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Evaluation of Protective Properties of *Elaeis oleifera* Fruit Extract on Renal Parameters of Dichlorvos-Induced Nephrotoxocity in Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author EON designed the study. Author IE performed the statistical analysis and wrote the protocol. Author FB wrote the first draft of the manuscript. Authors AEBC and NB managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Evaluate the protective effects of palm oil on renal parameters after dichlorvos toxicity in albino rats.
Study Design and Methodology: The study consisted of 3 phases: The acute study which lasted for 24 hours, the sub-acute study which lasted for 14 days and the sub chronic study which lasted for 30 days. The design and treatment pattern is shown below. Phase 1: Acute Study. Group 1: No DDVP, No palm oil for 24 hours (Negative control), Group 2: 30 mg/kg of DDVP without palm oil (positive control), Group 3: 30 mg/kg of DDVP and 100 mg/kg palm oil for 24 hours (treatment group). Phase 2: Sub-Acute (14 days) Study. Group 4: No DDVP, No palm oil for 14 days (Negative control), Group 5: 10 mg/kg of DDVP without palm oil daily for 14 days (positive control), Group 6: 10 mg/kg of DDVP and 100 mg/kg of palm oil daily for 14 days (positive control). Phase 3: Sub-Chronic (30 days) Study. Group 7: No DDVP, No palm oil for 30 days (Negative control), Group 8: 10 mg/kg of DDVP without palm oil daily for 30 days (positive control), Group 9: 10 mg/kg

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of DDVP and 100 mg/kg palm oil daily for 30 days (treatment group). All administration was done orally. After the period of treatments, the rats were sacrificed after 18 hours of fast. Whole blood samples (5 mls) were collected into lithium heparin bottle and spun at 3500 rpm for 5 minutes to obtain plasma samples. Samples obtained were used for the determination of Na^+, K^+, HCO_3, urea, and creatinine while renal tissues obtained were used for histopathological examinations.

Results: Significantly higher values were seen in urea in the dichlorvos treated rats over a period of 24 hours, 14 days, and 30 days as compared to rats co-treated with palm oil and the control. Creatinine indicated significantly higher over a period of 24 hours while non-significant increases were observed in the dichlorvos treated rats over a period of 14 days and 30 days. More so, significantly higher values were seen in potassium in the dichlorvos treated rats over a period of 24 hours and 14 days, while significantly higher values in potassium were seen after period of 30 days as compared to rats co-treated with palm oil and the control. Sodium and chloride did not indicate significant difference over the period of 24 hours, 14 days, and 30 days. Histological examination of the renal tissue indicated structural distortions dichlorvos treated rats over a period of 24 hours, 14 days and 30 days while significant improvements in the structural integrity of the kidney were observed in rats co-treated with palm oil.

Conclusion: Results obtained indicated that palm oil showed a protective effect in ameliorating the nephrotoxicity induced by dichlorvos as shown by the histological examination and decreased values of creatinine and urea as well as potassium in palm oil treated rats.

Keywords: Dichlorvos; palm oil; fruit extract; renal nephropathy; renal parameters.

1. INTRODUCTION

The intake of pharmacological substances by man has increased tremendously and these may be presented today in the form of food and food constituents, medicines, beverages, and other industrial and household products [1]. These chemicals or pharmacological substances may result in chronic toxicity in the living system when used over a long period of time or acute toxicity may also occur when large quantities capable of eliciting immediate toxic effect are used. Acute toxicity is defined as the unwanted effect(s) that occurs either immediately or at a short time interval after a single or multiple administration of such substance within 24 hours. The unwanted (or adverse) effect is an effect that produces functional impairments in organs and/or biochemical lesions, which could alter the functioning of the organism in general or individual organs [1].

One of such toxic substances is dichlorvos (*2, 3-dichlorovinyl dimethyl phosphate*) commonly referred to as sniper in Nigeria. Dichlorvos is one of the classes of insecticides referred to as organophosphates, used to control households and stored products from insects [2]. It is effective against mushroom flies, aphids, spider mites, caterpillars, thrips, and white flies in greenhouse, outdoor fruits, and vegetable crops [2,3]. Therapeutically, dichlorvos is used as a fumigant or to treat a variety of parasitic worm infections in dogs, livestock, and humans. It acts against insects as both a contact and a stomach poison [3]. These chemicals act by interfering with the activities of cholinesterase, an enzyme that is essential for the proper working of the nervous systems of both humans and insects [2].

Dichlorvos self-poisoning is an important clinical problem in the developing world, and kills an estimated 200,000 people every year [4]. In Nigeria, especially the northern part, dichlorvos is traded under different names such as Nuvan, Sniper, ata-pia-pia (Hausa), and is handled and used as a household insecticide indiscriminately. The most popular source of organophosphate insecticide or pesticide in Nigeria is the locally made variety called 'Otapiapia' [2]. The main active ingredient in 'otapiapia' is dichlorvos or *2, 2-dichlorovinyl dimethyl phosphate* (DDVP); with most containing between 5-10% w/v and are readily available for purchase on the streets [5]. 'Sniper', which is another common variety of DDVP has a more refined packaging and is available in stores nationwide at a higher cost. Organophosphate poisoning may occur through ingestion, inhalation, or penetration through the intact skin; and may be suicidal, accidental or homicidal. Most of the cases are accidental [6,7]. The World Health Organization (WHO) noted that pesticide poisoning is now the most common method of suicide worldwide [8,9,10]. About two million people attempt suicide and one million accidental poisoning cases occur each year worldwide [11]. Suicide accounts for an estimated 849,000 deaths worldwide [8] and approximately 300,000 deaths in the developing world every year [12]. In 2019, about 7 cases of

self-poisoning with dichlorvos have been recorded in Nigeria where the drug is used as a therapy against depression among youth and teenagers.

