

**SJMLS - 9(2) - 037****Protective Effect of *Garcinia Kola* Seed and Honey Mixture Against Cadmium-Induced Nephrotoxicity in Albino Rats**Francis Agwanyeokhai Anyia\*<sup>1</sup>, Davies G. Tamuno-Emine<sup>2</sup>, Kemzi Nosike Elechi-Amadi<sup>2</sup>, Ojoye Ngoye Briggs<sup>2</sup>Department of Medical Laboratory Science, Faculty of Allied Health Science, College of Health Sciences, University of Port Harcourt, Choba-Port Harcourt, Rivers State <sup>1</sup>, Faculty of Medical Laboratory Science, Rivers State University, Port Harcourt <sup>2</sup>.

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<https://dx.doi.org/10.4314/sokjmls.v9i2.37>**Abstract**

In this study, *Garcinia kola* Seed (GKS), Honey and a mixture of both were investigated for protective action against Cadmium acetate-induced nephrotoxicity in wistar rats. The GKS mixed with honey preparation was done by weighing 20g of powered GKS and 80g of honey, this mixture was diluted with distilled water at a weight/volume ratio of 1:4. Different groups of animals were fed GKS, Honey or a mixture of both (50mg/100g body weight and 100mg/100g body weight of GKS) in solution with normal saline or Cadmium acetate in solution with normal saline (induced group), Normal saline only (Control) was given for 14 days and then co-administered orally in this order: *Garcinia kola* seed and Cadmium acetate for 7 days (in different dose regimen of GKS 50mg/100g body weight and 100mg/100g body weight), *Garcinia kola* seed mixed with honey and cotreated with Cadmium acetate for 7 days and finally Honey and cadmium acetate for 7 days. In total, there were six (6) groups. Five rats were used for each group giving a total 30 wistar rats (25 animals for the study groups and 5 animals for the control). The renal function parameters were assessed by the following battery of tests including; Plasma total protein, Albumin, Sodium, Potassium, Bicarbonate, Urea and Creatinine. The histology of the kidney was done for one animal per group using the Haematoxylin and Eosin (H & E) stain, and their micro pictograph reported ( $\times 400$  magnification) was reported. The results obtained were analyzed using the SPSS Sheff and Duncan and presented as Mean  $\pm$  SEM, at a (P = 0.05). The results from the study showed

that the total protein, albumin, Urea, and Bicarbonate levels were significantly lowered and the potassium level was significantly high in the renal function indices. These show that Cadmium is a very toxic heavy metal causing serious renal cell damage evident in renal acidosis. The histological findings in the kidney also show a clear distortion and cellular damage. The GKS, Honey or a mixture of both were found to exhibit a partial protective characteristic against Cadmium acetate-induced kidney function damage as evidenced by the ability of each test material to mitigate the decrease in plasma total protein and albumin, and an increase in sodium and potassium, urea and creatinine. These findings were consistent with the results of the histological studies of the kidney from the GKS, Honey and a mixture of both using in the rats in the various groups.

**Introduction**

The age-long system of herbal medicine is being revived by the day-to-day practice for its long-lasting curative effect, easy availability, natural way of healing and less side effects, so that today herbal medicine is gaining importance and expanding throughout the world (Prete *et al.*, 2012). Mankind is known to eat and drink plant parts either as food or medicines for a long time. Traditional Medicine is still recognized as the preferred primary healthcare system in many communities, with over 60% of the world's population and 80% in developing countries depending directly on Medicinal plants for their medical purposes. This is due to a number of reasons including affordability, accessibility and low cost (Mintah *et al.*, 2019). Traditional

Medicine is known by many different names including Complementary and Alternative Medicine, Botanical Medicine, etc.

*Garcinia kola* Seed also known as bitter kola is a seed from the *Garcinia kola* tree. It grows in sub-Saharan Africa. It is within the family of Clusiaceae. *Garcinia kola* (Heckel), an angiosperm belongs to the family Guttiferae. It has a bitter taste on chewing and has smell like raw coffee. Its usage in traditional medicine has been long documented and reviewed. Its clinical uses include hepatoprotection against Cadmium toxicity [3], Viral infection treatment (Tamuno-Emine *et al.*, 2016) and cleansing agent (Essien and Akintonwa, 1990). These effects as reported are due to the presence of bioflavonoids such as tannins, cardiac glycoside and phenols.

Honey is a semi-solid fluid that is produced from bees. Honey has been used in traditional Medicine both for dietary and curative purposes. Many researchers have investigated the chemical and biological properties of honey; including its antibacterial, bacteriostatic, inflammatory and wound healing activities (Abdel-Moneim and Wafaa, 2007). Honey is a natural antioxidant which contains flavonoids, ascorbic acid, tocopherols, catalase and phenolic compounds. This work is to provide evidence for a synergistic antioxidant effect, hence scavenging and eliminating free radical properties.

