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# Assessment of the Ameliorative Potential of Water-Based Leaf Extracts from *Talinum triangulare*, *Vernonia amygdalina*, and *Ocimum gratissimum* on Liver Damage Caused by Dichlorvos in male wistar Rats

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Abstract: The increasing human exposure to dichlorvos (DDVP) and its toxicity is a growing public health concern. This work was aimed at studying the therapeutic effect of Vernonia amygdalina, Ocimum gratissimum, Talinum triangulare extracts against DDVP induced hepatotoxicity in rats. Total of 50 male adult rats of average weight 272g were placed in ten groups (n=5) and orally administered normal saline (normal control), 8.0mg/kg body weight DDVP (positive control), 8.0mg/kg body weight DDVP + 200mg/kg and 400mg/kg body weight of the plant extracts and 20 mg/kg body weight and 40 mg/kg bodyweight of vitamin C for 28 days. DDVP significantly (P<0.05) increased Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) and Gamma glutamyl transferase (GGT) when compared with normal control. Treatment with aqueous leaf extracts of Vernonia amygdalina, Ocimum gratissimum, Talinum triangulare significantly reversed the distortions in the values of biochemical parameters when compared with positive control. Photomicrographs obtained from histological examination of liver tissues corroborate these findings. Aqueous leaf extracts of Vernonia amygdalina, Ocimum gratissimum, Talinum triangulare may have therapeutic potentials to DDVP induced hepatotoxicity in rats.

**Keyword:** DDVP, hepatotoxicity, *Vernonia amygdalina, Ocimum gratissimum, Talinum triangulare.* 

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# **INTRODUCTION**

Dichlorvos also known as 2, 2-dichlorovinyl dimethyl phosphate (DDVP) is a very evaporative organophosphate insecticide, called Ota-piapia in South-East Nigeria, vended as sniper in Nigeria (Owoeye et al., 2012; and Okoroiwu et al., 2018). Surveys have shown that it is the most commonly used organophosphate insecticide in Nigeria and other developing countries (Binukumar and Gill, 2010; and Dhanajayan et al., 2020). DDVP is employed as a domestic and farm insecticide to get rid of mites and insect pests of plants and animals (Celik, 2008; Ogutcu, 2008; Das, 2013; and Dhan Dhanajayan et al., 2020). It has molecular formula C<sub>4</sub>H<sub>7</sub>CL<sub>2</sub>O<sub>4</sub>P, vapor pressure of 1.2 X 10<sup>-2</sup>mmHg at 20°C, density of 1.415g/ml at 25°C, boiling point 74.1°C and molecular weight of 220.98g/mol. Dichlorvos exposure may happen via food, air, or water because it can efficiently be absorbed through these several means

(Raheja and Gill 2002; Okoroiwu et al., 2018; and Anderson et al., 2023). It has been well documented that acute and chronic vulnerability of rodents and other animal species to DDVP has resulted in hepatic, nephrotic, cardiologic, neurologic, genotoxic, carcinogenic, immunologic, metabolic, reproductive, respiratory, dermal and other systemic toxicities and even death (Binukumar et al., 2010; Okoroiwu et al., 2018; Izah and Richard, 2020). It has also been showed that the major poisonous effect of DDVP is due to its inhibition of acetylcholinesterase activity, causing irreparable injury to DNA and membrane lipid peroxidation (Ajiboye, 2010; Antonijevic et al., 2016; Mostafalou et al., 2017; Aminu et al., 2018; Imam et al., 2018; and Enve et al., 2021).

Africa naturally has various herbs with nutritional and medicinal value. In developing countries like Nigeria, over 80% of individuals depend on

