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Anti-oxidant Activity of Novel Compound (AVO) Derived from L-arginine

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Abstract

The present study aimed to investigate the potential effect of AVO in alleviating Hematological and serum biochemical alterations induced by cadmium chloride toxicity in rats. 32 adult male rats were divided in to 4 groups (8 rats each for each group: Control group received normal saline by i.p injection and served as a control. Treated group I received AVO daily at 72 mg/kg body weight (B.W.) by ip injection. Treated group II administered $CdCl_2$ daily at dose 225 mg/kg B.W. by i.p injection. Treated group III administered of $CdCl_2$ daily at a dose of 225 mg/kg B.W. by i.p injection and after one hour of $CdCl_2$ administration the treated rats given the new compound AVO at dose 72 mg/kg B.W. by i.p injection. The obtained results indicated that cadmium chloride possesses a deleterious effect on blood cytology, induce oxidative damage, hepato-renal dysfunction, increase of MDA and cause clear changes on the sexual hormone. The administration of AVO with cadmium chloride minimized the hazard effects of cadmium chloride, it improved RBCs count, PCV, Hb concentration ,total and differential WBCs count and blood indices Diminished the level of serum malondialdehyde (MDA).Moreover, it ameliorates the activities of AST, ALT, ALP lipid profiles, bilirubin, urea, uric acid, protein and glucose

1 Introduction

Cadmium is a one of the dangerous heavy metal which is released from various industrial[1], causing contamination of the ambient air, water, and soils [2]. Chronic ingestion of Cd in food or inhalation in air would result in accumulate of Cd in the body specially into the liver and kidney, causing liver and renal diseases[3], testicular damage, disorders of the respiratory and nervous system[4-5], induces immunological effects[6], Osteomalacia ,Osteoporosis ,increased into blood pressure and atherosclerosis[7-9]. Furthermore Cd is known to induce anemia, reduce the RBCs count, hematocrit value as well as hemoglobin concentration[10],[11] reported the conformational changes of the rat hemoglobin which exposed to cadmium, by conversion it to methmoglobin (MetHb), which is a dysfunctional form of hemoglobin[12].

Antioxidants are substances that prevent damage from the cells which caused by unstable molecule called free radical that cause cell damage. Antioxidants substances are interacting with these free radicals and stabilize it; therefore, they act as a protector of the cell from being damaged by these molecules[13]. L-arginine is one of the semi - essential amino acid,normally the body produces it in enough amounts. But, dietary supplementation may be needed in some conditions such as oxidation by heavy materials[14]. L-arginine has important biological functions for the body[15], it can act as an improver defense mechanism of the body and increase resistance to the gastric carcinoma[16], reduce the inflammatory response [17].In sickle cell transgenic mice, L-arginine returns erythrocyte density to the normal state[18], make it save from oxidative stress[19], and improves function of microvascular[20]. L-arginine is a precursor of nitric oxide (NO), and all these therapeutic effects of L-arginine may attribute to NO, which is act as an endothelial relaxing factor, inhibit aggregation of platelets, and reduce neutrophils interaction with endothelium, by neutralizing superoxide radicals[21].

Other substances that acts as an antioxidant is Vanillin (4-hydroxy-3-methoxybenzaldehyde), which is the major compound of vanilla beans[22]. Vanillin considered as potent

antioxidant compound [23]. Synthetic structures of vanillin used as phytomedicine or manufactory of the drugs [24]. Synthesized compound (Schiff-base) was synthesized by reaction of L-arginine and Vanillin in presence of glacial acetic acid.

2 Materials and Methods

2.1 Cadmium chloride-induce methemoglobuline in hemolysate

Blood samples were obtained from healthy individuals by puncture of the vein, and kept in ethylene diamine tetraacetic acid (EDTA) containing tubes; then centrifuged at 2500 rpm for 10 minutes to remove plasma and the buffy coat of white cells. Washed the erythrocytes thrice with phosphate buffer saline (PBS, pH 7.4) and lased by suspending in 20 volumes of 20 mM Phosphate Buffer (PB, pH 7.4) to yield the required hemolysate concentration of 1:20. Finally, the lysate was centrifuged at 3000 rpm for 10 minutes to remove the stroma. The reaction was intiated by the addition of 1.5 ml freshly prepared hemolysate, 1.0 ml of different concentrations of L-arginine derivatives (20µM, 10µM and 5µM) each time were added concomitantly with 0.1 ml cadmium chloride, and the formation of MetHb was monitored spectrophotometrically at 631 nm for 50 minutes using computerized UV-visible spectrophotometer[25].

