Effects of aqueous extracts of *Costus afer* and Vit. C supplement on renal damage in male mice subjected to sub-chronic exposure of Dichlorvos

ABSTRACT

Aim: To evaluate effects of aqueous extracts of *Costus afer* and Vit. C supplement on renal damage in male mice subjected to sub-chronic exposure of Dichlorvos

Study Design: The study was a completely randomized design employing relevant statistical tools for analysis and interpretation.

Place and Duration of Study: The study was carried out in the Department of Animal and Environmental Biology, Rivers State University. The experiment lasted for 35 days between April and June 2023.

Methodology: A total of 30 male mice were randomly selected into 6 groups (n=5). Group A was the control, B received Dichlorvos@25mg/kg/bw/day, C & D received 100% vitamin C and *Costus afer* at 250mg/kg/bw/day only. Groups E & F were coadministered Dichlorvos with Vitamin C and 100% extract of *Costusafer*at 250mg/kg/bw/day respectively. At the end of the treatment period, blood samples were collected for analysis. Total protein was analyzed using the spectrophotometric method of biuret, Bradford and erythrosine – b, albumin was estimated, creatinine and urea was done using enzymatic method. Histological sections of the kidney were mounted on slides, stained with hematoxylin and eosin (H&E). Photomicrographs were generated.

Results: All kidney biomarkers examined increased significantly (p=0.05) in group B administered Dichlorvos only but decreased in groups administered the vit C and *Costusafer* respectively. Histopathological analysis of the kidney shows the regular structure of the kidney architecture in group A. Glomeruli shrinkage, necrosis and degeneration of nephrons were observed in group B, while less pronounced changes were observed in groups C-F co-administered with Vit C and *Costus afer.* However, group E shows fully regenerated nephrons while few depleted nephronic cells were still observed in group F despite coadministration of *Costus afer*.

Conclusion: Based on the results, Vit C supplement shows more potent, therapeutic properties against kidney damage in male mice subjected to sub-chronic exposure of Dichlorvos compared to*Costusafer*.

Keywords: *antioxidant, Costus afer, Dichlorvos, Nephrons, Sub-chronic*

1. INTRODUCTION

The use of plant and plant parts as sources for the treatment of different ailments have been a long tradition. Many indigenous plants have become the base for the development of medicine- a natural blueprint for the development of new drugs [1,2]. Majority of medicinal plants, maybe herbaceous, trees, weeds, shrubs and fruits possess phytochemical constituents, which produce definite and diverse physiological ,as well as, pharmacological responses in living systems. Over 25% of prescribed medications in industrialized countries

are directly or indirectly derived from plants, despite the notable advancements in synthetic organic medicinal compounds over the twentieth century[3]. New medications are frequently out of reach of the poor in emerging nations, particularly in West Africa. Thus, according to [4] up to 80% of the population employs medicinal herbs as a form of treatment.

According to [5,6,7] medicinal plants are natural resources that are typically accepted and are thought to have fewer adverse effects. *Costus afer* is also known as a bush cane or ginger lily. In tropical Africa, it is frequently used as a medicine to treat inflammation, arthritis, rheumatism, hepatic disorders, hemorrhoids, cough, epileptic attacks, miscarriages, helminthic, diuretics, and laxatives as an antidote for poison [8, 9,10]. The rhizome *Costus afer* locally known as Mbritem in Ibibio is a delicacy for children as it grows wild by the roadside. Children simply cut the stem, debark it and chew to quench thirst. *Costus afer* contains alkaloids, saponins, flavonoids, anthraquinones, cardiac glycosides, terpenoids, phenolic compounds and tannins which act as antioxidants. Vitamin C, is a natural water-soluble antioxidant which react rapidly with O_2 to play an antioxidant role in cytosol and extracellular matrix. It has been reported that vitamin C also scavenges peroxynitrite very effectively to prevent the formations of nitrotyrosine, nitrostryphophan and nitrated lipids [11] vit C protects kidney function and renal arterial reactivity against ischemia/reperfusion injury(IRI). [12]

Pesticides are designed in order to boost crop yields by protecting crops from undesirable species of weeds, insects and fungi, prevent vectors and vector-borne diseases in the environments. [13]. Although the benefits of their applications have been acknowledged, their adverse health effects are worldwide phenomenon that annually result in unintentional poisoning among exposed and non target organisms. Dichlorvos (DDVP), an organophosphates pesticides have been in use for over five decades. Due to its quick breakdown and evaporation, the presence of DDVP in the environment as a result of unintentional spills or direct application to soil or water is associated with long-term effects. Consequently, it has a complicated impact on organisms.[14] reported that people die every year because Dichlorvos pesticide is been used for self-poisoning, an important clinical problem in the developing world. when the body is exposed to pesticides, it becomes imperative for organs such as the kidney to detoxify such substances. In a disease state of the kidney, its detoxifying capacity is impaired. However, the search for natural remedy against the destructive effects of pesticides gives the shove for this study. Thus, the study investigated the effect of aqueous leaf extract of *Costus afer and* Vit C on Dichlorvos-induced nephrotoxicty.

