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Effects of "Reed A Dream" Insecticide on Selected Biomarkers of Kidney, Liver, and Lipid Profile in Wistar Rats

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Article Info	Abstract
Received: 10-04-2022 Revised: .04-06-2022 Accepted: 10-06-2022 Keywords insecticides, kidney, liver, Reed A Dream (RD), toxicity	This study investigated the effects of "Reed A Dream" (RD) insecticide on selected biomarkers of kidney, liver, and lipid profile in Wistar rats. Twenty-five (25) rats of both sexes (weighing 50-114 g) were divided into 5 groups, comprising of control (0.5mL water), N-hexane only (0.5mL), RD(50% vol/vol) + N-hexane, RD(25% vol/vol) + N-hexane, and RD (12.5% vol/vol) + N-hexane, also labelled as groups 1-5 respectively. After 2 weeks of oral daily administration, the animals were sacrificed; blood, liver, kidney, and heart tissues were taken for biochemical and histopathological investigations. A nonsignificant decreased (p \geq 0.05) in plasma concentration of total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) in groups 3-5, a significant decrease (p \leq 0.05) in albumin (groups 3-5); a non-significant increased in creatinine, gamma glutamyltranspeptidase (GGT) in groups 3-4 was observed, as compared with the control group; Moreover, a significant increased (p \leq 0.05) in urea in groups 3-5 when compared with the control group. Histology of the liver tissue (Group 3) revealed a central vein with mild congestion, while kidney tissues (groups 3-5) showed visible congestion of glomerulus, and heart tissue (group 3-5) showed interstitial deposition of collagens, as compared with the control group. RD insecticide caused acute nephrotoxicity and mild hepatotoxicity in Wistar rats on oral exposure.

1. Introduction

The use of insecticides in homes to control crawling and flying insects has been a popular practice for decades. In Africa, where malaria due to mosquito bite is a public health concern, governments, nongovernmental organizations (NGOs), international donors, and individuals have devised means of combating mosquitoes through various programmes. These include the distribution of insecticide treated nets, environmental spraying of insecticides, encouraging communities to clear their surrounding bushes, cleaning water drainage, and the draining of stagnant water that serves as home for mosquitoes. In Nigeria, some families prefer the use of either powdered, liquid, or aerosolized insecticides in their homes. There is an increasing concern over the persistent exposure of human beings to these insecticides, since it can possibly result in insecticide poisoning [1]. According to Ki-Hyun [2], the most severe





form of pesticide poisoning may arise from oral exposure. Oral exposure to insecticides could be either by accident, carelessness, or for intentional reasons [2, 3]. Gilden [4] stated that the most prevalent incidence of unintentional oral intake occurs during the process of moving pesticides from their initially marked vessel to an unlabelled one or during the packaging of foodstuff. In considering intentional ingestion of insecticides, several cases have been reported in Nigeria, for instance, cases where young people have committed suicide bv ingesting insecticide [5, 6]. In addition, children are at risk of insecticide toxicity if it is kept in drinking bottles or after drinking water from an insecticide-tainted bottle [6]. Workers handling insecticides or equipment containing insecticides for agricultural application can also ingest them if they do not wash their hands properly before eating, as reported by the United State Environmental Protection Agency (USEPA) [7].

Furthermore, in animal studies, Albert et al. [8] reported a progressive increase in mortality of albino rats exposed to "Raid" aerosolized insecticide as the concentration the insecticide increases. They also discovered that Raid insecticide may bio-accumulate in tissues in the following sequence: lipid > muscle > liver > brain. According to Albert et al. [8], the indicators of toxicity indicated no significant impact in the brain; however, substantial reduction was reported in glucose-6-phosphatase and lactic acid dehydrogenase levels in the muscles and liver. The authors concluded that Raid insecticide can inhibit several critical metabolic processes as a result of its constituents accumulating in the tissues.

The severity of effects from exposure to insecticides depends on concentration,

route of exposure, ease of absorption, metabolic fate, and persistency of exposure $[\underline{2}, \underline{6}]$. Although, there are three well-established major routes of human exposure to insecticides including skin, oral ingestion, and inhalation. The effects may be acute, showing immediately after the exposure or chronic, showing only after prolonged use [7]. In Nigeria, some of the major factors leading to a preponderance of adverse health impacts associated with insecticides use include the fact that the most deadly insecticides are used because they are cheaper than newer and safer insecticides. Moreover, legislation and the lack poor of enforcement of available legislation, improper and unsafe application of insecticides. and lack of adequate information, knowledge, and awareness of the inherent danger of persistent exposure to insecticides also create adverse health outcomes. These factors demonstrated the need for more studies which may guide the regulatory authorities in the country to formulate better policies that can properly regulate the sale and use of insecticides in homes.

