EFFECT OF VIRGIN COCONUT OIL ON THE KIDNEY OF WISTAR RATS EXPOSED TO A TOXIC DOSE OF PARAQUAT

Joseph Nwafor*, Joy Umudu*, Augustine Oviosun and Obinna Uchewa***

*Department of Anatomy, Faculty of Basic Medical Science, Alex Ekwueme Federal University Ndufu Alike, Ikwo AE FUNAI , Ebonyi State, Nigeria.,
**Department of Anatomy, Faculty of Basic Medical Science, Edo University, Iyamho EUI , Edo State, Nigeria.

ABSTRACT Background and objective: Paraquat toxicity to bodily organs is a public health concern, especially in climes were there no safe remedies. This study investigated the effect of Virgin Coconut Oil (VCO) on the kidney of Wistar rats exposed to a toxic dose of paraquat. **Materials and Methods:** Four groups (A, B, C and D) of six adult Wistar rats were used. Group A is the normal control. Group B rats received an oral dose of 12.75mg/kg b.wt of paraquat without treatment. Groups C and D were pre-treated respectively, with 5 and 10 ml/kg b.w.t of VCO before exposure to paraquat. The experiment lasted for 24 days. At the end of the administration, the rats were sacrificed. The kidney was harvested for routine histology, and blood was collected via cardiac puncture for biochemical analysis. **Results:** Group B had a significant ($p \leq 0.05$) increase in mean values of potassium (5.41±0.16), urea (5.15±0.07), creatinine (59.00±8.34) and MDA (2.94±0.07) levels when compared to group A. Group D shows a significant ($p \leq 0.05$) decrease in these parameters. Except for potassium, group C also showed a decrease in the parameters mentioned above when compared to group B. In comparison to groups B and D, C had a significant decrease in SOD (4.85±2.05). VCO preserved the kidney cortex. **Conclusion:** The finding of this study has shown that the toxic effects of paraquat on kidney function could be moderately reversed by phyto-oxidants present in virgin coconut oil. Thus, virgin coconut oil has anti-oxidative and nephroprotective benefits against paraquat nephrotoxicity. However, this may be dose-dependent.

KEYWORDS Paraquat, Virgin Coconut Oil, Kidney, Toxicity

Introduction

Nephrotoxicity can be defined as the adverse effect of substances on renal function [1]. The substances that cause kidney injury are called nephrotoxicants. These substances can include moulds and fungi, cancer therapeutics such as cisplatin, antibiotics such as aminoglycosides, metals such as mercury, arsenic and lead, and drugs of abuse such as cocaine. Paraquat dichloride (PQ) is a non-selective contact herbicide for broadleaf weed control. It is used widely in modern agricultural practice worldwide and is emerging rapidly in the Nigerian's agricultural industry. Paraquat is not only toxic to plants but adversely affect human and farm animals by accumulating in the lungs, liver, kidneys and heart [2]. Animal exposure occurs by accidental or intentional swallowing, or through damaged skin or inhalation. Studies on paraquat toxicity have shown that chronic exposure can lead to lung and liver damage, kidney failure, and Parkinsonian lesions in addition to fibrosis stress and damage [3]. Paraquat toxicity occurs through excess production of free radicals which results in oxidative stress and damage [4,5]

Plants represent a largely untapped source of natural active compounds that may serve as leads for the formulation of synthetic drugs or herbal remedies in the management of many adverse health conditions. Virgin Coconut Oil (VCO) could be

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[1]Department of Anatomy, Faculty of Basic Medical Science, Alex Ekwueme Federal University Ndufu Alike, Ikwo AE FUNAI , Ebonyi State, Nigeria; Email: jnwafor49@gmail.com

seen as a naturally processed, chemically-free and additive-free product, gotten from fresh coconut meat or it's derivative (coconut milk and coconut milk residue) which has not undergone any further chemical processing after extraction. It is the purest form of coconut oil that is milky in colour, contains natural Vitamin E and with very low free fatty acid content (even without refining) and low peroxide value. Therefore it retains more of the natural active components, which include polyphenols which have been proven to boost the antioxidant defence system [6]. Coconut oil has been extensively used throughout history owing to its medicinal properties and has served man as an important food for thousands of years [7]. Over the years, VCO has been consumed as potential food, and has health benefits but has been reported to possess anti-inflammatory, antibacterial and antioxidant properties [8]. Since virgin coconut oil (VCO) contains antiviral, antibacterial, antifungal, anti-inflammatory, antidiabetic, antiobesity, antiulcerogenic, analgesic, antipyretic, and antioxidant properties, it could be developed as therapeutic by investigating these properties against paraquat-induced nephrotoxicity.