2. MATERIALS AND METHODS

2.1 Experimental location

The study was carried out in the animal house, Department of Animal and Environmental Biology of Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria. GPS 4º47'50"N6º58'49"E.

2.2 Animal Care and Management

Thirty adult male Swiss mice (22.37±3.24g) were purchased from the animal house of Animal and Environmental Biology in Rivers State University, Nkpolu-Oroworukwo Port Harcourt, Rivers State, Nigeria. The mice were housed in wire mesh cages under standard conditions (12hrs:12hd) and allowed to acclimatize for 14 days before the commencement of the experiment. The mice were fed with standard pellet and clean cool water *ad libitum*. All experiments were conducted according to the institutional protocols of animal care at Rivers State University, Port Harcourt, and the standard procedure for ethical treatment of Laboratory animals.

2.3 Experimental Design

A total of thirty male mice were separated into 6 groups (A-F) of 5 mice each. Group A served as the control and received water and standard pellet only. Group B received the 25mg/kg/bw/day of Dichlorvos only. Group C received the 100% of Vitamin C at 250mg/kg/bw/day. Group D received the 100% aqueous extract of *Costusafer*at 250mg/kg/bw/day. Group E received 100% of Vitamin C at 250mg/kg/bw/day and 25mg/kg/bw/day of Dichlorvos. Group F received 100% aqueous extract of *Costusafer*at 250mg/kg/bw/day, standard pellets, water and 25mg/kg/bw/day of Dichlorvos.

2.3.1. Biochemical Analysis of Kidney Biomarkers

Blood samples were collected individually by cardiac puncture into sterile tubes and the serum separated at 2500g for 10min and stored for further analysis. Total protein was analyzed using the spectrophotometric method of biuret, Bradford and erythrosine – b, creatinine and urea was done using enzymatic method [15,16], Total bilurubin (TB) [17], Creatinine were determined as reported in [18]. Albumin (ALB) concentration was assayed according to Sigma Diagnostics based on the procedure of [19].

2.3.2.Histopathological Evaluation of the Kidney

Known weight of the kidney was fixed in Bouin's fixative and processed according to the protocol described by [20,21] sectioned with a Digital Microtome Model A O Spencer No. 820 at 5um thick and stained with Hematoxylin and Eosin (H &E) Photomicrographs were generated with a digital Microscope Biosphere Miller B with an image processor DN2 – Microscopy Image processing Software [20] at X400 magnification.

3. RESULTS AND DISCUSSION

The level of biomarkers for nephrotoxicity in Swiss mice exposed to *Costusafer*, Vit C supplement and DDVP is displayed in Table 1. Group A(control) had a value of 74.5±2.43g/dL for total protein. This value significantly ($p<0.05$) decreased to 52.1±1.69g/dL in group B administered DDVP only. All other groups (C-F) had 13.1%, 11.6%, 11.9%, and 11.03% increase respectively when compared with group B. The level of Albumin significantly ($p<0.05$) decreased to 30.2±2.21g/dL when compared with other groups including the control group. Also recorded is the level of total bilirubin which increased significantly ($p<0.05$) with over 50% of the value recorded in group C administered vitamin C supplement only but had 34.2% increase when compared with group A.

The level of creatinine and urea significantly ($p<0.05$) increased in group B administered DDVP only compared with other groups. The concentration of all biomarkers recorded significantly ($p<0.05$) decreased in group C administered Vit. C only, compared to group D administered *Costusafer* only. Moreover, there was a significant increase in the level of all biomarkers recorded in group F coadministered DDVP and *Costusafer* compared to group E coadministered DDVP and Vit. C, although values were still lower than that recorded in group B animals.

