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Research Article

Cucumis Sativus Aqueous Extract Exhibited Nephroprotective and Hepatoprotective potentials in Cadmium-Induced Toxicity in Male Wistar Rats

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ABSTRACT

Background: Chronic kidney disease (CKD) is a growing public health concern worldwide, affecting an estimated 10% of the global population. Hence, this study investigated the nephroprotective potential of *Cucumis sativus* extract on cadmium-induced toxicity in male Wistar rats.

Method: Sixteen rats weighing 100 to 180g were grouped into four groups (A to D) four rats each. Group A was control, Group B-D received 1.5mg/kg bw of cadmium, cadmium + 1mg/kg bw of *C. sativus*, and cadmium +2mg/kg bw of *C. sativus* respectively. The administration was done using gavage for 14 days, thereafter, blood samples and the liver were collected for biochemical assays. IBM SPSS statistical software version 28, was used to analyze the data (P-value=<0.05)

Results: Urea significantly ($p<0.001$) increased in treatment groups compared to control, and significantly($p<0.001$) decreased in the group treated with cadmium+2mg/kg *C.Sativus* compared to cadmium control. Creatinine significantly ($p<0.001$) increased in all treated groups than in control. Reduced glutathione significantly ($p<0.001$) reduced in cadmium control than control and increased in cadmium+*C. sativus* (1mg/kg) and cadmium+*C. sativus* (2mg/kg) than cadmium control. Glutathione peroxidase did not reveal any significant statistical difference amongst groups. Catalase significantly ($p<0.001$) decreased in treated groups than control. Superoxide dismutase in treatment groups significantly ($p<0.001$) decreased than control and increased in extract-fed rats than cadmium control. Malondialdehyde in treatment groups significantly ($p<0.001$) increased to the control and decreased in extract-fed groups than cadmium control. Aspartate aminotransferase significantly ($p<0.001$) increased across treated groups than control. Alanine aminotransferase significantly ($p<0.001$) increased significantly in treatment groups than in the control. Alkaline phosphatase in treatment groups significantly ($p<0.001$) increased compared to the control and decreased in extract-fed groups than in the cadmium control.

Conclusion: It Based on these results, we conclude that C. sativus may possess nephroprotective agents that resulted in reduced serum urea, creatinine, and kidney oxidative markers, especially in the cadmium+C. sativus (2mg/kg) treated group.

Nephroprotective and Hepatoprotective Effect of Aqueous Extract of *Cucumis sativus* in Cadmium-Induced Toxicity in Male Wistar Rats

1. Introduction

Chronic kidney disease (CKD) is a growing public health concern worldwide, affecting an estimated 10% of the global population (Arora and Meena, 2017). While conventional treatments for CKD can be effective, they can also be expensive, invasive, and come with a host of side effects. As such, there is increasing interest in exploring natural and safe alternatives for promoting kidney health (Arora and Meena, 2017). Cucumber is a widely consumed vegetable shown to possess a range of potentially beneficial properties, including antioxidant, anti-inflammatory, and diuretic effects (Ofoego, 2019). Studies have suggested that the bioactive compounds found in cucumbers may help protect against renal damage and improve kidney function in animal models (Ofoego, 2016). Ahmed *et al.,* (2016) reported that cucumber peel extract had significant nephroprotective effects.