Different substances have been used to provide a neutralizing effect for dichlorvos and other pesticide poisonings. Atropine has proven to be effective in the management or as an effective antidote for pesticide poisonings [13]. Other substances such as oxime (pralidoxin) can also be used although effectiveness varies markedly according to the organophosphate used and the ingested dose. Traditionally, substances such as charcoal, induction of emesis, ingestion of milk, and coconut water are commonly used for primary intervention and most of them have been reported to mitigate the toxic effect of toxic substance taken orally [14]. In Nigeria where there is an increase in substance toxicity especially with dichlorvos, different substances have been used by different persons so as to neutralize or limit the effects of these toxic substances thus preventing death and other adverse effects that may arise from the intoxication of such toxic substances. One of these neutralising or antidotal substances commonly used locally is palm oil is readily available, cost effectiveness and reduced side effect.

Palm oil is an edible vegetable oil derived from the mesocarp (reddish pulp) of the palm fruit, primarily from the African oil palm *Elaeis guineensis*, and to a lesser extent from the American oil palm *Elaeis oleifera* [15]. Locally, the red palm fruits were purchased in the market or harvested from farm lands. The processing of the ripe oil palm fruits involves boiling at 100°c for at least 2 hours, pounding the soft mesocarp to produce pulp marsh, immersed in hot water and stirred continuously thoroughly, filtration to remove fibers and the seeds and then boiling the filtrate for 3 - 4 hours to obtain palm oil which is set on top of the aqueous portion of the boiled filtrate after cooling.

In this study, we intend to evaluate the biochemical (renal) and histological effects of locally made palm oil on acute, sub-acute and sub-chronic toxicity poisoning with dichlorvos in order to provide evidence based information in the use of locally made palm oil for quick intervention in the management of dichlorvos poisoning. Dichlorvos poisoning in Nigeria has been one of the major source of poisoning and it is on the increase ranging from suicide cases, contamination, and food poisoning.

2. MATERIALS AND METHODS

2.1 Materials

Bucket centrifuge (MPW, Poland), tissue embedder (LEICA EG 1160), rotatory microtome (LEICA RM 2125 RTS) and albino rats, urea and creatinine bio-reagents were obtained from Randox Diagnostics, United Kingdom. 100 ml of 2, 3-dichlorovinyl dimethyl phosphate (DDVP) was purchased from Saro's Agro Science with concentration of 1.0 g/ml. All other chemical used for the analysis of renal parameters were of good quality and analytical grade.

2.2 Experimental Animals

Sixty three (63) albino rats consisting of males and females that weighed 150 g were selected for the study. The animals were obtained from University of Port Harcourt College of Health Sciences. They were transported in well ventilated wired cage to the animal house of Medical Laboratory Science Department, Rivers State University, Port Harcourt. During this study, the rats were maintained with a 12 hour light/dark cycle and were provided access to solid poultry chow as feed and water from the tap.

2.3 Collection and Preparation of Palm (Elaeis guineensis) Oil Extract

The Red Palm fruits were purchased from Oil Mill market Port Harcourt, Rivers State. It was identified and confirmed to be *Elaeis guineensis* by a Botanist in the Department of Applied and Environmental Biology, Rivers State University. The ripe oil palm fruits (40 kg) were boiled at 140±15°C in a pot for 2 hours in 5 liters of water to make the fruit soft (pulp) for manually pounding. The pulp was immersed in hot water and stirred continuously thoroughly. The fibers and the seeds were filtered out using a basket and a sieve (weight of fibers and seeds: 25 kg). The filtrate of about 1 liter in volume (with mass of 15 kg) were poured into a stainless steel cooking pot and boiled again at 140±15°C for 5 hours. The mixture was allowed to cool. The palm oil which set on top of the aqueous potion of the boiled filtrate was scooped into a fresh container. This implies that 1.0 ml of the palm oil has an approximate mass of 0.015 kg which is equivalent to 0.1 kg/kg (or 100 mg/kg) bodyweight of the rats weighing 0.15 kg.

2.4 Pilot Study and Dose Determination

Pilot study was carried out to determine the LD_{100} and LD_{50} of dichlorvos administered orally after

allowing 14 days of acclimatization, according to method used by Elekima et al. 2017. An aggregate of 18 rats weighing around 150 g were employed in the determination of LD_{100} of dichlorvos administered orally. The rats were classified into 6 groups with 3 rats in each group and labeled 1, 2, 3, 4, 5, and 6 were treated with 0 mg/kg, 20 mg/kg, 40 mg/kg, 60 mg/kg, 80 mg/kg and 100 mg/kg dichlorvos (sniper) respectively. The LD_{50} of the dichlorvos administered orally was obtained using the arithmetic method of Karber after determining the LD_{100} from the pilot toxicity study described by Elekima et al. [16]. The arithmetic method of Karber for calculating LD_{50} was given as follows:

$$LD_{50} = LD_{100} - \frac{(Sum\ of\ dose\ difference \times mean\ dead)}{Number\ of\ rats\ per\ group}$$

2.5 Experimental Design and Treatment Pattern

The study consisted of 3 phases: the acute study which lasted for 24 hours, the sub-acute study which lasted for 14 days and the sub chronic study which lasted for 30 days. The design and treatment pattern is shown below.

