Original Research

Investigating the Ameliorative Potential of the *Aloe barbadensis* Aqueous Fraction on Oxidative Stress Markers and Biochemical Parameters in Cadmium-Intoxicated Rabbits

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Abstract

This study was conducted to evaluate the possible protective effects of the flavonoid-rich fraction of *Aloe barbadensis* leaf skin on cadmium (Cd)-induced toxicity in male albino rabbits. Cadmium is a notable environmental pollutant due to its wider range of toxic manifestations. The aqueous fraction of aloe extract (AAF) showed higher phenolics, flavonoids, and antioxidant capacity among other fractions, suggesting its rationale use in this study. Twenty-four rabbits were randomly divided into four groups, including the control group (receiving only vehicle), the Cd group (receiving Cd, 5.1 mg/kg/day), the AAF groups (receiving AAF, 200 mg/Kg/day), and the Cd+AAF group (receiving the same concentrations as the Cd and AAF groups). Oral treatment over a period of 40 days significantly increased ($p<0.05$) biochemical marker enzymes, including transaminases (AST, ALT), alkaline phosphatase, γ-glutamyl transferase, creatinine, and urea, while total bilirubin (Tb) and albumin were decreased on days 10, 20, 30, and 40 in the Cd group as compared to control. A significant decrease ($p<0.05$) in enzyme levels and increases in Tb and albumin for Cd+AAF were observed as compared to Cd-treated rabbits. Contents of superoxide dismutase, catalase, and vitamins C and E in liver and kidney tissues were significantly increased ($p<0.05$), while cadmium content was significantly decreased ($p<0.05$) for Cd+AAF rabbits as compared to Cd-intoxicated rabbits. Values of all the parameters in only the AAF group were near to control. The histopathological studies for liver and kidney have also supported the Cd+AAF group markedly reducing the toxicity of Cd in both tissues to near normal. Thus, the results suggest that the flavonoid-rich fraction of AAF may

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act as a natural protective agent against Cd toxicity via suppressing oxidative stress due to higher antioxidant activity.

Introduction

It is evident that increasing anthropogenic activities modified the cycle of heavy metals and metalloids globally, including toxic elements like cadmium. Thus an ample opportunity for exposure to cadmium is possible in and outside the workplace [1-2]. Concentrations of Cd in the surrounding environment are increasing due to its diverse sources and prolonged biological half-life of 10-30 years. It is nonessential and known to be toxic even at low concentrations [3-4]. Both acute and chronic exposure to Cd results in damage to plants, animals, and humans, including injury to the liver, lungs, testes, renal dysfunction, anemia, osteoporosis, DNA damage, neurodegenerative diseases, intestinal damage, and immunosuppressive and pro-inflammatory effects mainly due to oxidative stress [4-9]. Many approaches have been employed to reduce cadmium toxicity, including antioxidant therapy, chelation therapy, and the use of synthetic antioxidants. These therapies have their own disadvantages, such as chelation with ethylenediaminetetraacetic acid (EDTA), which can lead to osteoporosis due to excretion of calcium from the body. Therefore, there is dire need to use some benign therapies such as ethnomedicines. Traditionally, ethnomedicines are being used extensively in the Indo-Pak region and elsewhere, mainly due to their cost-effectiveness, easy accessibility, and perceived fewer side effects [10-11]. The mechanism of action of these herbal drugs is different in many respects from that of the synthetic drugs/pure compounds. This may be characterized as a polyvalent action and understood as an additive or potentiate.

In this study, a famous plant known as *A. barbadensis* Miller (Family Liliaceae) was selected (to deal with oxidative stress on the basis of its higher antioxidant activity) to prevent/reduce toxicity associated with cadmium. For this purpose we used the skin of mature aloe leaves. This plant is commonly used in folk medicine as it is antibacterial, antioxidant, antidiabetic, anticarcinogenic, hypocholesterolemic, hepatoprotective, and nephroprotective [12-17]. The polyphenolics and flavonoids reported so far in the aloe skin include sinapic acid, quercitrin, kaempferol, apigenin, catechin, quercetin, epicatechin, syringic acid, chlorogenic acid, gentisic acid, caffeic acid, ferulic acid, rutin, miricetin, and vanillic acid [18]. The possible protective activity of *Aloe barbadensis* aqueous extract against Cd-induced renal and hepatotoxicity has not been reported so far. Therefore, in this study, *Aloe barbadensis* methanolic extract was sequentially partitioned to obtain different fractions, out of which an aqueous fraction termed as aloe aqueous fraction (AAF) was utilized to evaluate its protective effects against Cd-induced sub-chronic oxidative kidney and liver injury models in male rabbits.

Material and Methods

Collection, Identification, and Extraction of Plant Material

Fresh leaves of *Aloe barbadensis* were collected from the Botanical Garden of the University of the Punjab, Lahore, during September-October. Identification and authentication were performed by the Department of Botany, GC University, Lahore. Fresh leaves were washed with distilled water, sliced into smaller pieces, air-dried, ground, and extracted with aqueous ethanol, 30:70 v/v (plant material to solvent ratio, 1:20 w/v) using an ultrasonic bath for six hours at room temperature, then filtered through Wattman filter paper No. 1, and evaporated to dryness using a rotary evaporator (Edolph Rotary, Germany) according to Sytkiewicz et al. [19]. The residue was extracted three times for maximum yield. The crude extract, thus obtained, was suspended in double-distilled water and successively partitioned with petroleum spirit and chloroform to get the respective fractions by using a rotary evaporator. The remaining aqueous fraction was lyophilized using Labconco, FreeZone 2.5, USA. Out of three fractions and crude extract, the aqueous fraction showed higher phenolic and flavonoid contents and the highest antioxidant potential. Thus the aqueous fraction was retained in a glass bottle, covered with aluminum foil, and kept in refrigerator until further use.