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medicinal herbs in folk medicine practices to manage or cure different diseases (Fokunang et al., 2011 and Salmeron et al., 2020). These medicinal herbs also called medicinal plants contain phytochemicals that possess therapeutic abilities that are beneficial to humans and other animals. For several years, these plants with healing abilities have been employed in natural medicine practices for disease treatment and prevention, and also as precursors for drug production (Salmeron et al., 2020). The search for drugs that are more effective, inexpensive and without adverse reactions is the focus of most laboratories around the world, and there has been restored concern in medicinal herbs in the late years as they seem otherwise effective without serious adverse reactions from various studies reported (Ladeji et al., 2003 & Chebaibi et al., 2020). The development of herb derived medications in present-day medicine has been linked to the utilization of phytochemicals extracted from medicinal plants that possess therapeutic potentials, and have been employed in indigenous medicine practices to restore health (Sandberg et al., 2001; Igoli et al., 2003; Gershenzon et al., 2022). Vernonia amygdalina (Bitter leaf), Ocimum gratissimum (Scent leaf), Talinum triangulare (water leaf) among others, are medicinal plants traditionally employed in the management and prevention of numerous ailments in indigenous medicine practices (Ladeji et al., 2003; Suba et al., 2004; Tiamiyu et al., 2019; Akara et al., 2021; and Henrrietal et al., 2021). The therapeutic effectiveness of these plants has been traced to its content of some phytonutrients, used by plants as frontline defenses against diseases. These phytonutrients from various studies, are present in the leaves, stems, barks and roots plants. Some of these well documented of phytochemicals in the literature are flavonoids. phenolics, alkaloids, phlobatannin, saponins, tannins, bitters, cardiac glycosides, cyanogenic glycosides, phenols, minerals, polysaccharides, proanthocyanins, vitamins and volatile oils (Kennedy et al., 2011; and Gershenzon et al., 2022). Investigations on some of these medicinal plants also showed that, the major therapeutic potency of these phytonutrients were detoxifying agents, antioxidants, neuropharmacological agents, anti-cancer agents and immunity-potentiating agents (Kennedy et al., 2011; Agunloye et al., 2018; and Ojha et al., 2020).

The aim of this research work was to assess the the ameliorative potential of *Vernonia amygdalina*, *Ocimum gratissimum*, *Talinum triangulare* leaf extracts against Dichlorvos induced liver toxicity in male Wistar rats.

## **MATERIALS AND METHOD**

#### Plant Materials/preparation of extracts

Fresh leaves of *Vernonia amygdalina*, *Ocimum gratissimum*, and *Talinum triangulare* used in this study were collected from Amassoma, Southern Ijaw Local Government Area of Bayelsa State, Nigeria. They were identified by Professor Kola Ajibesin, Department of

Pharmacognosy, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

The fresh leaves of the experimental plants were rinsed with clean water in order to get rid of dust and dirt, and then air dried by constantly exposing the leaves to air, and turning the leaves at intervals to avert fungal growth. The leaves of Vernonia amygdalina and Ocimum gratissimum (Scent leaf) were dried completely in 13 days while the leaves of Talinum triangulare were completely dried in 33 days. The dried leaves of the experimental plant materials were milled using an electric blender, and 250g of each of the milled plant materials was soaked in one (1) litre of distilled water for three (3) days, with intermittent stirring. Thereafter, filtration was done to obtain the filtrate using a sterile cheese cloth. The filtrate was then evaporated in a rotary evaporator to obtain a thick paste, which was further dried in a freeze dryer and then kept in the refrigerator until used (Aiwaguore et al., 2019).

#### **Experimental Animals**

In total, 50 adult-male Wistar rats whose mean weight was 272 g were obtained and utilized in this investigation. The rats were obtained from The Department of Pharmacology, Faculty of Basic Clinical Medicine, University of Portharcourt, Rivers State, Nigeria and used for the investigation. They were housed in humane, well-ventilated cages in the animal house of the Department of Pharmacology, Faculty of Basic Clinical Sciences, College of Health Sciences, Niger Delta University. They were provided a standard diet and water ad *libitum*. Strict adherence to standard norms was maintained when caring for and using these animals.

#### **Chemicals/Reagents**

Dichlorvos (DDVP, 100% solution with 100g per liter of 2, 2-dichlorovinyl-dimethyl phosphate commonly known as sniper (commercial grade), was bought from an agricultural chemical supplier in Amassoma, Bayelsa State, Nigeria, and used in this study. The biochemical kits for ALT, AST, ALP, and GGT used in the investigation were sourced from Randox<sup>™</sup> Laboratories Ltd., United Kingdom.

