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Effect of Combined Fruit Juice of *Citrullus lanatus* and *Cucumis sativus* on Cadmium Induced Hepatotoxicity and Nephrotoxicity in Male Wister Rats

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Original Research Article

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Abstract: The protective effect of fruit juice of Cucumber and Watermelon on selected biochemical parameters of cadmium induced toxicity on male albino rats was investigated. Forty male rats were divided into eight groups. Group VIII served as normal control while group VII was positive control that was administered with Cadmium only. Groups I to VI received high and low doses of Cucumber and Watermelon juice respectively. Apart from the normal control group, other groups were fed with lard 14days before treatment commenced. Doses of 0.8mg/kg body weight-high dose and 0.4mg/kg body weight-low dose for Cucumber and Watermelon was administered respectively. At the 4th and 6th week, biochemical parameters were assayed. The testes, liver and kidney were submitted for histopathological examination. Results show that pretreatment with Cucumber and Watermelon juice caused a decrease in aspartate transaminase and alkaline phosphatase in the treatment groups after 4 weeks compared to positive control value (35.66±13.32U/L) and (516.66±23.02) respectively. Alanine transaminase values reduced in treatment groups compared to positive control group (16.33±2.31U/L). Urea and Creatinine levels were significantly ($P< 0.05$) reduced in treatment groups compared to positive control of 11.58 ± 1.24mmol/L and 161.4±3.17µmol/L respectively after 4 weeks of pretreatment. Electrolytes were reduced compared to positive control. The group that received low dose of the combination of Cucumber and Watermelon extract showed more significant change than other groups indicating possible dose dependent effect.
Keywords: Cadmium, Water melon, Cucumber, Hepatotoxicity and Nephrotoxicity

INTRODUCTION

It is recognized in recent years that environmental problems have exponentially increased, due to the growing needs of human and population [1]. Humans are exposed to harmful environmental contaminants at different stages of life especially in reproductive stage. A number of these contaminants are heavy metals and are toxic. Cadmium (Cd), one of the foremost toxic metals widely dispersed in environmental and occupational settings has been found to reduce male fertility [2].

Cadmium enters the atmosphere via several ways [3, 4], through erosion, volcanic activity, river transport and weathering [5]. As an alloy, in electroplating of other metals and as a pigment which contaminate air, water and land. The extensive use in the manufacture of alkaline batteries and plastics, and the major source of cadmium available in the rural regions is because of human activities like phosphate fertilizing, fuel combustion and waste burning [5].

Bioaccumulation is the process which cadmium enters the food chain in different animals and human tissues [6]. The usual means for cadmium exposure are smoking, breathing of contaminated air and eating contaminated seafood and water [3]. Reports confirm that kidney, liver, testes, ovary, prostrate are organs damaged by cadmium exposure [7, 8, 9]. Low dose of 1-2 mg/kg body weight of cadmium can cause damage to testes without causing such effects on other organs in the body [10].

Chronic cadmium toxicity can lead to renal failure, nephrotoxicity and testicular cancers among others [11] with the most marked effect in rats given a single parenteral dose being testicular necrosis [12]. Cadmium exerts its toxic effect by generating reactive oxygen species (ROS) which interferes with antioxidant in cells [13].

Another mechanism of cadmium toxicity is interference of preoxidant and antioxidant stability by generating reactive oxygen species [14]. It is proven that tissue levels of lipid peroxide is an indicator in oxidative stress [15]. Furthermore, investigations have recorded acute cadmium exposure is linked to elevated lipid peroxidation in sex organs in males and other organs [16, 14].

Watermelon (*Citrullus lanatus*) is a fruit with a juicy pulp that is red or pink with many seeds. In Nigeria watermelon fruit is consumed more owing to recent appreciation on the wellbeing benefits. The juice comprises of vital carotenoids like lycopene, carotene and β-carotene which counteract free radicals effect in the human [17]. The part mostly consumed in Nigeria is the fleshy pulp. The seeds are mostly thrown away by consumers but in some areas it is crushed into flour and included in bread baking [18]. The seeds are very rich in proteins and fats [11].

Watermelon fruit contains water and sugar in 91% and 6% respectively, and is stumpy in fat. Citrulline an amino acid is also contained in the rind of watermelon. Watermelon pulp contains carotenoids, including lycopene[19, 20].

