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AMELIORATIVE EFFECT OF ALLIUM *SATIVUM* AND JUSTICIA *CARNEA* EXTRACTS CO-ADMINISTRATION ON ACUTE CADMIUM CHLORIDE-INDUCED CHANGES ON LIVER FUNCTION PARAMETERS OF ALBINO RATS

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ABSTRACT

Background: Cadmium is a dangerous environmental and industrial pollutant of growing concern that negatively affects several tissues and organs in human and animals. This study investigated the ameliorating effect of combined aqueous extracts of Allium *sativum* and Justicia *carnea* on cadmium-induced hepatotoxicity and oxidative stress in female albino rats. **Methods**: Twenty-five (25) female albino rats weighing between 90 - 160g were randomly divided into five (5) groups (A –E) of five (5) each. Group A rats were gavaged with 10mL/kg body weight of normal saline while groups B – E were gavaged orally with 25mg/kg body weight of cadmium chloride for seven consecutive days. Groups C, D and E were also treated with the aqueous extract of Allium *sativum*, Justicia *carnea* and a combination of both extracts respectively daily for the next fourteen days. After the last day of treatment, the animals were sacrifice and blood samples collected via cardiac puncture for determination of cadmium (Cd^{2+}) (by atomic absorption spectrophometry), liver function parameters: Total protein (TP), Albumin (ALB), Total bilirubin (TB), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Molondialdehyde (MDA), superoxide dismutase (SOD) and Glutathione peroxide (GPx) using spectrophotometric method. **Results**: The result showed a statistically ($p<0.05$) reduction in the level of total protein and albumin (66.80 ± 2.77 Vs 74.80 ± 3.83; 33.98 ± 2.33 Vs 41.86 ± 2.07), and a non-significant and a non-significant ($p>0.05$) increase in the concentration of AST, ALP, and TB (30.60 ± 25.52 Vs 9.60 ± 3.65; 194.60 ± 49.00 Vs154.00 ± 67.94; 10.48 ± 3.94 Vs 7.500 ± 3.03 respectively) in the cadmium intoxicated rats compared to the control. ALT (115.60 ± 19.64 Vs 24.20 ± 13.14) was significantly ($p<0.05$) increased in the cadmium treated group compared to the control. SOD (194.60 ± 13.54 Vs 249.40 ± 9.79) and GPx (7789.60 ± 214.64 Vs 9124.80 ± 589.35) were significantly reduced while MDA level (6.84 ± 0.29 Vs 2.93 ± 0.09) was significantly elevated in the cadmium intoxicated rats. However, post treatment of the rats with the extracts of Allium *sativum*, Justicia *carnea* and a combination of both extracts to groups C, D and E respectively had a mitigating effect on cadmium induced hepatotoxicity. This is reflected by an improvement in the levels of the studied parameters when compared with the cadmium treated group. **Conclusion:** Acute exposure to cadmium chloride caused alterations in some liver function parameters and oxidative stress markers in male albino rats. Allium *satium* and Justicia *carnea* either used alone or co-administered had an ameliorating effect on cadmium toxicity. However, coadministration of both extracts exerts a synergistic effecton the adverse effect of cadmium.

KEYWORDS: Cadmium chloride, Allium *sativum*, Justicia *carnea*, liver function parameters and oxidative stress markers.

INTRODUCTION

Heavy metals are naturally occurring in the earth's crust and are considered as constant environmental pollutants due to the inability to be degraded or destroyed easily.[1] Cadmium (Cd) is a heavy metal and is an important component of batteries, cadmium pigments and plating.[2] It is also used as stabilizers for plastics and chemical, metal coatings, alloys, and serves as a barrier to control neutrons in nuclear reactions, television picture tubes and semiconductors.

Cadmium is spread throughout the environment mainly as a result of pollution from a variety of sources.[3] Indirectly cadmium is delivered as toxin from the earth crust through volcanic eruption, mining and the use of phosphate fertilizers.[4] Acute and chronic human exposures to cadmium (Cd) occur through food, air,

water, industrial products and by occupational exposure.[5] Cadmium (Cd) exposure and its accumulation in mammalian systems may cause severe damage to the nervous and reproductive systems, gastrointestinal tract and mucous tissues.

Several ailments associated with cadmium toxicity include anaemia, osteoporosis, increased blood pressure, brain disorders, myocardial dysfunctions, proteinuria, pulmonary oedema and death,[6,7] skin related diseases, malfunctioning of foetus which includes ablephary, club foot, exencephaly, micrognathia, non-hypertrophic emphysema, irreversible renal tubular injury, eosinophilia, chronic rhinitis and microphthalmia,[8,9,10] In neonatal and adult animals, cadmium exposure has been observed to causes alterations in the neurotransmitter level of brain affecting behaviour the animals. Various studies suggest cadmium is neurotoxic but the exact mechanisms involved in the neurotoxicity are not well understood.[11]

The liver is a very important organ and it plays key roles in various metabolic pathways, such as detoxification process, breakdown of red blood cells and in the synthesis of proteins and hormones.[12] Although the liver is involved in these diverse metabolic pathways, it is still susceptive to a lot of injuries (from infections) and metabolic assaults (from toxic xenobiotics). These injuries and assaults manifest as liver damage and could progress to hepatic failure. Several researchers have investigated liver disease and hepatic failure.[13,14,15] It is reported that, exposure to chemicals (eg cadmium chloride) is one of the risk factors that may increase the chances of hepatic damage [16,17,18] The liver is the organ most sensitive to cadmium toxicity both through environmental and occupational sources of exposure. Cadmium exposure induces hepatotoxicity which depends on the amount and duration of exposure. The main mechanism in hepatotoxicity is considered to be caused primarily by the binding of cadmium to thiol groups in the mitochondria, leading to mitochondrial dysfunction and related injury.[19] The activities of serum ALT, AST and ALP enzymes and level of bilirubin are known to be the major indicators for evaluating the functional integrity of the liver.[1]

Cadmium exposure generates free radicals such as superoxide radicals, hydroxyl radicals and nitric oxide.[20] Liver, kidney and brain tissues are highly vulnerable to oxidative damage due to their high consumption of oxygen and poorly developed antioxidant defense mechanism.[21] Oxidative stress has been proposed as a method for cadmium toxicity in a number of tissues such as kidney, liver and brain.[22] Oxidative stress plays a major part in the development of chronic and degenerative ailments such as cancer, autoimmune disorders, rheumatoid arthritis, cataract, ageing, cardiovascular and degenerative disease.[23,24] Cadmium exerts its toxic effects via oxidative damage to cellular organelle by inducing the generation of excessive reactive oxygen species (ROS) that results in the decrease in intracellular GSH content as it combines with thiol groups of enzymes involved in antioxidant mechanisms such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and exerts inhibitory effect on the activities of these antioxidants.[25,27] Cadmium has been reported to form cadmium-selenium complexes in the active centre of GPx and shows the inhibition of enzyme activity. The mitochondrial complex III of the electron transport chain has also been reported to be inhibited by Cd and increases production of ROS thereby damaging mitochondrial membrane.[28] Cadmium-induced oxidative stress could result in DNA damage or mutations, lipid peroxidation (LPO) and oxidation of proteins [29, 30]. Cadmium also inhibits the activity of antioxidant enzymes, such as catalase, manganese-superoxide dismutase, and copper/zinc-dismutase.[31]

Medicinal plants have been documented as having beneficial properties for the management of various ailments. These plants have been demonstrated to possess phytochemicals which are the active components responsible for their pharmacological actions.[32] and secondary metabolites that can protect humans against diseases.[33] Allium *sativum* (garlic) and Justicia *carnea* are among such documented medicinal plants.