Cadmium is a heavy metal that does not have a known physiological function and it is considered a toxicant. Cadmium is an environmental pollutant as a result of the rapid development of industries and modern technologies. Low level exposure to Cadmium may lead to damage to the Kidneys, liver, skeletal system and Cardiovascular system, as well as to the deterioration of sight and hearing. Cadmium is also known to have strong teratogenic and mutagenic effects. Cadmium even at low doses has adverse effects on both human male and female reproductive system and it affects pregnancy or its outcome (Genchi *et al.*, 2020). Cadmium is responsible for the generation of reactive oxygen species, glutathione depletion, lipid peroxidation, protein

cross-linking, DNA damage, culminating ultimately in oxidant-induced cell death (Arruebarrena *et al.*, 2023). The average level of cadmium in Warri River waters between 1986 and 1991 was 0.3mg/L (Egbore, 1994). This is 60-fold above the maximum allowable level in drinking water. Naturally this is worrisome (Asagba and Obi, 2004).

The kidneys are an important organ in the body with each in the right and left side of the body (Crook, 2006). The functional unit of the kidneys are the nephrons. The main functions of the kidneys include ultrafiltration, selective reabsorption, permeability, release of hormones and water/PH balance. It is well established that the kidneys are adversely affected by Cadmium toxicity and it is the main site for cadmium toxicity (Orisakwe, 2016). Since the herbal remedies considered in this study are rich in antioxidants, it is believed that by the administration of the remedies either orally or by ingestion will ameliorate the effects of cadmium on the body or organ (Abdel Baset *et al.*, 2012). This work is to provide evidence for a synergistic antioxidant effect, hence scavenging and eliminating free radicals' properties which is the main cause of sicknesses.

## Materials and Methods

### (a) Laboratory animals

A total of 30 albino rats aged 2-4 months and weighing 150-200g, were used for this study. The rats comprised of male and female rats, and were purchased from a local farm in Choba, Port Harcourt, Rivers State. The animals were kept in the Animal house, Department of Human physiology, University of Port Harcourt. The animals were kept in a spacious and well-ventilated cage at room temperature and allowed to acclimatize for 21 days. The acclimatization period was allowed before initiation of the experiment. The animals were fed with commercial rat feed and given clean water *ad libitum*. All the animals received humane care according to the criteria outlined in the guide for the care and use of laboratory animals prepared by the National Academy of Science and published by the National Institute of Health.

- (b) **Honey:** The Honey used was a pure and unadulterated commercial product (Havillah Natural Forest honey, NAFDAC No 007654). It was purchased from a shop in Port Harcourt, Rivers State. A 1:4 dilution of the honey was used for this study; that is 25g honey dissolved in 100ml normal saline. The honey used was of nutritional grade.
- (c) **Plant Material:** The seed of plant used for this study was that of *Garcinia kola* Seed. It was purchased from a local trader in the Choba Market, Port Harcourt. The brown testa of the seeds was carefully removed, after which each seed was cut into small pieces, allowed to air dry and then grinded into smooth and fine powder. It was grinded using the QASA electric blender (QASA SA 230). For the solvent preparation, 25g of the pulverized seed was mixed with 100ml of normal saline. The seeds were properly identified and authenticated in the Department of Plant Science and Biotechnology, University of Port Harcourt by Dr. Wosu who is a Plant Scientist.
- (d) **Chemicals:** Cadmium acetate was obtained from the Postgraduate Research Laboratory, Department of Biochemistry, University of Port Harcourt. All chemicals and reagents used were of analytical grade. The Cadmium acetate preparation used was made thus: 50g of the salt was measured and allowed to air dry. Then 20g of the salt was weighed and dissolved in 100ml normal saline. The preparation was allowed to dissolve fully before use.
- (e) **Study Design:** The Nephroprotective study is reported thus: At the end of the acclimatization period, the animals were divided into six (6) groups of five (5) rats each, as follows:
- Group I (Normal control): The rats received orally 0.5ml/100g body weight of normal saline once daily.
  - Group II (Positive control): Rats were fed orally with 200mg/kg Cadmium acetate once daily for two (2) weeks, that is 14 days.
  - Group III: Rats received orally 50mg/100g body weight of *Garcinia kola* seed at a constant volume of 0.2ml/100g body weight for 14 days and then cotreated with Cadmium acetate 200mg/kg body weight from the 14th to 21st day.
  - Group IV: Rats received orally 100mg/100ml body weight of *Garcinia kola* seed at a constant volume of 0.4ml/100g body weight for 14 days and then treated with cadmium acetate 200mg/kg body weight for the 14th to 21st day.
  - Group V: Rats received orally 50mg/100g body weight of *Garcinia kola* seed mixed with honey at a constant volume of 0.2ml/100g body weight for 14 days and then cotreated with Cadmium acetate 200mg/kg body weight for 14th to 21st day.
  - Group VI; Rats in this group received orally honey (50mg/100ml body weight) at a constant volume of 0.2ml/100g body weight for 14 days and then cotreated with cadmium acetate (200mg/kg body weight) for 14th to 21st day.
- The doses of the cadmium acetate and the extracts used in this study were obtained from the pilot study conducted for the purpose of this study (not reported).
- At the end of the experimental period, the animals were fasted for 6 hours and sacrificed under chloroform anesthesia. After each sacrifice, blood was collected by cardiac puncture.
- Sample Collection:** Seven (7 mls) of blood sample was collected from each animal by cardiac puncture, and put into blue-capped lithium heparin bottle. All the blood samples collected were centrifuged at 3,000 rpm for 5 minutes using the automated centrifuge Model AU 380. The plasma obtained was put a clean plain bottle used for the study.
- Laboratory Analyses:** The renal function test profile include: Electrolytes (Sodium, Potassium and Bicarbonates), Urea, Creatinine, Total protein and Albumin.
- Plasma Sodium/Potassium analysis was done using the Ion selective autoanalyzer. Plasma Bicarbonate analysis was done using the back titration method. Plasma Urea was estimated

using the Diacetyl monoxime method (DAM). Plasma Creatinine was estimated using the Jaffe-slot colorimetric method. Plasma Total Protein was estimated using the biuret method. Plasma Albumin was estimated using the dye method “Bromcresol Green”.