The liver is a vital inner organ that performs numerous functions which include secretion of bile, excretory function, synthetic function, metabolic function, hemopoietic function, hemolytic function, heat production, defense and detoxification functions (Guyton and Hall, 2012), clotting factors and albumin synthesis (Palmer, 2004). The human liver consists of numerous enzymes that aid in necessitating chemical processes in the body (Palmer, 2004). The most used indicators of liver functions are alanine aminotransferase (ALT), aspartate aminotransferase (AST) (Giboney, 2005), and alkaline phosphatase concentration (Schlaeger, Haux, and Kattermann, 1982). Increased levels of ALT and AST are indications of hepatocellular disease, active cirrhosis, metastatic liver tumor, toxic hepatitis, severe, pancreatitis, myocardial infarction (heart attack), trauma, severe burns, acute hemolytic anemia; crushing injuries and shock (Adeyemi *et al.,* 2015). Alkaline phosphatase is commonly used to access obstruction in the biliary system (Adeyemi *et al.,* 2015). Alkaline phosphatase increase is majorly detected in biliary tumors, gallstone disease, alcohol abuse, and drug-induced hepatitis (Schlaeger *et al.,* 1982). Cadmium is a general toxic agent that has been reported to cause various physiologic adverse effects (Chin-Chan *et al.,* 2015). Industrial development has increased cadmium (Cd) pollution. Cadmium contributes to environmental pollution through industrial activities like battery manufacturing, electroplating, plastic, and paint production. Moreover, Cd is released during the smelting of zinc and lead ores (Friberg and Vahter, 1983; Robards and Worsfold, 1991). It has been reported that soluble Cd salts entering the body through various routes can cause damage to different tissues such as the liver, kidney, pancreas, testes, brain, and lungs (Yang and Shu, 2015). Cadmium exposure can lead to various health problems in humans. This metal is also classified as a Group I carcinogen for humans by the International Agency for Research on Cancer (Zhai *et al.,* 2015). Hence, this study investigated the nephroprotective potential of Cucumis sativus extract on cadmium-induced nephrotoxicity in male Wistar rats.

2. Methodology

Procurement of Test Substances

2.1 Chemicals: The cadmium chloride was purchased from Sigma-Aldrich Limited Germany with EC number 233-296-7. Xylazine and ketamine (Guangzhou JHD Chemical Reagent Co., Ltd. Shantou Guangdong, China).

2.2 Extraction of *Cucumis sativus*

Matured Cucumber fruits were harvested from farmland at IkotAbasi local government area of Akwa Ibom state, Nigeria. It was ensured that they were free from any blemishes or bruises that could affect the quality of the extract. The fruits were washed thoroughly with clean water to remove dirt. Thereafter, cucumber fruits were chopped into tiny pieces using a food processor to increase the surface area and facilitate the release of the contents. After which, the chopped cucumber pieces were immersed in 250ml of distilled water and heated to boiling point for 30 minutes, and then the suspension was filtered using a Whatman filter paper and concentrated using a vacuum evaporator (Labotech International Co., Ltd, Tokyo, Japan) at a temperature of 60 °C. Method used by Duke, (2001).

2.3 Laboratory Animals

Sixteen (16) male Wistar rats weighing between 100 to 180g were employed for this study. The animals were housed in the Department of Physiology Animal House, University of Calabar, Nigeria. Normal animal cages (435 x 290 x 150mm) with wood flakes as bedding were used in housing the animals (4 rats per cage). They were given ad libitum access to feed (AEC Agrosystem Limited, Port Harcourt, Rivers State, Nigeria) and fresh water, and exposed to 12/12-hr dark/light phase. They were acclimatized for 7 days and kept in line with laid-down ethics for animal care approved by the National Committee for Research Ethics in Science and Technology (NENT), 2018. Before the commencement of this research, ethical approval was obtained from the University of Calabar animal ethics committee, which aligned with the standard guidelines for the use of laboratory animals outlined by the World Health Organization. The study was permitted with ethical clearance registration reference number 040PHY3719.

2.4 Experimental Design and Administration of Cadmium and *Cucumis sativus*

The animals were arbitrarily allotted into 4 separate groups (n = 4). At the end of 7 days of acclimatization, a solution of Cadmium with a density of 1.5mg/kg was given ad-libitum to induce Cd toxicity in the experimental animals for 4 days. Thereafter, *Cucumis sativus* aqueous extract administration commenced. The Cd and *Cucumis sativus* extract was given via oral means using gavage, once, daily, to animals in treatment groups (2 to 4), doses as described in Table 1, whereas the control group was given feed and 0.5ml normal saline as a vehicle throughout the experimental period. *Cucumis sativus* aqueous extract administration lasted for fourteen (14) days. Thereafter, the rats were euthanized under xylazine and ketamine anesthesia. Blood samples were collected from rats via ocular puncture and the left kidneys of each rat were harvested. Blood and tissue samples were stored at -80°C until biochemical analyses were performed.