#### **Experimental Procedure**

Fifty adult-male wistar rats were randomly divided into 10 groups of 5 and were treated as follows for 28 days:

- Group 1 (Normal Control 1): 1ml/kg body weight normal saline only.
- Group 2(Positive Control 2): 8.0mg/kg body weight DDVP only.
- Group 3:8.0mg/kg body weight DDVP + 200mg/kg body weight *Vernonia amygdalina* (bitter leaf) extract
- Group 4:8.0mg/kg body weight DDVP + 400mg/kg body weight *Vernonia amygdalina* (bitter leaf) extract

Group 5:8.0mg/kg body weight DDVP + 200mg/kg body weight *Ocimum gratissimum* (scent leaf) extract

Group 6:8.0mg/kg body weight DDVP + 400mg/kg body weight *Ocimum gratissimum* (scent leaf) extract

Group 7:8.0mg/kg body weight DDVP + 200mg/kg body weight *Talinum triangulare* (water leaf) extract

Group 8:8.0mg/kg body weight DDVP + 400mg/kg body weight *Talinum triangulare* (water leaf) extract

Group 9: (Standard control 1): 8.0mg/kg body weight DDVP + 20mg/kg body weight Vitamin C Group 10: (Standard control 2): 8.0mg/kg body weight DDVP + 40mg/kg body weight Vitamin C.

All administrations were per oral and the doses of the extracts used were derived from a study previously conducted to determine the lethal dose (LD50) of the three plants.

#### **Collection of Samples**

Blood samples were collected via submandibular vein puncture. Blood samples were taken from the sub-mandibular vein of the Wistar albino rats on days 0, 1, 7, 14 and 21. They were kept to stand for 30 minutes in order to obtain serum for the estimation of biochemical parameters. On day 28, all the Wistar albino rats were sacrificed after anesthesia with the aid of chloroform and blood samples, obtained via heart puncture was collected and utilized for biochemical parameter evaluation; additionally, the liver of the Wistar rats was taken for histopathological examinations.

#### **Biochemical Assays**

All liver marker enzyme activities were assayed following instructions in the biochemical kit (Randox brand) leaflets.

#### **Histological Preparation and Analysis**

Each Wistar albino rat was euthanized, liver removed, and it was then perfused with saline solution. Samples were obtained from the liver, stored in a 10% neutral buffered saline solution, and ready for histological examination. Hematoxylin and eosin (H and E) staining, sectioning and wax embedding were the steps involved in this process to find any histological variations or alterations, such as lesions or injuries, in each section, the stained sections were then inspected under a microscope at magnifications of X100 and X400 (Mehranjani *et al.*, 2009).

#### **Statistical Analysis**

Data were put through analysis using one-way analysis of variance (ANOVA) followed by group comparisons conducted using post-hoc Tukey's test with the aid of the Statistical Package for the Social Sciences (SPSS) Version 20, IBM, USA. All values were presented as the mean  $\pm$  standard error of the mean (S.E.M.) P value of less than 0.05 (P < 0.05) was considered statistically significant.

# **R**ESULTS

Results of study in treated albino rats (8.0mg/kg body weight DDVP) administered plant extracts and vitamin C are shown in the figures and plates below.

The data on serum ALT activities (U/L) of controls and DDVP exposed rats treated with aqueous leaf extracts of *V.A.,O.G.,T.T* and Vitamin C for 28 days are presented in Fig 1.

The study's findings demonstrated that, in contrast to the normal control group, the experimental rats' exposure to 8.0 mg/kg body weight DDVP (positive control) considerably (P<0.05) raised their serum ALT activity. When contrasted to the positive control group (8.0 mg/kg body weight DDVP only), treatment with 200 mg/kg body weight and 400 mg/kg body weight of aqueous leaf extracts of V.A., O.G., and T.T.; and 20 mg/kg body weight and 40 mg/kg body weight vitamin C, respectively, significantly decreased the serum ALT activities in a dose-dependent way.

The data on serum AST activities (U/L) of controls and DDVP exposed rats treated with aqueous leaf extracts of *V.A.*, *O.G.*, *T.T* and Vitamin C for 28 days are presented in Fig 2.

The study's findings demonstrated that in contrast to the normal control group, the experimental rats' exposure to 8.0 mg/kg body weight DDVP (positive control) considerably (P<0.05) raised their serum AST activity. When contrasted with the positive control group (8.0 mg/kg body weight DDVP only), treatment with 200 mg/kg body weight and 400 mg/kg body weight of aqueous leaf extracts of V.A., O.G., and T.T.; and 20 mg/kg body weight and 40 mg/kg body weight vitamin C, respectively, significantly decreased the serum AST activities in a dose-dependent way.

The data on serum ALP activities (U/L) of controls and DDVP exposed rats treated with aqueous leaf extracts of *V.A., O.G., T.T* and Vitamin C for 28 days are presented in Fig 3.