Phase 1: Acute Study: Group 1: No DDVP, No palm oil for 24 hours (Negative control).
Group 2: 30 mg/kg bodyweight of DDVP without palm oil (positive control).
Group 3: 30 mg/kg bodyweight of DDVP and 100 mg/kg bodyweight of palm oil for 24 hours (treatment group).

Phase 2: Sub-Acute (14 days) Study: Group 4: No DDVP, No palm oil for 14 days (Negative control).
Group 5: 10 mg/kg bodyweight of DDVP without palm oil daily for 14 days (positive control).
Group 6: 10 mg/kg bodyweight of DDVP and 100 mg/kg bodyweight of of palm oil daily for 14 days (positive control).

Phase 3: Sub-Chronic (30 days) Study: Group 7: No DDVP, No palm oil for 30 days (Negative control).
Group 8: 10 mg/kg bodyweight of DDVP without palm oil daily for 30 days (positive control).
Group 9: 10 mg/kg bodyweight of DDVP and 100 mg/kg bodyweight of palm oil daily for 30 days (treatment group).

2.6 Collection and Preparation of Samples

At the end of the 24 hours, 14 days, and 30 days of treatment for the respective groups, the rats fasted for 18 hours before specimens were collected. The animals in all groups were anaesthetized with chloroform, blood samples (5 ml) were obtained by cardiac puncture, poured into lithium heparinized containers, and centrifuged at 4000 rpm for 5 minutes to obtain plasma samples. The plasma samples after centrifugation were aliquoted into a plain bottle for analysis of electrolytes, urea and creatinine while renal tissues were excised and collected into 10% formol saline for histological examination.

2.7 Biochemical and Histological Analysis

Urea was analysed using berthelot enzymatic colorimetric method as documented by Patton and crounch [17]. The estimation of creatinine was done using jaffe's enzymatic method as documented by Vaishya et al. [18]. The estimation of electrolytes (Na^+, K^+, and Cl^-) were performed using Ion Selective Electrode (ISE) analyser as described Bucks and Linder [19]. The renal tissues fixed in 10% formal saline were prepared for histological examination by dehydrating in increasing grades of alcohol and clearing in two changes of xylene, embedment in paraffin wax, trimming and sectioning at 3um. The sections were attached to slides, dewaxed in xylene, stained in Haematoxylin and Eosin (H&E) and examined under electronic microscope.

2.8 Statistical Analysis

Data obtained from evaluation of parameters were presented as mean ± SD. Statistical comparison between groups were done using one-way ANOVA method while Turkeys multiple comparisons (post hoc tests) was used to obtain specific significant differences among the various groups. Analysis was computed with SPSS for windows version 23. Differences were considered significant at $P<0.05$.

3. RESULTS

3.1 Determination of LD_{100} and LD_{50} of *2, 3 Dichlorovinyl diphosphate*

After the administration of 2, 3 dichlorovinyl diphosphate in the pilot study, the treated rats were monitored within 24 hours for signs of toxicity such as restlessness, pupil constriction, respiratory distress, and convulsion. The minimum dose that caused 100% death was

seen as the LD100 and it was seen to be 80 mg/kg for dichlorvos orally treated rats (Table 1).

The mean lethal dose (LD_{50}) for dichlorvos treated rats was determined using the arithmetic method of Karber after LD_{100} determination. Applying the arithmetic method of karber,

$$LD_{50} = LD_{100} - \frac{(Sum\ of\ dose\ difference\ \times mean\ dead)}{Number\ of\ rats\ per\ group}$$

Where,

LD_{100} = 80 mg/kg (Table 1)
Sum of dose difference × mean dead = 110 (Table 1)
Number of rats per group = 3 (Table 1)

Therefore:

$$LD_{50} = 80\ \text{mg/kg} - \frac{110}{3}$$
= 80 mg/kg – 55 mg/kg
= 25 mg/kg

Thus, the mean lethal dose (LD_{50}) for dichlorvos treated rats is 25 mg/kg bodyweight of rat.

3.2 Results of Renal Parameters of Rats given 30 mg/kg Diclorvos Orally over a Period of 24 hours

Results obtained after oral administration 30 mg/kg diclorvos over a period of 24 hours indicated significant differences in K+, urea, and creatinine. However, no significant differences were recorded in Na+ and Cl-. When potassium was evaluated, significantly increased values were seen in the dichlorvos treated animals without palm oil (group 2) against rats given dichlorvos and those treated with palm oil at $p<0.05$. However, non-significant increase was seen between rats not given dichlorvos (group 1) and rats given dichlorvos (group 2) as well as between group 1 and 3 at $p<0.05$. Furthermore, when urea and creatinine were evaluated, significant increase in urea and creatinine were observed in animals given dichlorvos without palm oil treatment (group 2) compared to rats given dichlorvos and were treated with palm oil as well as the negative control (group 1). No significant difference was seen between group 1 and 3 at $p<0.05$ (Table 2).