Table 1. Study design and Administration

Groups	No of rats	Treatment
Group A (control)	4	Feed + 0.5ml of normal saline as a vehicle throughout the experiment
Group B (cadmium control)	4	1.5mg/kg bw of Cadmium
Group C	4	1.5mg/kg bw of cadmium + 1mg/kg bw of *Cucumis sativus*
Group D	4	1.5mg/kg bw of cadmium +2mg/kg bw of *Cucumis sativus*

2.5 Determination of serum Urea

Principle: Urea was measured using the Urease-Berthelot Method (mmol/L) (Richterich and Kuffer 1974). Urea in serum was hydrolyzed to ammonia in the presence of urease. The ammonia was then measured photometrically by Berthelot's reaction.

2.6 Determination of serum Creatinine

Principle: Creatinine was measured using the Direct End-Point Method (umol/L) (Kaplan and Szabo 1983). Creatinine reacts with picric acid in an alkaline solution to form a colored complex. The amount of complex formed is directly proportional to the creatinine concentration.

2.7 Kidney Antioxidant Assessment

The left kidney of each rat was harvested and homogenized using a Potter-Elvehjem homogenizer. Twenty percent (1/5 w/v) of tissue homogenate was placed in 50 mm Tris–HCl buffer (pH 7.4) with 1.15% potassium chloride and centrifuged at 10,000 rpm at 4°C for 10 minutes. The supernatant was obtained for catalase (CAT) assay with hydrogen peroxide as substrate. Reduced Glutathione (GSH) was assayed at 412 nm using the method of Luchese *et al.,* (2009). Glutathione peroxidase (GPx) was assayed using hydrogen peroxide as substrate (Lucchese *et al.,* 2009). Superoxide dismutase (SOD) was assayed using the method described by Misra and Fridovich (1972). Malonyldialdehyde (MDA) was assessed in thiobarbituric acid reacting substances (TBARS) as explained by Meenakshi, *et al.,* (2007).

2.8 Assessment of Serum Liver Enzyme Markers

2.8.1 Assessment of Serum Alkaline Phosphatase

Alkaline phosphatase was measured according to a standard procedure (Bergmeyer and Bernt 1974). Briefly, P-nitrophenyl phosphate was hydrolyzed to phosphate and p-nitrophenol in the presence of ALP. A calculated amount of sample 0.01ml in a test tube was mixed with reagent (0.5ml) containing substrate p nitrophenyl phosphate and kept at room temperature. The solution was mixed, and the initial absorbance was read after one minute. The reaction was allowed to stand for three minutes and absorbance read again at 405nm. Alkaline phosphatase activity was calculated from the equation:

UL = 2760 x ΔA nm/minute micro

Where UL = Unit of alkaline phosphatase affinity

ΔA = Change in absorbance

Okhawa, *et al.,* (1979). Afterward, the mixed reaction generated 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of 20% acetic acid solution matched to pH 3.5 with sodium hydroxide, and 1.5 ml of 0.8% water solution of thiobarbituric acid was added to 0.2 ml of 10%(w/v) of homogenate. The mixture was moved up to 4.0 ml using distilled water and heated at 95°C for 60 mins. Almost 1.0 ml of distilled water and 5.0 ml of the mixture of n-butanol and pyridine (15:1 v/v) was added and centrifuged ice cooling at 4000rpm. The crude layer was discarded and absorbance was summed at 532 nm and added with results obtained from MDA standards. The concentrations were calculated from absorption values as normal absorption. Method used by Mobisson *et al.,* (2023)