The study's findings demonstrated that, in contrast to the normal control group, the experimental rats' exposure to 8.0 mg/kg body weight DDVP (positive control) considerably (P<0.05) raised their serum ALP activity. When contrasted with the positive control group (8.0 mg/kg body weight DDVP only), treatment with 200 mg/kg body weight and 400 mg/kg body weight of aqueous leaf extracts of V.A., O.G., and T.T.; and 20 mg/kg body weight and 40 mg/kg body weight vitamin C, respectively, significantly decreased the serum ALP activities in a dose-dependent way.



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Fig 2: Comparison of AST activities of normal control, positive control (8.0mg/kg body weight DDVP only), and DDVP exposed rats (8.0mg/kg body weight DDVP) treated with aqueous leaf extracts of *Vernonia amygdalina*, *Ocimum gratissimum*, *Talinum triangulare* and Vitamin C for 28 days. P value less than 0.05 (P<0.05) was regarded as significantly different (P<0.05) was determined. The data is reported as Mean ± SEM





Fig 3: Comparison of ALP activities of normal control, positive control (8.0mg/kg body weight DDVP only), and DDVP exposed rats (8.0mg/kg body weight DDVP) treated with aqueous leaf extracts of *Vernonia amygdalina*, *Ocimum gratissimum, Talinum triangulare* and Vitamin C for 28 days. p value less than 0.05 (P<0.05) was regarded as significantly different (P<0.05) was determined. The data is reported as Mean ± SEM

The data on serum GGT activities (U/L) of controls and DDVP exposed rats treated with aqueous leaf extracts of V.A., O.G., T.T and Vitamin C for 28 days are presented in Fig 4.

The study's findings demonstrated that, in contrast to the normal control group, the experimental rats' exposure to 8.0 mg/kg body weight DDVP (positive

control) considerably (P<0.05) raised their serum GGT activity. When contrasted with the positive control group (8.0 mg/kg body weight DDVP only), treatment with 200 mg/kg body weight and 400 mg/kg body weight of aqueous leaf extracts of V.A., O.G., and T.T.; and 20 mg/kg body weight and 40 mg/kg body weight vitamin C, respectively, significantly decreased the serum GGT activities in a dose-dependent way.



Fig 4: Comparison of GGT activities of normal control, positive control (8.0mg/kg body weight DDVP only), and DDVP exposed rats (8.0mg/kg body weight DDVP) treated with aqueous leaf extracts of *Vernonia amygdalina*, *Ocimum gratissimum*, *Talinum triangulare* and Vitamin C for 28 days. P value less than 0.05 (P<0.05) was regarded as significantly different (P<0.05) was determined. The data is reported as Mean ± SEM

#### **Results of Liver Histopathology**

Plates 1 to 6 showed photomicrographs of histological segments of liver of normal control, positive control (8.0mg/kg body weight DDVP only), 8.0mg/kg body weight DDVP + 400mg/kg body weight *Vernonia amygdalina* aqueous leaf extract, 8.0mg/kg body weight DDVP + 400mg/kg body weight *Ocimum gratissimum* aqueous leaf extract, 8.0mg/kg body weight DDVP + 400mg/kg body weight *Talinum triangulare* aqueous leaf extract, and 8.0mg/kg body weight DDVP + 40mg/kg body weight vitamin C respectively for 28 days.



**Plate 1: Normal Control:** Photomicrograph (H&E X400) of the liver showing the centrilobar area of the central vein (CV): hepatocytes (HC) with kupfer cells within the sinusoids (SS) draining via the central vein.

Tissue appears normal (arrows). **Diagnosis:** Normal liver tissue.

**Plate 2: Positive Control (8.0mg/kg DDVP only):** Photomicrograph (H&E x400) of the liver showing diffused lymphocytic activities within the central vein and surrounding liver parenchymal (arrows) **Diagnosis:** Inflammation of the liver tissue.

**Plate 3: 8.0mg/kg DDVP + V.A (400mg/kg):** Photomicrograph (H&EX400) of the liver architecture showing minimal congestion of the central vein, sinusoids with kupfer cells and hepatocytes (arrows): tissue appears normal **Diagnosis:** Normal appearance of the liver parenchyma. V.A (400mg/kg) showed protection against DDVP induced toxicity in the liver as the histopathological photomicrograph showed normal appearance of the liver parenchyma

Plate 4: 8.0mg/kg DDVP + O.G (400mg/kg): Photomicrograph (H&E X 400) of the liver showing mild sinusoidal dilation and lymphocytic activities in zone1 of the liver parenchyma (arrows). **Diagnosis:** mild inflammation of the liver tissue.