2.2 In-vivo antioxidant activity

2.2.1 Animal and experimental design

Rats were divided into 4 groups (8 rats in each group) as following:

Control group: In this group, 8 male rats and were injected I.P with 0.9% normal saline (N.S) 0.5 ml daily for 28 days.

Treated group 1: Group consisted of 8 male rats which were injected intraperitoneally (I.P) with AVO only 0.5 ml daily for 28 days.

Treated group 2: Group consisted of 8 male rats were injected I.P with 225 mg/kg $CdCl_2$ daily for 28 days.

Treated group 3: 8 male rats and 10 female rats were injected I.P with 1/10 of LD_{50} (72) mg/kg AVO complex daily after one hour of 225mg/kg $CdCl_2$ administration for 28 days.

Blood samples were collected from the heart by heart puncture disposable syringes of 5cc capacity were wsed. After anesthesia of the rats, blood collected and analyzed according to Sood (1996)[26]

- 4 ml of blood was poured into a tube containing the ethylene diamin tetra acetic acid (EDTA) as an anticoagulant for RBC, Hb, PCV and WBC, differential WBC analysis.
- 6 ml of blood was poured into test tubes free from anticoagulant to isolate blood serum to estimate the biochemical parameters such as glucose, total cholesterol, HDL – cholesterol, LDL – cholesterol and triglycerol, VLDL, GOT, GPT, uric acid, follicular stimulating hormone, leutinizing hormone and testosterone.

2.2.2 Hematological tests

Erythrocytes count, Hb, PCV, MCH, MCHC, WBC and deferential WBC.

2.2.3 Biochemical test

Estimated Serum AST, ALT, ALP lipid profiles, bilirubin, urea, uric acid, protein, MDA and glucose were evaluated.

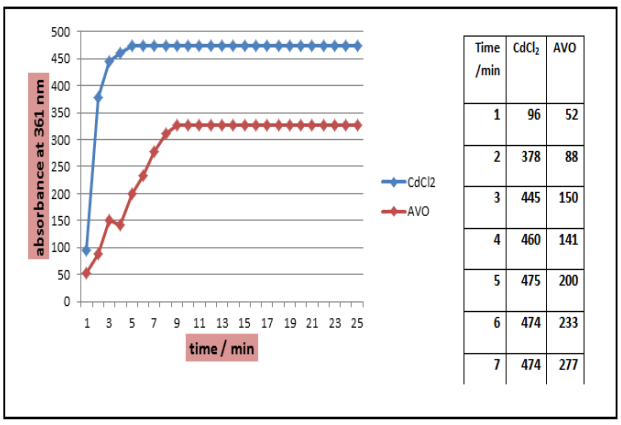
2.3 Statistical analysis

Computerized SPSS (Statistical Package for Social Sciences) (V.13) program were used for analysis of results of the present study. The data were expressed as mean ± standard error (mean ± SE).Least significant difference test (LSD) was used to test the difference between means (groups); P≤ 0.05 was considered significant [27].

3 Results and Discussion

3.1 In-vitro antioxidant activity

$CdCl_2$ caused rapid oxidation of hemoglobin (Hb) to methemoglobin (MetHb). Using AVO complex in a dose dependent manner, the oxidation process delayed. The results showed that the time required to convert the hemoglobin to methemoglobin was 6 minutes in the absence of the AVO complex, whereas using of 20 µM of AVO complex the time was increased to 9 minutes (Fig 1).