In addition, when 10 mg/kg diclorvos orally over a period of 14 days were evaluated, results of renal function parameters of rats given 10 mg/kg diclorvos orally over a period of 14 days indicated significant differences in K+, urea, and creatinine. However, non significant differences were seen in Na+ and Cl-. Potassium indicated a significantly higher values in dichlorvos treated rats without palm oil supplements (group 2) compared to rats given dichlorvos and were treated with palm oil at $p<0.05$. However, non-significant increase was seen between Rats not given dichlorvos (group 1) and rats given dichlorvos (group 2) as well as between group 1 and 3 at $p<0.05$. Again, when urea were analyzed, significant increments in urea were observed in rats given dichlorvos without palm oil treatment (group 2) compared to rats given dichlorvos and were treated with palm oil (group 3). No significant difference was seen between group 1 and 3 as well as between group 1 and group 2 at $p<0.05$ (Table 3). When creatinine was analyzed, significant increment was also seen in rats given dichlorvos without palm oil treatment (group 2) against negative control (group 1). However, non-significant increases were seen in creatinine in rats given dichlorvos without palm oil treatment compared to rats given dichlorvos and were treated with palm oil (group 3) at $p<0.05$ (Table 3).

Finally, when rats given 10 mg/kg diclorvos orally over a period of 30 days were considered, results of renal function parameters again indicated significant differences in K+, urea, and creatinine but no significant differences were recorded in Na+ and Cl-. Potassium showed significantly elevated values in rats given dichlorvos only (group 2) against negative control (group 1). However, non-significant increases were seen in creatinine in rats given dichlorvos without palm oil treatment compared to groups given diclorvos and were treated with palm oil (group 3) at $p<0.05$ (Table 4). Furthermore, when urea was investigated, significantly elevated values were observed in rats given dichlorvos only (group 2) against rats given dichlorvos and were treated with palm oil (group 3). Again, significant reductions were sighted in palm oil treated rats compared to negative control (group 1) at $p<0.05$ (Table 4). When creatinine was evaluated, significant elevations were recorded in rats given dichlorvos without palm oil treatment (group 2) against negative control (group 1). However, non-significant increases were seen in creatinine in rats given dichlorvos without palm oil treatment compared to rats given dichlorvos and were treated with palm oil (group 3) at $p<0.05$ (Table 4).

Table 1. Determination of minimum dose that caused 100% death (LD_{100}) and mean lethal dose (LD_{50}) for dichlorvos treated rats administered orally

Groups	Dose (mg/kg)	Number of rats	Number of death	Number alive	Average time of death (Minutes)	Dose difference	Mean dead	Dose difference mean dead
1	0	3	0	3	0	0	0	0
2	20	3	0	3	0	20	0	0
3	40	3	0	3	0	20	0	0
4	60	3	1	2	7	20	0.5	10
5*	80 *	3	3	0	5	20	2.0	40
6	100	3	3	0	4	200	3.0	60
								∑110

*Mean dead = Average number of dead animals in 2 successive doses, * = Minimum Dose that caused 100% death (LD_{100})*

Table 2. Renal parameters of rats given 30 mg/kg dichlorvos orally over a period of 24 hours

Parameters	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	Urea (mmol/L)	Creatinine (µmol/L)
Group 1 (n=5) (Negative control)	154.20 ± 2.77^{a}	5.76 ± 1.03^{a}	103.00 ± 1.87^{a}	4.20 ± 0.94^{a}	53.06 ± 10.85^{a}
Group 2 (n=5) (Positive control)	159.80 ± 4.71^{ab}	6.50 ± 0.37^{ab}	106.60 ± 1.52^{ab}	6.88 ± 0.43^{bc}	75.04 ± 5.34^{bc}
Group 3 (n=5) (Treatment)	150.20 ± 4.55^{ab}	4.76 ± 0.15^{ac}	104.20 ± 0.84^{ab}	3.57 ± 1.03^{ad}	58.18 ± 8.49^{ad}
p-value	0.056	0.04	0.07	<0.001	0.004
F-value	2.393	9.368	1.754	4.531	9.087
Remark	NS	S	NS	S	S

PostHoc: Na+ & Cl- : Values with identical superscript (a) in the same column are insignificant when Group 1 and other groups were compared. Again, values with identical superscript (b) in the same column are insignificant when Group 2 was compared with groups 3. K+: Values with identical superscript (a) in the same column are insignificant when Group 1 and other groups were compared. However, values with un-identical superscripts (b, c) in the same column differ significantly when Group 2 and group 3 were compared. Urea & Creatinine: Values with un-identical superscripts (a, b) in the same column differ significantly when Group1 and group 2 were compared. Further, values with un-identical superscripts (c, d) in the same column differ significantly when Group2 and group 3 were compared at P<0.05. NS= Non Significant, S= Significant

Table 3. Renal parameters of rats given 10 mg/kg dichlorvos orally over a period of 14 days