**Histology:** Three (3) rats from each group were picked at random for the histology study. The kidneys of each rat picked were carefully collected via the abdomino-thoracic dissection into a plain bottle containing formalin. The harvested kidney was then sent to the Histology Laboratory, Department of Anatomy, University of Port Harcourt. The kidneys were properly fixed, processed, sectioned and stained using the haematoxylin and eosin stains. The photomicrographs were prepared and interpreted accordingly.

**Statistical analysis:** the data from this study were analyzed using Statistical Package for Social Sciences (SPSS), with ANOVA being the tool used to compare the means of the different groups. The results were presented as Mean ± SEM, and p = 0.05 was used to determine statistical significance.

**Results**

The effect of *Garcinia kola* Seed, Honey and a mixture of both on Plasma Sodium, Potassium, Bicarbonate, Urea, Creatinine, Total Protein and Albumin on rats fed Cadmium acetate are shown in tables 1-3. These parameters are known as renal function makers. Tables 1, 2 and 3 are presented below:

**Table 1: Effect of GKS On Renal Function Indices of Rats Fed Cadmium Acetate.**

Indices/Groups	Normal Control	Cd only	GKS 1 + Cd	GKS 2 + Cd
Sodium	142.8 ± 0.73	141.6 ± 0.51	148.8 ± 2.13*	147.60 ± 2.01*
Potassium	10.04 ± 0.67	12.2 ± 0.58 *	14.26 ± 2.02 *	14.36 ± 2.02*
Bicarbonate	23.40 ± 0.40	12.2 ± 0.63 *	12.0 ± 0.45*	14.2 ± 0.86*
Urea	6.58 ± 0.15	5.26 ± 0.14 *	5.22 ± 0.51	5.38 ± 0.62*
Creatinine	67.00 ± 2.00	71.0 ± 4.3	69.0 ± 4.85	61.0 ± 3.67*
Total Protein	70.8 ± 0.58	73.4 ± 4.4	57.2 ± 1.20 *	56.60 ± 0.40 *
Albumin	35.2 ± 0.37	35.8 ± 2.22	28.80 ± 0.49 *	27.0 ± 0.63 *

Note:

Where all values are expressed as Mean ± SEM for rats across the groups.

the superscript (\*) represents statistically significant across the groups.

GKS 1 represents rats fed GKS at 50mg/100g body weight.

GKS2 represents rats fed GKS at 100mg/100g body weight

Cd represents Cadmium acetate.

**Table 2: Effect of Honey on some renal function parameters on rats fed Cadmium acetate.**

Indices/ Groups	Normal Control	Cadmium only	Honey plus Cadmium
Sodium	142.8 ± 0.73	141.6 ± 0.51	148.0 ± 1.10*
Potassium	10.04 ± 0.67	12.2 ± 0.58*	12.02 ± 0.96
Bicarbonate	23.40 ± 0.40	12.0 ± 0.63*	13.60 ± 0.40*
Urea	6.58 ± 0.15	5.26 ± 0.14*	5.40 ± 0.41*
Creatinine	67.00 ± 2.00	71.0 ± 4.3	51.0 ± 4.3*
Total Protein	70.8 ± 0.58	73.4 + 4.4	54.2 ± 1.66*
Albumin	35.2 ± 0.37	35.8 ± 2.22	30.2 ± 0.97*

All values are expressed as Mean ± SEM for rats across the groups.

Where the superscript (\*) represents statistically significant across the groups.

Cd represents Cadmium acetate.



**Table 3.0: Effect of Garcinia kola seed mixed with honey on some renal function indices of rats fed Cadmium.**

Indices/ Groups	Normal Control	Cadmium only	GKS mixed with Honey
Sodium	142.8 ± 0.73	141.6 ± 0.51	144.6 ± 1.66*
Potassium	10.04 ± 0.67	12.2 ± 0.58	13.6 ± 1.61*
Bicarbonate	23.4 ± 0.4	12.0 ± 0.63*	13.6 ± 0.4*
Urea	6.58 ± 0.15	5.26 ± 0.14	4.96 ± 0.70*
Creatinine	67.0 ± 2.0	71.0 ± 4.30	60.0 ± 6.67
Total Protein	70.0 ± 0.58	73.4 ± 4.4	57.4 ± 0.97*
Albumin	35.2 ± 0.37	35.8 ± 2.22	26.8 ± 1.37

Note:

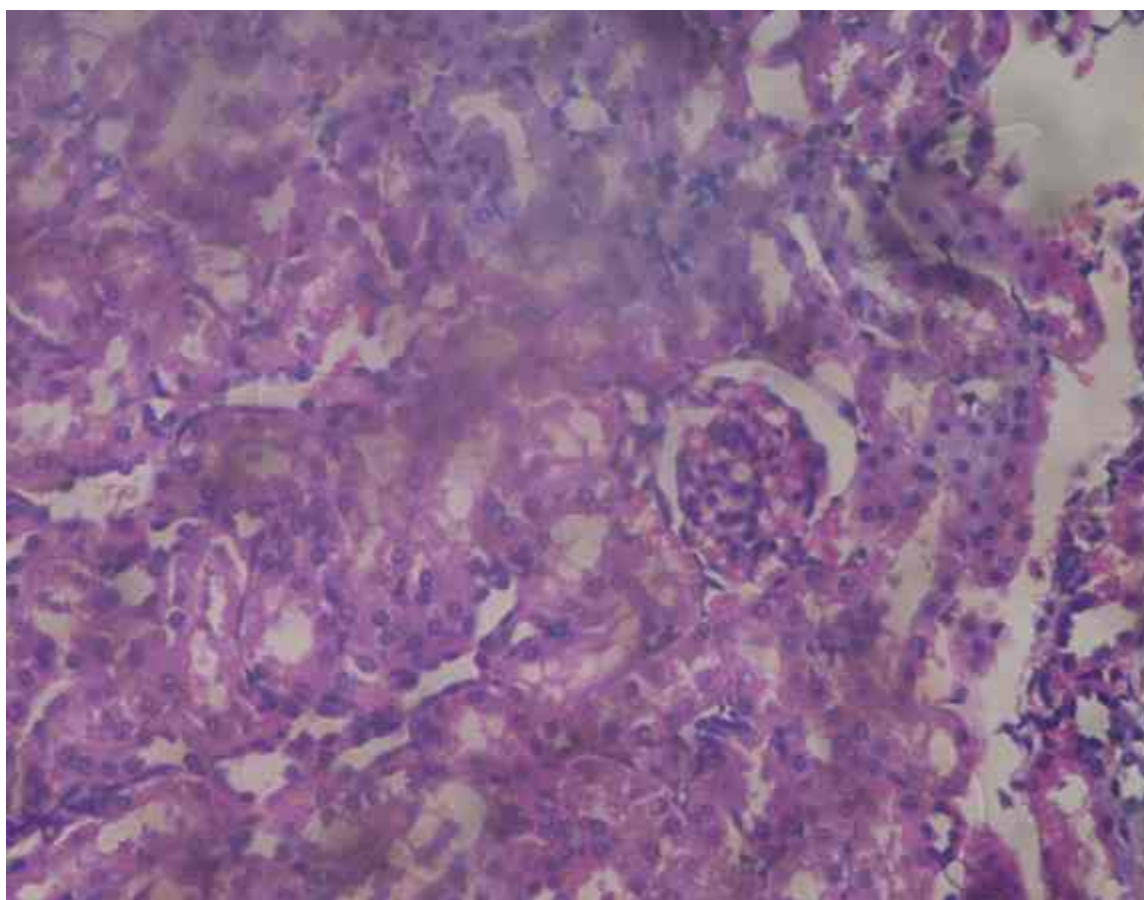
All values are expressed as Mean ± SEM across the groups.

where the superscript (\*) means statistically significant across the groups.

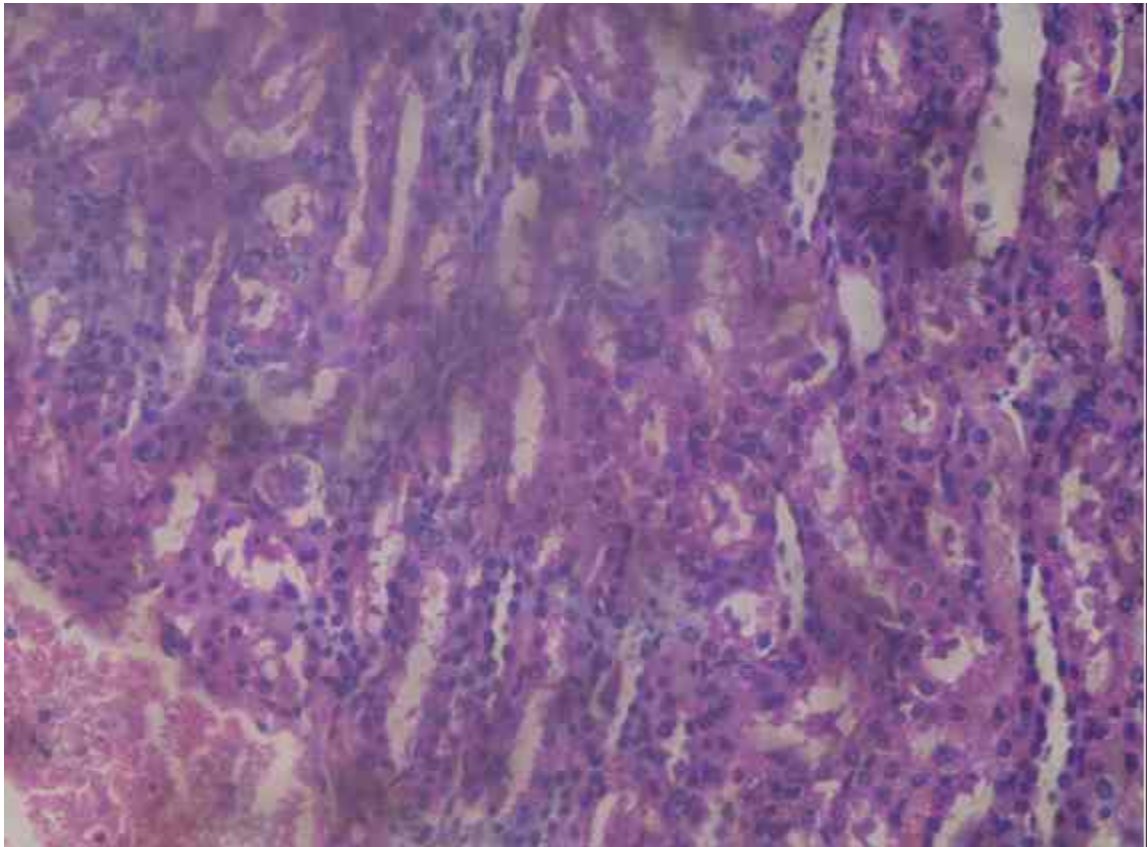
Cd represents Cadmium acetate.