Cucumis sativus also known as Cucumber is domicile in the family Cucurbitaceae. Cucumber is initially from Southern Asia, but now many different varieties are sold in the market. Cucumbers are usually more than 90% water [21].The human olfactory response to cucumbers varies; some say it has a bitter taste while others say it's tasteless or water taste [22].

MATERIALS AND METHODS

Procurement of Samples

Cucumis sativus (Cucumber) and *Citrullus lanatus* (Watermelon) used for this study were purchased from Choba market, Rivers State, Nigeria.

Experimental Animals

Forty Wistar male albino rats of body weight 150-250g were acquired from the Animal house of the Department of Biochemistry, University of Port Harcourt, Nigeria. They were housed separately in cages and grouped into eight groups (I-VIII). The rats were fed with grower's mash (Top feeds) and water ad libitum for a duration of 2 weeks before the commencement of the study.

Preparation and Extraction of the Samples

The fruits were washed and the bark removed, the pulp and seeds were blended without adding water, the juice was sieved and put in a water bottle and preserved in a refrigerator before use. Fresh fruit juice was blended every two days.

Experimental Design

The experiment lasted for 42 days; the rats were grouped into eight groups. The choice of dose of administration of the two samples; Water melon and Cucumber was adopted from the method of Georgina *et al* 2011. All the rats in the test groups were initially fed with diets comprising 600 g of grower's mash (Top feeds) mixed with 60 g of lard.

Group I **--**treated with cucumber juice (0.8 ml – High dose/kg body weight)

Group II**--** treated with water melon juice (0.8 ml-High dose/kg body weight)

Group III **–** treated with Cucumber and Water melon juice (0.8 ml-High dose/kg body weight)

Group IV**–** treated with Cucumber and Water melon juice (0.4 ml-High dose/kg body weight) Group V **--** treated with water melon juice (0.4 ml-High dose/kg body weight)

Group VI **--** treated with cucumber juice (0.4 ml-High dose/kg body weight)

Group VII were fed with Lard (high fat diet) in order to induce hyperlipidemia and served as positive control, Group VIII rats served as normal control. Animals in group's I-VII were treated with 3mg/kg BW, 24 hours prior to their sacrifice while group VIII was not treated with cadmium.

Sacrificing of Animals

The animals were sacrificed and blood samples were collected in anti-coagulant bottles and centrifuged at 3000 rpm for 10 minutes to obtain serum. The serum obtained was stored using for further analyses.

Biochemical Assay

Estimation of liver enzymes; Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) activities were determined by enzymatic methods with commercial test kits (Randox Laboratories, Crumlin, England. Serum electrolytes (Na^+, K^+ and Cl^-), were determined using automated ion selective electrode, while Urea and Creatinine were measured following the protocol stated in Randox test kit(Randox Laboratories, Crumlin, England) .

HISTOLOGICAL ANALYSIS

The organs (liver and kidney) were harvested from the treated and control rats and placed in 10% formaldehyde. Dehydration was done with Isopropyl alcohol and these tissues were subjected to a series of increasing concentrations of Isopropyl alcohol (60%) for two hours, 80% alcohol for two hours, 95% alcohol (overnight) and absolute alcohol (100%) for two hours, in which the water is replaced by Isopropyl alcohol. These tissues were infiltrated with paraffin and were left to equilibrate using an incubator for one hour at 60°C. These tissues were mounted on the microtome for sectioning after the decantation, solidification of paraffin around these tissues; the paraffin was thereafter trimmed out. The sections were attached to microscope slides and these slides were labeled, properly washed and allowed to dry and the slides were dipped in an adhesive solution and allowed to dry overnight. The slides were then stained with hematoxylin and the sections were mounted on a cover slip after adding 2 drops of resin and left for 24 hours. The histological slides were examined under a microscope and interpreted.

STATISTICAL ANALYSIS

One way analysis of variance was performed using SPSS 21 version. The values were presented as MEAN ± SD.