Allium *sativum* (garlic) contains several enzymes, amino acids, minerals such as selenium and organosulfur compounds which are responsible both for garlic's pungent odour and its many medicinal properties [34]. It is consumed as a spice because it has many health benefits due to the many diverse bioactive compounds it contains such as organic sulfides, saponins, phenolic compounds, and polysaccharides[35,36] Allicin is a sulfur-containing compound extracted from garlic with antioxidant properties[37] The antioxidants in garlic are responsible for the anti-thrombotic, hypo-cholesterolemic and anti-hypertensive properties[38] The bulb of garlic has been used as a carminative, anti-septic, expectorant, anti-helmintic and diuretic.[39] It is also responsible for the cadioprotective, anticancer, anti-inflammatory, immunomodulatory, anti-diabetic, anti-obesity,and antibacterial properties of garlic.[40,41]

Justicia *carnea* is a flowering plant, widely distributed in various parts of Africa.[42] In Nigeria, the shrubs of *J. carnea* are grown around homes and in some cases are used for fencing. In the local parlance, Justicia *carnea* is called "hospital too far" in some parts of Nigeria. Traditionally, several species of Justicia are used in the management of inflammation, gastrointestinal disorders, respiratory tract infection, fever, pain, diabetes, diarrhea, liver diseases, rheumatism and arthritis.[43,44] The plant has been reported to also possess anti-inflammatory, anti-allergic, anti-tumoral, anti-viral and analgesic activities.[45] Most of the medicinal properties exhibited by the plant extracts are associated with their bioactive constituents mainly phenols and flavonoids.[46] It has also

been reported to be rich in both macronutrients and trace elements of which calcium and iron are in high quantity.[7] Cadmium mediates its toxicity by generation of reactive oxygen species leading to peroxidation and subsequently oxidative stress. Use of antioxidants has proven to be beneficial in ameliorating oxidative stress. The use of extracts from medicinal plants has been demonstrated to be effective in the treatment of several ailments associated with oxidative stress. Justicia *carnea* extract has been shown to possesses protective ability against cellular damages arising from free radical-mediated complications.[48] The bulbs of garlic have been investigated for its antioxidant potential in ameliorating cadmium induced hepatoxicity in previous studies in rats.[49] However, there is a dearth of literature on the effect of Justicia *carnea* and the combined effects of garlic and Justicia *carnea* in cadmium induced hepatotoxicity and oxidative stress in rats. This study therefore seeks to evaluate the protective the co-administration of Allium *sativum* and Justicia *carnea* on cadmium induced hepatotoxicity and oxidative stress in adult Wistar rats.

MATERIALS AND METHOD

Management of Experimental Animals

Chemical

Cadmium chloride (Sigma Aldrich, USA)
Reagent 1 (17.5% trichloroacetic acid)
Reagent 2 (70% trichloroacetic acid)
Reagent 3 (0.6% Thiobarbituric acid)

Preparation of Aqueous Extract of Garlic (Allium *sativum*)

Justicia *carnea* plant was collected from the botanical garden of the department of pharmacognosis in NDU, Amassoma. Fresh Allium *sativum* bulbs were purchased from the local market at Swali in Yenagoa, Bayelsa State Nigeria. The garlic aqueous extract was prepared as a modification of that according to the method of Ghiasi,[50] The bulbs were peeled to remove the scales. They were then washed with distilled water to remove any dirt that might be present. The water was allowed to drain off the bulbs. Thirty (50g) gram of garlic was crushed in a blender and added to 100 ml distilled water. The resultant homogenized mixture was filtered three times using a cheese cloth, and then centrifuged at 2,000 rpm for 10 minutes. The clear supernatant was quickly collected and kept in dark bottles. It was stored at 2 – 8^{o}C in a refrigerator until used. Based on the weight of the starting material (50 g per 100 ml), the concentration of prepared garlic is considered to be 500 mg per ml.[50]

Preparation of Aqueous Extract of Justicia *carnea*

Justicia *carnea* plant was collected from the botanical garden of the department of pharmacognosis in NDU, Amassoma. The leaves were plucked off from the stem of the plant and were washed with distilled water to remove any dirt that might be present. The water was allowed to drain off the leaves. The leaves were then cut into smaller pieces. Five-hundred (500g) gram of the leaves were crushed in a blender and macerated in 750 ml distilled water. The resultant homogenized mixture was filtered three times using a cheese cloth, and then centrifuged at 3,000 rpm for 10 minutes. The supernatant was collected and stored at 2 – 8^{o}C in a refrigerator until used. The concentration of the Justicia *carnea* extract was calculated from the formula:

Concentration of the *Justicia carnea* extract = $\frac{WL - WR}{VD}$

where
WL = Weight of fresh Leaves
WR = Weight of leaves Residue after filtration
VD =volume of distilled water used

Animal Treatment and Experimental Design

All procedure for animal handling and treatment were approved by Ethics committee office of the Niger Delta University, Amassoma. Twenty-five adult female albino rats (*Rattus norvegicus*) (10weeks old) and weighing between 90-160g were procured from the animal house of the Department of pharmacology Niger Delta University, NDU, Amassoma. The rats were moved to the animal house of the department of Medical Laboratory Science, NDU, Amassoma in well aerated laboratory cages in a room under standard conditions of temperature range of 25 ± 5^{O}C and a 12/12 hours of light and dark schedule. The rats were allowed to acclimatize to the laboratory environment for a period of two (2) weeks before the commencement of the experimental protocol. The rats were randomly divided into five (5) groups (n=5/group) as follow:

GROUP A: Normal Control (NC): Rats were given 10mL/kg body weight normal saline daily for twenty-one days by oral gavage to reflect the effect of gavage on the animals

GROUP B: Positive Control (PC) - This comprised rats to which cadmium chloride (25mg/kg b wt) was administered by oral gavage for seven consecutive days.

GROUP C: The rats in this group were gavaged with cadmium chloride (25mg/kg b wt) for seven consecutive days and then followed by daily oral administration of Allium *sativum* (500mg/kg body weight/day) for the next 14 days.