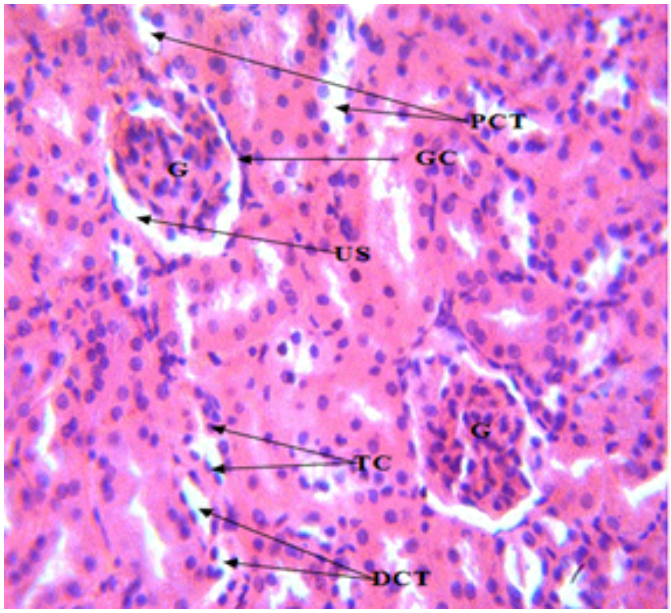

Figure 1: Normal control section of kidney shows normal architecture of the kidney cortex with G (Glomerulus), glomerular capsule (GC), TC (Tubular cells), PCT (Proximal convoluted tubules), DCT (Distal convoluted tubules). (x400) (H/E Stain).

Materials and Methods

Experimental Animals

Twenty-four (24) male Wistar rats weighing 100-150g were obtained from the animal house section of the Alex Ekwueme Federal University Ndufu-Alike Ikwo (AE-FUNAI), Ebonyi State, Nigeria. The rats were housed in cages at the animal house of the Department of Anatomy, AE-FUNAI. They were acclimatized for two weeks prior to experimental use and allowed free access to standard vital feeds produced by Grand cereals Ltd, and water. The constant environmental condition was maintained with proper ventilation and a good source of light (12h light -12h dark and 24ºC ± 30ºC).

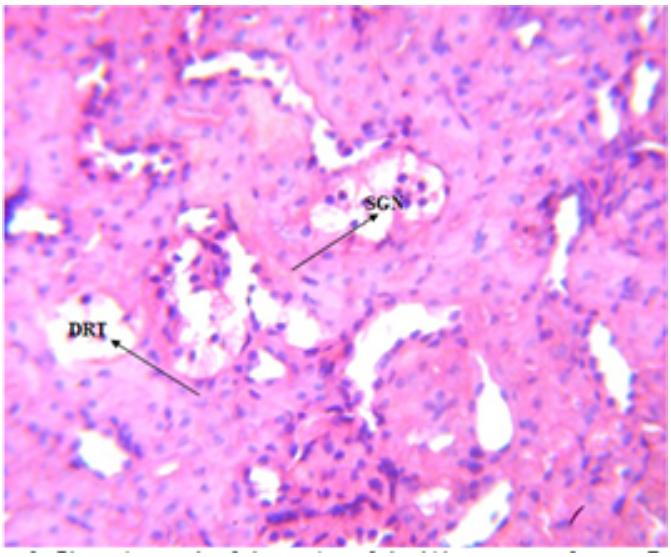

Figure 2: Section of the kidney cortex of group B animals administered with Paraquat showing dilated renal tubules (DRT) with dispersed/scanty tubular cells, presence of severe glomerular Necrosis (SGN). (x400) (H/E Stain).