Table−1a: Effects of different concentrations of cucumber and watermelon on liver enzymes activity of cadmium induced testicular damage in Wistar albino rats after two (2) weeks of treatment

Groups	Alkaline Phosphatase (U/L)	Aspartate Transaminase (U/L)	Alanine Transaminase (U/L)
NC	248.50±57.28	18.50±4.95	10.50±9.19
PC	438.00±108.89[a]	32.00±4.24[a]	14.50±3.53
GRP 1	265.50±20.51[b]	28.50±3.54[a]	13.50±3.53
GRP 2	269.50±105.36[b]	29.80±17.68[a]	10.50±9.19
GRP 3	310.50±202.94[a,b]	26.00±4.24[a,b]	12.50±4.94
GRP 4	268.50±152.03[b]	27.00±11.31[a,b]	12.50±6.36
GRP 5	317.00±258.80[a,b]	30.50±21.92[a]	11.50±12.02
GRP 6	383.50±111.02[a,b]	29.00±14.14[a]	13.50±6.36

Data expressed as mean±SD, n=5
"X" shows significant difference between week 2 and week 4
"a" shows significant difference when compared to normal control(NC)
"b" shows significant difference when compared to positive control(PC)
NC=Normal control; PC=Positive control; GRP 1=High concentration of whole extract of Cucumber; GRP 2= High concentration of whole extract of Watermelon; GRP 3=High concentration of whole extract of combination of Cucumber and Watermelon; GRP 4= Low concentration of whole extract of combination of Cucumber and Watermelon; GRP 5=Low concentration of whole extract of Cucumber; GRP 6= Low concentration of whole extract of Watermelon.

Table−1b: Effects of different concentrations of cucumber and watermelon on liver enzymes activity of cadmium induced testicular damage in Wistar albino rats after four (4) weeks of treatment

Groups	Alkaline Phosphatase (U/L)	Aspartate Transaminase (U/L)	Alanine Transaminase (U/L)
NC	245 .00±57.20	18.80±4.95	10.3±9.195
PC	516.66±23.02[a,x]	35.66±13.32[a]	16.33±2.31
GRP 1	257.0±113.85 [b]	24.00±7.01	8±2.13[a,x]
GRP 2	252.50±21.9 [b]	26.06±.12	11.5±3.54
GRP 3	249.33±111.1 [b X]	21.66±14.74[b]	9.66±2.31
GRP 4	244.74±7.77[b]	20.5±10.61[b]	10.43±2.82
GRP 5	256.09±41.0[b,X]	27.66±10.96	12.1±8.88
GRP 6	275.44±72.12[a,b,X]	26±10.98[a]	11.2±2.81

Data expressed as mean±SD, n=5
"X" shows significant difference between week 2 and week 4
"a" shows significant difference when compared to normal control(NC)
"b" shows significant difference when compared to positive control(PC)
NC=Normal control; PC=Positive control; GRP 1=High concentration of whole extract of Cucumber; GRP 2= High concentration of whole extract of Watermelon; GRP 3=High concentration of whole extract of combination of Cucumber and Watermelon; GRP 4= Low concentration of whole extract of combination of Cucumber and Watermelon; GRP 5=Low concentration of whole extract of Cucumber; GRP 6= Low concentration of whole extract of Watermelon.

Table-2a: Effects of different concentrations of cucumber and watermelon on urea and creatinine levels of cadmium induced testicular damage in Wistar albino rats after two(2) weeks and four((4) weeks of treatment

Groups	Urea (mmol/L)		Creatinine (µmol/L)	
	WK 2	WK4	WK 2	WK4
NC	2.68±.28	2.67±0.27	94.30±29.20	93.85±29.21
PC	10.06±6.43[a]	11.58±1.24[a]	73.60±8.44	161.4±3.17[a,x]
GRP 1	8.29±2.25[a]	3.89±1.3[b,x]	82.49±4.12	98.77±0.52[b]
GRP 2	9.34±1.07[a]	3.63±1.09[b,x]	87.50± 2.49	98.6±0.85[b]
GRP 3	7.24±4.28[a]	3.04±0.09[b,x]	68.47±2.31[a]	103.44±23.14[x]
GRP 4	7.75±1.66[a]	3.01±0.46[b,x]	77.18±3.38	93.51±10.05[b]
GRP 5	10.21±0.06[a]	3.67±1.26[b,x]	69.26±1.68[a]	96.5 0±13.46[b,x]
GRP 6	8.24±3.87[a]	3.48±1.33[b,x]	70.46±0.50	94.80±6.51[b]