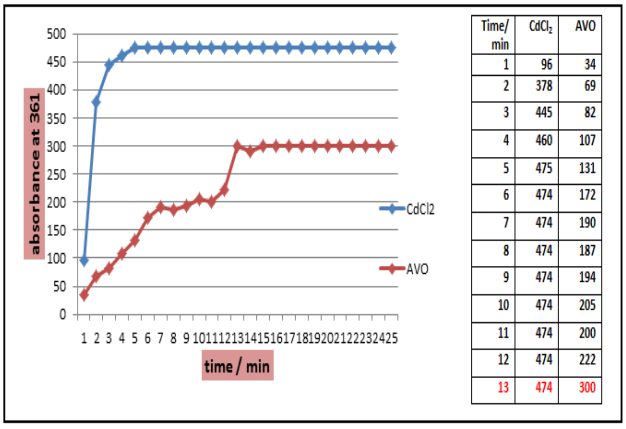


Time /min	$CdCl_2$	AVO
1	96	52
2	378	88
3	445	150
4	460	141
5	475	200
6	474	233
7	474	277

Fig 1: Inhibition of Cadmium-induced methemoglobin formation by AVO 20 µM at 9 min, in hemolysate

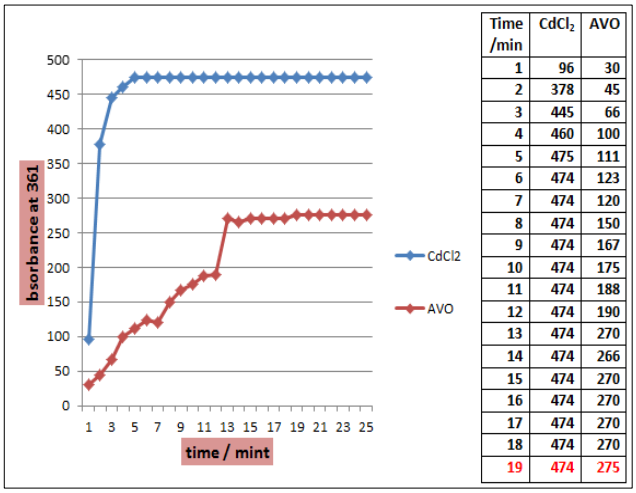
It seeded that not all Hb convert to MetHb. That means, when the complex was added along with cadmium at 0 min, the formation of methemoglobin was been inhibited to wide extent. This observation also appeared in (Fig 2) in which the time required to convert the hemoglobin to methemoglobin was 13 min when using 15 µM concentration of AVO complex and that there was increased the inhibition of the conversion of Hb to MetHb. The best result of study is at concentration of 5 µM concentration of AVO complex in which it protected

hemoglobin from oxidation by sodium nitrite to great extent, the conversion time of Hb to MetHb reached to 19 minutes compared with other AVO concentrations and $CdCl_2$ samples (Fig 3), also see Fig 4.



Time/ min	$CdCl_2$	AVO
1	96	34
2	378	69
3	445	82
4	460	107
5	475	131
6	474	172
7	474	190
8	474	187
9	474	194
10	474	205
11	474	200
12	474	222
13	474	300

Fig 2: Inhibition of Cadmium-induced methemoglobin formation by AVO 10 µM at13 min, in hemolysate



Time /min	$CdCl_2$	AVO
1	96	30
2	378	45
3	445	66
4	460	100
5	475	111
6	474	123
7	474	120
8	474	150
9	474	167
10	474	175
11	474	188
12	474	190
13	474	270
14	474	266
15	474	270
16	474	270
17	474	270
18	474	270
19	474	275

Fig 3: Inhibition of Cadmium-induced methemoglobin formation by AVO 5 µM at 19 min, in hemolysate

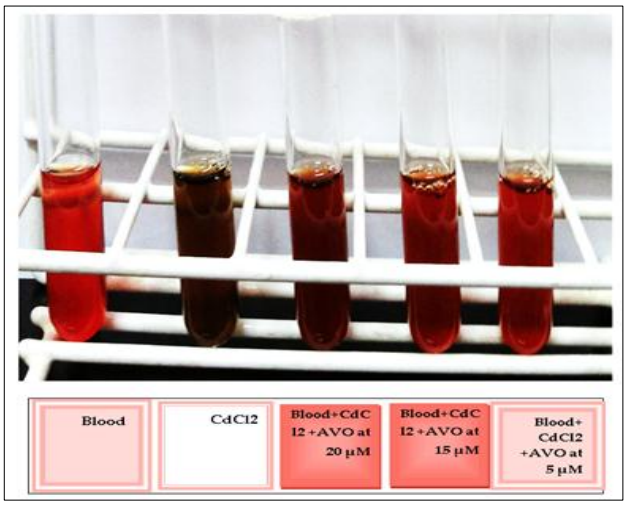


Fig 4: Inhibition of Cadmium-induced methmoglobin formation by AVO at 20, 15, 5 µM in hemolysate