In Vitro Determination of Total Phenolics, Flavonoids, and Antioxidant Contents

The total phenolic contents of crude extract, petroleum spirit, chloroform, and aqueous fractions were determined as described by Liu et al., based on Folin-Ciocalteu's reagent method [20]. All the results were stated as mg gallic acid equivalents (GAE) per gram of sample. Total flavonoids were measured using the aluminum chloride method as described by Liu et al. [20]. Results of total flavonoid content were stated as mg catechin equivalents (CE) per gram of dry weight of sample. Total antioxidant capacity was determined using the phospho-molybdenum method by Prieto et al. [21], and stated as mg BHT equivalents (BHTE) per gram of sample.

Identification of Quercetin by RP-HPLC

Aloe aqueous fraction (AAF) was prepared for HPLC identification by mixing a freeze-dried fraction (100 mg) in 2 mL of methyl alcohol, uniformly homogenized by vortex at room temperature in darkness, and filtered through a 45 μm nylon syringe filter prior to injection.

Chromatographic identification was performed on an LC-9 system equipped with a vacuum degasser, a thermostat column compartment, fixed loop injector (20 μL), a binary pump, and UV-visible detector set at 373 nm – which all was connected to CSW32 software. The separation was performed with a reverse-phase C18 column. The mobile phases were water (0.1% formic acid) and acetonitrile, with flow rate of 0.6 mL/min.

Animals

In this study we used male albino rabbits (*Oryctolagus cuniculus*), aged 22-26±2 weeks, weighing 1.6-2.2±0.1 kg, bred in the Pakistan Council of Scientific and Industrial Research, Lahore-Pakistan animal house. The rabbits were fed on a routine rabbit diet and had free access to fresh drinking water. They were housed in individual cages and were handled under standard laboratory conditions of 12 h light:dark cycle at 24±3ºC and relative humidity of 50±10%. Before the commencement of the experiment all the rabbits were administered ivermectin (0.1 mL/Kg b.wt., s.c.) followed by a seven-day adjustment period. The study protocols were approved (18 June 2014) by the Advance Studies and Research Board (AS&RB) of the University of the Punjab, Pakistan. Experimental protocols were in agreement with the National Institute of Health (NIH) guidelines for the care and use of laboratory animals [2]. Body weights were recorded every four days until the end of the experiment.

Selection of Cadmium and AAF Dosage

LD50 of cadmium (as cadmium chloride) was determined to be 61.34 mg/Kg b.wt. in male albino rabbits (data not shown). The 1/12th dose of LD50 of cadmium (as cadmium chloride) was selected, i.e., 5.1mg/Kg to induce toxicity in rabbits. To evaluate the appropriate dose of aloe aqueous fraction (AAF), three different concentrations (i.e., 50, 100, and 200 mg/Kg b.wt.) were administered orally for 10 days along with $CdCl_2$ (5.1mg/Kg) daily. Various biochemical parameters like AST, ALT, ALP, Cr, and Urea were estimated in serum (data not shown). Results of these parameters suggest that 200 mg/Kg b.wt./day was found to be an appropriate dose to conduct this study.

Experimental

The experimental design has been constructed in accordance with that of Prabu et al. [1]. Rabbits were divided at random, into four groups (each containing six animals):

1. Control: non-treated animals received the vehicle only orally.
2. Received only an aqueous fraction of the leaf extract (AAF, 200mg/Kg/day, orally).
3. Received cadmium in normal saline at a dose of 5.1mg//Kg/day orally.
4. Rabbits orally received 5.1mg/Kg/day cadmium and 200mg//Kg/day aqueous extract.

This experimental study was conducted for 40 days, representing a sub-chronic model according to Bae et al. [22].

Blood and Tissue Sampling

Before commencement and after every 10th day of the experiment, approximately 5 ml of blood was collected from the marginal ear vein of each rabbit from all groups. Samples were divided into two aliquot: one of 2 ml in EDTA containing a tube for CBC (data not shown), and the other of 3 ml in a plain dry vacutainer to separate serum after centrifuging (1,000 rpm, 10 min.) for biochemical analysis.

On day 40 dissection of all rabbits from each group was performed to remove liver and kidneys, which were rinsed with cold normal saline, dried, and weighed individually. Small portions of left kidneys and caudal parts of livers were preserved for histopathological examinations, and about one gram of each tissue was minced and homogenized (10 times the weight of tissue) in a buffer maintained at pH 7.4 and cold centrifuged (10,000 g for 10 min). The resulting clear supernatant was collected for additional estimations.

Estimating Serum Hepatic and Renal Marker Enzymes

The activities of serum hepatic and renal markers like aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), Gamma glutamyl transferase (GGT), urea, creatinine, albumin, total protein (TP), and total bilirubin (TB) were assayed using a chemistry analyzer (Microlab-300, Merck) according to the standardized procedures using diagnostic kits purchased from Merck (Pvt.) Ltd., Pakistan.