Toxic Chemical

Paraquat dichloride was purchased under the trade name paraeforce, a product of Nanjing Redsun Biochemical Company Ltd from an agrochemical store in Abia state, Nigeria.

Plant Source and preparation

Fresh coconuts (30) were purchased from a farm in Abakaliki, Ebonyi State, Nigeria. Virgin coconut (VCO) was extracted by the wet method [9]. The solid matured coconut was crushed and made into a viscous slurry, water about 500ml was added and to obtain coconut milk. The coconut milk was left for 24hours for gravitational separation of the milk. The upper oily phase was decanted and heated for 10 minutes. The resultant VCO was sieved and stored under room temperature.

Experimental Protocol

The weighing and observations were done before and after the administration of the extract respectively. The weights of the animals were estimated at procurement, during acclimatization, at the commencement of the experiments and once within a week throughout the period of the experiment, using an electronic analytical and precision balance. At the end of the experiment, the animals were sacrificed by cervical dislocation after 12 hours of fasting. The kidney was immediately harvested and weighed. It was then homogenized in phosphate buffer and sampled for assessment of kidney reduced SOD, lipid peroxidation using MDA and for histological evaluations.

Experimental Design

The rats were randomly assigned into 4 groups, containing 6 rats each. Group A served as negative control was treated with 5 ml/kg b.w.t. normal saline daily for 24 days. Group B served as positive control, received 5ml/kg b.w.t. normal saline and 12.75 mg/kg b.w.t of paraquat on the 22nd, 23rd and 24th days (last three days) of the experiment. Group C was administered with 5ml/kg b.w.t of VCO for 21 days, followed with 12.75

Table 1 Potassium, Urea, Creatinine levels, and Oxidative stress markers in paraquat-induced renal injury (means ± SD).

	Group A	Group B	Group B	Group C
Potassium	4.62±0.87	5.41±0.16*^{c}	5.70±2.27	4.66±0.01^{b}
Urea	3.60±0.28	5.15±0.07*^{c}	4.75±0.63^{c}	3.35±0.63^{b}
Creatinine	44.25±4.17	59.00±8.34*^{c}	56.05±4.17	44.25±12.51^{b}
SOD	13.33±2.10	11.66±0.80**^{d}	4.85±2.05 ^{b},^{d}	16.05±1.76^{a}
MDA	1.74±0.46	2.97±1.09*	2.65±1.15^{a},^{c}	1.85±0.31^{b}

*,**represent significant increases or decreases respectively at ($p \leq 0.05$) when values are compared with the normal control (Group A).

^{a},^{b} represent significant increases or decreases respectively at ($p \leq 0.05$) when the values are compared with positive control (Group B).

^{c},^{d} represent significant increases or decreases respectively at ($p \leq 0.05$) when the values are compared with Group D.

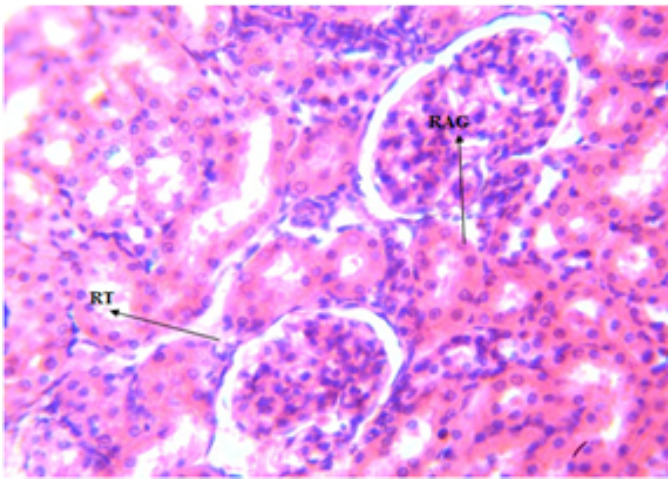

Figure 3: Section of the kidney cortex of group C animals administered with Paraquat and treated with low dose VCO showing RAG (relatively dilated glomerulus) with, RT (Renal Tubules) with mild persistent presence of tubular cells. (x400) (H/E).

