
65. Veljkovic AR, Nikolic RS, Kocic GM, et al (2012) Protective Effects of Glutathione and Lipoic Acid against Cadmium-Induced Oxidative Stress in Rat's Kidney. http://dx.doi.org/103109/0886022X2012723661 34:1281–1287. https://doi.org/10.3109/0886022X.2012.723661
66. Waisberg M, Joseph P, Hale B, Beyersmann D (2003) Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology 192:95–117. https://doi.org/10.1016/S0300-483X(03)00305-6
67. Wang MG, Li WH, Wang XY, et al (2017) CaMKII is involved in subcellular Ca2+ redistribution-induced endoplasmic reticulum stress leading to apoptosis in primary cultures of rat proximal tubular cells exposed to lead. Oncotarget 8:91162. https://doi.org/10.18632/ONCOTARGET.20035
68. Wätjen W, Biometals DB-, 2004 undefined (2004) Cadmium-induced apoptosis in C6 glioma cells: influence of oxidative stress. Springer 17:65–78. https://doi.org/10.1023/A:1024405119018
69. WeerasakSamee S (2007) Simultaneous Determination of Gallic acid, Catechin, Rutin, Ellagic Acid and Quercetin in Flower Extracts of Micheliaalba, Caesalpinia pulcherrima and Nelumbo nucifera by HPLC. Thai Pharm Health Sci J 2:131–137
70. Werner M, Gabrielson D, chemistry JE-C, 1981 undefined (1981) Ultramicro determination of serum triglycerides by bioluminescent assay. academic.oup.com 27:268
71. Yamamoto Y, Biofactors SY-, 1999 undefined Plasma ubiquinone to ubiquinol ratio in patients with hepatitis, cirrhosis, and hepatoma, and in patients treated with percutaneous transluminal coronary reperfusion. content.iospress.com
72. Yang Z, He Y, Wang H, et al Protective effect of melatonin against chronic cadmium-induced hepatotoxicity by suppressing oxidative stress, inflammation, and apoptosis in mice. Elsevier
73. Zhang J, Wang Y, Fu L, et al (2019) Chronic cadmium exposure induced hepatic cellular stress and inflammation in aged female mice. Journal of Applied Toxicology 39:498–509. https://doi.org/10.1002/JAT.3742