Parameters	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	Urea (mmol/L)	Creatinine (µmol/L)
Group 4 (n=5) (Negative control)	154.20±2.77^{a}	5.76±1.03^{a}	103.00±1.87^{a}	4.20±0.94^{a}	53.06±10.85^{a}
Group 5 (n=5) (Positive control)	152.40±3.13^{ab}	5.52±0.89^{ac}	105.00±3.74^{ab}	5.55±0.44^{ab}	82.86±6.11^{bc}
Group 6 (n=5) (Treatment)	154.40±5.32^{ab}	3.56±0.58^{bd}	105.60±1.82^{ab}	3.33±1.88^{ac}	70.58±4.56^{bc}
p-value	0.681	0.003	0.299	0.045	<0.001
F-value	0.397	10.01	1.337	4.067	14.14
Remark	NS	S	NS	S	S

Post-Hoc: Na+ & Cl- : Values with identical superscript (a) in the same column are insignificant when Group 1 and other groups were compared. Again, values with identical superscript (b) in the same column are insignificant when Group 2 was compared with groups 3. K+: Values with un-identical superscripts (a, b) in the same column differ significantly when Group 1 was compared with other groups. Again, values with un-identical superscripts (c, d) in the same column differ significantly when Group 2 against group 3 were compared. Urea: Values with identical superscript (a) in the same column are insignificant when Group 1 was compared with group 2. However, values with un-identical superscripts (b, c) in the same column differ significantly when Group2 against group 3 were compared. Creatinine: Values with un-identical superscripts (a, b) in the same column differ significantly when Group 1 against other groups were compared. But, values with identical superscript (b) in the same column are insignificant when Group 2 against groups 3 were compared at P<0.05. NS= Non Significant, S= Significant

Table 4. Renal parameters of rats given 10 mg/kg dichlorvos orally over a period of 30 days

Parameters	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	Urea (mmol/L)	Creatinine (µmol/L)
Group 7 (n=5) (Negative control)	153.00±1.87^{a}	5.64±1.13^{a}	105.20±5.26^{a}	3.90±0.44^{a}	56.86±13.16^{a}
Group 8 (n=5) (Positive control)	151.80±6.53^{ab}	2.94±0.50^{bc}	106.20±5.85^{ab}	5.50±0.41^{bc}	76.60±3.47^{bc}
Group 9 (n=5) (Treatment)	148.60±6.23^{ab}	3.50±1.34^{bc}	102.80±8.58^{ab}	3.05±0.48^{bd}	67.40±0.73^{ac}
p-value	0.427	0.004	0.720	<0.001	0.007
F-value	0.913	9.158	0.338	39.503	7.878
Remark	NS	S	NS	S	S

Post-Hoc: Na+ & Cl- : Values with identical superscript (a) in the same column are insignificant when Group 1 was compared with other groups. Again, values with identical superscript (b) in the same column are insignificant when Group 2 against groups 3 were compared. K+ & Creatinine: Values with un-identical superscripts (a, b) in the same column differ significantly when Group 1 was compared with other groups. However, values with identical superscript (c) in the same column are insignificant when Group2 against group 3 were compared. Urea: Values with un-identical superscripts (a, b) in the same column differ significantly when Group1 was compared against group 2. Again, values with un-identical superscripts (c, d) in the same column differ significantly when Group 2 was compared against group 3 at P<0.05. NS= Non Significant, S= Significant

3.3 Histologic Evaluation of Renal Tissues

The histologic findings of the kidneys at the different duration of exposure over periods of 24 hours, 14 days, and 30 days are shown below (Figs. 1- 3).

4. DISCUSSION

The present evaluated the effects of palm oil on renal parameters after dichlorvos exposure over periods of 24 hours, 14 days, and 30 days in albino rats. From our results, it was shown that over the periods of 24 hours, 14 days, and 30 days of exposure of albino rats to dichlorvos and subsequent treatments in some groups with palm oil, there were significant increases seen in potassium, creatinine, and urea in the rats were given dichlorvos without treatment compared to rats that were given dichlorvos and treated with palm oil. However, 30 days of exposure indicated significantly lower values in potassium in dichlorvos treated rats without palm oil compared to rats given palm oil.

The significant increases seen in creatinine and urea across the various phases of treatment could be attributed to nephrotoxic effects of dichlorvos. As reported by Uroko et al. [20], Urea and creatinine are sensitive markers of kidney function especially when the glomeruli are affected. Urea is the end product of protein catabolism, formed by deamination of amino acids in the liver and transported to the kidney where it is excreted as urine. Therefore, increased blood urea concentration is correlated with poor filtrative function of the kidney probably due to loss of glomerular function with a corresponding increase in creatinine. More so, the significant rises seen in the level of creatinine further suggest impairment of the glomerular function and tubular damage of the kidneys. Our finding is in agreement with results obtained