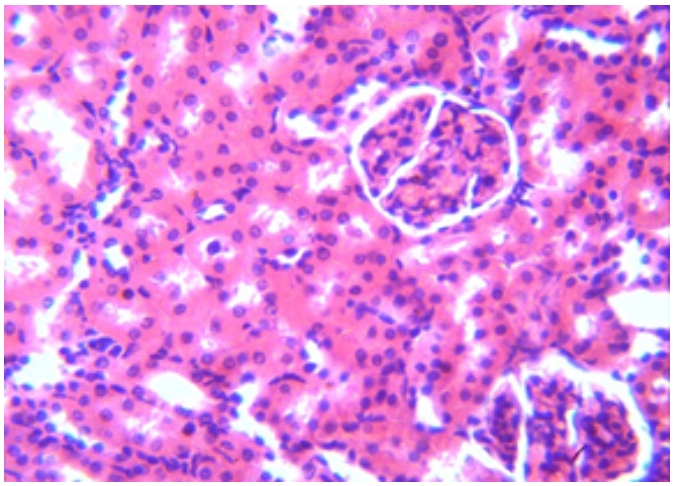

Figure 4: Section of the kidney cortex of group D animals administered with Paraquat and treated with high dose VCO shows DRT (Dilated renal tubules) with better population density of renal tubular cells and moderately preserved Glomerulus (MPG). (x400) (H/E).

mg/kg b.w.t. of paraquat on the 22nd, 23rd and 24th days of the experiment. Group D were treated with 10 ml/kg b.w.t. of VCO for 21 days, followed with 12.75 mg/kg b.w.t. of paraquat on the 22nd, 23rd and 24th days of the experiment.

Biochemical Analysis

Blood biochemical parameters including serum urea, creatinine level, MDA, and SOD were assayed using the commercially available kits.

Assay of SOD and MDA

Kidney tissues were immediately harvested and cooled on ice and homogenized in 4 volumes (w/v) of ice-cold 0.1 M KH2 PO4 buffer containing 1 mM EDTA adjusted to pH 7.4 and centrifuged at 10,000 for one hour at 4º C. The supernatant was collected and kept -80 º C for subsequent determination of MDA and SOD [10,11].

Assay of Serum Creatinine, Urea and Potassium level

Creatinine was determined by the modified method of Heinegard and Tiderstrom [12]. Potassium was estimated by adopting a previously described principle and procedure [13]. Urea was estimated using the Berthelot reaction method as earlier described [14].

Histological Evaluation

Tissues from all groups were subjected to histology, following standard routine procedures. The kidney was fixed in 10% buffered formaldehyde for 24 hours and processed for paraffin wax embedding; Sections were cut on a rotary microtome at 5 μm thickness. The slides were viewed under a microscope for both quantitative and qualitative evaluation. Photomicrographs were taken and interpreted.

Data Analysis

All data obtained from this was study was expressed as mean ± standard deviation of the number of experiments (n=6). One-way analysis of variance (ANOVA) was used for comparison between means of treated groups and control group. Analysis of data was done using statistical package for social sciences (SPSS)/ PC computer program (version 23.0 SPSS, Cary, NC, USA).

Ethical Approval

Guidelines relating to National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978) were adhered to following research approval by the Animal and ethics committee of AE-FUNAI, Ebonyi state, Nigeria.

Physical Observation

The animals treated with paraquat generally showed decreased food intake, weakness, lethargy with a reduction in locomotive activities. These observations are associated with toxicity and were more pronounced in group B. The lower feed consumption could be possibly due to the direct adverse effect of paraquat on the gastrointestinal tract, which is corrosive and strong irritant to the gastric ulceration and gastritis.