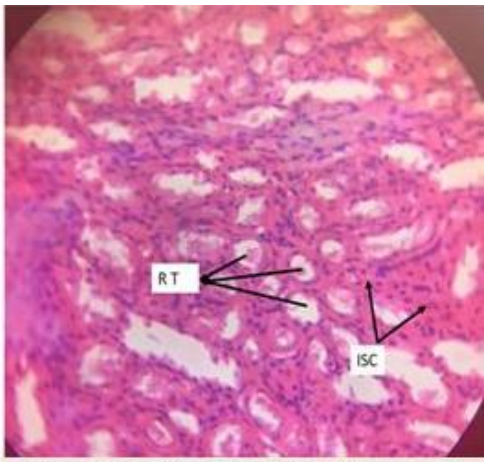
Table 1: Kidney damage biomarkers in mice exposed to aqueous extracts of *Costusafer, Vit C and DDVP*

Groups	Total protein(g/dL)	Albumin(g/dL)	Total Bilirubin (µmol/L)	Creatinine(mg/dL)	Urea (mg/dL)
A	74.5± 2.43a	48.5±1.38b	7.3±2.13b	88.5±4.01ab	3.9±0.46c
B	52.1±1.69d	30.2±2.21d	11.1±2.02a	108.7±3.21a	6.46±1.33a
C	68.2±2.31b	50.8±2.11a	5.85±1.83c	68.5±3.01d	4.21±2.21ab
D	60.2±2.17ab	43.01±2.33ab	6.65±2.06ab	73.8±2.93c	4.62±1.32ab
E	62.01±1.52ab	47.26±1.93b	4.06±2.16d	88.4±3.61ab	4.75±2.10ab
F	57.5±2.35c	36.24±2.18c	5.18±1.79c	93.7±4.82b	5.41±2.22b

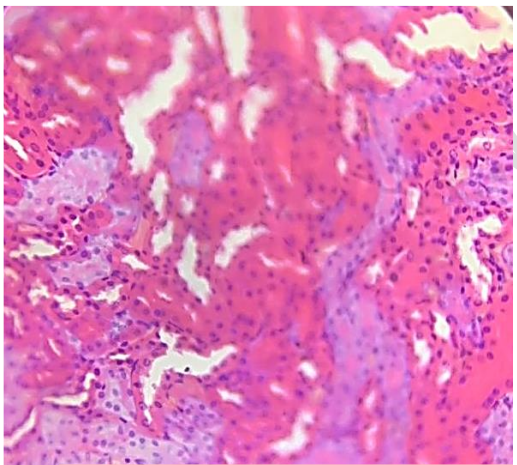
*values are Mean ± SD. Values with the same superscript are not significantly different, whereas those with different superscript are significantly different ($p<0.05$)

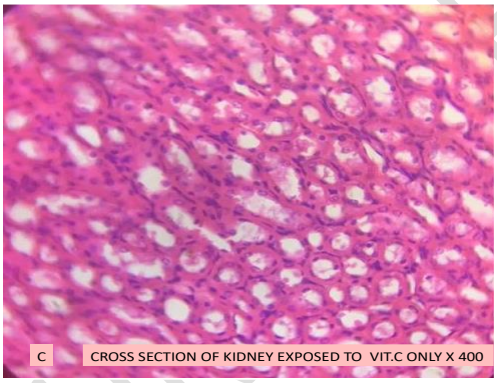
The results of Histopathological evaluation of the Kidney of Swiss mice exposed to DDVP and *Costus afer* are shown in Plate 1A-1F. Plate 1 shows a section of the kidney of Swiss micein group A (control) stained with H&E. The cross section shows normal architecture of the kidney with numerous glomeruli. Also visible are renal tubules, blood vessels and healthy nephrons. Plate 1B shows inflammation, fibrosis, and necrosis of kidney tissues, depletion of nephrons.Plate 1C shows kidney section exposed to Vitamin C only shows normal

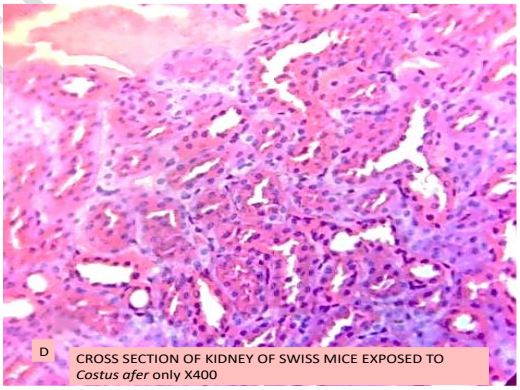
architecture of nephrotic cells equivalent to the control kidney section. Plate 1D shows Kidney section of Swiss mice exposed to *Costus afer* only shows minimal nephrotic damage . Plate 1E shows the cross section of kidney in male mice exposed to DDVP and Vit C supplement with fully regenerated nephrons without inflammation, necrosis or fibrosis. Plate 1F is cross section of kidney in male mice exposed to DDVP and *Costus afer* still showing massive depletion of nephrotic cells and presence of inflammation.