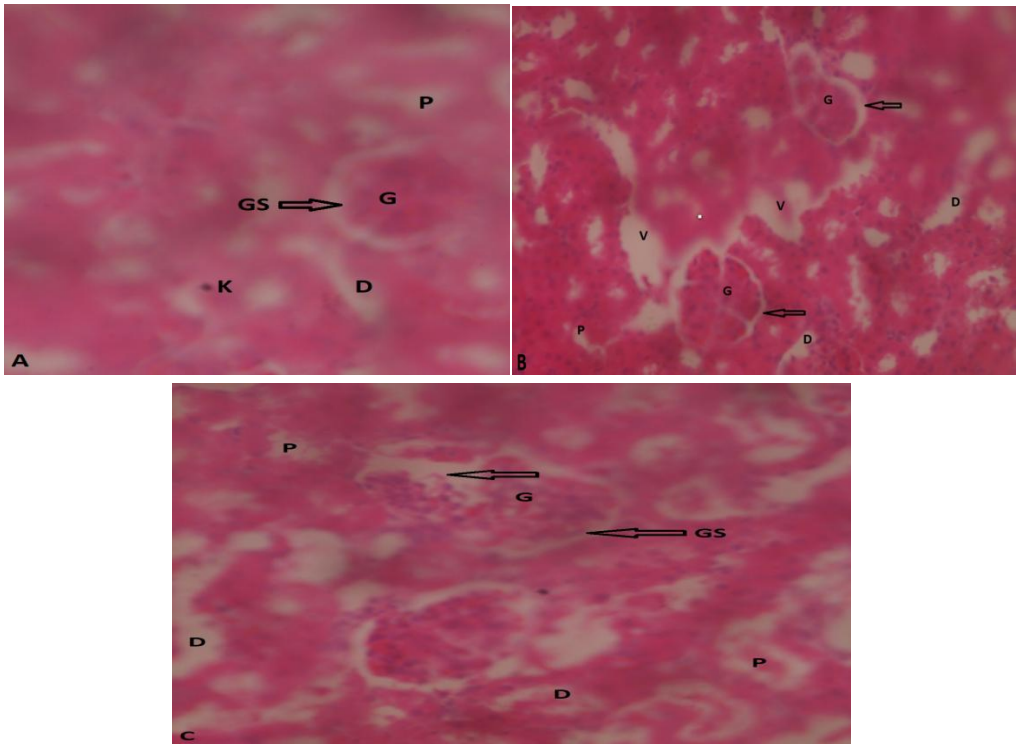

Fig. 1. Histological examination of renal tissue over a period of 24 hours exposure

Plate (A): Negative Control group: Glomerulus (G) and glomerular space (GS), proximal (P) and distal tubules (D) appear normal. Plate (B): Positive Control group: Glomerulus (G) shows distortions with vacuolated areas (arrow) and hypercellurized mesangials cells. The glomerular spaces (GS) were observed to be normal while the proximal (P) and distal tubules (D) showed tubular cast (blockage). Plate (C): Palm oil Treatment group: Hypercellularization of the mesangial cells of the glomeruli (G), normal glomerular spaces with distortions (arrow) were seen, while the proximal (P) and distal tubules (D) were observed to be normal with loss of parenchymal materials owing to vacuolation (V). H&E stain. X400

by Achuba et al. [21]. Achuba et al. [21], reported increase in urea and creatinine level when diesel-contaminated diets was fed to rats. They also mentioned that significantly lower values of urea and creatinine were observed when rats were fed with palm oil as compared to rats given diesel contaminated diets only. Our results concur with the findings of Khan et al. [22]. Khan et al. [22], reported that palm oil have ameliorative nephrotoxic effect against lipid-induced renal injury. However, our finding in the sub-chronic study with respect to urea disagrees with the reports of Agina et al. [23]. Agina et al. [23], reported no difference in urea levels when 4 mg/kg body weight dichlorvos was administered for 28 days. The variation in results could be attributed to the dose of dichlorvos administered. In another related work, Tan et al. [24], reported significant reduction in creatinine level in diabetic nephropathy patients in a clinical trial in Malaysia when palm oil extract (Tocotrienol-rich vitamin E) was used as a supplement at a dose of 200 mg/kg bodyweight twice daily. Tan et al. [24], further reported significant improvement in renal recovery of the diabetic patients with nephropathy.

When electrolytes were evaluated, significant increments were observed in potassium in the dichlorvos treated rats compared to rats treated alongside with palm oil in the acute and sub-acute phases of the study. However, a significant reduction was observed in dichlorvos treated rats compared to rats treated alongside palm oil in the sub-chronic phase. The non-significant difference in sodium as well as the significant fall in potassium in the acute phase in the palm oil treated rats compared to dichlrvos treated rats is also in line with the finding of Imafidon and Okunrobo [25]. Imafidon and Okunrobo [25], reported a significant reduction in potassium level as well as, no significant difference in sodium in rats treated with 10% freshly prepared palm oil for 6 weeks compared to controls. The significant higher values seen in the acute and sub-acute phases could be a result of electrolyte disturbances induced due to metabolic acidosis probably through oxidative stress on the kidneys induced by dichlorvos. The fact that sodium and chloride were not significantly affected (especially chloride), acidosis due to the hyperchloraemic effect is ruled out. The increase in potassium alongside increase in urea and creatinine suggest more of renal derangements affecting predominately the glomeruli thus affecting filtration with minimal tubular derangements affecting re-absorption of substances. However, the significant fall in potassium observed in the sub-chronic study alongside increased urea and creatinine levels could suggest an extension of the nephrotoxic effect of dichlorvos from the glomerular region to the tubular region. The reduction in potassium further suggests renal tubular derangements like tubular acidosis. In tubular acidosis especially distal tubular acidosis, there are loss of electrolytes through the tubules due to inabilities of the tubules (lamina) cells to re-absorb valuable or useful materials such as electrolytes back into the system which is usually accompanied with polyuria and glycosuria. However, ameliorative effects were seen in the potassium levels when palm oil treatments were given to the rats initially given dichlorvos. This perhaps might further imply that the palm oil supplementation could be slightly effective in ameliorating glomerulonephritis with loss of parenchymal materials due to dichlorvos poisoning in a local or emergency setting before medical intervention.