According to present findings, the free radicals liberation due to expose of cadmium chloride on erythrocyte lysate, leads to oxidative damage and production of MetHb[11]. The tertiary or quaternary conformation of the Hb molecule is related to its molecular stability against auto-oxidation[28,29]. It implies that the injected of cadmium to the rats effect on the auto- oxidation rate of hemoglobin and oxygen (HbO_2) to MetHb which is occurring especially on the tertiary conformation change of molecule Hb. This conformational change effect on the protein moiety of Hb, its reflect the stability of hemoglobin against normal or auto-oxidation [11].

The study that achievement by Gray *et al* [30] were demonstrated that the highly significant increase in the Met-Hb,S-Hb, and Hb-co concentration in exposed rats to cadmium represents an evidence of the inactivation of the couple enzymes related to elevated of oxidizes Hb. Moreover, propose that the administration of cadmium to the rats causing defect in enzymes activity, which involved the system of Met-Hb reductase within erythrocytes. Met-Hb reductase means oxidation and reduction disorders[30]. Attia *et al* [31] demonstrated the role of cadmium in formation of metHb in the blood of rats in vivo by induced oxidative stress.

Oxidation of hemoglobin by cadmium was used as *in vitro* model to assess the concentration and time dependent protection of AVO compound cadmium-induced Hb oxidation. Also the present study indicated the pharmacological effect of AVO; it prolonged the time of MetHb formation at 5, 10, 20 µm concentration. Ahmed *et al,* [32] reported that the L-arginine evokes antioxidant activity in vitro. Other study demonstrated the antioxidant effect of L-arginine in low dose orally, it increased RBC resistance to lysis by osmotic by increased activity of antioxidant enzymes, but also it caused reduced density of RBC in Sickle cell anaemia disease [33]. The results that obtained by Nigris *et al* [34], demonstrate that disturbed shear stress modulates eNOS activity in both *in vitro* and *in vivo* and show that prolonged therapeutic intervention with antioxidants and L-arginine can normalize this proatherogenic disequilibrium.

Other study by Ho *et al* [35], provided the effect of vanillin in vitro and in vivo against oxidative stress [36], suggest that the aldehyde group of vanillin may be important for its anti-metastatic activity and the effect on cell growth [37], were found the antioxidant activity of vanillin and ethyl-vanillin when given orally to the mice; they prevent the oxidative stress of the plasma, and [38] suggest that this particular derivative has an efficient antioxidant activity.

3.2 In-vivo antioxidant activity

In this study, the data showed a significant change in the blood parameters of the male rats treated with $CdCl_2$ as shown in the tables 1 and 2, including a significant decrease in the RBC, Hb,

platelets, PCV, MCV, MCH, MCHC, and a significant decreased in lymphocyte percentage; and a significant increase in the WBC, neutrophil, monocyte, eosinophil and basophil, compared with the control group. Those findings were agreed with the results of previous studies [10,39-42], considering that exposure to cadmium led to decrease in RBC count, Hb concentration, PCV percentage and total WBC count and its differentiation number, in addition, induced anemia in rats as well as in rabbits.

Table 1: effects of AVO on $CdCl_2$ treated rats

Group	RBCs×10^6/µL	Hb (g/dL)	Platelets ×10^3/µL	PCV (%)	MCV(fL)	MCH(pg/ cell)	MCHC(g/d)
Control	6.93± 0.15 a	11.62 ± 0.28 a	546.25 ± 9.9 a	31.58 ± 0.69 a	45.61± 0.67 a	16.90 ± 0.31 a	36.81 ± 0.50 a
AVO (72mg/kg)	6.91± 0.08 a	11.79 ± 0.19 a	551.12 ± 8.5 a	31.68 ± 0.33 a	45.69± 0.3 a	17.08 ± 0.35 a	37.39 ± 0.77 a
$CdCl_2$ (225mg/kg)	4.79 ± 0.18 b	6.92 ± 0.18 b	329.62 ± 8.28 b	19.95 ± 0.22 b	43.24± 1.48 b	14.53 ± 0.43 b	34.77 ± 1.17 b
AVO(72mg/kg) +$CdCl_2$ (225mg/kg)	5.263 ± 0.23 a	9.43 ± 0.39 c	437.62 ± 11.35 c	24.92 ± 0.79 c	47.76± 1.71 c	17.97 ± 0.32 a	37.98 ± 1.11 c
LSD	1.64	2.18	108	4.97	4.51	1.07	3.2