**Histological Presentation:**

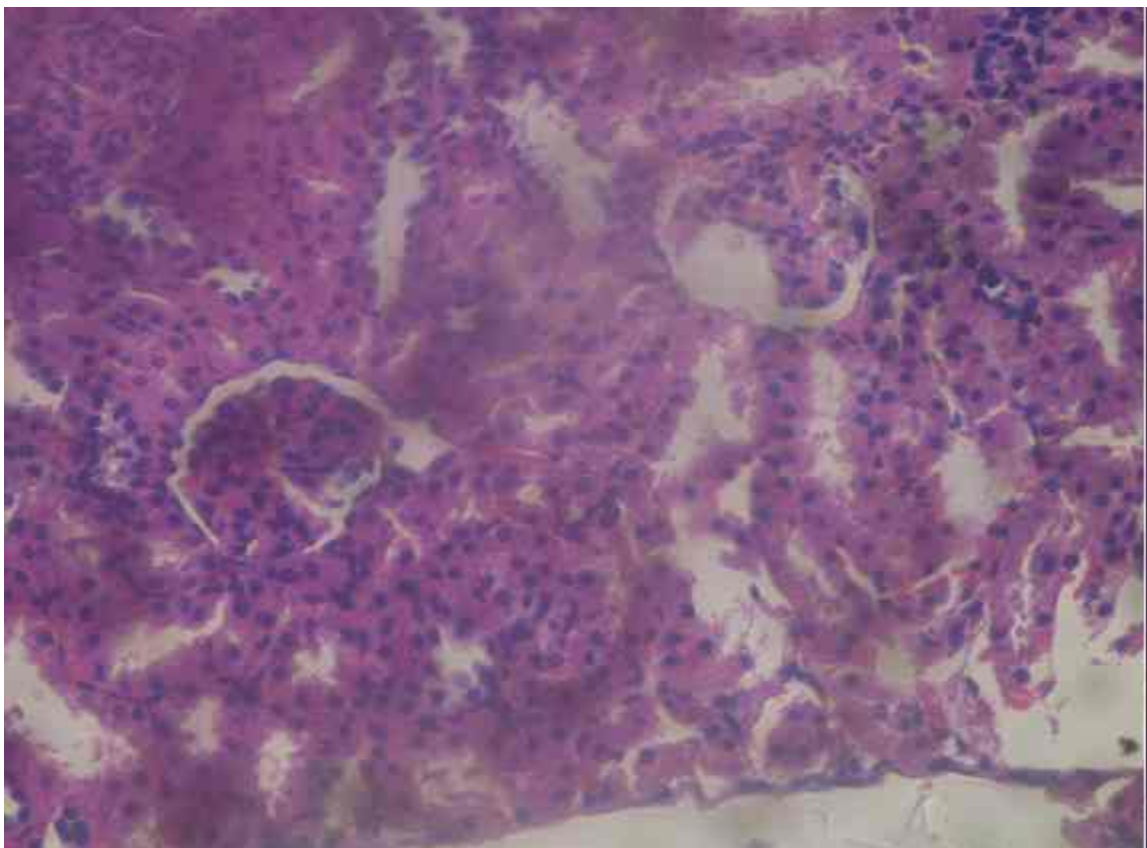
The histological presentation of the kidney cells is shown below. The kidney cells were stained with hematoxylin and eosin; and their micropictograph reported at ×400 magnification.