Biochemical Results

Table 1 shows that the mean values for Urea level varied significantly across and within the group at ($p \leq 0.05$). When mean values were compared between groups, it was observed that there was a significant ($p \leq 0.05$) increase in the level of urea in Group B animals that received paraquat without treatment when their values were compared to Group A. However, on treatment with 10ml/kg VCO, Group D showed a significant drop in urea level when values were compared with those of groups B. A similar decrease in urea level was equally observed in group D when their mean values were compared with those of group B. For potassium electrolyte level, there was a significant ($p \leq 0.05$) decrease in groups A, B, and D, when compared across the groups. However, on treatment with a low dose of VCO and paraquat (5ml/kg b.w.t VCO + paraquat), group C showed a significant increase in potassium level. Furthermore, the result also showed that there was a significant ($p \leq 0.05$) increase in creatinine level of rats in group B when compared to groups A and D. Evaluation of Oxidative stress markers, shows that Group B had significant ($p \leq 0.05$) increase in the level of MDA with a concomitant decrease in SOD level when values were compared with group A. Comparing values between Group B and Pre-treatment groups, the significant decrease in the combative level of SOD may account for the sustained increase in MDA level. However, the rats in group D had significantly ($p \leq 0.05$) decreased the level of MDA due to significant ($p \leq 0.05$) increase in SOD level.

Histological results

The kidney tissues for each group of animals (A, B, C and D) presented as micrographs were interpreted for the basis of comparison between the respective groups.

Discussion

Paraquat is a very toxic herbicide used widely across the globe. Regardless of its administration route, paraquat is rapidly distributed in most tissues, with the highest concentration found in the kidneys, where it produces early and severe nephrotoxicity [15]. Oxidative stress has key role in the toxicity of paraquat and leads to lipid peroxidation, body cell damage, and apoptosis [16]. Presently, virgin coconut oil (VCO) is gaining wide popularity in the scientific field and among the public. It is believed that virgin coconut oil is more beneficial than usually obtained copra oil since the mode of extraction retains more biologically active components such as vitamin E and polyphenols with combative roles in oxidative stress [17]. The histological results of kidney in normal control showed normal architecture of the kidney cortex, which evidently suggest the well-being and functionality of the kidney.

In contrast, there was a loss of tissue architecture in Group B. The necrotic zone around the glomerulus indicates renal damage and possible impairment in ultrafiltration of urine. The relative preservation of the glomerulus and renal cells responsible in transport as seen in VCO pre-treated groups suggest restorative changes from the damage caused by paraquat. The fairly normal histoarchitectural presentations of the kidney of groups C and D suggests a protective potency of VCO. However, this was much more evident in group D. Potassium is an intrinsic component of the sodium-potassium pump and its normal physiologic estimate is of concern. Hyperkalemia may affect the functional integrity of this channel, consequently leading to homeostatic imbalances. It is often associated with renal failure, dehydration shock and adrenal insufficiency. Abnormal measures of urea and creatinine may equally downregulate the activity of ion receptor channels and trigger biochemical pathways associated with nephrotoxicity. The increment in serum renal markers, such as creatinine that is related to abnormal excretion rate of the kidney as seen in group B was moderately prevented with VCO anti-oxidant therapy as documented in other previous studies [18, 19].

There are antioxidants systems against oxidative agents in the human body. Their activities regulate the level of lipid peroxidation, indexed by the amount of tissue MDA. Spontaneous increment in MDA suggests depletion in the body antioxidant defence system in combating free radicals. In this present study, the SOD level was decreased significantly in Group B, which is in accordance with previous studies that paraquat may deplete the SOD level in kidney tissue [20]. Nevertheless, virgin coconut oil administration led to an improved activity of SOD, which is consistent with a similar study [21]. Therefore, these effects of virgin coconut oil on renal SOD in paraquat-induced nephrotoxicity may be due to its antioxidant properties.

Conclusion

In conclusion, this study proposes a protective role of virgin coconut oil against renal damage induced by paraquat in rats because not only did it decrease the number of renal inflammatory cells but also elevates kidney activities of SOD with minimal lipid peroxidation.

Authors' contributions

Joseph Nwafor is liable for the conception, design of this piece of research work and he is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity

of the work are appropriately investigated and resolved. Joy Umudu earned authorship being responsible for Data acquisition, data analysis and interpretation. Austine Oviosun drafted the article and critically revised it to ascertain and improve its intellectual content. Obinna Uchewa ratified the final approval of the version to be published.

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Patient informed consent

Experimenntal research with wistar rats.

Ethics committee approval

The study was approved by the ethics committee.

Conflict of interest

Authors declare no conflict of interests. There was no funding applied for this article.

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