GROUP D: The rats in this group were gavaged with cadmium chloride (25mg/kg b wt) for seven consecutive days and then followed by daily oral administration of Justicia *carnea* (496mg/kg body weight) for the next 14 days.

GROUP E: The rats in this group were gavaged with cadmium chloride (25mg/kg b wt) for seven consecutive days and then followed by daily oral administration of mixture of Allium *sativum* (500mg/kg body weight) and Justicia *carnea* (496mg/kg body weight) for the next 14 days.

Collection, Preparation and Preservation of Specimens Blood Samples for Biochemical Assays

At the completion of the experiment, the rats were anaesthetized to death by inhalation with diethyl ether

and then sacrificed. Blood samples were collected via cardiac puncture from each anaesthetized rat into heparinized and plain sample containers. The heparinized blood was stored at 2 – 8^{o}C and used for the estimation of cadmium. The blood in the plain glass tubes were allowed to cloth properly at room temperature and centrifuged at 3,000rpm for 5miutes to obtain serum. The clear serum was collected in sterilized disposable plastic tubes and stored at 2 – 8^{o}C until use. The serum was used for the assay of total protein, albumin, AST, ALT, ALP, TB, SOD, GPx and MDA. All analysis was carried out within a week of sample collection.

Determination of Blood Cadmium
This was determined by Atomic Absorption spectrophotometry as described by Anetor et al.[51]

Determination of Selected Biochemical Parameters
The serum concentration of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), Total Bilirubin, albumin, SOD, and GPx were assayed using test kits manufactured by Randox Diagnostics, United Kingdom. Serum MDA was estimated using Analytical grade reagents.

Estimation of Serum Total Protein: The total protein concentration was determined using the method described by Lowry et al.[52] Principle**:** The cupric ions in the reagent react with peptide bonds in protein in an alkaline solution to produce a blue-violet or purple coloured complex. The absorbance of the colour is directly proportional to the concentration of protein in the sample and is measured in a spectrophotometer at a wavelength of 540nm. Briefly,10µl of distilled water was dispensed into the test tube labeled blank, 10µl of the standard solution was pipetted into the test tube labeled standard, and 10µl of the sample was added to the sample test tube and 10µl of the quality control serum was added to the test tube labeled quality control. Then 500µl of Reagent was added to each of the four test tubes. The solution was mixed and incubated for 30min at room temperature. The absorbance of each tube was measured at a wavelength of 540 nm.

Estimation of Serum Albumin: The serum albumin content was determined according to the method of Doumas et al.,[53] Principle: The measurement of serum albumin is based on its quantitative binding to the indicator 3,3',5,5'-tetrabromo-m cresol sulphonephthalein (bromocresol green, BCG). The albumin-BCG-complex absorbs maximally at 578 nm. The absorbance being directly proportional to the concentration of albumin in the sample. Briefly, one hundred microlitter of distilled water, standard, quality control sera and test were dispensed into their respective tubes. Then three thousand microlitter of the bromocresol green (BCG) was added to each tube. The content of each tube was mixed and incubated for 5 minutes at +20 to +25°C. The absorbance of the sample (Asample) and the standard (Astandard) were measured against the reagent blank.

Determination of Alanine Aminotransferase: Alanine aminotransferase was determined according to the method described by Rietman-Frankiel,[54] Principle**:** ALT catalyzes the amino conversion reaction between alanine and α-ketoglutaric acid to produce pyruvic acid and glutamic acid at pH 7.4 and 37. Then phenylhydrazine was added to form phenylhydrazone with pyruvic acid. Phenylhydrazone is reddish brown under alkaline conditions. ALT activity can be calculated by measuring the absorbance values at 510 nm. Briefly, 0.1 milliliters of sample was added to the test; 0.5 milliliters of reagent 1 was added to both the test and blank and 0.1ml of distilled water was added to the blank. The tubes were properly mixed and incubated for exactly 30min at 37^{O}C. After incubation, 0.5ml of Reagent 2 was added to both test and blank. The content of both tubes was mixed and allowed to stand for 20min at 20-25^{0}c. Five (5) mililiters of sodium hydroxide was then added to the tubes and mixed and the absorbance of sample ($A_{sample)}$ was read against the reagent blank after 5 minutes at 546 nm wavelength.

Determination of Aspartate Aminotransferase.
Alanine aminotransferase was determined according to the method described by Rietman-Frankiel, (54). Principle**:** AST/GOT enables alpha-ketoglutaric acid and aspartic acid to displace amino and keto groups to form glutamic acid and oxaloacetic acid. Oxaloaceticacids candecarboxylate itself to form Pyroracemic acid during the reaction. Pyroracemic acid reacted with 2,4-dinitrophenylhydrazine (DNPH) to form 2,4, dinitrophenylhydrazoneproducing reddish brown in alkaline solution. Measure the absorbace values and calculate the enzyme activity. In brief, 0.1 milliliters of sample was added to the test; 0.5 milliliters of reagent 1 was added to both the test and blank and 0.1ml of distilled water was added to the blank. The tubes were mixed properly and incubated for exactly 30min at 37^{O}C. After incubation, 0.5ml of Reagent 2 was added to both test and blank. The content of each tube was mixed and allowed to stand for 20min at 20 – 25^{O}C. 5mililitersof sodium hydroxide was then added to the tubes and mixed and the absorbance of sample ($A_{sample)}$ was read against the reagent blank after 5 minutes at 546 nm wavelength.

Determination of Alkaline Phosphatase: The method of Bowers and McComb's Method.[55] was employed for the determination of serum Alkaline Phosphatase. Principle: Alkaline phosphatase in the sample catalyzes the hydrolysis of colorless p-nitrophenyl phosphate (p-NPP) to give p-nitrophenol and inorganic phosphate. At the pH of the assay (alkaline), the p-nitrophenol is in the yellow phenoxide form. The rate of absorbance increase at 404 nm is directly proportional to the alkaline phosphatase activity in the sample. Briefly, 10 microliters of the serum samples were added to the test tubes and 500 microliters of alkaline phosphatase working reagent was added to the test tubes, mixed and read immediately at 405nm wavelength.

Estimation of Total Bilirubin: Total bilirubin concentration was estimated using the method described by Jendrassik-Grof's.[56] Principle: The total bilirubin concentration is determined in presence of caffeine by the reaction with diazotized sulphanilic acid to produce an intensely coloured diazo dye (560 – 600nm). The intensity of colour formed by this dye is proportional to the concentration of total bilirubin. Briefly, 200 microliters of reagent 1 was added to both the test and blank and 10 microliters of reagent 2 was added to the test only; 1000 microliters of reagent 3 was added to the test and blank and 200 microliters of the sample was also added to both test and blank. The tubes were mixed properly and incubated for 10 minutes at 20-25^{O}C. After the incubation, 1000 microliters of reagent 4 was added to the test and blank; the tubes were mixed properly and incubated at 20-25^{O}C for exactly 5 minutes. The absorbance of sample ($A_{sample)}$ was read against the sample blank at 578 nm wavelength.