Estimating Urine Renal Marker Enzymes

Before commencement and after every 10th day of experiment, the urine was collected on a 24h basis using metabolic cages for each rabbit from all groups. Creatinine and urea levels were determined by spectrophotometric methods using commercial diagnostic kits.

Determining Antioxidant Enzymes and Vitamins C and E in Tissues

Superoxide dismutase (SOD) activity was measured in tissue homogenates using the method of Kakkar et al.

[23] with a spectrophotometer at 560 nm. Catalase activity (CAT; EC 1.11.1.6) was assayed in tissue homogenates by determining the rate of decomposition of H_2O_2 at 240 nm [24]. The levels of ascorbic acid (vitamin C) and alpha-tocopherol (vitamin E) were assayed in the kidney and liver homogenates according to the methods of Omaye et al. and Desai, respectively [25-26].

Determining Cadmium Contents in Tissues

The tissue samples of kidney and liver were oven-dried, weighed accurately, digested with nitric acid and hypochloric acid, and estimated by an atomic absorption spectrophotometer (Perkin Elmer Analyst 800). The calibration curve of cadmium was constructed using 01, 02, and 03 ppm standards (Perkin Elmer). Bovine liver (NIST:1577b) was used as standard/certified reference material to ascertain the results of tested tissues.

Histopathological Studies

The left kidney and caudal part of liver tissue samples were fixed for 24 hours in buffered 10% formalin, washed under tapwater for 12 hours, then dehydrated by passing through different mixtures of ethanol and water, cleaned in xylene, and embedded in soft white paraffin. Rotary microtome was used to prepare tissue sections (5-6 mm thick) and stained with haematoxylin and eosin (H&E) dye with neutral DPX medium for microscopic observations.

Statistical Analysis

All the data were expressed as mean values ± standard deviation (S.D.) for six rabbits per group. The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS 16.0 version and the individual comparisons were obtained by Duncan's Multiple Range Test (DMRT). Values were considered statistically significant when $p < 0.05$.

Results and Discussion

Determination of Total Phenolics, Flavonoids, and Antioxidant Contents

Antioxidant-rich compounds were extracted from leaf skin in aqueous methanol, and fractions of petroleum ether, chloroform, and water were obtained from sequential partitioning of crude extract. In this study, the amount of extraction yield (g/100 g of dry wt.), TPC (mg GAE/g of dry wt.), TFC (mg CE/g of dry wt.), and TAOC (mg BHTE/g of dry wt.**)** significantly varied between the various fractions of the leaf extracts and was dependent on the solvents used for fractionation. Yield, TPC, TFC, and TAOC of aqueous fraction were the highest, i.e., 13.7±2.6, 98.5±4.8, 46.2±2.1, and 5.8±1.7, respectively, and petroleum ether fraction showed the lowest values (Table 1). Our findings are in close agreement with Miladi and Sultana et al. [27-28]. The differences in TPC, TFC, and TAOC values may be attributed to the use of different solvents for extraction. It has been described in several studies that higher levels of phenolics may be associated with the use of more polar solvents for extraction [29]. Flavonoids and polyphenols are produced as natural secondary metabolites and possess rich antioxidant properties. The flavonoids are mainly comprised of six sub-classes that show some structural differences. These are capable of interacting and scavenging free radicals, which initially damage cells and result in various diseases. Our results clearly illustrate that phenolic and flavonoid compounds like quercetin (identified by HPLC in leaf skin as shown in Fig. 1) are most probably the major contributors to the observed higher antioxidant properties of the AAF.

Changes in Body Weight of Rabbits

Fig. 2 shows the variation in body weight among control, Cd-, Cd+AAF-, and AAF-treated rabbits during 40 days of study. Careful measurement of weight was performed on an electronic balance and was recorded after every four days until the end of the experiment. Cd-receiving group shows the decreasing trend in weight

Table 1. Percentage yields, total phenolics, flavonoids, and antioxidant capacity of *Aloe barbadensis* Miller leaf skin extract.

Extract/Fraction	Percentage yields (g/100g of DW)	Total phenolics (mg GAE/g of DW**)**	Total flavonoids (mg CE/g of DW)	Total antioxidant capacity (mg BHTE/g of DW)
Aqueous methanol	9.8±1.9	81.3±3.1	32.5±1.8	3.2±0.9
Pet. Ether fraction	21.4*±0.8	11.3±1.9	5.4±0.8	1.1±0.5
Chloroform fraction	14.3*±0.5	24.4±2.2	9.1±1.1	2.1±0.8
Aqueous fraction	64.2*±2.6	98.5±4.8	46.2±2.1	5.8±1.7