2.8.2 Assessment of Serum Aspartate Aminotransferase and Alanine Aminotransferase

Serum AST and ALT levels were determined using an endpoint colorimetric diagnostic kit (Randox Laboratories, UK) based on Reitman and Frankel's method (Reitman and Frankel, 1957). The pyruvate produced by the transamination reaction between L-alanine and ketoglutarate reacts with 2, 4, dinitrophenyl hydrazine to give a colored hydrazone, and was used to measure alanine aminotransferase activity. The oxaloacetate hydrazone formed with 2, 4 dinitrophenyl hydrazine was used to measure AST. Both ALT and AST were read at 540nm wavelength (Reitman and Frankel, 1957). Method used by Mobisson *et al.,* (2019).

2.9 Statistical analysis

All results are presented as mean ± SEM, n=4. One-way analysis of variance (ANOVA) was utilized in comparing the differences within groups, followed by post hoc multiple comparisons. IBM Computer software SPSS version 28.0 and Excel analyzer were used for the analysis. The level of significance was set at $p<0.05$.

3. Results

3.1 Comparison of serum urea and creatinine concentration in control and different experimental groups.

As shown in Table 2 below, there was a significant ($p<0.001$) increase in serum concentration of urea in treated rats compared to control. However, the rats treated with cadmium + 2mg/kg *C. Sativus* significantly ($p<0.001$) decreased compared to cadmium control. The serum concentration of creatinine was significantly ($p<0.001$) increased in all treated rats compared to control. However, there was a significant ($p<0.01$) decrease in the group treated with cadmium+ *C. sativus* (2mg/kg) compared to the group treated with cadmium +1mg/kg *C. sativus* and cadmium control.

Table 2 Comparison of serum urea and creatinine concentration in control and different experimental groups.

Parameters	Group A(control)	Group B (Cd control)	Group C (Cd+1mg/kg C.S)	Group D (Cd+2mg/kg C.S)
Urea (mmol/l)	3.47±0.40	5.55±0.12***	5.22±0.16***	4.62±0.08**, g
Creatinine (mmol/l)	73.75±5.60	115.75±1.54***	110.25±3.27***	92.00±1.22**, g, ###

Values are expressed as mean ± SEM, n=4, **=p<0.01, ***=p<0.001 vs control, g= p<0.001 vs cadmium, ### = p<0.001 vs cadmium + CS (1mg/kg).

3.2 Comparison of kidney oxidative stress markers in control and experimental groups

As shown in Table 3 below, the kidney-reduced glutathione concentration in the cadmium control group was significantly ($p<0.001$) reduced compared to the control. However, groups treated with cadmium+CS (1mg/kg) and cadmium+CS (2mg/kg) significantly ($p<0.001$) increased compared to cadmium control. The concentration of kidney glutathione peroxidase did not reveal any significant statistical difference among groups. The concentration of kidney catalase in the treatment groups was significantly ($p<0.001$) decreased compared to the control group. However, there was a significant ($p<0.05$) increase in groups treated with cadmium+CS (1mg/kg) and cadmium+CS (2mg/kg) compared to cadmium control. The concentration of kidney superoxide dismutase in the treated groups was significantly ($p<0.001$) decreased compared to control. However, groups treated with cadmium+CS (1mg/kg) and cadmium+CS (2mg/kg) significantly ($p<0.001$) increased compared to cadmium control. Although, groups treated with cadmium+CS (2mg/kg) significantly ($p<0.001$) increased compared to groups treated with cadmium+CS (1mg/kg). The concentration of kidney malondialdehyde in treated groups was significantly ($p<0.001$) increased compared to control. However, groups treated with cadmium+CS (1mg/kg) and cadmium+CS (2mg/kg) significantly ($p<0.001$) decreased compared to cadmium control. Although, groups treated with cadmium+CS (2mg/kg) significantly ($p<0.001$) decreased compared to groups treated with cadmium+CS (1mg/kg).