Measurement of Antioxidant Enzymes and Malondialdehyde

The activity of the superoxide dismutase (SOD) was measured by method of Yi-Sun et al,[57] glutathione peroxidase (GPx) by Paglia and Valentine (58) and MDA by Yoshika et al.[59] The enzyme levels were determined using commercial test kits according to the manufacturer' instructions.

Estimation of superoxide dismutase (SOD): SOD activity was assayed according to the method of Yi-Sun et al.[57] In this method, xanthine-xanthine oxidase system was used to generate a superoxide flux, and nitroblue tetrazolium (NBT) was used as an indicator of superoxide production. SOD activity was then measured by the degree of inhibition of the reaction unit of enzyme provides 50% inhibition of NBT reduction. The enzyme activity was expressed as U/ml**.**

Estimation of superoxide dismutase (SOD)

SOD activity was assayed according to the method of Yi-Sun et al.[57] Principle**:** In this method, xanthine-xanthine oxidase system was used to generate a superoxide flux, and nitroblue tetrazolium (NBT) was used as an indicator of superoxide production. SOD activity was then measured by the degree of inhibition of the reaction unit of enzyme provides 50% inhibition of NBT reduction. The enzyme activity was expressed as U/ml. Xanthine ad xanthine oxidase generate super oxide radicals which reacts with 2-(4-iodophenyl)-3-(4-nitrophenol)- 5-phenyl tetrazolium chloride (I..N.T) to form a red formazan dye. The super oxide dismutase activity is the measured by the degree of inhibition of this reaction. One unit of SOD is that which causes a 50% inhibition of the rate of reaction of INT uder the conditions of the assay. Three test tubs were labeled as "Ransod sample diluents", "Standard", and 'diluted sample. Fifty microlitres (50µl) of each of "Ransod sample diluents", "Standard, and 'diluted sample" were dispensed into their respective tube. One thousand and seven hundred microliters (1,700µl) of the mixed substrate was added to all the tubes. The content of each tube was well mixed and two hundred and fifty microliters (250µl) Xanthine oxidase was then added to all the tubes. The tubes were mixed again and the initial absorbance (A_1) was read after 30 seconds. The final absorbance (A_2) of the tubes were read after 3 minutes. The absorbance was measured at a wavelength of 505nm. The activity of SOD was then calculated from the formula below:

Calculation

$$\text{A/min of standard/sample} = \frac{(\Delta A2 - A1)}{3}$$

Calculation of % inhibition

$$\text{Standard} = 100 - \frac{(\Delta A_{std}/\text{min} \times 100)}{(A_{SI}/\text{min})}$$

$$\text{Sample} = 100 - \frac{(A_{std}/\text{min} \times 100)}{(A_{SI}/\text{min})}$$

Estimation of Glutathione peroxidase: Glutathione peroxidase is by the method according to Paglia and Valentine (58). The principle is based on the fact that GPx catalyse the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and nicotinamide adenine dinucleotidephosphate (NADPH), the oxidized glutathione was immediately converted to the reduced form with concomitant oxidation of NADPH to $NADP^{+}$. Briefly, 10µL of the haemolysate was mixed with 500µL of reagent R1 and 20µL cumene R2. The absorbance was the measured at 340nm and the GPx activity was calculated as state in the manufacturer's instruction (Ransel –Randox Lab Antrim, UK). The enzyme activity was expressed as U/ml.

Determination Malondialdehyde: Lipid peroxidation products were measured as an index of MDA production, in blood by the method of Shah and Walker's [59]. Principle: Malondialdehyde in serum was separated and determined as conjugate with TBA. Serum proteins were precipitated by TCA and then removed by centrifugation. The MDA – TBA complex was measured at 534 nm. Briefly, 2 test tubes (18 x 150 mm Pyrex) were labeled as test and blank. 1ml of the serum was added into the tube labeled test and 1ml distilled water into the tube labeled blank. 1ml each of reagent 1, 2, and 3 were added to both the blank and test and mixed. The tubes were incubated in boiling bath for 15 minutes, allowed to cool, at room temperature for 20 minutes. Then the tubes were centrifuged at 2000 rpm for 15 minutes and the supernatant layer was read at 534 nm. The concentration of MDA (nmol/ml) was calculated by using the following formula:

Concentration of the test= Abs (test) –Abs (blank) / 1.56 x 1000000

Statistical Analysis

Graph pad prison 5.0 statistical software version and microsoft excel 2003 was used. Data obtained were presented in tables as mean and standard deviation (Mean ±SD). Comparison of result between control and

test was done using student's t-test and all post hoc testing were done using Bonferroni multiple comparison. Level of significance was determined at a probability level of $p< 0.05$. All post hoc testing were done using Bonferroni multiple comparison.

RESULTS

Table 4.1 shows the effect of cadmium on some liver function parameters following acute exposure (7days) to cadmium chloride in adult female albino rats. There was a significant reduction in the serum levels of total protein and albumin in the cadmium intoxicated rats compared with the control group (74.80 ± 3.83 Vs 66.80 ± 2.77 and 41.86 ± 2.07 Vs33.98 ± 2.33 respectively). Serum AST, ALP and TB were nonsignificantly ($p>0.05$) elevated and ALT was significantly ($p<0.05$) increased in the cadmium group compared with the control group. The cadmium concentration was found to significantly ($P<0.00$) elevated in the cadmium poisoned group when compared with the control (0.01 ±0.01Vs 0.16±0.03).

Table 4.2 showed some selected serum liver function parameters on acute exposure to cadmium chloride in adult Albino rats and later treated with garlic bulb extract. There was a significant increase in the concentration of ALT and nonsignificant increase in AST, ALP and TB compared with their respective control group. Post treatment with the garlic extract resulted in significant reduction elevation in the levels of serum total protein and albumin to near normal. The serum enzymes ALT, AST and ALP were found to be elevated in the cadmium treated group. Post administration of the garlic extract caused a significant reduction in ALT concentration and a nonsignificant reduction in the ALP and AST levels. TB was also elevated in the cadmium group. The concentration of total bilirubin was restored upon garlic administration. Also, garlic administration caused a significant reduction in cadmium level in the garlic treated rats.

Table 4.3 represented the effect of cadmium chloride and Justicia *carnea* on some selected biochemical parameters of adult Albino rats on acute exposure to cadmium chloride. The result showed significant increase in total protein and albumin; and a nonsignificant reduction in ALP, AST and TB. ALT was significantly reduced. Post treatment with Justicia *carnea* extract showed a nonsignificant reduction in the levels of TB, ALP and AST, and a significant decrease in ALT. The Justicia *carnea* extract also restored the levels of total protein and albumin to near normal. The blood cadmium concentration was also significantly reduced by the Justicia *carnea* extract.