Values are expressed M ± SE. Different letters indicate significant difference at (P≤0.05); a = means control group value, b = means Cd treated group that compared with control group, c = means AVO + Cd treated group that compared with control group. Depend on the M ± SE

Table 2: effects of AVO on $CdCl_2$ treated rats on total and differential leukocyte count

Group	Leuko (×10^3/µL)	Neut%	Lympho%	Mono%	Esino%	Baso%
Control	5.83 ± 0.31 a	23.61± 0.3 a	66.73± 0.53 a	8.08± 0.24a	1.4± 0.02 a	0.33± 0.03 a
AVO (72mg/kg)	5.46 ± 0.26 a	23.86± 0.47 a	66.67± 0.39 a	7.93± 0.13a	1.38± 0.02a	0.3± 0.02 a
$CdCl_2$ (225mg/kg)	16.75 ± 0.39 b	30.58± 0.36 b	57.41 ± 0.47 b	9.93 ± 0.33 b	1.58± 0.03 b	0.6± 0.03 b
AVO (72mg/kg) +$CdCl_2$ (225mg/kg)	11.35 ± 0.44 c	26.68± 0.29 c	61.92± 0.48 c	9.51± 0.21 c	1.45± 0.01 c	0.5± 0.02 a
LSD	5.4	2.82	4.51	1.42	0.13	0.1

Values are expressed M ± SE. Different letters indicate significant difference at (P≤0.05); a = means control group value, b = means Cd treated group that compared with control group, c = means AVO + Cd treated group that compared with control group. Depend on the M ± SE

Blood is most sensitive to the toxicity of metals or even toxicity by drugs[43,44], previous experiments showed that chronic administration of Cd cause damage in RBCs of rats and goldfish by destroying of cell membranes due to increase induced of lipid peroxidation, in addition to modified in the antioxidant defense mechanism, energy metabolism and finally anemia induced[39,45,46]. Cd caused increased lipid peroxide concentration in the blood of rats with formation of ROS [47]. As a result, clear disturbances of the antioxidant defense system occurred[48,49]. The membrane of RBC consists of highly amounts of polyunsaturated fatty acids, that have highly susceptible to peroxidation caused by released of free radical. Eventually, peroxidation of lipid membrane results in hemolysis [50]. Incidence of lipid peroxidation causes different effects of toxicity, including disturbance in membrane fluidity and function, dysfunction of mitochondrial and Golgi apparatus, and finally inhibition of an antioxidant enzymes. MDA is a main end product of lipid peroxidation and is a frequently indicator of toxicity processes. MDA can cross-link with membrane contains of RBC[51,52]. On the other hand Horiguchi *et al* [53] found that the chronic cadmium intoxication caused anemia. Cadmium is known to reduce red blood cell count and hematocrit value as well as Hb concentration[45,54]. The reduction in the Hb content may occur from increase rate of RBC destruction or from decrease rate of RBC formation in rat by hypo induction of erythropoietin from kidney [53].In addition, the decrease in Hb content my attributed to increase activity in bone marrow, which result in increased formation of RBC with impaired integrity and can be easily destruction in the circulation[55].

Treatment with $CdCl_2$ followed by AVO at 72 mg/kg appears to have significant improvements on the erythrocytes and MCH. However there are significant changes in the other blood parameters, which include: Hb, PCV, platelets, MCV, MCHC, and in leukocytes counts and differential (Table 1, 2).

Also, found that the L-arginine normalizes density of RBC[18]. In addition, L-arginine act as protector against oxidative stress[19], improving function of microvascular[20]. In humans with Sickle cell disease or sickle cell anemia (SCA), L-arginine increases NO synthesis [56].

On the other hand Bor-Kucukatay *et al*[57],as in literature review, were mention that NO can clearly affect RBC deformability and therefore suggest the important effect of NO in maintaining normal erythrocyte deformability. RBC has the ability to deform (i.e., RBC deformability) is an importance activity for the maintenance of normal circulation, it essential in allowing the passage of erythrocytes in narrow capillaries of the microcirculation and thus reduces viscosity of blood in large blood vessels[58].