Data expressed as mean±SD, n=5
"X" shows significant difference between week 2 and week 4
"a" shows significant difference when compared to normal control(NC)
"b" shows significant difference when compared to positive control(PC)
NC=Normal control; PC=Positive control; GRP 1=High concentration of whole extract of Cucumber; GRP 2= High concentration of whole extract of Watermelon; GRP 3=High concentration of whole extract of combination of Cucumber and Watermelon; GRP 4= Low concentration of whole extract of combination of Cucumber and Watermelon; GRP 5=Low concentration of whole extract of Cucumber; GRP 6= Low concentration of whole extract of Watermelon.

Table-2b: Effects of different concentrations of cucumber and watermelon on electrolyte levels of cadmium induced testicular damage in Wistar albino rats after two (2) weeks and four(4) weeks of treatment

Groups	Sodium (mEq/L)		Potassium (mEq/L)		Chloride (mEq/L)	
	WK 2	WK4	WK 2	WK4	WK 2	WK4
NC	103.39±7.66	103.98±7.65	5.00±.39	5.05±0.35	102.87±3.21	102.87±3.21
PC	108.38±7.18	128.75±7.03[a,x]	4 .58±.86	7.50±0.34 [a]	92.36±4.69	108.54±3.17
GRP 1	95.30±22.25	104.25±11.24	4.09±2.07	5.00±2.37	97.69±15.56	98.33±0.61
GRP 2	101.78±7.89	106.75±6.29	3.91±.03	5.22±1.46	98.35±.16	101.08±24.72
GRP 3	104.07±7.89	105.74±15.88	3.64±1.63	4.55±1.27	105.75±.64	105.28±4.62
GRP 4	112.27±7.06	103.96±1.74	3.75±.28	4.61±1.13	112.15±4.87	102.22±0.45
GRP 5	91.95±23.54	101.13313.16	3.84±1.01	4.57±0.37	104.90±.57	101.03±2.62
GRP 6	117.19±2.87	98.42±30.52[x]	4.44±1.75	4.65±0.14	110.00±2.12	104.71±2.42

Data expressed as mean±SD, n=5
"X" shows significant difference between week 2 and week 4
"a" shows significant difference when compared to normal control(NC)
"b" shows significant difference when compared to positive control(PC)
NC=Normal control; PC=Positive control; GRP 1=High concentration of whole extract of Cucumber; GRP 2= High concentration of whole extract of Watermelon; GRP 3=High concentration of whole extract of combination of Cucumber and Watermelon; GRP 4= Low concentration of whole extract of combination of Cucumber and Watermelon; GRP 5=Low concentration of whole extract of Cucumber; GRP 6= Low concentration of whole extract of Watermelon.

PHOTOMICROGRAPH OF THE LIVER AND KIDNEY

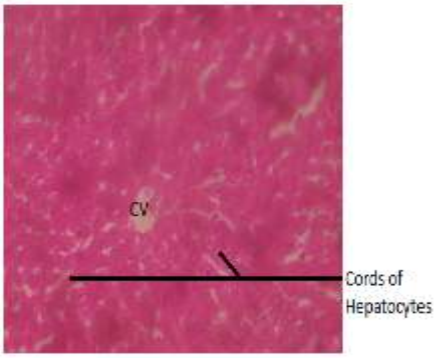

Plate-1.1: Photomicrograph of rat liver in normal control group showing normal hepatocytes. H &E X400

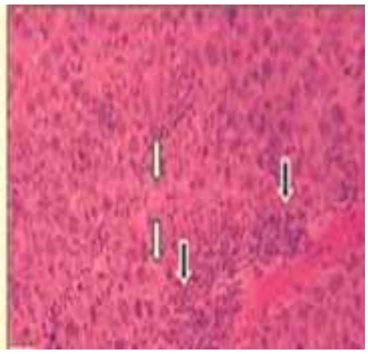

Plate-1.2: Photomicrograph of rat liver fed lard and untreated showing infiltration of inflammatory cells in the hepatic tissue (arrowed).H &E X400