Table 4.4 showed the effect of cadmium chloride and a mixture of garlic and Justicia *carnea* on some liver function parameter on acute exposure to cadmium chloride. The result revealed a reduction in serum total protein and albumin in the cadmium intoxicated. Serum AST, ALP, AST and TB were elevated by cadmium toxicity. Post treatment with the plant extract caused a reduction in the levels of serum AST, ALP, AST and TB to near normal. The total protein and albumin levels were found to be improved.

Table 4.5 represents the effect of acute cadmium exposure on some oxidative stress parameter in the control (group A) rats and the cadmium treated rats (group B). There was a significant reduction in the activities of SOD and GPx. The MDA levels was also significantly elevated in the cadmium group B compared to the control group A.

Table 4.6 showed the effect of cadmium and Allium sativum (garlic) on the activities of some antioxidants in acute cadmium exposure in rats. SOD and GPx activities were significantly increased post treatment of the rats with garlic when compared with the cadmium treated (group C Vs group B). There is a significant elevation in MDA concentration in the cadmium treated group B compare with the control group A.

Table 4.7 shows the effect of cadmium and Justicia *carnea* on some oxidative stress parameters in albino rats. there effect of the Justicia *carnea* resulted in a significant elevation in the activities of the studied antioxidants in group D compared with the cadmium treated group A rats.

Table 4.8 shows the effect of cadmium chloride and Allium *sativum* plus Justicia *carnea* extract on selected oxidative stress parameters upon acute exposure to cadmium. SOD, GPx and MDA were significantly elevated in the Justicia *carnea* and garlic treated groups compared with the cadmium treated group.

Table 4.1: Toxicological Assessment of Oral Cadmium Poisoning on Some Liver Parameters in Adult Albino Rats on Acute Exposure to Cadmium Chloride.

Parameters (Units)	GP A	GP B	*t* statistic	*p* value	Remark
ALP (IU/l)	154.00 ± 67.94	194.60 ± 49.00	-1.08	.31	NS
TB (mg/dl)	7.500 ± 3.03	10.48 ± 3.94	-1.34	.22	NS
ALT (IU/l)	24.20 ± 13.14	115.60 ± 19.64	-8.65	.00	S
AST (IUl/l)	9.60 ± 3.65	30.60 ± 25.52	-1.82	.14	NS
TP (g/l)	74.80 ± 3.83	66.80 ± 2.77	3.78	.01	S
ALB (g/l)	41.86 ± 2.07	33.98 ± 2.33	5.65	.00	S
CAD (µg/dl)	0.01 ± 0.01	0.16 ± 0.03	-10.79	.00	S

Key: GP A = Negative Control, GP B = Pb treated, ALP = alkaline phosphatase, TB = total bilirubin, ALT = alanine transaminase, AST = aspartate aminotransferase, TP = total protein, ALB = albumin, CAD = cadmium, NS = Not Significant, S = Significant.

Table 4.2: Effect of Cadmium and Allium Sativum on Some Liver Function Parameters on Acute Exposure to Cadmium Chloride in Albino Rats.

Parameters (Units)	GP A	GP B	GP C	*F* statistic	*p* value	Remark
ALP (IU/l)	154.00 ± 67.94	194.60 ± 49.00	160.20 ± 71.27	0.59	.57	NS
TB (mg/dl)	7.500 ± 3.03	10.48 ± 3.94	8.56 ± 5.97	0.57	.58	NS
ALT (IU/l)	24.20 ± 13.14$^{\alpha}$	115.60 ± 19.64$^{\beta}$	88.00 ± 19.63$^{\gamma}$	34.92	.00	S
AST (IUl/l)	9.60 ± 3.65	30.60 ± 25.52	16.20 ± 1+9.02	1.69	.23	NS
TP (g/l)	74.80 ± 3.83$^{\delta}$	66.80 ± 2.77	69.80 ± 3.42	7.19	.01	S
ALB (g/l)	41.86 ± 2.07$^{\eta}$	33.98 ± 2.33$^{\varphi}$	38.84 ± 3.29	11.53	.00	S
CAD (µg/dl)	0.01 ± 0.01$^{\tau}$	0.16 ± 0.03	0.10 ± 0.06$^{\chi}$	19.26	.00	S

Key: GP A = Negative Control, GP B = Pb treated, GP C = Vitamin E treated, ALP = alkaline phosphatase, TB = total bilirubin, ALT = alanine transaminase, AST = aspartate aminotransferase, TP = total protein, ALB = albumin, CAD = cadmium, NS = Not Significant, S = Significant. All post hoc testing were done using Bonferroni multiple comparison. $^{\alpha}$Significant difference observed in ALT concentration between GP A and GP B, p = .00. $^{\beta}$Significant difference observed in ALT concentration between GP B and GP C, p = .01. $^{\gamma}$Significant difference observed in ALT concentration between GP C and GP A, p = .00. $^{\delta}$Significant difference observed in TP concentration between GP A and GP B, p = .01. $^{\eta}$Significant difference observed in ALB concentration between GP A and GP B, p = .00. $^{\varphi}$Significant difference observed in ALB concentration between GP B and GP C, p = .04. $^{\tau}$Significant difference observed in CAD concentration between GP A and GP B, p = .00. $^{\chi}$Significant difference observed in CAD concentration between GP C and GP A, p = .01.

Table 4.3: Effect of Cadmium chloride and Justicia *carnea* on Some Liver Function Parameters in Acute Exposure to Cadmium Chloride in Albino Rats.

Parameters (Units)	GP A	GP B	GP D	*F* statistic	*p* value	Remark
ALP (IU/l)	154.00 ± 67.94	194.60± 49.00	171.20 ± 35.93	0.75	.49	NS
TB (mg/dl)	7.500 ± 3.03	10.48 ± 3.94	8.94 ± 4.11	0.80	.47	NS
ALT (IU/l)	24.20 ± 13.14$^{\alpha}$	115.60± 19.64	52.40 ± 35.93	17.76	.00	S
AST (IUl/l)	9.60 ± 3.65	30.60 ± 25.52	18.00 ± 26.05	1.25	.32	NS
TP (g/l)	74.80 ± 3.83$^{\delta}$	66.80 ± 2.77	68.00 ± 4.12$^{\gamma}$	7.09	.01	S
ALB (g/l)	41.86 ± 2.07$^{\eta}$	33.98 ± 2.33	37.38 ± 1.79$^{\varphi}$	18.11	.00	S
CAD (µg/dl)	0.01 ± 0.01$^{\tau}$	0.16 ± 0.03	0.13 ± .02$^{\chi}$	66.43	.00	S