When histological changes in these renal tissues were evaluated, it was also shown that rats were given dichlorvos over the period of 24 hours, 14 days and 30 days indicated several forms of histological changes such as distortion of the glomerulus, vacuolation, and hypercellurized and hypertrophied mesangials cells. In addition, the proximal and distal tubule appears distorted in most cases and in some cases the presence of tubular cast (obstructions) were observed (Fig. 1B, Fig. 2D, and Fig. 3H). On the other hand, rats that were initially given dichlorvos and later treated with palm oil indicated normal glomerulus and glomerular space with hypercellularized mesangial cells (Fig. 1C) while in some instances the glomerulus appears mildly distorted while the proximal and distal tubule appears normal (Fig. 2F). Again, the glomerular space in some cases appears distorted at some point. In addition, normal glomerular and distorted proximal and distal tubules were also seen (Fig. 3I). Generally, the structural and functional changes seen in the palm oil treated rats were much milder compared to the rats given dichlorvos which may probably indicates recovery. The histological results further explain why significant falls in urea, creatinine and potassium were seen in rats treated with palm oil against rats given dichlorvos. Our findings also concur with the reports of Achuba et al. [21]. Achuba et al. [21], reported an improvement in the degenerative changes in rats treated with palm oil compared to the distortions of renal tissue seen when disel-contaminated diets was fed to rats. Our finding also agrees with the reports of Olatunde et al.

[23]. Olatunde et al. [23], reported that DDVP caused significant renal parenchymal loss and thus a reduction (10%) in the maximum glomerular diameter and 18% reduction in the maximum weight of the renal corpuscle when compared with unexposed rats. Olatunde et al. [23], further reported that palm oil treatment significantly improved the parenchymal integrity of the kidney and therefore elevated a maximum glomerular diameter by 23% and maximum width of the renal corpuscle by 20%. Again, Ekpo et al. [26], also documented normal structural integrity viz-a-viz normal weight of the kidneys when rats were fed with 10% freshly prepared palm oil for 90 days against oxidized palm oil. In addition, Khan et al. [22], also documented that tocotrienol extracted from palm oil has ameliorative nephrotoxic effect against lipid-induced acute renal injury.

The ameliorating role played by palm oil could be because palm oil naturally consists of tocopherol and tocotrienol which is known as palm oil tocotrieniol rich fraction (TRF). Palm oil tocotrieniol rich fraction, because of it anti-oxidative properties which act similarly to vitamin E could have been responsible for the effective ameliorating factor caused by various organophosphate compounds including dichlorvos. Moreover, palm oil also contains compounds such as carotenoids, vitamin A, squalene, and coenzyme Q10. These compounds having antioxidants and free radicals scavenging properties in addition to tocotrieniol rich fraction of palm oil could have synergistically ameliorated the damaging effects of dichlorvos-generated free radicals capable of causing toxic effect and renal oxidation stress related damages.