*weight of fraction/weight of crude extract*100

Data are presented as means ± standard deviation (n = 3); GAE: Gallic acid equivalents; CE: Catechin equivalents; BHTE: butylated hyhroxy toluene equivalents; DW: dry weight.
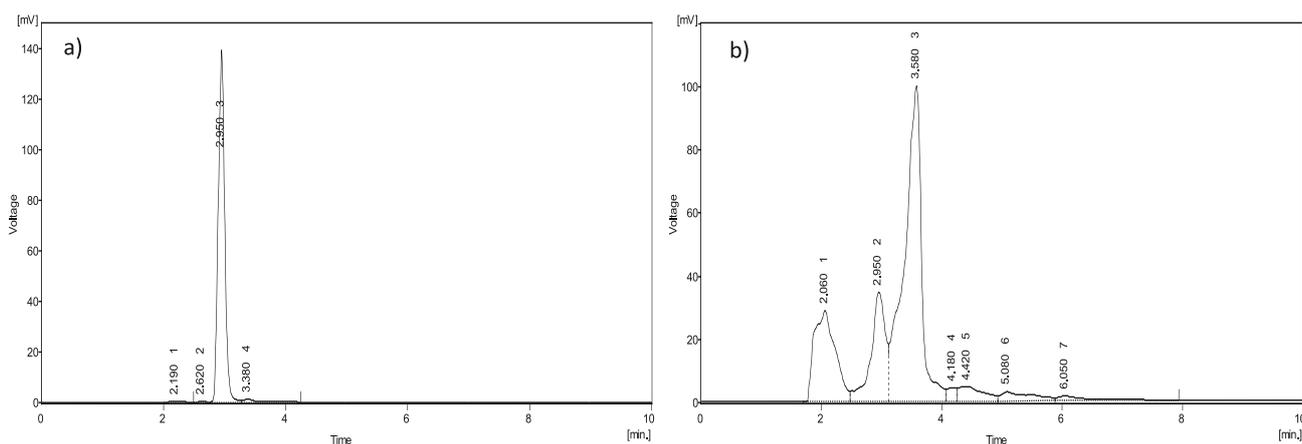
Fig. 1. a) Quercetin standard b) sample of aloe vera aqueous fraction (AAF).

gain from start to finish as compared to the control group as suggested by Sajjad et al. [30], whereas increases in weights of the cadmium-plus-AAF group was better when compared to only the Cd-receiving group. Values of only AAF-treated rabbits were in agreement with the control group and did not show significant variations in weights as shown in Fig. 2. The aloe aqueous fraction may have affected the overall health of animals due to the presence of polyphenols and flavonoids, or due to its high antioxidant activity, which may have resulted in counteracting the damage caused by $CdCl_2$.

Status of Serum Hepatic Marker Enzymes

The aim of the present study was to evaluate the possible protective effects of Aloe aqueous fraction (AF) on hepatic toxicity due to oxidative stress induced by cadmium in male rabbits for 40 days. Table 2 shows the serum liver functional enzyme levels and the bilirubin of the control, cadmium, cadmium-plus-AAF, and the AAF
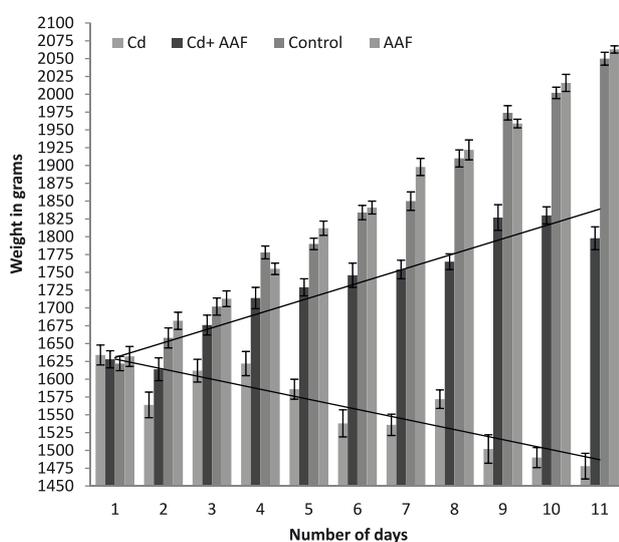

Fig. 2. Variation in body weights of male albino rabbits from the start and days 0-40 of the experiment (mean ± SD, n = 6).

groups. Orally administered cadmium chloride may result in abnormal hepatic functions, in a progressive manner – from day "0" to days 10, 20, 30, and 40 of the experiment. The levels of serum enzymes such as transaminases (ALT, AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), and total bilirubin (TB) were gradually increased to show a significant incremental trend ($p<0.05$) in cadmium-intoxicated rabbits when compared to the control group. Our findings are in close agreement with Renugadevi and Prabu et al. [31-32] regarding Cd intoxication significantly raising the serum total bilirubin and liver enzymes. Administering aqueous fraction of aloe (AAF, 200 mg/kg/day) plus Cd (5.1 mg/kg/day) significantly lowered ($p<0.05$) the levels of liver enzymes and bilirubin when compared to the Cd-receiving group. Levels of liver functional enzymes and total bilirubin remained at satisfactory levels in the AAF group when compared to control rabbits as shown in Table 2.

The raised levels of liver enzymes may indicate leakage from the hepatic cells in the bloodstream and loss of hepatic histoarchitecture. Higher levels of transaminases and alkaline phosphatase are the deci_ sive factors for identifying liver injury in cadmium-intoxicated rabbits as described by Williamson et al. [33]. Serum GGT, an enzyme bound to membrane, is the most renowned indicator of cellular and tissue injury by lethal substances, and its level is also significantly increased in cadmium-intoxicated rabbits. In this study we raised levels of serum liver markers indicating that cadmium had caused functional and structural injury to the cellular membranes, and augmented membrane permeability, resulting in the leakage of liver enzymes into the blood stream. The serum level of bilirubin was also increased in cadmium-treated animals, suggesting that increased levels of serum bilirubin is a strong marker of liver dysfunction.