Key: GP A = Negative Control, GP B = Pb treated, GP C = Vitamin E treated, ALP = alkaline phosphatase, TB = total bilirubin, ALT = alanine transaminase, AST = aspartate aminotransferase, TP = total protein, ALB = albumin, CAD = cadmium, NS = Not Significant, S = Significant. All post hoc testing were done using Bonferroni multiple comparison. $^{\alpha}$Significant difference observed in ALT concentration between GP A and GP B, p = .00. $^{\beta}$Significant difference observed in ALT concentration between GP B and GP D, p = .01. $^{\delta}$Significant difference observed in TP concentration between GP A and GP B, p = .01. $^{\gamma}$Significant difference observed in TP concentration between GP D and GP A, p = .04. $^{\eta}$Significant difference observed in ALB concentration between GP A and GP B, p = .00. $^{\varphi}$Significant difference observed in ALB concentration between GP D and GP A, p = .02. $^{\tau}$Significant difference observed in CAD concentration between GP A and GP B, p = .00. $^{\chi}$Significant difference observed in CAD concentration between GP D and GP A, p = .00.

Table 4.4: Effect of Cadmium chloride and Allium *sativum* plus Justicia *carnea* on Some Liver Function Parameters on Acute Exposure to Cadmium Chloride in Albino Rats.

Parameters (Units)	GP A	GP B	GP E	*F* statistic	*p* value	Remark
ALP (IU/l)	154.00 ± 67.94	194.60± 49.00	157.00 ± 39.25	0.90	.43	NS
TB (mg/dl)	7.500 ± 3.03	10.48 ± 3.94	7.22 ± 3.83	1.24	.32	NS
ALT (IU/l)	24.20 ± 13.14$^{\alpha}$	115.60± 19.64$^{\beta}$	58.80 ± 30.96	21.05	.00	S
AST (IU/l)	9.60 ± 3.65	30.60 ± 25.52	9.80 ± 12.56	2.66	.11	NS
TP (g/l)	74.80 ± 3.83	66.80 ± 2.77	70.40 ± 12.34	1.38	.29	NS
ALB (g/l)	41.86 ± 2.07$^{\eta}$	33.98 ± 2.33$^{\varphi}$	39.59 ± 0.79	23.85	.00	S
CAD (µg/dl)	0.01 ± 0.01$^{\tau}$	0.16 ± 0.03$^{\gamma}$	0.09 ± 0.06$^{\chi}$	21.85	.00	S

Key: GP A = Negative Control, GP B = Pb treated, GP C = Vitamin E treated, ALP = alkaline phosphatase, TB = total bilirubin, ALT = alanine transaminase, AST = aspartate aminotransferase, TP = total protein, ALB = albumin, CAD =

cadmium, NS = Not Significant, S = Significant. All post hoc testing were done using Bonferroni multiple comparison. ᵅSignificant difference observed in ALT concentration between GP A and GP B, p = .00. ᵝSignificant difference observed in ALT concentration between GP B and GP E, p = .01. ᵑSignificant difference observed in ALB concentration between GP A and GP B, p = .00. ᵠSignificant difference observed in ALB concentration between GP B and GP E, p = .00. ᶻSignificant difference observed in CAD concentration between GP A and GP B, p = .00. ᵞSignificant difference observed CAD concentration between GP B and GP E, p = .04. ᵡSignificant difference observed in CAD concentration between GP E and GP A, p = .01.

Table 4.5: Toxicological Assessment of Oral Cadmium Poisoning on Some Oxidative stress Parameters in Adult Albino Rats on Acute Exposure to Cadmium Chloride.

Parameters (Units)	GP A	GP B	*t* statistic	*p* value	Remark
SOD (U/mL)	249.40 ± 9.79	194.60 ± 13.54	7.34	.000	S
GPx (U/L)	9124.80 ± 589.35	7789.60 ± 214.64	4.76	.001	S
MDA (nmol/mL)	2.93 ± 0.09	6.84 ± 0.29	-28.94	.000	S

Key: GP A = Negative Control, GP B = Cadmium treated, SOD = Superoxide dismutase, GPx = Glutathione peroxidase, MDA = Malondialdehyde, NS = Not Significant, S = Significant.

Table 4.6: Effect of Cadmium and Allium Sativum on Some Oxidative Stress Parameters on Acute Exposure to Cadmium Chloride in Albino Rats.

Parameters (Units)	GP A	GP B	GP C	*F* statistic	*p* value	Remark
SOD (U/mL)	249.40± 9.79ᵅ	194.60± 13.54ᵝ	215.40± 6.19ᵞ	36.17	.000	S
GPx (U/L)	9124.80± 589.35ᵠ	7789.60± 214.64ᶻ	8798.80± 319.10	14.68	.001	S
MDA (nmol/mL)	2.93 ± 0.09ᵡ	6.84 ± 0.29ᵚ	3.88 ± 0.20ᵟ	481.35	.000	S

Key: GP A = Negative Control, GP B = Cadmium treated, GP C = Garlic treated, SOD = Superoxide dismutase, GPx = Glutathione peroxidase, NS = Not Significant, S = Significant. All *post hoc* testing were done using Bonferroni multiple comparison. ᵅSignificant difference observed in the SOD concentration between GP A and GP B, p = .000. ᵝSignificant difference observed in the SOD concentration between GP B and GP C, p = .023. ᵞSignificant difference observed in the SOD concentration between GP C and GP A, p = .001. ᵠSignificant difference was observed in the GPx concentration between GP A and GP B, p = .001. ᶻSignificant difference observed in the GPx concentration between GP B and GP C, p = .006. ᵡSignificant difference was observed in the MDA concentration between GP A and GP B, p = .000. ᵚSignificant difference was observed in the MDA concentration between GP B and GP C, p = .000. ᵟSignificant difference was observed in the MDA concentration between GP C and GP A, p = .000.

Table 4.7: Effect of Cadmium chloride and Justicia *carnea* on Some Liver Function Parameters in Acute Exposure to Cadmium Chloride in Albino Rats.

Parameters (Units)	GP A	GP B	GP D	*F* statistic	*p* value	Remark
SOD (U/mL)	249.40 ± 9.79ᵅ	194.60 ± 13.54ᵝ	217.00± 14.40ᵞ	23.40	.000	S
GPx (U/L)	9124.80±589.35ᵠ	7789.60± 4.64ᶻ	8637.80± 386.53	12.62	.001	S
MDA(nmol/mL)	2.93 ± 0.09ᵡ	6.84 ± 0.29	3.93 ± 0.12	587.00	.000	S

Key: GP A = Negative Control, GP B = cadmium treated, GP D = *Justicia carnea* treated, SOD = Superoxide dismutase, GPx = Glutathione peroxidase, NS = Not Significant, S = Significant. All *post hoc* testing were done using Bonferroni multiple comparison. ᵅSignificant difference observed in SOD concentration between GP A and GP B, p = .000. ᵝSignificant difference observed in SOD concentration between GP B and GP D, p = .050. ᵞSignificant difference observed in the SOD concentration between GP D and GP A, p = .005. ᵠSignificant difference observed in the GPx concentration between GP A and GP B, p = .001. ᶻSignificant difference was observed in the GPx concentration between GP B and GP D, p = .025. ᵡSignificant differences were observed across all pairwise comparison of MDA concentration, p = .000 each.