**Plate 1: Kidney section (Group I, Normal Control).**

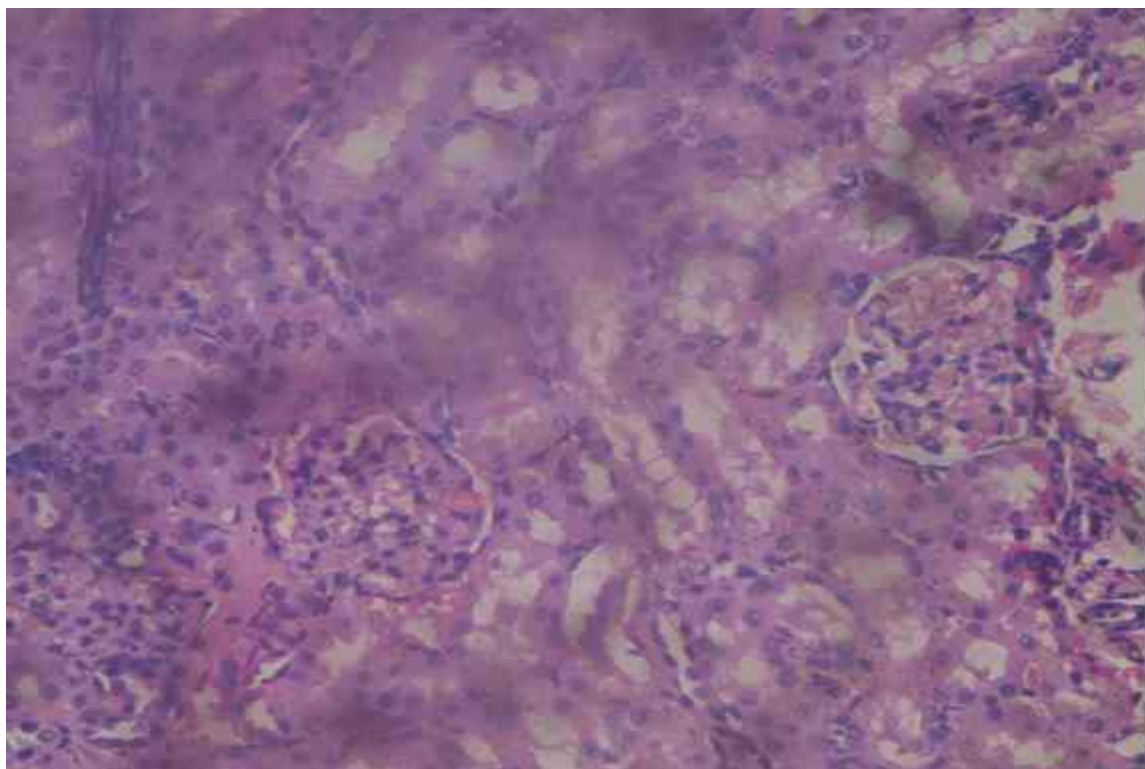


**Plate II: Kidney Section: Group II, Cadmium acetate treated only**

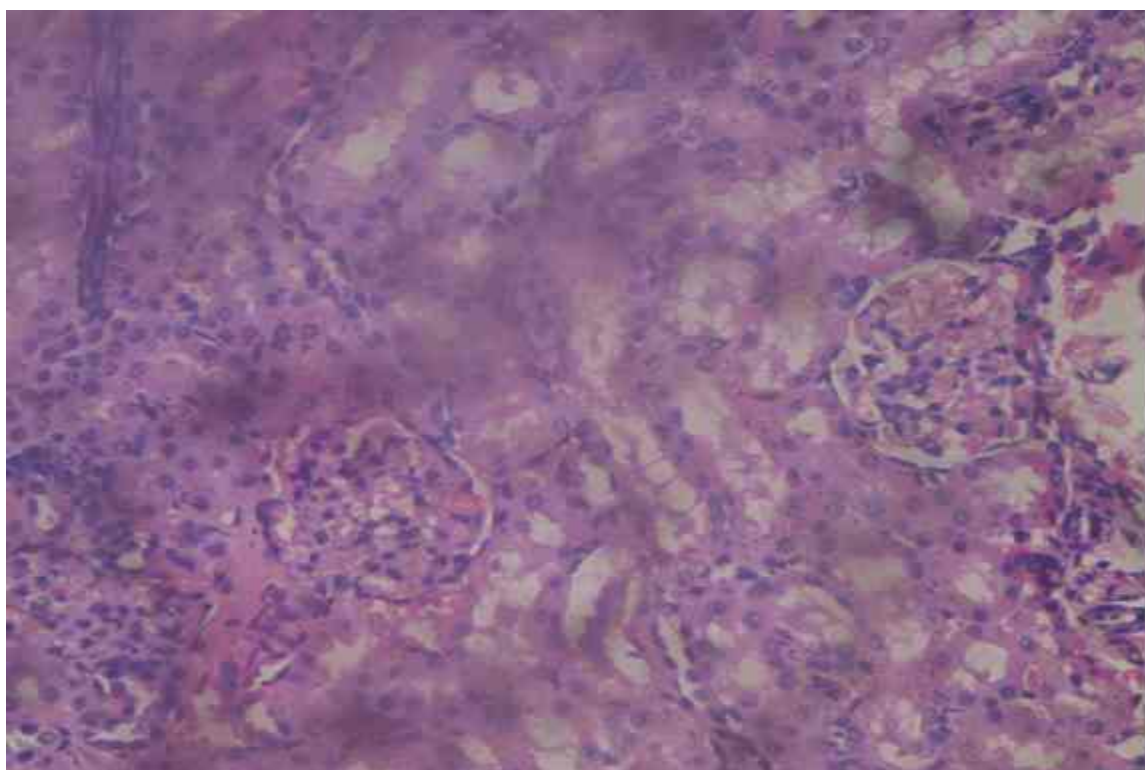


**Plate III: Kidney section, Group III for GKS at 50mg/100g body weight cotreated with Cadmium acetate.**

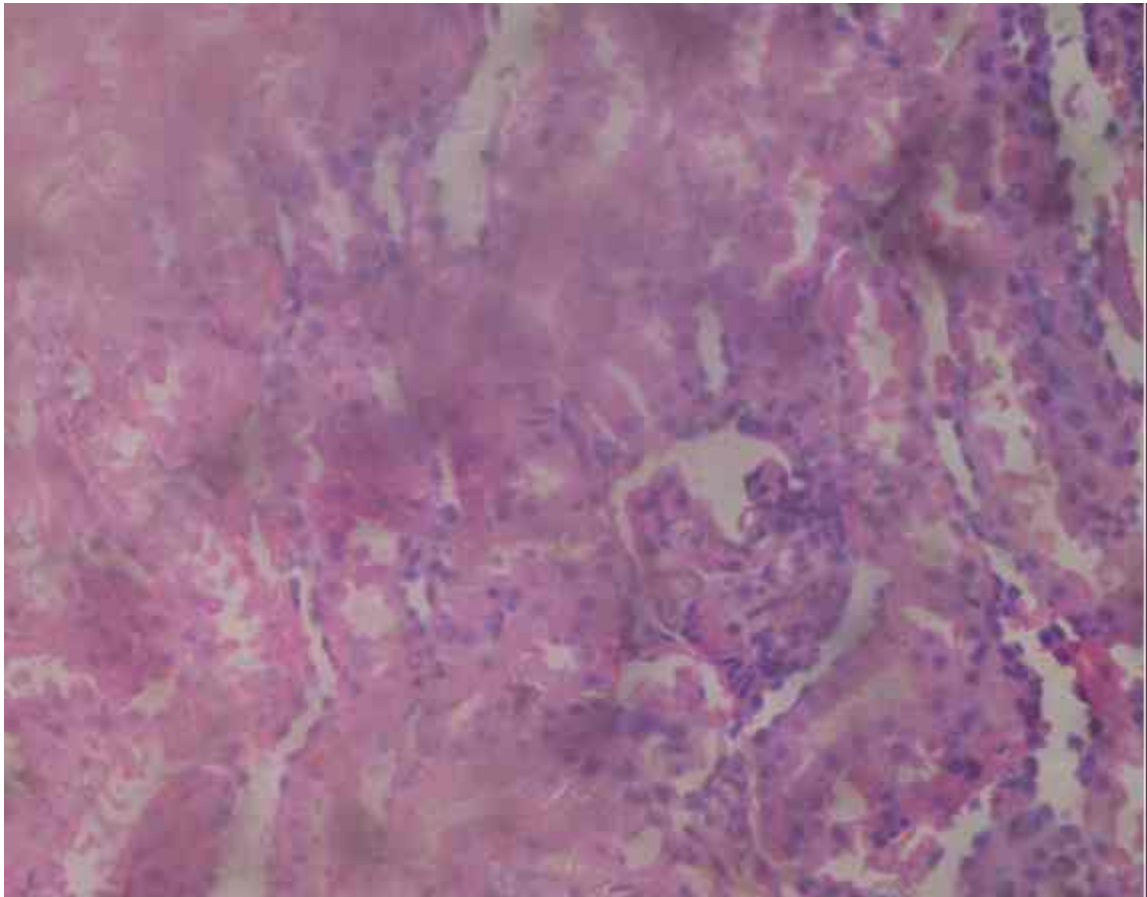




**Plate IV: Kidney section, Group IV for GKS at 100mg/100g body weight and cotreated with Cadmium acetate.**



**Plate V: Kidney section, Group V. *Garcinia kola* seed mixed with honey and cotreated with Cadmium acetate.**



**Plate VI: Kidney section (Group VI). Honey cotreated with cadmium.**

**Plate I:** The rats in this group received normal saline. The renal Corpuscle showed a well delineated Cellular Matrix. There was clear evidence of a well-organized supportive tissue. There is also observed a Clear distinction of Cellular boundaries. The Renal corpuscle are intact.

**Plate II:** Rats were fed with Cadmium acetate orally. The renal Corpuscle expressed a high degeneration in the Cellular Matrix. There was also an encroached interlobular duct with nuclear materials and Cellular debris. There was evidence of infiltration and inflammation in the Cells. Cadmium expresses as a Kidney poison.

**Plate III:** The rats receive orally Garcinia kola Seed at a low dose of 50mg/100g body weight and Cadmium at a dose of 100mg/100g body weight. The renal corpuscles showed a distorted Cellular Matrix but it is more or less similar to the Control. There was evidence of a well-filled fluid interlobular lumen as a result of inflammatory response. There is also an encroached capillary with Cellular debris. The renal corpuscle is intact.

**Plate IV:** The rats received Garcinia kola Seed and Cadmium. The Garcinia kola Seed was at a dose concentration of 100mg/100g body weight and the Cadmium was at a dose concentration of 100mg/100g body weight. The renal corpuscle expressed an encroached interlobular duct and these ducts were fluid-filled. This is evidence of inflammation. The renal cell was intact and didn't express any cellular necrosis. Garcinia kola Seed exhibited Reno protection against Cadmium poisoning. The renal Corpuscle are stable.

**Plate V:** The rats were fed with a mixture of Garcinia kola Seed with Honey and Cadmium. The renal cells showed a well delineated lobular septum. The renal corpuscle was intact and free from any fluid or debris. There was no evidence of degeneration or cell death. The cell integrity was maintained. This mixture exhibited Reno protective effect against Cadmium poisoning.