Hepatic damage can be protected either by strong antioxidant activity or by inhibiting the generation of free radicals [34]. Several results of various studies have also proved the protective effects of plants extracts on cadmium-induced hepatotoxicity [2, 35-36].

Table 2. Values of serum hepatic markers in male albino rabbits at the start, 10th, 20th, 30th and 40th day of experiment (mean ± S.D, n = 6).

Treatment	Days				
	0	10	20	30	40
ALT (IU/L)					
Cadmium	53.0±4.0aa	70 ±6.11ab	95±6.06ac	132±5.24ad	188±8.35ae
Cd + AAF	49±4.92ba	61±7.4bc	76±6.79bd	100±6.77be	133±7.75bf
AAF	49±3.69ba	52±4.9ba	51±5.4ba	55±4.6aa	59±5.1aa
Control	54±4.5aa	56.4±5.8aa	60±4.1aa	48±4.7ba	59.1±3.5aa
AST (IU/L)					
Cadmium	70±4.3kl	90.0±5.3hi	118.0±5.9fg	157.0±7.2cd	215.0±6.1a
Cd + AAF	71±5.6kl	86±7.04ij	107±9.13gh	134±8.85ef	173±8.95c
AAF	65±4.8l	71±4.9kl	69±5.1kl	62±5.40l	72±4.06kl
Control	69±5.4kl	73±4.2kl	76±5.3kl	79±5.4il	75±6.1kl
ALP (IU/L)					
Cadmium	126±3.25cb	148.0±5.08cc	177.0±4.81cd	216.0±6.63ce	270.0±7.47cf
Cd + AAF	121±3.02da	136±5.55db	156.5±6.28dc	185.5±5.89de	221±7.83df
AAF	123±3.2da	120±4.8da	131±3.35db	122±4.72da	128±3.60cb
Control	125±3.72cb	122±2.54da	128±2.79cb	132±5.37db	119±4.35da
GGT (IU/L)					
Cadmium	2.10±0.28ef	2.67±0.63eg	3.68±1.07eh	4.37±1.62ei	5.75±1.02ej
Cd + AAF	1.87±0.19fa	2.2±0.40 fb	2.6±0.78fc	3.3±1.14fd	4.2±0.93fe
AAF	1.77±0.12fa	1.9±0.14fa	2.07±0.15ef	1.83±0.12fa	2.32±0.12fb
Control	2.05±0.19ef	1.93±0.16fa	2.12±0.12ef	2.1±0.23ef	1.81±0.22fa
Total Bilirubin (mg/dL)					
Cadmium	0.21±0.02gh	0.49±0.05gi	0.68±0.07gk	0.92±0.18gl	1.43±0.27gm
Cd + AAF	0.22±0.02gh	0.41±0.04gi	0.60±0.09ic	0.84±0.11id	1.09±0.31if
AAF	0.29±0.03ia	0.33±0.02ia	0.26±0.02ia	0.31±0.03ia	0.22±0.03gh
Control	0.20±0.02gh	0.24±0.02ia	0.31±0.03ia	0.27±0.02a	0.21±0.03gh

Means not sharing similar letter (for each parameter) in a row or in a column are statistically significant (P<0.05). Small letters represent comparison among interaction means of one parameter only.

Status of Urine and Serum Renal Marker Enzymes and Serum Proteins

Table 3 shows the urine and serum renal functional enzymes, serum albumin, and total proteins of control, cadmium, cadmium-plus-leaf extract, and leaf extract groups of adult male albino rabbits. Oral administration of cadmium chloride caused abnormal renal functions in a progressive manner from day "0" to days 10, 20, 30, and 40 of the experiment. The levels of serum enzymes like creatinine and urea were gradually increased to show a significant incremental trend (p<0.05) in cadmium-intoxicated rabbits when compared to the control group as described by Renugadevi et al. [31]. The administration of the aqueous fraction of aloe (AAF, 200 mg/kg/day) plus Cd (5.1 mg/kg/day) significantly decreased (p<0.05)

these levels in serum when compared to only Cd-treated rabbits, while creatinine and urea levels were significantly decreased in urine samples in the Cd-group when compared to control as suggested by Mohamed and Thangapandiyan et al. [36-37]. The cadmium-plus-aqueous fraction of aloe resulted in significant improvement of these values when compared to the Cd group. Serum levels of albumin and total proteins were also decreased in the Cd group when compared to control – contrary to the results of Ige, who demonstrated an increase in serum albumin in cadmium-intoxicated rats. But our findings are in accordance with the results of Mohamed et al. [36, 38].

Cadmium plus aqueous extract significantly restored these levels when compared to only the Cd-treated group. Levels of urine and serum renal functional enzymes and serum albumin and total proteins remained at satisfactory

Table 3. Values of creatinine, urea (in urine), albumin, creatinine, total proteins, and urea (serum) in male albino rabbits at the start and on days 10, 20, 30, and 40 of the experiment (mean ± S.D, n = 6).