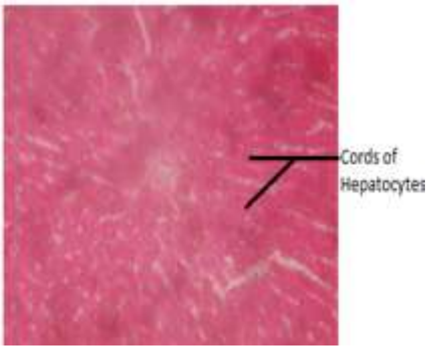

Plate-1.3: Photomicrograph of rat liver treated with high concentration of cucumber juice showing normal cords of hepatocytes radiating away from the central vein. H&E X400

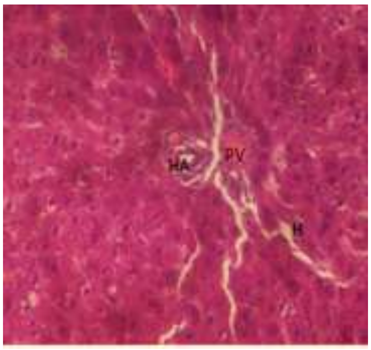

Plate-1.4: Photomicrograph of rat livertreated with high concentration of watermelon showing normal cords of hepatocytes and portals triad (portalvenule, hepatic arteriole and bile duct). H &E X400

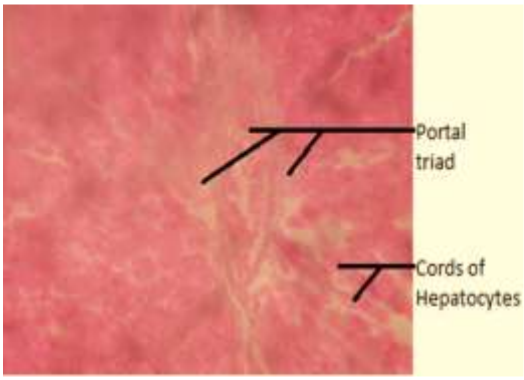

Plate-1.5: Photomicrograph of rat liver treated with high concentration of cucumber and watermelon showing normal cords of hepatocyte, portal venule, hepatic arteriole. H &E X400

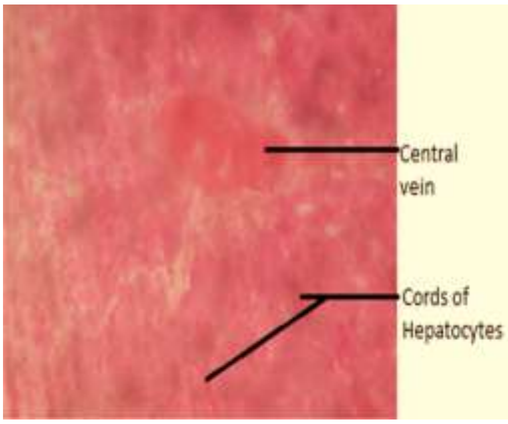

Plate-1.6: Photomicrograph of rat liver treated with low concentration of cucumber and watermelon showing congested central venule and normal cords of hepatocytes. H &E X400

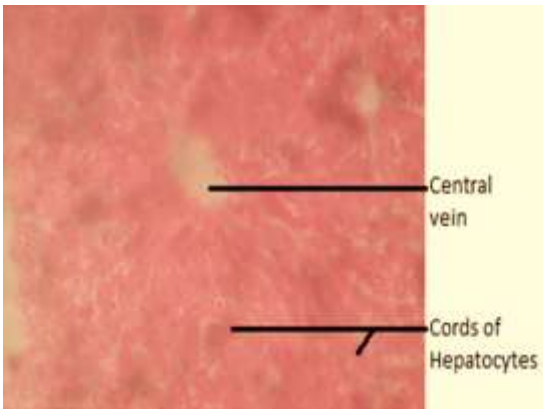

Plate-1.7: Photomicrograph of rat liver treated with low concentration of cucumber showing normal cords of hepatocytes radiating away from the central vein. H &E X400