The insecticide "Reed A Dream" (RD), manufactured by Yuanlong (Fujian) Commodities. China and marketed in Nigeria by Cu-Bas International Nigeria (Limited) is used in most parts of Nigeria to control insects including mosquitoes, cockroaches, flies, and other crawling insects, especially in major cities of the country. However, there is a lack of documented studies regarding the toxicity caused by this insecticide on direct exposure or through contamination of food in homes. The effects of persistent exposure to this insecticide can be more severe in children, who may easily ingest it when not properly stored or when used indiscriminately in homes. This is a major

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concern as insecticides reportedly have adverse effects on human health [1]. Moreover, there are rising number of cases of suicide committed by the ingestion of insecticides among teenagers in some cities in Nigeria. Thus, there is a need for independent studies to ascertain the threats posed by this insecticide when used in homes. Hence, this study investigated the effect of RD insecticide on selected biomarkers of kidney, liver, and lipid profile in Wistar rats.

2. Materials and methods

Test facility

Twenty-five Wistar rats of both sexes weighing 50-114 g were divided into five groups of five rats each and maintained at the Department of Biochemistry, University of Port Harcourt animal breeding facility. The rats were fed standard rat food and given free access to water. Protocols defining animal usage stated in the World Medical Association (WMA) [9] handbook regarding the care and use of laboratory animals were followed.

Source of Animals and Insecticide

The Department of Biochemistry, University of Port Harcourt animal breeding facility provided the Wistar rats used for this study. Commercial grade RD aerosolized insecticide was purchased from a grocery store in Choba, Rivers State, Nigeria.

Test procedure

The stock was prepared using 300ml of RD aerosolized insecticide which was dissolved in 300ml N-hexane to give 1:1 dilution (50% vol/vol). From the initial dilution, a 1:2 (25% vol/vol) and 1:4 (12.5% vol/vol) dilution of RD to N-hexane were prepared and orally

administered to animals. Animals in groups 1-5 were given water and rats chow only, N-hexane only, 50% vol/vol, 25% vol/vol, and 12.5 % vol/vol RD insecticide, respectively.

Analytical procedure

The plasma obtained from different treatments after sacrifice was subjected to biochemical analysis using Spectrumlab 23A. Urea and creatinine concentrations were determined following the method described by Fawcett and Soctt [10] and Spencer [11] respectively. Magnesium and Potassium ion, albumin, total bilirubin, High Density Lipoprotein-Cholesterol (HDL-C) and triglyceride determination were by Tietz [12] method while Hochstrasser and Skeggs [13] method was determine used chloride ion to concentration. Trinder's approach [14] was used to detect sodium ion concentration. The concentrations of total protein and bilirubin were determined using Nowotny's technique [15]. The activities of transaminases (ALT and AST) were determined using the Reitman and Frankel method [16]. ALP, GGT, and total cholesterol were determined using the methods suggested by Plummer [17], Theodorsen and Stromme [18], and Allain et al. [19] methods respectively. The approach of Friedewald et al. [20] was used to estimate low density lipoproteincholesterol (LDL-C). To avoid postmortem deterioration, liver, kidney, and heart tissues were obtained immediately sacrifice for histopathological after examination using the procedure stated by Windsor [21].

Data analysis

Statistical analysis was performed using SPSS version 20.0 (IBM, U.S.A). The data were analysed using one-way analysis of variance (ANOVA) and significant

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differences were determined using the least significant difference (LSD) for the post hoc test of multiple comparisons at $p \le 0.05$. The mean and standard error of the mean (M \pm SEM) were used to report the results.

3. Results

Table 1 shows a significant increase ($p \le 0.05$) in plasma urea concentration in RD (50 percent vol/vol) + N-hexane, RD (25 percent vol/vol) + N-hexane, and RD (12.5% vol/vol) + N-hexane test groups as compared to the control group. It also shows a significant increase (p 0.05) in plasma creatinine concentration in RD (12.5% vol/vol) + N-hexane test group when compared with the control group. Electrolyte concentrations (Mg²⁺, Cl⁻, and Na⁺) are also increased, albeit non-significantly.