Treatment	Day				
	Baseline	10	20	30	40
Urea in urine (mg/dL)					
Cadmium	135±15aa	115±18ab	96±19ac	82±16ad	69±20ae
Cd+AAF	133±18aa	121±15ba	111±18bc	94±19bd	83±18be
AAF	138±17aa	130± 14aa	135±17aa	136±15aa	132±18aa
Control	130±19aa	133±17aa	127±18aa	136±19aa	140±16aa
Creatinine in urine (mg/dL)					
Cadmium	2.9± 0.3ca	2.32±0.25cb	1.91±0.3cd	1.55±0.2ce	1.3±0.15cf
Cd+AAF	2.8± 0.4ca	2.4± 0.25cb	2.15±0.3da	1.9±0.15db	1.7±0.2bc
AAF	2.4±0.25cb	2.6±0.3cb	2.5±0.4cb	2.9±0.25ca	2.8±0.3ca
Control	3.1±0.2ca	2.9±0.25ca	2.7±0.4ca	3.2±0.35ca	2.6±0.2cb
Serum Albumin (mg/dL)					
Cadmium	3.90±0.5ea	3.5±0.4eb	2.9±0.5ec	2.2±0.3ed	1.8±0.4ef
Cd+AAF	3.7±0.4ea	3.4±0.5ea	3.41±0.3ea	2.72±0.5ec	2.12±0.45ed
AAF	4.1±0.5ea	3.6±0.4ea	3.5±0.35ea	3.9±0.5ea	3.7±0.4ea
Control	3.4±0.6ea	3.7±0.5ea	3.6±0.3ea	3.5±0.4ea	3.9± 0.5ea
Serum Total proteins (mg/dL)					
Cadmium	7.50±0.7fa	6.9±0.5fb	6.1±0.4fc	5.1±0.6fd	3.9±0.5fe
Cd+AAF	7.3±0.6fa	7.1±0.4fa	6.4±0.5ga	5.8±0.35gb	4.6±0.5gc
AAF	6.9±0.8fb	7.1±0.5fa	7.6±0.7fa	7.1±0.6fa	7.9±0.6fa
Control	7.2±0.5fa	6.9±0.4fb	7.4±0.5fa	7.3±0.6fa	7.8±0.4fa
Serum Creatinine (mg/dL)					
Cadmium	1.6±0.26ha	2.1±0.25hb	2.7±0.3hc	3.51±0.2hd	4.62±0.3he
Cd+AAF	1.5±0.15ha	1.6±0.2ha	1.8±0.15ha	2.3±0.19ia	2.8±0.1hc
AAF	1.8±0.3ha	1.4±0.2ha	1.3±0.1ha	1.6±0.3ha	1.5±0.2ha
Control	1.4±0.2ha	1.3±0.15ha	1.8±0.2ha	1.5±0.1ha	1.7±0.2ha
Serum Urea (mg/dL)					
Cadmium	35±2.5ja	45±3.6jb	54± 4.2jc	69±3.8jd	91±4.6je
Cd+AAF	36±3.5ja	43.5±2.8jb	48.4±4.0ka	59±4.5kb	73±3.9kc
AAF	38±3.8ja	37±2.8ja	31±3.2ja	33±2.9ja	39±3.6ja
Control	33±4.1ja	34±3.5ja	40±2.5ja	37±3.8ja	35±2.9ja

Means not sharing similar letter (for each parameter) in a row or in a column are statistically significant (P<0.05). Small letters represent comparison among interaction means of one parameter only.

levels only in the AAF-administered group when compared to control rabbits (Table 3). Prolonged exposure to cadmium may result in damage to various organs and systems, predominantly renal tissue [39]. Kidneys are more susceptible to injury because of greater perfusion and a large number of combinations are excreted by renal tubular cells. Urea is the major metabolic product obtained from protein metabolism that contains nitrogen.

It is well-documented that Cd hinders the incorporation of amino acids into proteins, leading to increased levels of urea. Creatinine is a waste product of creatine phosphate by muscle metabolism and it usually signifies impaired renal function or renal disease. As the kidneys become impaired, the creatinine level in serum will rise due to poor creatinine clearance by the kidneys. Thus the urea and creatinine levels in both serum and urine samples

were considered indicators of renal functioning (because hyperuricemia is a renal prognostic factor). Administration of an antioxidant-rich fraction protects the renal function from cadmium-induced toxicity as shown by significantly restoring the serum and urine urea and creatinine. The present study supports the findings of Morales et al. [40]. Various results suggest that plant extracts may prevent nephrotoxicity by suppressing the oxidative stress caused by cadmium [36, 39, 41-43].

Determining Antioxidant Enzymes and Vitamins C and E in Tissues

Table 4 shows the enzymatic anti-oxidants, i.e., superoxide dismutase and catalase and non-enzymatic antioxidants, vitamins C and E in liver and kidney homogenates of Cd, Cd-plus-AF, AF, and control groups of adult male albino rabbits. Orally administered cadmium chloride caused abnormal renal and hepatic functions in a progressive manner from day "0" to 40 of the experiment, resulting in significantly decreased enzymatic and non-enzymatic antioxidants in liver and kidney samples in only the Cd-receiving group when compared to control. The cadmium-plus aqueous fraction of aloe resulted in significant improvement of these values when compared to the Cd group. Levels of these enzymatic (SOD, CAT) and non-enzymatic (vitamins C and E) antioxidants in tissue homogenates remained at satisfactory levels only in the AAF-administered group when compared to control rabbits (Table 4).