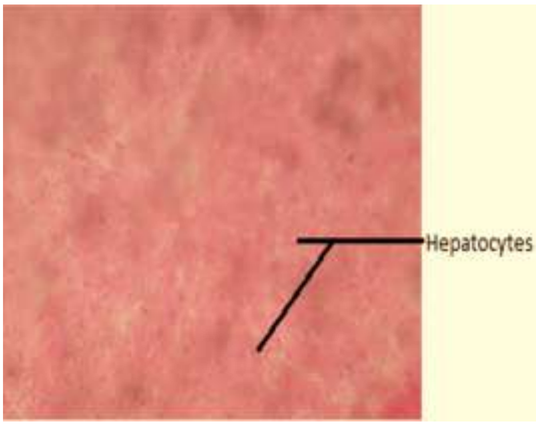

Plate-1.8: Photomicrograph of rat liver treated with low concentration of watermelon showing normal cords of hepatocytes radiating away from central vein. H &E X400

Kidney

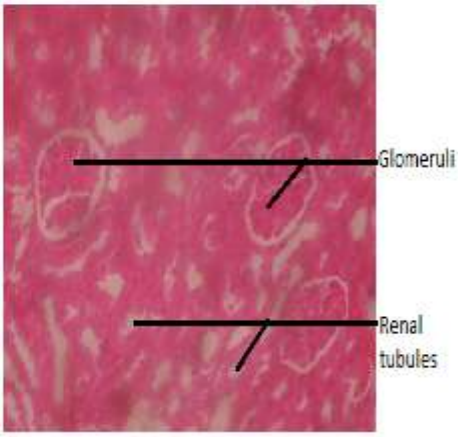

Plate 2.1: Photomicrograph of kidney of normal control rat showing normal renal tubules and glomeruli. H &E X400

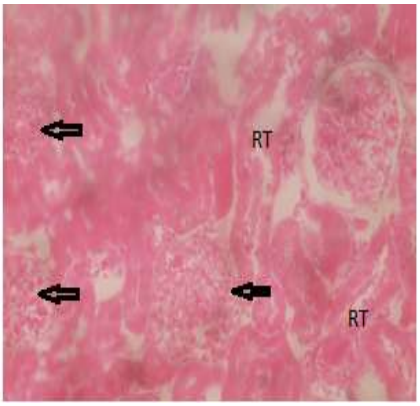

Plate-2.2: Photomicrograph of rat kidney fed lard and untreated showing obliterated capsular spaces in the glomeruli (arrowed). H &E X400

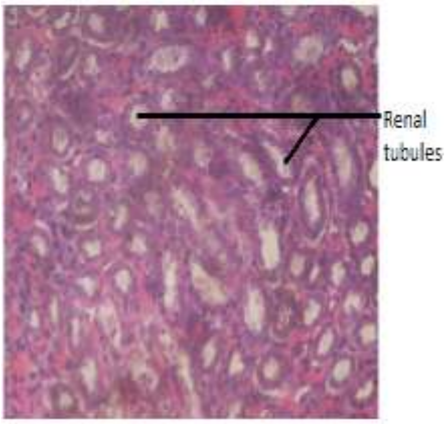

Plate-2.3: Photomicrograph of rat kidney treated with high concentration of cucumber showing normal renal tubules. H &E X400

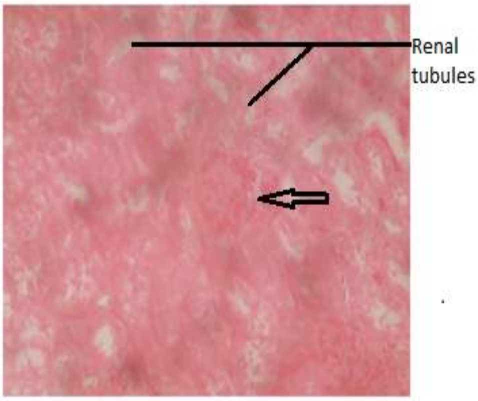

Plate-2.4: Photomicrograph of rat kidney treated with high concentration of watermelon showing obliterated capsular space in a glomerulus. H &E X400

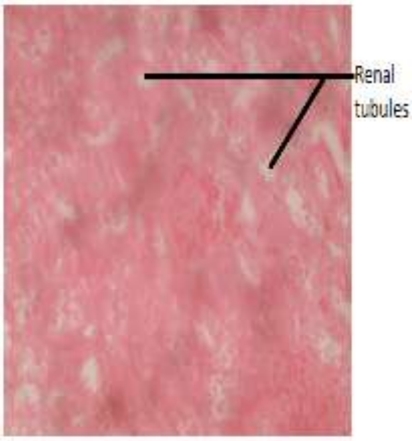

Plate-2.5: Photomicrograph of rat kidney treated with high concentration of cucumber and watermelon showing normal renal tubules. H &E X400