The amount of total protein (Table 2) decreased ($p \ge 0.05$) in RD (50% vol/vol) + N-hexane, RD (25% vol/vol) + N-hexane, and RD (12.5% vol/vol) + N-hexane test groups. A significant decrease in albumin concentration in RD (50% vol/vol) + Nhexane, RD (25% vol/vol) + N-hexane, and RD (12.5% vol/vol) + N-hexane test groups was also observed, contrasted by a significant increase ($p \le 0.05$) in total bilirubin concentration in RD (50% vol/vol) + N-hexane, RD (25% vol/vol) + N-hexane, and RD (12.5% vol/vol) + N- hexane test groups as compared with the control group. AST, ALT, and ALP activities of RD (50% vol/vol) + N-hexane, RD (25% vol/vol) + N-hexane, and RD (12.5% vol/vol) + N-hexane test groups showed a non-significant decrease ($p \ge 0.05$) compared with the control group. However, GGT activity non-significantly increased in RD (50% vol/vol) + N-hexane and RD (25% vol/vol) + N-hexane test groups, while a non-significant decrease was observed in RD (12.5% vol/vol) + N-hexane test groups as compared with the control group.

Table 3 reveals a non-significant increase $(p \ge 0.05)$ in HDL-C levels in RD (50% vol/vol) + N-hexane and RD (25% vol/vol) + N-hexane test groups. It also depicts a non-significant decrease $(p \ge 0.05)$ in LDL-C, triglyceride, and cholesterol levels in RD (50% vol/vol) + N-hexane, RD (25% vol/vol) + N-hexane, and RD (12.5% vol/vol) + N-hexane test groups in relation to the control group.

The effects of RD insecticide on the liver, heart, and kidney weight of Wistar albino rats (Table 4) include a significant increase ($p \le 0.05$) in liver weight in RD (50% vol/vol) + N-hexane (1:1 dilution) test group, as opposed to a significant decrease ($p \le 0.05$) in kidney and heart weight in RD (12.5% vol/vol) + N-hexane (1:4 dilution) test group compared with the control group.

Table 1. Plasma Concentration of Urea, Creatinine, Mg^{2+} , K^+ , Cl^- and Na^+ in Wistar Albino Rats Orally Exposed to RD Insecticide

Treatments	Urea (mmol/L)	Creatinine (mmol/L)	Mg ²⁺ (g/dL)	K ⁺ (mmol/L)	Cl - (mmol/L)	Na ⁺ (mmol/L)
Control (Normal food and	1.79±0.21	116.89±5.77	2.22±0.11	5.49±0.30	97.54±5.44	153.47±1.25
Water) N-Hexane Only (0.5	4.90±1.20	132.70±6.09	2.40±0.12	5.68±0.70	99.79±8.81	154.04±0.32

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Treatments	Urea (mmol/L)	Creatinine (mmol/L)	Mg ²⁺ (g/dL)	K ⁺ (mmol/L)	Cl ⁻ (mmol/L)	Na⁺ (mmol/L)
mL) RD (50% vol/vol) + N-Hexane	4.17±0.44ª	138.56±12.35	2.41±0.16	5.12±0.93	105.78±8.92	153.11±2.14
(1:1 dilution) RD (25% vol/vol) + N-Hexane	4.73±1.20ª	142.33±12.78	2.14±0.09	4.87±0.54	105.94±7.55	160.49±4.88
(1:2 dilution) RD (12.5% vol/vol) + N-Hexane	3.36±0.87	154.75±10.53ª	2.36±0.08	5.61±0.63	101.27±7.97	153.81±3.30
(1:4 dilution)						

Values are reported as Mean \pm Standard Error of Mean (M \pm SEM) (n =5), ^a superscript indicates statistical significance difference (p \leq 0.05) compared with control and between treatment groups.

Table 2. Plasma Concentration of Total Protein, Bilirubin, Albumin, AST, ALT, ALP, and GGT in Wistar Albino Rats Orally Exposed to RD Insecticide