3.3 Serum biochemical

The other results are presented in the table (3), indicated an absent of effect of AVO on ALP, AST and ALP, when given alone compared with the control group, whereas injection of cadmium chloride resulted in a significant increase in the levels of AST, ALT and ALP levels. The liver is one of the target organs to cadmium, whether after acute or chronic exposure [59]. Liver damage caused by cadmium leads to releases of ALT, AST and ALP enzymes into the extracellular fluid and increased their levels into the plasma.

Table 3: The ameliorative effects of AVO on $CdCl_2$ treated rats on serum alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphotase (ALP)

Group	ALT (U/L)	AST (U/L)	ALP (IU/L)
Control	9.5 ± 0.29 a	19.31 ±0.34 a	32.76 ± 0.60 a
AVO (72mg/kg)	9.38 ± 0.14 a	19.18 ± 0.29 a	32.18 ± 0.61a
$CdCl_2$ (225mg/kg)	20.12 ± 0.63b	45.07 ± 0.58 b	57.91 ±1.10 b
AVO (72mg/kg) + $CdCl_2$ (225mg/kg)	12.83 ± 0.6 c	28.11 ± 0.43a	39.39 ± 1.57 c
LSD	3.28	8.8	6.62

Values are expressed M ± SE. Different letters indicate significant difference at (P≤0.05); a = means control group value, b = means Cd treated group that compared with control group, c = means AVO + Cd treated group that compared with control group. Depend on the M ± SE

Also Rikans, and Yamano [60], were founded that oxidative stress intensification after cadmium administration in the liver are responsible for the increase of ALT, AST and ALP activity. Cadmium hepatotoxicity is probably affected in two ways: first by occurrence of inflammatory state, second by direct toxic action of cadmium on hepatocyte[61]. Toxicity of cadmium started with released of ROS[62], releasing of ROS lead to induction of oxidative stress unless it was overcome with endogenous antioxidants. Thus, overproduction of ROS may be contributed to the decrease levels of antioxidants or to the direct action of cadmium on peroxidation[63].

Tkachenko and Kurhalyuk[64], Reported that L-arginine has hepato-protection ability against lead toxicity by decreasing lipid hydroperoxidase, and increase glutathione antioxidant defense system in the liver. Injection of L-arginine and synthesis of NO has benefit effect in inhibits the lead toxicity[65]. L-arginine-NO pathway seems to be a good couple as a solder body protects against invading of metal toxicity like lead, it has several properties[66]. It's thought that NO may reveal its protection effect by inhibit the fenton reaction, through binding to ferrous iron, result in prevention of hydroxyl radical formation [67]. It seems that NO has the ability to reduce the formation of ferryl heme that result from the reaction of hemoglobin with peroxides, in addition to this mechanism, NO may interfere with the hydroperoxides detoxication[66] .On the other hand Wang and Liu [68] reported role of NO in immunological liver destroyed in mice. The hepato-protective effect of vanillin is agreed with recent studies reported b [69, 70].

A study by Ho *et al* [35] suggested the protective effects of vanillin in-vitro and in-vivo on oxidative stress. This action of vanillin is may attributed to polyphenol substance which is the main component of vanillin, its known as scavenging superoxide and then preventing lipid peroxidation in the membranes[71,72].

Also there are modified in the data of lipid profiles after treatment with cadmium (Table 4), there was a significant increase in the TC, TG, LDL, VLDL, levels, and a significant decrease in the level of HDL when animal exposures to cadmium chloride. These results were agreed with[73-76]. Cd causes alterations in metabolism of lipids and lipoprotein, which may eventually, leads to atherosclerosis[77,78]. This might be attributed to the impairment of liver function caused by the

disturbance in antioxidant defense mechanism in Cd intoxicated rats [79]. The degree of hypercholesterolemia is directly increased with increase severity of liver injury in toxic condition which attributes disorder in lipid homeostasis[80]. HDL fraction transports both free and esterified cholesterol from the peripheral tissues to the liver, where the metabolized of cholesterol is occur into bile acids and then excreted[81]. This good type of cholesterol has an important role in reducing level of cholesterol in circulation and peripheral tissues therefore inhibiting thermogenesis. Toxicity of cadmium causes decrease in the level of HDL due to liver injury.