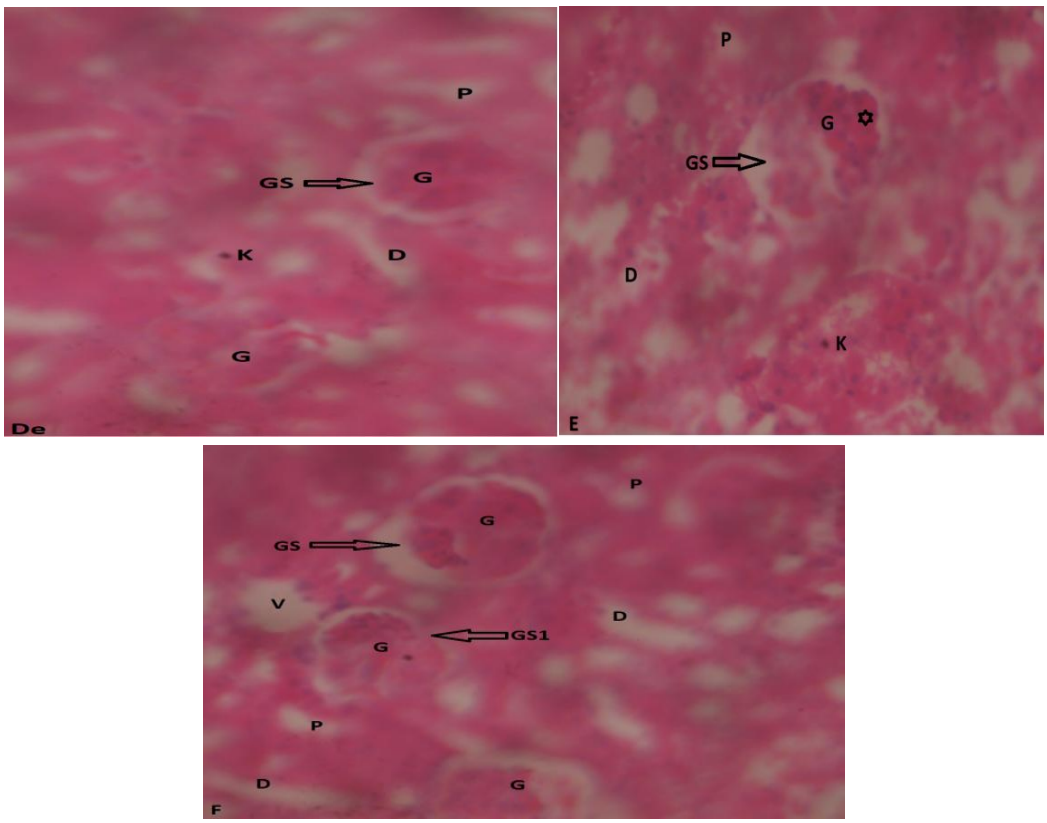

Fig. 2. Histological examination of renal tissue over a period of 14 days exposure

Plate (De): Negative Control group: Glomerulus (G), glomerular space (GS), proximal (P), and distal tubules (D) were observed to be normal. Plate (E): Positive Control group: Glomerulus (G) appears distorted with hypertrophic areas (asterisk) of mesangials cells. Distortions of the glomerular spaces (GS) were also seen. The proximal (P) and distal tubules (D) are normal. Plate (F): Palm oil Treatment group: Slight distortion of the glomerulus (G) were seen. The glomerular spaces appeared dilated (GS) and obstructed (GS1) at some points with presence of mild vacuolation (V) while the proximal (P) and distal tubules (D) were observed to be normal. H&E stain. X400

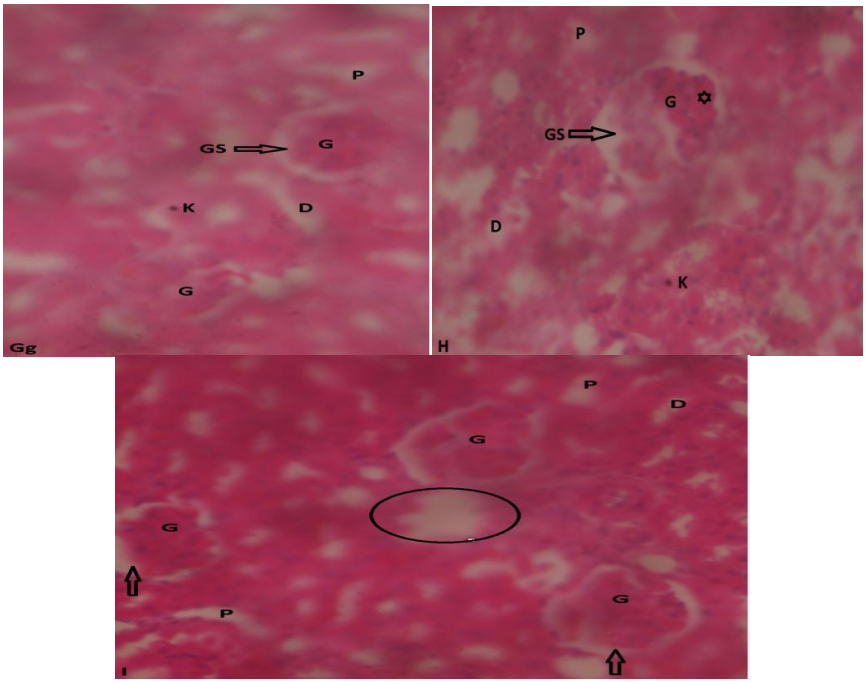

Fig. 3. Histological examination of renal tissue over a period of 30 days exposure

Plate (Gg): Negative control group: Normal structural appearance of the glomerulus (G), glomerular space (GS), proximal (P) and distal tubules (D) were seen. Plate (H): Positive Control group: Glomerulus (G) appears distorted with hypertrophic areas (asterisk) of mesangials cells. Distortions were also seen within the glomerular spaces (GS). Structural distortions of proximal (P) and distal tubules (D) were also observed. Plate (I): Palm oil Treatment group: Normal glomerulus (G) structure was observed. However, distortion of the glomerular spaces (arrows), proximal (P), and distal tubules (D) were seen. Mild loss of parenchymal materials was also observed in the circled area. H&E stain. X400

5. CONCLUSION

Results obtained indicated that palm oil showed a protective effect in ameliorating the nephrotoxicity induced by dichlorvos as shown by the histological examination and decreased values of creatinine and urea as well as potassium in palm oil treated rats.

6. RECOMMENDATION

It is recommended that palm oil be given in case of poisoning with dichlorvos due to its ameliorative nephrotoxic effects. We also recommend further research and verification of the use of palm oil as ameliorating agent in cases of poisoning.

7. LIMITATION OF THE STUDY

The study did not cover a longer duration in order to fully evaluate palm oil supplementation on dichlorvos poisoning or nephrotoxicity on long-term basis.

CONSENT

It is not applicable.

ETHICAL APPROVAL

We hereby declare that the Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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