Enzymatic antioxidants such as SOD and CAT may establish a supportive and defensive group against reactive oxygen species (ROS). Superoxide dismutase, being a metallo-protein, may catalyze the dismutation of superoxide radicals. Catalase, being a hemeprotein, may catalyze the reduction of H_2O_2 to H_2O and O_2, thus defending the cells from oxidative destruction by H_2O_2 and OH^-. SOD activity may decrease either owing to the opposed effect of Cd with Cu and Zn, which are considered

significant metals due to their activity or inactivation of superoxide dismutase by lipid peroxidation induced by Cd. It has been reported that the decline in CAT activity by Cd might reflect the reduced absorption of Fe, a vital trace metal essential for the CAT activity [31]. Our results for this study are in accordance with Renugadevi and Waisberg et al. [44-45], who also showed that SOD and CAT activities are decreased by cadmium intoxication. The deficiency in the antioxidant defensive system may be considered an acute event in cadmium-induced hepato-renal toxicity. Cadmium exposure may be characterized by depletion of non-enzymatic antioxidants (vitamins C and E) in tissues and in the bloodstream. These are the main non-enzymatic antioxidants showing synergetic effects in scavenging reactive oxygen species or free radicals. Our findings are in accordance with Sk and Valko et al. [46-47], who showed that the level of vitamin C (ascorbic acid) and vitamin E (alpha-tocopherol) are significantly reduced during Cd intoxication, leading to the development of Cd-induced hepatic and renal impairment.

Administration of plant extracts in cadmium-intoxicated rabbits may protect the enzymatic and non-enzymatic antioxidants by means of either their metal chelation activity or by their antioxidant activities, which might reduce the exploitation of these antioxidants, thus reinstating their levels to near normal as described by Sakr et al. [48].

Determining Cadmium Content in Tissues

Table 4 also shows the cadmium contents in liver and kidneys of the Cd, Cd-plus-AF, AF, and control groups of rabbits. Orally administered $CdCl_2$ resulted in the accumulation of cadmium in renal and hepatic tissues, while a significant decrease in Cd contents was observed for the Cd-plus-aqueous fraction of the aloe group in liver and kidney samples when compared to the Cd group. Accumulation of cadmium in liver is a renowned result

Table 4. Catalase (CAT, μmol H_2O_2/min), Superoxide dismutase (SOD, units/mg protein), ascorbic acid (Vitamin C, μmol/mg tissue) and alpha tocopherol (Vitamin E, μmol/mg tissue) and cadmium content (μg/g) in liver and kidney of male albino rabbits on 40th day of experiment (mean ± S.D, n = 6).

ORGANS	Parameters	Control	AAF	Cadmium	Cd + AAF
KIDNEY	CAT	51.3±1.6[a]	50.6±1.9[a]	22.4±1.43[b]	41.2±1.63[c]
	SOD	11.71±0.78[a]	11.14±0.81[a]	5.32±0.52[b]	8.97±0.63[c]
	Vitamin C	0.95±0.07[a]	0.93±0.09[a]	0.48±0.04[b]	0.69±0.05[c]
	Vitamin E	0.62±0.05[a]	0.61±0.0⁵a	0.21±0.03[b]	0.33±0.03[c]
	Cd content	2.54±1.1[a]	2.71±0.9[a]	138.93±6.8[b]	114.37±6.1[c]
LIVER	CAT	68.4±1.9[a]	70.1±2.1[a]	27.8±1.5[b]	50.36±1.7[c]
	SOD	8.72±0.60[a]	8.21±0.65[a]	3.6±0.28[b]	5.92±0.47[c]
	Vitamin C	1.51±0.11[a]	1.49±0.1[a]	1.02±0.08[b]	1.31±0.07[c]
	Vitamin E	0.93±0.06[a]	0.91±0.07[a]	0.49±0.04[b]	0.63±0.05[c]
	Cd content	1.32±0.89[a]	1.48±0.8[a]	86.7±4.5[b]	65.21±3.9[c]

[a,b,c] Values not sharing a common superscript letter (a, b, c) in a row differ significantly at $p < 0.05$. (Least Significant Difference)