Treatmen ts	Total Protein (g/L)	Albumin (g/L)	Total Bilirubin (mmol/L)	AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L)
Control (Normal food & Water)	61.06±12. 01	41.74±2.55	2.77±0.79	147.11±8.64	46.89±13. 18	43.26±7.9 6	18.05±1. 37
N-Hexane Only (0.5 ml)	37.42±13. 56	25.19±4.32	4.32±1.24	138.32±3.20	21.51±1.6 6	25.28±2.3 5	17.70±0. 85
RD (50% vol/vol) + N-Hexane (1:1 dilution)	54.95±6.1 3	31.98±2.11 ^a	5.05±1.46ª	130.78±10.6 3	22.60±2.4 3ª	25.73±4.6 8ª	20.88±2. 40
RD (25% vol/vol) + N-Hexane (1:2 dilution)	34.01±5.9 9	26.47±2.94 _{a,b}	13.14±5.68 _{a,b}	100.73±3.45 _{a,b}	22.15±2.8 1ª	26.71±5.4 4ª	18.39±3 12
RD (12.5% vol/vol) + N-Hexane (1:4 dilution)	40.25±8.8 9	35.93±3.39 b	8.33±2.58ª	111.09±8.39 _{a,b}	22.41±2.2 7ª	23.94±3.3 5ª	16.42±1 06

Values are reported as Mean \pm Standard Error of Mean (M \pm SEM) (n =5). ^{a,b} superscripts indicate statistical significance difference (p \leq 0.05) compared with control and between treatment groups.

Treatments	HDL-C	LDL-C	Triglyceride	Total
	(mmol/L)	(mmol/L)	(mmol/L)	Cholesterol
				(mmol/L)
Control (Normal food and Water)	1.78±0.06	0.73±0.13	1.63±0.09	3.25±0.10
N-Hexane only (0.5 mL)	1.54±0.17	0.58±0.17	1.13±0.17	2.64±0.31
RD (50% vol/vol) + N- Hexane (1:1 dilution)	1.82±0.48	0.54±0.12	1.41±0.43	3.01±0.75
RD (25% vol/vol) + N- Hexane (1:2 dilution)	1.87±0.44	0.36±0.13	1.18±0.27	2.70±0.45
RD (12.5% vol/vol) + N- Hexane (1:4 dilution)	1.78±0.17	0.48±0.23	1.10±0.15	2.74±0.39

Table 3. Lipid Profile of Wistar Albino Rats Orally Exposed to RD Insecticide

Values are reported as Mean \pm Standard Error of Mean (M \pm SEM), n = 5.

Table 4: Weight of Liver, Heart, and Kidney of Wistar Albino Rats Orally Exposed to RD Insecticide

Treatments	Liver (g)	Kidney (g)	Heart (g)
Control (Normal and Water)	4.88±0.06	0.44±0.02	0.52±0.06
N-Hexane only (0.5 mL)	4.60±0.31	0.54 ± 0.02	0.46 ± 0.02
RD (50% vol/vol) + N-hexane (1:1 dilution)	5.12±0.39 ^a	$0.54{\pm}0.04^{a,b}$	0.42±0.05
RD (25% vol/vol) + N-hexane (1:2 dilution)	4.70±0.18	$0.32 \pm 0.05^{a,b,c}$	$0.34{\pm}0.04^{a}$
RD (12.5% vol/vol) + N-hexane (1:4 dilution)	4.22±0.39 ^a	0.48±0.02°	0.46±0.06

Values are reported as Mean \pm Standard Error of Mean (M \pm SEM), n =5. ^{a,b,c} superscripts indicate statistical significance difference (p \leq 0.05) compared with control and between treatment groups.

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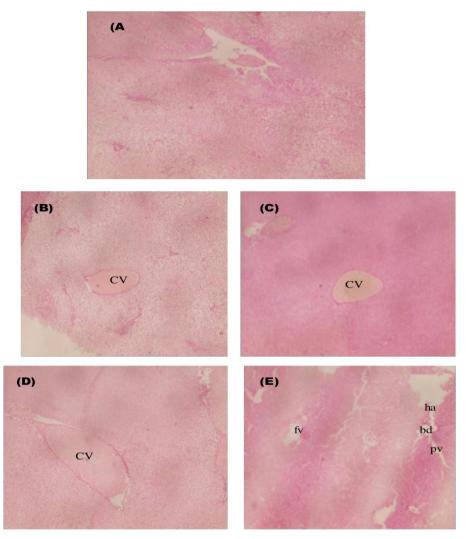


Plate 1: Photomicrograph liver sections. (A) Control (normal food and Water) (B) N-Hexane Only (0.5 ml). (C) RD (50% vol/vol) + N-Hexane. (D) RD (25% vol/vol) + N-Hexane and (E) RD(12.5% vol/vol) + N-Hexane (H&E X100).

Plates 1(A) and (B) show the central vein (CV) of normal hepatocytes architecture.

Plate 1(C) shows central vein with mild liver congestion. Plate 1(D) indicates severe liver congestion and an enlarged central vein. Plate 1(E) shows hepatic artery (ha), bile duct (bd), portal vein (pv), and hepatoccytes (ha) with enlarged fat vacuoles (fv).