NO synthesis in the cardiovascular system may provide a pathogenic factor in many diseases, it may cause increase in blood pressure, kidney failure, diabetes mellitus, increase cholesterol in the blood[82, 83].

The hypolipemic effect of L-arginine is due to an increase in NO level and a decrease oxidation fatty acid[84], Whereas hypolipidimic effect of AVO may due to vanillin[85,86]. Vanillin is a very a potent scavenger of free radical[87,88]. Administration of vanillin orally, results in an increase in both vanillin concentration and its antioxidant activity in the plasma and therefore helps in the prevention and inhibition of free radical which induced lipid peroxidation in the membranes[37].

Table 5 revealed that the cadmium chloride caused a significant increase in urea level when the animal injected with cadmium chloride compared with control, the same conclusion was an observation by Ulaiwi *et* al[89], Aktoz *et al* [90] and Kari *et al* [91].

Table 4: Ameliorative effects of AVO on $CdCl_2$ treated rats on serum lipid profile

Group	TC mg/dL	TG mg/dl	HDL mg/dl	VLDL mg/dL	LDL mg/dL
Control	55.38 ± 0.65 a	36.45 ± 0.78 a	39.41 ± 0.54 a	7.29 ± 0.15 a	8.71 ± 0.67 a
AVO (72mg/kg)	55.15 ± 0.85 a	35.81 ± 0.78 a	39.47 ± 0.57 a	7.2 ± 0.16 a	8.51 ± 0.94 a
$CdCl_2$ (225mg/kg)	82.82 ± 1.53 b	69.36 ± 1.57 b	26.66 ± 0.77 b	13.84 ± 0.31 b	41.69 ± 1.82 b
AVO (72mg/kg) + $CdCl_2$ (225mg/kg)	67.36 ± 1.7 c	44.58 ± 1.44 c	37.35 ± 0.88 c	8.91 ± 0.28 a	21.08 ± 2.24 c
LSD	11.97	8.77	2.05	1.62	12.36

Values are expressed M ± SE. Different letters indicate significant difference at (P≤0.05); a = means control group value, b = means Cd treated group that compared with control group, c = means AVO + Cd treated group that compared with control group. Depend on the M ± SE

Table 5: Ameliorative effects of AVO on $CdCl_2$ treated rats on serum level of total bilirubin, urea, uric acid, total protein

Group	Bilirubin mg/dL	Urea mg/dl	Uric acid mg/dL	Protein g/dl
Control	0.33 ± 0,01 a	29.78 ± 0.3 a	3.03 ± 0.15 a	5.87 ± 0.14 a
AVO (72mg/kg)	0.32 ± 0.02 a	28.38 ± 0.51a	2.88 ± 0.12 a	5.96 ± 0.03 a
$CdCl_2$ (225mg/kg)	0.65 ± 0.05 b	41.75± 1.34 b	3.35 ± 0.19 a	5.33 ± 0.16b
AVO + $CdCl_2$ (225mg/kg)	0.40 ± 0.01 c	32.72 ± 0.43 c	3.02 ± 0.018 a	5.74 ± 0.06 a
LSD	0.07	2.93	0.46	0.41

Values are expressed M ± SE. Different letters indicate significant difference at (P≤0.05); a = means control group value, b = means Cd treated group that compared with control group, c = means AVO + Cd treated group that compared with control group. Depend on the M ± SE

The elevation of urea level is an indicator for kidney disorders, whereas urea is the principle end product of protein catabolism and accelerated amino acid deamination for gluconeogenesis, therefore, it could be the cause of elevated levels of urea, also uric acid is a marker for varied biochemical disorders. For instance, elevated serum uric acid concentration indicated renal insufficiency[92].

Cd is as an extremely toxic environmental contaminator agent that causes oxidation by the release of ROS such as hydroxyl radicals, superoxide anions and hydrogen peroxide[93]. These ROS cause lipid peroxidation. Lipid peroxidation plays an important role in Cd induced renal damage. This finding is in agreement with report by Onwuka *et al* [54], suggested that Cd induces oxidative stress by increasing lipid peroxidation.