Table 4.8: Effect of Cadmium chloride and Allium *sativum* plus Justicia *carnea* on Some oxidative Stress Parameters on Acute Exposure to Cadmium Chloride in Albino Rats.

Parameters (Units)	GP A	GP B	GP E	*F* statistic	*p* value	Remark
SOD (U/mL)	249.40 ± 9.79ᵅ	194.60 ±13.54	213.80 ± 9.88ᵝ	30.78	.000	S
GPx (U/L)	9124.80± 589.35ᵞ	7789.60 ± 214.64ᶻ	8555.00± 300.33	13.92	.001	S
MDA (nmol/mL)	2.93 ± 0.09ᵡ	6.84 ± 0.29	3.79 ± 0.28	377.43	.000	S

Key: GP A = Negative Control, GP B = cadmium treated, GP E = Garlic + *Justicia carnea* treated, SOD = Superoxide dismutase, GPx = Glutathione peroxidase, NS = Not Significant, S = Significant. All *post hoc* testing were done using Bonferroni multiple comparison. ᵅSignificant difference observed in the SOD concentration between GP A and GP B, p

= .000. [β]Significant difference observed in the SOD concentration between GP E and GP A, p = .001. [γ]Significant difference observed in the GPx concentration between GP A and GP B, p = .001. [=]Significant difference observed in the GPx concentration [≠]Significant differences were observed across all pairwise comparison of MDA concentration, p = .000 each.

DISCUSSION

Cadmium is a dangerous environmental and industrial pollutant of growing concern that negatively affects several tissues and organs in human and animals. It is known to promote an early oxidative stress and contributes to the development of serious biochemical and pathological alterations as it is non-biodegradable and it slowly eliminated from tissues.[60] The treatment strategies for cadmium toxicity include chelation and antioxidant therapies.[61]

In the current study, the blood cadmium level was significantly ($p<0.05$) elevated in cadmium treated rats compared with the control. This observation was consistent with the study of Andjelkovic *et al.,*[62] Andjelkovic and coworkers in their study of the effect of acute cadmium in rat blood observed a significantly higher blood cadmium concentration compared to the unexposed groups. The increase in the blood cadmium level could be attributed to the accumulation of cadmium in tissue (blood) as cadmium is non-biodegradable. This could also be attributed to saturation of cadmium binding site in the blood. Following treatment with garlic, (Group C), Justicia *carnea* (Group D) and a combination of both extracts (Group E), there was a reduction in the blood cadmium level in all the groups (C, D and E). The reductive effect could be attributed to the antioxidant properties of garlic and Justicia *carnea*. The reductive effect of garlic could be attributed to complex formation between cadmium and garlic. Garlic combine with heavy metals (cadmium inclusive) in the body and promote their excretion through the bile to the faeces. The heavy metals harbor positive charges to which the organosulfur components of garlic can bind.[63] The reduction in cadmium by Justicia *carnea* could also be attributed to the antioxidant in the phytochemicals contained in the leaves of the plants [64]. Administration of both extracts also reduced the effect of cadmium. This was more profound compared to either the use of garlic or Justicia *carnea* alone.

The liver mainly functions in the detoxification of harmful chemical substances or compounds that are injurious to the tissues and organs. It is also involved in the synthesis of substances needed by the body. The liver synthesize the total albumin and thus could serve s a useful marker to evaluate the status of hepatic damage.[65,66] Previous investigation has revealed that cadmium adversely influence liver function even at very low concentration indicating that it is a prominent hepatotoxicant.[67] In the current study, rats administered cadmium chloride by oral gavage for seven consecutive days demonstrated a reduction in serum total protein and albumin. This finding is consistent with the study by Kumar and Sharma,[65] and Oyinloye, *et al*,[68] The observed reduction in serum total protein and albumin could be the consequences of mitochondrial and cytosolic dysfunction.[69]

Treatment of the cadmium intoxicated rats with garlic, Justicia *carnea* and a combination of both extracts (to group C, D and E respectively) for fourteen consecutive days was observed to improve the serum levels of total protein and albumin towards the control value (table 2). The finding that the garlic extract administration improved the protein profile was in consonance with the study by Sharma and Vijaya,[70] They treated mice exposed to a single dose of cadmium with a chronic dose of garlic extract.

The improvement in the levels of total protein and albumin by these plants' extracts could be attributed to the activity of the abundant antioxidants they contained. Antioxidants have proven valuable in mitigating cadmium toxicity.[71] Garlic and Justicia *carnea* are known to possess abundant amount of antioxidant.[50,72] which can scavenge the reactive oxygen species generated by cadmium toxicity thereby allowing the regeneration of damaged liver cells.[73] In this study, it was observed that the combined extract caused a better improvement in the total protein and albumin concentrations compared with the effect of the individual extracts. Some researchers have reported that supplementation of combined antioxidant nutrients have proven to have a more protective effect compared with the use of the individual antioxidants in mitigating ROS.[74,75] Xhyrel et al.,[75] studied the effect of combining vitamin, C and E synergy on lead toxicity in rats and reported that the lowest blood lead level was observed that the combination of the antioxidants proved more efficient than vitamin C or E in all of the studied parameters despite the same total international unit dose used. The better improvement observed in the combined extract administration could be attributed to the additive effect produced by the antioxidants.

In the current study, total bilirubin was significantly elevated in the cadmium intoxicated rats. The finding of elevated total bilirubin in the cadmium treated rats was in agreement with the study by Kumar and Sharma.[65] The elevated total bilirubin could be due to the biliary tract dysfuntion which makes hepatocytes unable to uptake bilirubin or it could be due to altered bilirubin excretion. Overproduction of bilirubin may also contribute to the elevated bilirubin level. Garlic, Justicia *carnea* and their combination mitigated the toxic effect of cadmium as manifested by the reduction in the level of total bilirubin in groups C-E. Also, the co-administration of both extracts produced a better ameliorating effect on

cadmium toxicity. The improved hepatic excretory and synthetic functions are suggestive of the hepatoprotective ability of garlic and Justicia *carnea*.