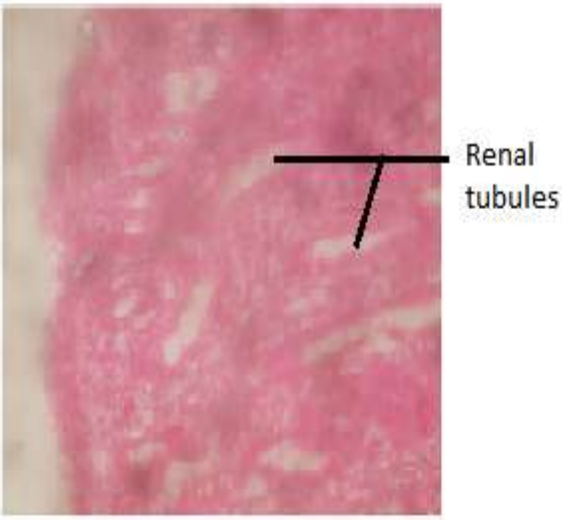

Plate-2.6: Photomicrograph of rat kidney treated with low concentration of cucumber and watermelon showing normal renal tubules. H &E X400

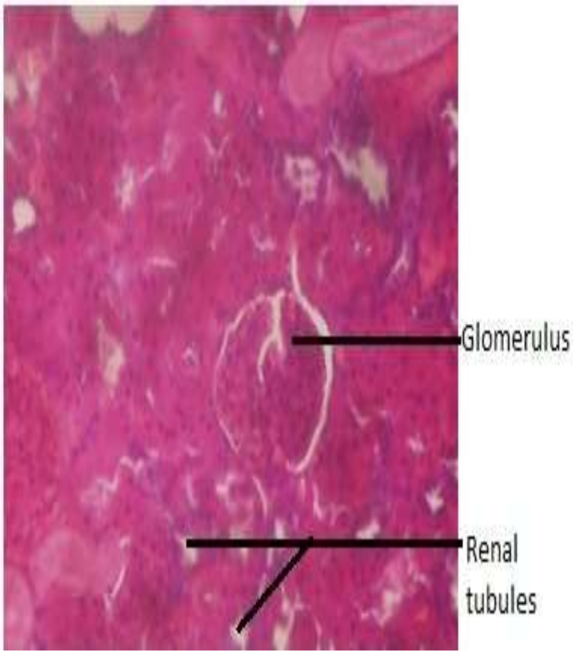

Plate-2.7: Photomicrograph of rat kidney treated with low concentration of cucumber showing normal renal tubules. H & E X400

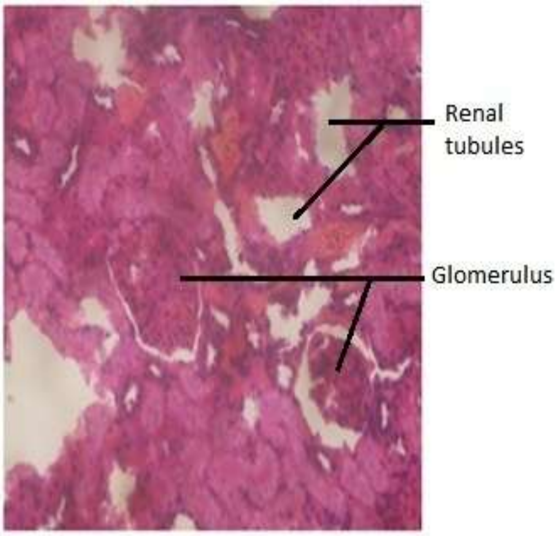

Plate-2.8: Photomicrograph of rat kidney treated with low concentration of watermelon showing normal renal tubules. H & E X400

DICUSSION

Liver disease is linked with leakage of membrane contents of the cytosolic hepatocyte which causes elevation in serum enzymes namely; AST, ALT and ALP as markers [23]. Exposure to cadmium after 4 weeks in treated groups caused an elevation in aspartate transaminase and alanine transaminase levels in the first 2 weeks of pretreatment, After 4 weeks of administration of whole juice of cucumber and watermelon the aspartate transaminase and alanine transaminase values were reduced compared to positive control group which increased both in weeks 2 and 4 as shown in tables 4.1a and 4.1b above. This suggests that continuous administration of the juice helped in ameliorating cadmium effect on the liver. Alkaline phosphatase of positive control group was increased when compared to normal control after 2 weeks and continually increased after 4 weeks.