Table 3: Comparison of Kidney oxidative stress markers in control and experimental groups

Parameters	Group A(control)	Group B (Cd control)	Group C (Cd+1mg/kg C.S)	Group D (Cd+2mg/kg C.S)
GSH ug/ml	1.62±0.09	0.67±0.01***	1.38±0.09[c]	1.58±0.14[g]
GPx ug/ml	0.06±0.00	0.28±0.24	0.06±0.00	0.07±0.00
CAT u/g	1.15±0.02	0.52±0.01***	0.77±0.07**, [a]	0.83±0.10**, [f]
SOD ug/ml	0.50±0.01	0.16±0.01***	0.27±0.01***, [c]	0.39±0.01**, [g, ###]
MDA umol/ml	0.22±0.01	0.60±0.01***	0.49±0.00***, [b]	0.34±0.02***, [g, ###]

Values are expressed as mean ± SEM, n=4, **=p<0.01, ***=p<0.001 vs control, a= p<0.05 vs cadmium, b=p<0.01 vs cadmium, c= p<0.001 vs cadmium, f= p<0.01 vs cadmium, g= p<0.001 vs cadmium, ### = p<0.001 vs cadmium + CS (1mg/kg).

3.3 Comparison of liver enzyme markers of control and experimental groups

In Table 3 below, the serum concentration of aspartate aminotransferase (AST) in treatment groups was significantly (p<0.001) increased compared to the control. The serum concentration of Alanine aminotransferase (ALT) in treatment groups was significantly (p<0.001) increased compared to control. However, groups treated with cadmium+CS (1mg/kg) and cadmium+CS (2mg/kg) significantly (p<0.001) decreased compared to cadmium control. The serum concentration of Alkaline phosphate (ALP) in treatment groups was significantly (p<0.001) increased compared to control. However, groups treated with cadmium+CS (1mg/kg) and cadmium+CS (2mg/kg) significantly (p<0.001) decreased compared to cadmium control.

Table 4: The comparison of liver enzyme markers of control and experimental groups

Parameters	Group A(control)	Group B (Cd control)	Group C (Cd+1mg/kg C.S)	Group D (Cd+2mg/kg C.S)
AST u/l	40.00±1.41	76.75±7.11***	80.25±1.31***	76.75±4.11***
ALT u/l	19.75±3.70	53.00±1.29***	37.50±1.89***, [c]	33.25±1.25**, [g]
ALP u/l	30.00±0.81	38.75±0.85***	36.00±0.40***, [a]	34.25±0.47**, [g]

Values are expressed as mean ± SEM, n=4, **=p<0.01, ***=p<0.001 vs control, c=p<0.001 vs cadmium, a=p<0.05 vs cadmium, g= p<0.001 vs cadmium

4. Discussions

The increasing rate of chronic kidney disease (CKD) in the world at large has elicited concern about its cause and possible remedies. Chronic kidney disease affects about 10% of the world's population. Though, orthodox treatments for CKD can be effective, but can be expensive and invasive with numerous adverse effects. This has triggered interest in the use of natural and safe alternatives to promote kidney health (Arora and Meena, 2017). Cucumber is a commonly consumed vegetable with evidence of healthy beneficial properties, including antioxidant, anti-inflammatory, and diuretic effects (Arora and

Meena, 2017). Hence, this study investigated the nephroprotective potentials of *Cucumis sativus* aqueous extract in cadmium-induced toxicity in male Wistar rats.