Monitoring the serum activities of Alanine aminotransferase (ALT) and aspartate transaminase (AST) has been employed in order to assess liver function [65]. Increase in the activities of these enzymes in plasma is an indication of their leakage from tissue into plasma following hepatic lesion responsible for the deterioration of the membrane permeability (layachi and kechrid, [76]. This damage and relaxation of the enzymes in blood is due to an accumulation of cadmium in the liver. In the current study, the activities of AST and ALP were non-significantly ($p>0.05$) elevated whereas ALT was significantly ($p<0.05$) elevated in the serum of the cadmium-induced hepatotoxicity group compared with the control group. The finding from this study is corroborated by the study of Adefegha *et al.*,[77] and Toppo et al.,[78] who reported the hepatotoxic effect of cadmium exposure in rats. Nashwa.[79] reported that the hepatotoxicity of cadmium could be due to inflammation leading to damage of the liver or direct destruction of cells of the liver. Treatment of the rats with the plant extracts has an attenuating effect on the elevated enzymes levels by reducing the concentrations in this study. The attenuating effect of garlic on AST, ALT and ALP is similar to reports by Padalko *et al.*,[80] The observed result indicates that garlic may have the ability to preserve the structural integrity of the tissues and protects tissue against the toxic effects of cadmium. The Justicia *carnea* extract also mitigated the toxic effect of cadmium by reducing the elevated levels of AST, ALT and ALP. The combined extract has a more ameliorating effect on the toxicity of cadmium as against either of garlic or Justicia *carnea* extract. Synergistic use of antioxidants has been proven to be more effective compared with the use of the individual antioxidants.[75] Xhyrel *et al.*,[75] observed that treatment of lead intoxicated rats with vitamin c or vitamin E proved to be less potent than the combination of both antioxidants in reducing the level of lead in the blood of exposed rats.

Cadmium is known to mediate its toxicity via the generation of excessive amount of reactive oxygen species.[81] which could result in oxidative stress. Oxidative stress is characterized, mainly by excessive triggering of free radicals that result in induction of high lipid peroxidation levels and the increased damaging of the cellular membranes.[82] Investigation revealed that cadmium interaction with bio-molecules initiates lipid peroxidation, resulting in oxidative stress associated with various cellular damages.[83] Oxidative injury and lipid peroxidation can be monitored by a measure of MDA level. Thus, MDA is considered a significant marker of the oxidative process in body cells. In the present study, there was an increase in MDA level in the cadmium treated rats (group B) compared to the control (6.84 ±0.09 Vs 2.93 ±0.29). Similar results were also reported by Alghasham *et al*[84] and Al-Baqami et al.[85] Andjelkovic *et al.*,[61] observed significant elevation in MDA level in plasma following single oral-dose (30mg/kg body weight). The observed increase in MDA in this study could be due to the excessive generation of reactive oxygen species due to cadmium which results in increased lipid peroxidation and oxidative stress. Following administration of garlic, Justicia *carnea* and combination of garlic and Justicia *carnea* extract, the MDA level was reduced in groups C (3.88±0.20 Vs 6.84 ±0.09), D (3.93 ± 0.12 Vs 6.84 ±0.09) and E (3.79 ± 0.28 Vs 6.84 ±0.09) compared with the cadmium exposed group B. The reduction in the Justicia *carnea* treated group suggest that the leave extract prevented the excessive formation of free radical and cause a reduction in the rate of lipid peroxidation. This is similar to a study by Udedi *et al.*,[86] They reported that the administration of ethanolic extract of the plant reduced MDA levels. The decrease level of MDA shows that Justicia *carenea* leaf extract can improve cadmium induced oxidative stress. The reduction in MDA level in the garlic treated group could be explained by the antioxidant properties of garlic. Garlic possesses an abundant amount of antioxidants (allicin, ally-trisulfide etc) which are known to scavenge reactive oxygen species (ROS) and consequently reduced the lipid peroxidation.[87] The co-administration of the garlic and Justicia *carenea* extract also caused a reduction in the MDA. However, the combined extracts (antioxidants) showed a more potent effect in ameliorating the toxic effect of cadmium compared to that of each individual plant extract. The possible explanation for the difference in the potency of the combined extract could be due to the synergistic effect of both plants' antioxidants. It has been reported that combining antioxidant has proven to be more effective and valuable in mitigating the toxic effect of a substance than the use of the individual antioxidants.[76] Layachi and Kechrid,[76] demonstrated that the synergistic use of the antioxidant vitamins C plus vitamin E proved more effective and potent as compared to vitamin C or vitamin E alone in ameliorating cadmium toxicity.

Cadmium toxicity impacts negatively on enzyme activities. Antioxidant enzymes such as SOD and GPx are known to form the first line of defense against ROS attack. The decrease activity of antioxidant enzymes may be due to the interaction of cadmium with the -SH groups of enzymes.[88] SOD is a crucial component of cellular antioxidant defense system, and is important for evading oxidative stress. The significant reduction in the level of SOD in the cadmium group may be accredited to a devastating oxidation alteration of enzymatic proteins and bio-membrane lipids by reactive oxygen species.[68] In the current study, there was a significant ($p<0.05$) reduction in the activities of SOD and GPx in the cadmium treated group compared to the control group (249.40 ±9.79 and 194.60 ±13.54 respectively). A similar observation was reported by other researchers.[88,89] The decreased in the activities of the antioxidants enzymes SOD and GPx, is suggestive

inhibition of the activities of these enzymes and also of the antioxidant defense- system been overwhelmed by the excess ROS generated due to the cadmium toxicity. SOD scavenges superoxide radicals thereby promoting cytoprotection against the free radical-induced damage. The reduction in SOD level in the current study could be due to inhibition of its activity by cadmium.[90] The interaction between cadmium and essential trace element may be one of the reasons for the decrease in SOD. Cadmium can occupy the Zn site in Cu/Zn-SOD and creates inactive form of the enzyme Cu/Cd-SOD.[89] GPx is an antioxidant that degrades hydrogen perioxide and requires selenium for its activity. The significant decrease in of GPx activity in the cadmium exposed rats in this study may be due to enchancement of peroxidative damage to polyunsaturated fatty acids which will result in higher lipid peroxidation.[89] It could also be attributed to competition by cadmium-methioneins.[88]

Garlic, Justicia *carnea* and the combination of both extracts had a reductive effect on cadmium-induced liver toxicity as manifested by the increase activities of the antioxidant enzymes – SOD and GPx – post treatment in groups C, D and E respectively compared with group B. The increase and restoration of the enzyme activities following the extracts treatment could be attributed to the presence of phenolic compounds. It has been demonstrated that phenolic antioxidant prevents oxidation and free radical change [91]. Enzyme induction by phenolic compounds has also been reported.[92]

CONCLUSION

The study demonstrated the ameliorating potential/effect of combined aqueous extract of allium sativum and Justicia *carnea* on some liver functions parameters and oxidative stress biomarkers. The combined extract of allium *sativum* and Justicia *carnea* had more effect on cadmium induced hepatotoxicity than either of Allium *sativum* or Justicia *carnea* extract alone

DECLARATION OF INTEREST

Authors report no conflicts of interest.

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