**Plate VI:** The rats were fed with Honey and Cadmium. The renal Corpuscle showed a clear Central lumen. The renal cells were intact and



there was no clear evidence of inflammation. There was evidence of encroached glandular acinus with debris. There was no cellular necrosis or cell death. The cellular content maintained their integrity and cellular structure. Honey expresses a Reno protective effect against Cadmium Toxicity.

### Discussion

Cadmium is a known environmental toxicant whose mechanism of damage include the production of free radicals that alter mitochondrial activity and genetic information (Deburbure and Bernard, 2006). In this study, animals fed with Cadmium only exhibited a significant reduction in plasma bicarbonate and urea levels and a significant rise in the potassium levels. This shows that Cadmium portrays renal acidosis which is characteristic with a reduction in bicarbonate and a rise in potassium. This confirms that Cadmium causes renal damage and that it has Nephrotoxic effect (Orisakwe *et al.*, 2017). Cadmium is known not to have any benefit in the mammalian system. It accumulates in plants and animals with a long half-life of 25-30 years. Cadmium expresses its toxicity by lipid peroxidation, DNA damage, Protein damage etc. Cadmium is also known to cause Oxidative damage by reducing the total antioxidant capacity leading to the damage to the organs of the human system. Cadmium is known as a grade I toxicant in the environment (Patrick, 2007). It binds irreversibly to the cells and tissues, thereby causing irreversible damage to the tissues and organs (Jarup, 2003). Cadmium has been reported to cause adverse damage to the Liver and Kidney (Asagba and Obi, 2004).

The present study focuses on the possible protective effect of *Garcinia kola* Seed, Honey and their mixture on oxidative damage generated by Cadmium induced Nephrotoxicity. Kidney function tests were done for the different studied groups to assess their status. It is known that GKS and Honey are rich in antioxidants. This is why they were chosen for this study. Both biochemical and histological changes induced by Cadmium with or without the chosen herbal remedies were investigated using the appropriate techniques. The Histological changes of kidney were examined by a microscope  $\times 400$  magnification.

In this work, the renal function indices studied include; Sodium, Potassium, Bicarbonate, Urea, Creatinine, Total Protein and Albumin in the group fed GKS 1 & II with Cadmium, Honey and Cadmium and then GKS+ Honey treated with Cadmium.

For the group fed *Garcinia kola* Seed at 50mg/100g body weight and cotreated with Cadmium acetate, a significant increase in Plasma Sodium and Potassium occurred while chosen herbal remedies were investigated using the appropriate techniques, there was a significant reduction in plasma bicarbonate, total protein and albumin as compared to the normal control. For the group fed *Garcinia kola* Seed at 100mg/100g body weight and cotreated with Cadmium acetate group, there was a significant increase recorded in plasma sodium and potassium while a significant decrease was observed in bicarbonate, urea, creatinine, total protein and albumin as compared to the normal control. The histological studies of the kidney shows that the *Garcinia kola* seed at 50mg/100g body weight and 100mg/100g body weight yielded a protective effect against Cadmium acetate. This confirms that *Garcinia kola* Seed has a nephroprotective effect against Cadmium acetate. However, for the dosage regimen of *Garcinia kola* Seed at 50mg/100g body weight and 100mg/100g body weight and cotreated with Cadmium, the pattern observed shows that at a higher dose there was slight significant change across the renal indices. So, at a higher dose, *Garcinia kola* seed renders a better renoprotection against Cadmium acetate toxicity. The protective effect of *Garcinia kola* seed against toxicity in other heavy metals and drug agents have been reported earlier. This shows that *Garcinia kola* Seed could be a powerful herbal agent which is rich in antioxidants. The ability of *Garcinia kola* Seed to render this effect is due to the fact that it is rich in bioflavonoids and phenolic compounds (Agada and Braide, 2009; Eminatedoki *et al.*, 2010; Tamuno-Emine *et al.*, 2010; Adesuyi *et al.*, 2012).

For the group fed Honey and cotreated with Cadmium acetate, there was a significant increase ( $p < 0.05$ ) in Plasma Sodium, and a significant decrease ( $p < 0.05$ ) in Plasma Bicarbonate, Urea, Creatinine, Total Protein and Albumin as compared to the Control. The Histological slides of the kidney for the group

fed honey and cotreated with Cadmium acetate show a well-delineated cell lining. This shows that honey exhibited a protective effect against Cadmium acetate. This is as reported by many researchers (Abdel-Moneim and Wafaa, 2007; Genchi *et al.*, 2020). They all reported that Honey is a powerful antioxidant that is rich in Vitamin C, A, E, Zinc, Phenolic compounds and fructose, etc.

Herbal Combo which is a mixture of two or more herbal plants or oilment is commonly used in Africa, Asia and in many parts of the world. In this present study, a mixture of *Garcinia kola* seed and honey was done and cotreated with cadmium acetate. For this group, there was a significant increase in Plasma Sodium and Potassium and a significant decrease in Plasma Urea, Total Protein and Albumin as compared to the Normal control. This shows that the *Garcinia kola* seed and Honey mixture renders a nephroprotective effect against Cadmium acetate toxicity. This effect is possible because *Garcinia kola* and honey are both rich in antioxidants, nutrients and trace elements (Ibrahim *et.al.*, 2014; Emine-Tamuno and Anyia, 2016).

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