The significant increase in serum urea and creatinine concentrations in treated groups could be possible indication of kidney damage caused by cadmium induction. It has been shown that the accumulation of urea and creatinine which are waste products of metabolism is a possible indication of renal dysfunction (Guyton and Hall 2012). Paul *et al.,* (2010), reported that elevated creatinine concentration indicates possible toxins that could lead to renal dysfunctions. The significant reduction in serum urea and creatinine in group treated with cadmium +C. S (2mg/kg) compared to cadmium untreated could be an indication of ameliorative effect of *Cucumis sativus*. Furthermore, Ofoego *et al.,* (2019), reported ethanolic extract of *Cucumis sativus* ameliorated kidney damage in male diabetic nephropathic rats. The significant decrease in kidney antioxidant markers in treated groups compared to control may be an indication of oxidative stress induced by cadmium (Gill *et al.,* 2009). However, the significant increase in glutathione, catalase, and superoxide dismutase in *Cucumis sativus* treated groups compared to the cadmium untreated group may be an indication of increased antioxidant activity (Gill *et al.,* 2009). The significant increase in malondialdehyde in treated groups could be an indication of possible lipid peroxidation caused by cadmium induction (Anbarasu *et al.,* 2011). Malondialdehyde is a marker for lipid peroxidation, therefore, its elevation is a pointer. The decreased levels of liver oxidative stress markers may suggest a link between drugs and hepatic oxidant levels (Anbarasu *et al.,* 2011; Anand *et al.,* 2009). The liver is a major organ targeted in drug toxicity. Radical species production, majorly reactive oxygen species, and reactive nitrogen species are suggested as early events of drug hepatotoxicity and as indicators of hepatotoxic potential (Sodhi *et al.,* 1998). It has been reported that many drugs may likely induce oxidative stress by altering cellular oxidant levels and lipid peroxidation (Wopara *et al.,* 2021).

The result showed that rats treated with cadmium and *C. sativus* at different doses had significantly increased serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) concentrations compared with the control. The increased serum liver enzyme markers in treated rats may likely be indicating hepatic damage caused by the toxic effect of cadmium (Giboney, 2005; Mobisson *et al.,* 2023). However, there was significant decrease in serum ALT and ALP in *C. sativus* treated, especially in 2mg/kg group compared to cadmium untreated. This could be linked to the ameliorative effect of *C. sativus*. The most commonly used indicators of liver functions are ALT and AST (Giboney, 2005) and ALP concentration. Variations in the concentration of these enzymes have many implications. Increased levels of ALT and AST are indications of hepatocellular disease, active cirrhosis, metastatic liver tumor, toxic hepatitis, severe pancreatitis, myocardial infarction (heart attack), trauma, severe burns, acute hemolytic anemia, and crushing injuries and shock (Adeyemi *et al.,* 2015). Alkaline phosphatase is commonly used to signify obstruction in the biliary system. Its increase is majorly detected in the biliary tumor, gallstone disease, alcohol abuse, and drug-induced hepatitis (Schlaeger *et al.,* 1982).

5. Conclusion

Administration of cadmium caused a significant increase in serum urea, creatinine, decreased antioxidant markers, and increased malondialdehyde and liver enzyme markers in male Wistar rats. These biochemical alterations could be due to the toxic effect of cadmium. However, *C. sativus* treatment had an ameliorative effect. Hence, if this study is to be applied to humans, moderate consumption of *C. sativus* is recommended especially in chronic kidney disease as it may possess nephroprotective potentials.

6. Declarations

6.1 Ethics approval

University of Calabar animal ethics committee permitted our research procedure with approval number 040PHY3719.

6.2 Availability of data and materials
All data generated or analyzed during this study are included in this article.

6.3 Competing interests
There are no competing interests. Each author has read the document and given their consent for publication.

6.5 Funding
There was no funding assistance from any private or public sector.

6.6 Authors' Contributions

Mobisson Samuel Kelechi and **OtoAbasi Sunday Abaka**, designed the study and wrote the study protocol. Mobisson Samuel Kelechi, **OtoAbasi Sunday Abaka** and **Agnes Igimi Odey** performed laboratory experiments and literature searches. Mobisson Samuel Kelechi drafted the manuscript; **Enene Esu Ukpai** worked on data analysis. Mobisson Samuel Kelechi performed the statistical analysis. All authors read and approved the final manuscript.

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