Alkaline phosphatase concentrations were elevated in treated groups in week 2 compared to normal control. After 4 weeks of administration, alkaline phosphatase of animal groups administered high and low concentrations of whole juice of cucumber and water malon respectively were reduced to the range of normal control. The reduction in liver enzymes in this study may be credited to the antioxidant property [24] of juice of cucumber according to Saidu *et al*, [25] who reported cucumber to be rich in alkaloids, glycosides, saponins, flavonoids, phenols, tannins and terpenes and also Nwankwo *et al*, [26] showed watermelon contains phytochemicals phenol, saponin, tannin, flavonoid and alkaloid and other constituents carotenoids, lycopene and citrulline. These results are in consonance with those obtained by Ibiam *et al*. [27]. The glomeruli of the kidney freely filters metabolic waste products urea and creatinine [28] and their serum levels are used to screen for normal kidney function [29, 30].

Cadmium caused a considerable increase in serum creatinine and urea levels at the positive control. While the whole extract of cucumber and watermelon tried to ameliorate the effect of cadmium in the first 2 weeks of administration in the treated groups, significant decrease was observed in week 4 compared to week 2 of urea level. Also, significant reduction was observed in treated groups of creatinine from values obtained in positive control after 4 weeks of administration of whole juice of cucumber and watermelon. After 4 weeks, creatinine levels increased significantly in positive control of creatinine compared to level of normal control. In treated groups, creatinine level decreased in week 2 when compared to normal control level, and increased to the range of normal control at week 4. This increase at week 4 was significant compared to levels of creatinine after 2 weeks. The result was consistent with those obtained by Liu *et al*, [7].

For electrolytes (sodium, potassium and chloride), there was an observed increase as a result of cadmium toxicity which was mostly seen in positive control group but in treated groups the juice was able to ameliorate the effects of cadmium. This result is in consonance with the study done by Onwuka *et al*.[31].

Microscopic examination using haematoxylene and eosin (H&E) stain shows sections of the liver in control rat group showing normal histologic structure of the renal tubules. The hepatic histology of cadmium chloride treated groups showed inflammatory cell infiltration due to the production of lipid peroxidation and free radicals induced by cadmium chloride, the biochemical observation of increase in liver enzymes in the cadmium treated group coincide with the histoarchitecture of the liver. This result is consistent with those obtained by El-Refaiy and Eissa, [32] where 3mg/kg of cadmium administration caused severe hepatocyte necrosis and inflammatory cell infiltration. Pretreatment groups liver histology showed features close to normal control rat liver which is largely due to the antioxidant and metal chelating effect of cucumber and watermelon which contains phytochemicals like flavonoids and phenols. Plate 1.6 liver histology showed a mild congestion of central vein and normal hepatocytes, this may be due to the response to injury caused by cadmium. This study is consistent with that of Renugadevi and Prabu, [33] where nangerin attenuated the effect of cadmium on liver cells and also the work of Arguelles *et al*, [34], grapefruit protected liver damage caused by cadmium chloride.

Histological examination of cadmium treated kidney showed occlusion of the bowman capsule, abnormal structure of the glomeruli of positive control rat when compared to the kidney proximal tubule of control rat whose renal glomeruli showed normal structure. Intraperitonal injection of cadmium induced kidney alterations evidenced by increase in serum urea and creatinine of cadmium treated groups and pretreatment groups. The nephrotoxic effect of cadmium was alleviated successfully by administration of cucumber and watermelon also seen in improvements of serum kidney markers by stimulating antioxidant system and suppressing oxidative stress. This result is in line with those obtained by Wang *et al*, [35] in which quercetin protected the kidney from cadmium induced damage.

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