L-arginine has improvement effect on the kidney function, it cause ameliorate the cadmium toxicity by decrease urea and uric acid levels. This result agreed with the result that finding by [94-97], in previous literature reviews. A study reported by Klahr[98] that the improvement activity of L-arginine supplementation on the GFR with reduction of macrophage infiltration. There are multiple mechanisms by which L-arginine could influence function and morphology of the transplanted kidney, it converted by arginine decarboxylase to the agmatine, which could enhance GFR [99]. L-arginine can also increase secretion of glucagon [100], which is known to increase the rate of GF[101]. The vasodilation effect of L-arginine is attributed to releasing of nitric oxide and L-citruline, which are released by nitric oxide synthase[102].

It was hypothesized that the supplementation with L-arginine inhibits the oxidative injury in kidneys[103]. Last studies demonstrated the effect of L-arginine in the treatment of kidney diseases like obstruction of ureter, nephritis that caused by special substance like cisplatin, and CsA[104].

On the other hand Ho *et al*[35] suggested the role of vanillin in prevent oxidative stress[105], were reported the renal protective effect of vanillin against toxicity and renal disorder induced by carbon tetrachloride.

A significant rise in the MDA levels following cadmium chloride administration (Table 6). Cd is as an extremely toxic environmental contaminator agent that causes oxidation by the release of ROS such as hydroxyl radicals, superoxide anions and hydrogen peroxide[93]. These ROS cause lipid peroxidation. Lipid peroxidation plays an important role in Cd induced tissue damage.

Table 6: The ameliorative effects of AVO on $CdCl_2$ treated rats on serum MDA and glucose

Group	MDA nmol/mL	Glucose mg/dL
Control	88.67 ± 0.74 a	94.75 ± 1.75 a
AVO (72mg/kg)	78.21 ± 1.05 a	94.15 ± 1.77 a
$CdCl_2$ (225mg/kg)	180.47 ± 1.47 b	162.22 ± 1.15 b
AVO (72mg/kg) + $CdCl_2$ (225mg/kg)	120.06 ± 1.89 c	119.82 ± 0.7 a
LSD	10.46	25.07

Values are expressed M ± SE. Different letters indicate significant difference at (P≤0.05); a = means control group value, b = means Cd treated group that compared with control group, c = means AVO + Cd treated group that compared with control group. Depend on the M ± SE

Thus, MDA can be used a good indicator of lipid peroxidation. The result of the current study was in agreement with earlier studies and suggested a massive release of reactive oxygen species (ROS) were produced due to exposure to Cd, which in turn may damage the cell membrane[31, 91,106].

Malondialdehyde (MDA) is one of the common markers for oxidative stress and its levels are indicative of the intensity of the oxidative stress [107].

It's reported that the administration of L-arginine cause decreased level of the MDA and increased antioxidants activity that results from damage of RBC membrane and inhibits of deformability of RBC is caused by peroxidation of the lipid, which is mediated through an increase level of MDA. Antioxidants such as vitamin E inhibit oxidative state by decrease MDA level and enhanced erythrocyte deformability [108-110]. A Study by Kehinde *et al* [33] showed that the low-dose of L-arginine orally can decrease MDA level, increased antioxidant activity and increased red blood cell resistance in SCD.

It appears from table 4 to 7 that the effect of cadmium chloride on glucose was increase sharply increased, this observation is in agreement with[111-112].

There are many mechanisms of hyperglycemic effect of cadmium. Cd may affect metabolism of the glucose by acting on a different organ including: pancreas, liver, adipose tissue and also adrenal gland. In current results Cd has a direct effect on the pancreas, consequently, may reduce the insulin release from pancreatic β-cells:-, therefore, glucose level went high significantly. According to Nilsson *et al*[113] study, Cd was rapidly taken up in pancreatic tissue ,they concluded that administration of a small amount of cadmium resulted in an enhanced rate of glucose-stimulated insulin release, whereas a high amount of cadmium (20μM) resulted in a significantly decrease rate of insulin release. Also the result shows a significant reducing in glucose level after treatment with L-arginine derivatives (AVO).

4 Conclusion

It can be concluded from current study that the activity of novel compound derived from arginine AVO acted as a strong potential of antioxidant agent. It has the ability to minimized the hazard effects of cadmium chloride, it improved blood picture parameters, total and differential WBCs count and blood indices, in addition, it was capable to reduced the level of serum MDA. Moreover, it ameliorates the activities of AST, ALT, ALP lipid profiles, bilirubin, urea, uric acid, protein and glucose and improved the sexual hormones

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