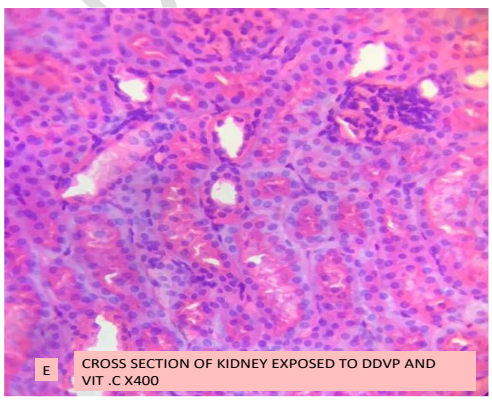


A: Cross section of control animals X400


B CROSS SECTION OF KIDNEY OF SWISS MICE EXPOSED DDVP ONLY X 400


C CROSS SECTION OF KIDNEY EXPOSED TO VIT.C ONLY X 400


D CROSS SECTION OF KIDNEY OF SWISS MICE EXPOSED TO *Costus afer* only X400


E CROSS SECTION OF KIDNEY EXPOSED TO DDVP AND VIT .C X400

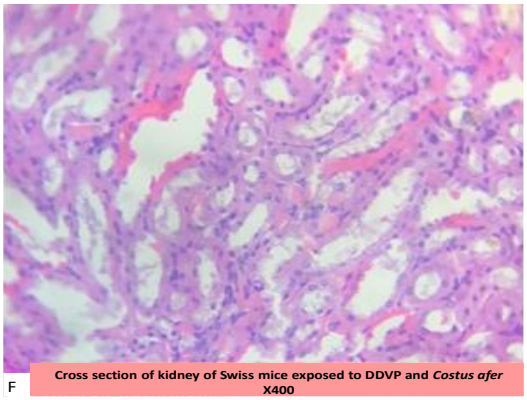

F **Cross section of kidney of Swiss mice exposed to DDVP and *Costus afer* X400**

Plate 1 : A-F:Section of the kidney of Swiss mice in group A -F stained with H&E
RT- renal tubules, ISC- interstitial cells,

DDVP, also known as dichlorvos, is a commonly used pesticide that is known to have toxic effects on various organs in animals, including the kidneys. Studies have shown that exposure to DDVP can lead to kidney damage in rats, which raises concerns about the potential health risks associated with this chemical. This observation is in agreement with the report of [21,22,23]

DDVP has also been shown to disrupt the balance of electrolytes and some metabolites in the kidneys. This can lead to abnormalities in the regulation of fluid and electrolyte balance, which is essential for maintaining proper kidney function. Disruption of these processes can lead to kidney dysfunction and ultimately kidney damage.

Elevated blood urea and creatinine resulting from increased breakdown of tissue or impaired excretion is correlated with an increased protein catabolism in the mammalian body. Results from this study showed a significant decrease in total protein concentrations in animals treated with DDVP, as urea is the end product of protein catabolism. Treatment with DDVP only as seen in group B caused significant increase in serum creatinine and urea. This indicates diminished ability of the kidneys to filter these waste products from the blood and excrete them in the urine.

Furthermore, DDVP has shown to cause histopathological changes in the kidneys of rats especially in group B animals. These changes included inflammation, fibrosis, degeneration of Nephrons and necrosis of kidney tissues, all of which can impair kidney function and lead to kidney damage.

However, Vit C supplement administration for 35 days caused a significant difference in kidney parameters, regeneration of Nephrons, reduction in kidney tissue inflammation as seen in group C and E compared to group D and F indicating the efficacy of vit C supplement over *Costus afer.* This agrees with [24] who reported protective effects of vit. C and A on oxidative renal tissue damage. [25 26,27,28] also reported reversal of all hepatic markers to near normal levels indicating the ameliorative effect using lycopene [27], herbal extract of Zingiberofficinale, Alstoniaboonei, aqueous seed of Leaguineensis and vitamin C supplementation as antioxidant on Dichlorvos induced- toxicity in animal studies.

4. Conclusion

Overall, the effect of DDVP on rat kidneys highlight the potential health risks associated with exposure to this pesticide while *Costus afer* ameliorated the adverse effects of DDVP in the Kidney of exposed male mice. It is important for regulators and policy makers to take these findings into consideration when assessing the safety of DDVP and other pesticides, and for individuals to take precautions to minimize their exposure to these chemicals. Additional research is needed to further understand the mechanisms by which DDVP affects the kidneys and to develop strategies to protect against its harmful effects.

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1.

2.

3.

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