and it is recognized to produce hepatic oxidative injury by increasing per-oxidation of membraneous lipids – an injurious process exclusively performed by free radicals. Although free radicals are not generated by cadmium directly, numerous radicals like superoxide, nitric oxide, and hydroxyl are generated indirectly, thus producing injuries consistent with oxidative stress [49], whereas the Cd-metallothionein (Cd-MT) complex formed in hepatic cells is released slowly by hepatic cells and circulates to the renal tissue. This Cd-MT complex is dissolved in renal cells, releasing free cadmium that is absorbed by proximal tubules. If renal metallothionein detoxification and defense systems are incredulous, free cadmium can cause injury to the renal proximal and distal tubules [50].
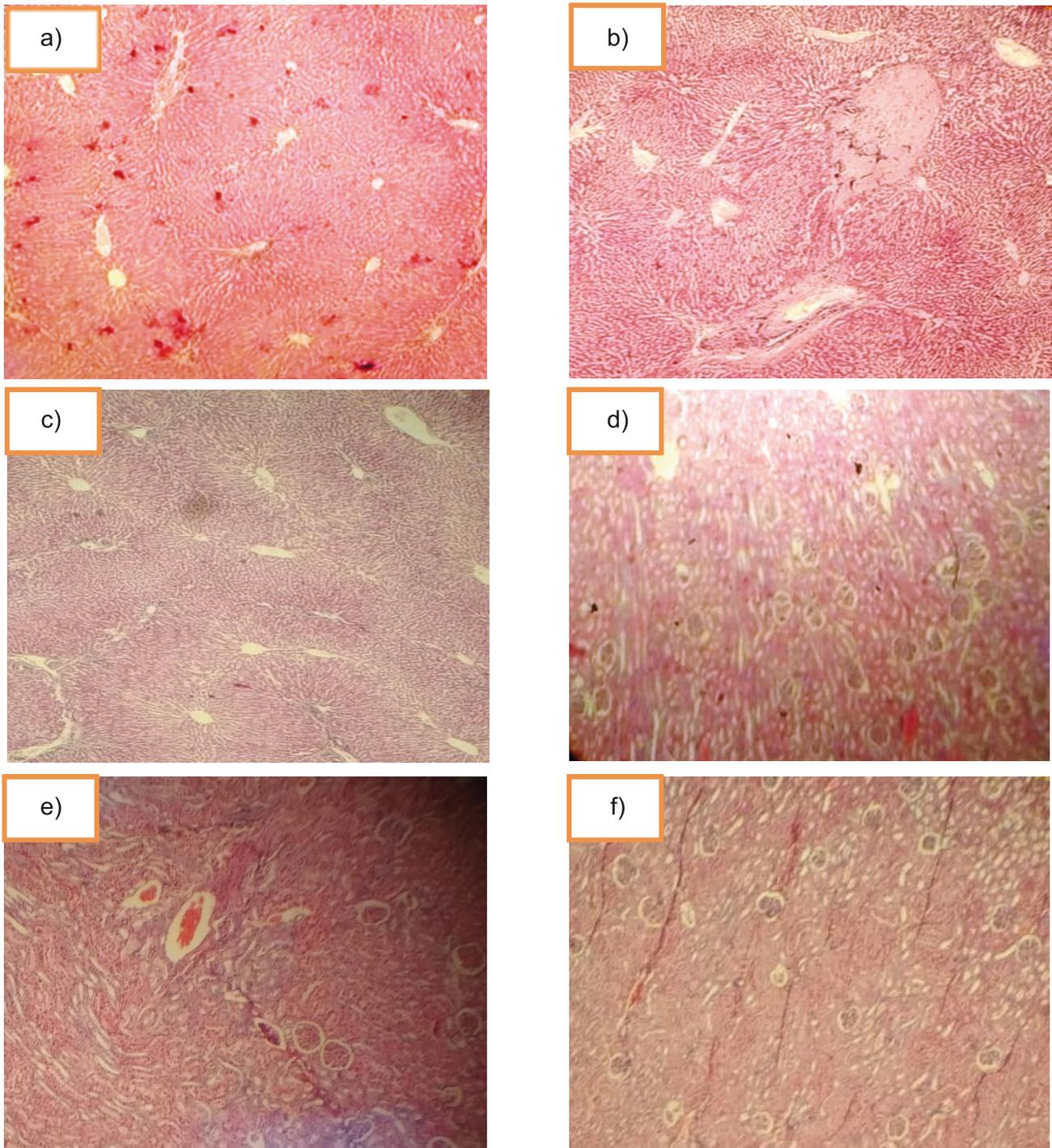

Fig. 3. Microphotograph of a) control group rabbit showing normal architecture of liver cells, b) $CdCl_2$-intoxicated rabbit liver with peri-portal infiltrations and vascular congestion, c) $CdCl_2$- and AAF-treated rabbit liver with near-normal liver cells, d) control group rabbit showing normal architecture of renal cells, e) $CdCl_2$-intoxicated rabbit kidney with swelling and glomerular congestion, and (F) $CdCl_2$- and AAF-treated rabbit with near normal renal cells.

Histopathological Studies

Histopathological changes in the kidneys and liver were evaluated in such a manner that the histopathologist responsible for interpretation was kept unaware of the treatment protocols. Histopathological studies of liver of rabbits revealed that oral cadmium intoxication may have caused severe hepatic damage, including steatosis, sinusoidal dilation, inflammation of the central vein, inflammatory cell infiltration, portal inflammation, portal fibrosis, and necrosis when compared to control. These changes were reduced in livers of the AAF-plus-Cd treated group.

Histopathological studies of kidneys revealed that intoxication with orally administered Cd resulted in severe kidney damage, including proximal and distal tubular damage, hydropic swelling, and degeneration of tubular epithelium when compared to control. Significant reversal of these changes was observed in the AAF-plus-Cd-treated group. The histoarchitecture of liver and kidney was almost normal in rabbits treated with AAF alone (Fig. 3).

Conclusion

Significant decreases in biochemical hepatic and renal marker enzymes – in contrast to decreases in total bilirubin and albumin – were observed in a progressive manner in the Cd+AAF group when compared to Cd-treated rabbits. Moreover, superoxide dismutase, catalase, and vitamins C and E in liver and kidney tissues were significantly increased, whereas cadmium content decreased for the Cd+AAF rabbits as compared to the Cd-intoxicated rabbits. The histopathological studies for liver and kidney have also supported findings that the Cd+AAF group noticeably lessened the toxicity of cadmium in both tissues at close to normal, thus suggesting that the flavonoid-rich fraction of AAF may act as a natural protective agent against Cd toxicity due to its high antioxidant activity.

Acknowledgements

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