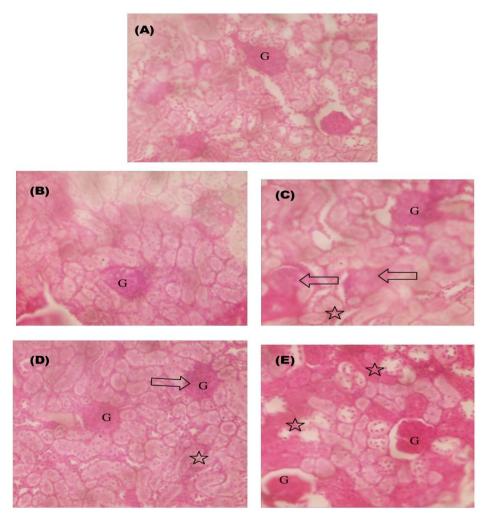


Plate 2: Photomicrograph of kidney sections. (A) Control (normal food and water) (B) N-Hexane only (0.5 ml) (C) RD(50% vol/vol) + N-Hexane (D) RD (25% vol/vol) + N-Hexane and (E) RD (12.5% vol/vol) + N-Hexane (H&E X400).

Plates 2 (A) and (B) show the normal architecture of glomerulus (G), tubules,

and blood vessels of kidney histology. Arrows in Plate 2 (C), (D), and (E) indicate visible congestion of glomerulus, while asterisks (*) show the areas of tubular necrosis.

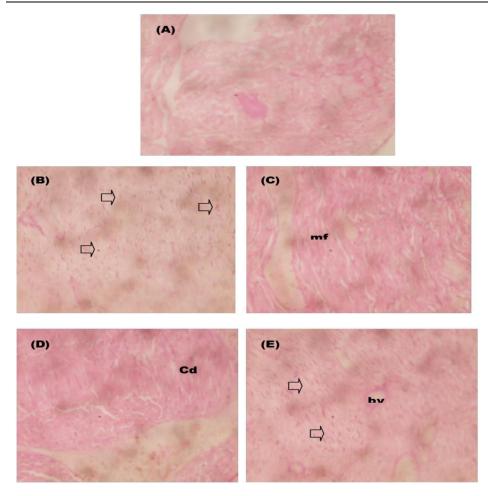


Plate 3: Photomicrograph of t heart sections. (A) Control (normal food and water) (B) N-Hexane only (0.5 ml) (C) RD(50% vol/vol) + N-Hexane (D) RD(25% vol/vol) + N-Hexane and (E) RD(12.5% vol/vol) + N-Hexane (H&E X400).

Discussion

Insecticides are insect-controlling agents that are either chemical or biological in nature [1]. Pyrethroid insecticides are synthetic organic chemicals derived from chrysanthemum flowers and widely used as both domestic and commercial Arrows in plates 3 (A) and (B) show the nucleus of normal heart histology. Arrows in Plate 3 (C) show myofibres. Plate 3 (D) shows interstitial deposition of collagens (cd). Arrows in Plate 3(E) show nucleus and blood vessels (bv).

pesticides [22]. According to the manufacturer, active ingredients of RD aerosolised insecticide are d-t-tetramethrin (0.2%), d-t-Phenothrin (0.2%), and d-t-allethrin (0.2%). These are classified as pyrethroids [23].

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The effects of exposure to pyrethroids in adult human beings have been reported by the United State Centre for Disease Control (CDC) [24]. It results in skin irritation. dizziness, twitching, and nervous disorders. However, dose range at which symptoms occur differs for different types of pyrethroids. Moreover, exposure to pyrethroid-based insecticide was also reported in several animal studies [25, 26]. It was observed to cause changes in physiological activities of the exposed animals. Donald and Michael [26] reported that these changes affected protein. important enzymes. other and biomolecules in several animals.

The results of our investigation (Table 1) demonstrated significant elevation of plasma urea in test groups, similar to the findings of Kingsley et al. [27]. They found that a substantial increase in urea concentrations in Wistar rats was subjected to dichlorvos-based pesticide preparations in south-eastern Nigeria. Although poor urea clearance alone may not be a definitive indicator of abnormal glomerular filtration rate. since overproduction can be influenced by a variety of non-renal variables, such as nutrition and urea cycle enzymes [28]. Additionally, elevated urea levels have been found to develop during late pregnancy [29] or as a result of consuming a high-protein diet [28]. Our findings also revealed a significant elevation in plasma creatinine concentration in test groups, compared to the control group, in a manner that conforms to the findings of Kingsley et al. [27]. Creatinine is a derivative of creatinine-phosphate in muscles [28, 29] and it is fairly constant. It is a definitive indicator of renal function [29]. The compound is cleared from blood circulation by the kidneys [30]. Elevated creatinine levels found in this study depict poor clearance by the kidneys, implying that RD insecticide may have caused impaired kidney function.

Furthermore, our study found a nonsignificant increase in electrolytes (Mg²⁺, Cl⁻, and Na⁺) concentrations in test groups, which is in line with the findings of Malekirad et al. [31]. They reported that individuals directly exposed to a wide range of insecticides have elevated urea, creatinine, electrolytes, and albumin levels. well plasma as as aminotransferases. Tierney et al. [32] described electrolytes as positively and negatively charged ions present in cells and extracellular fluids, such as blood plasma. Mg²⁺, Cl⁻, and Na⁺ elevations seen in this study also indicate poor renal function, since these ions are measured to assess renal, endocrine, and acid-base balance. They are components of the general metabolic processes as well [33].

Total protein in test groups (Table 2) showed a non-significant decrease, similar to a report by Pieper et al. [33], which may imply damage to hepatocytes membrane by RD active compounds, thereby interfering with protein synthesis in the liver. Albumin concentration in test groups was significantly increased in conformity with Green and Flamm [34]. Albumin was reported by Green and Flamm [34] to be a biomarker of synthetic function and it is a valuable guide to the severity of chronic liver disease. This present study finding is also in line with the finding of Malekirad et al. [31], who reported a significant increase in albumin in people that are occupationally exposed to insecticides.

Total bilirubin concentrations were found to be significantly higher in test groups. This rise might be attributed to hepatocyte dysfunction, blockage of biliary excretion into the duodenum in haemolysis, and

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errors in hepatic absorption or conjugation of bilirubin pigment seen in Gilbert's disease, as described by Mansour et al. [35]. The activities of AST, ALT, and ALP did not significantly increase in test groups. However, GGT activity was nonsignificantly elevated in test groups. GGT is a microsomal enzyme found in many organs (including the liver) and its activity can be induced by pharmaceuticals or other xenobiotics [29]. If ALP is normal, then elevated GGT level is a useful guide to hepatocytes toxicants [29].

Our findings regarding lipid profile (Table 3) showed a non-significant increase in HDL-C levels in test groups, as well as a non-significant decrease in LDL-C, triglyceride. and levels cholesterol compared with the control group. These findings suggest that RD insecticide may not have a significant influence on lipid profile in animals. Animal nutrition may have contributed to the non-significant rise in HDL-C seen in this study. HDL is produced in the liver, the intestines, and during the lipolysis of triglyceride-rich lipoproteins [36, 37]. It is measured in clinical chemistry by its cholesterol level (HDL-C). High HDL-C plasma concentrations have been linked to a decreased risk of cardiovascular diseases [38, 39]. Pamela et al. [38] stated that deficiencies or imbalances of lipid metabolism can lead to atherosclerosis and obesity.

There was also a significant increase in liver weight in RD (50% vol/vol) + Nhexane (1:1 dilution) test group, contrasted by a significant decrease in kidney and heart weight in RD (12.5% vol/vol) + Nhexane (1:4 dilution) test group compared with the control group. Mansour et al. [35] reported similar findings with five different insecticides (abamectin, carbosulfan, fenpropathrin, methomyl, and profenofos). Jayusman et al. [40] also reported on the effects of sub-lethal dosages of the insecticides phorate and fenitrothion in animal tests, suggesting that RD pesticide has a harmful effect on important internal organs.

The photomicrograph results in plates 1-3 showed histological changes similar to the findings of Idowu et al. [41], corroborating the biochemical results discussed above. The reported results also align with Mansour et al. [35] report of degenerative changes and granularity of hepatocytes with Kupffer cell activation, shrinking Bowman's capsule, degenerative changes in epithelium lining renal tubules, and necrotic effects associated with the desquamation of epithelium lining tubules in the liver and kidney tissues of rats exposed to insecticide preparations.

Conclusion

The current study established that "Reed A Dream" insecticide causes acute nephrotoxicity and mild hepatotoxicity in Wistar rats on oral exposure. Therefore, whenever the need arises to use this insecticide in homes to control insects, precaution must be emphasized to avoid oral ingestion.

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