Plate 5: 8.0mg/kg DDVP + T.T (400mg/kg): Photomicrograph (H&E X 400) of the liver with mild congestion of the central vein with glycogen degeneration of the liver parenchyma (arrows). Diagnosis: mild degeneration of the liver.

Plate 6: 8.0mg/kg DDVP + Vit C (40mg/kg): Diagnosis: mild degeneration of the liver parenchyma.

# DISCUSSION

Dichlorvos, generally known by its chemical name 2,2-dichlorovinyl dimethyl phosphate (DDVP), is an organophosphate insecticide widely utilized in agricultural, domestic, and industrial settings for pest control. Its mechanism of action is by the antagonism of acetylcholinesterase, an enzyme that is necessary for the nervous system to operate properly. By preventing the breakdown of acetylcholine, DDVP induces an accumulation of this neurotransmitter, leading to overstimulation of the nervous system and ultimately causing the death of the targeted pests (Enye *et al.*, 2021).

Despite its effectiveness, DDVP's use has raised significant concerns due to its potential toxicity to nontarget organisms, including humans. Exposure to DDVP can occur through inhalation, ingestion, or dermal contact, and has been linked to several detrimental health effects. Acute exposure may give rise to symptoms including dizziness, headaches, respiratory distress and nausea, while long term exposure has been linked to more severe outcomes, including neurological (Okoroiwu *et al.*, 2018) and hepatic damage (Zhao *et al.*, 2015).

Due to its widespread application and the associated health risks, the study of DDVP's toxicological impact on biological systems is crucial. Investigating potential protective agents, such as natural plant extracts and antioxidants, offers a promising avenue for mitigating the harmful effects of DDVP exposure. This study specifically explores the hepatoprotective potential of aqueous leaf extracts of Vernonia amygdalina, Ocimum gratissimum, Talinum triangulareand Vitamin C in countering DDVP-induced liver toxicity in albino rats, providing insights into possible therapeutic interventions for organophosphate poisoning. Findings from this study elucidate the protective effects of Vernonia amygdalina, Ocimum gratissimum, Talinum triangulare and Vitamin C against dichlorvos (DDVP)-induced hepatic toxicity in albino rats. The elevation of serum liver enzymes-alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gammaglutamyl transferase (GGT)-upon exposure to DDVP provides a clear indication of hepatocellular damage. These biomarkers are extensively used to assess liver function and integrity, where their increased activities are directly associated with liver injury (Meunier and Larrey, 2019).

Administration of DDVP led to significant (P<0.05) increase in serum ALT activities contrast with the normal control group, reflecting hepatic cell membrane damage and subsequent enzyme leakage into the bloodstream (Fig 1). This is agreements with Treatment with aqueous leaf extracts of *Vernonia amygdalina, Ocimum gratissimum, Talinum triangulare* and Vitamin C significantly mitigated this elevation in a dose-dependent manner. The reductions observed at 200mg/kg and 400mg/kg for the plant extracts and at 20mg/kg and 40mg/kg for Vitamin C suggest that these treatments promote hepatocyte stabilization and integrity.

Similarly, the serum AST activities were significantly elevated in the DDVP-only exposed group, indicating further hepatocellular damage (Fig 2) (Zhao *et al.*, 2015). The attenuation of AST activities by the plant extracts and Vitamin C in treated groups demonstrates their efficacy in reducing liver injury. The dose-dependent reduction implies an increased antioxidant defense mechanism and a potential reduction in lipid peroxidation, as supported by prior studies on the antioxidative compounds present in these plants (Gershenzon *et al.*, 2022).

The DDVP exposure also significantly increased serum ALP activities, which are associated with cholestasis and liver dysfunction (Fig 3). The plant extracts and Vitamin C effectively lowered ALP activities, indicating their role in enhancing bile flow and preventing cholestasis. The dose-dependent manner of this effect underscores the potential of these treatments in maintaining biliary function and integrity under toxic conditions.

Finally, the GGT activities were significantly elevated following DDVP exposure, a marker of oxidative stress and liver toxicity (Fig 4). The significant reduction in GGT activities upon treatment with *Vernonia amygdalina, Ocimum gratissimum, Talinum triangulare* and Vitamin C suggests a protective mechanism against DDVP-induced oxidative damage. This result is consistent with other findings that highlight the antioxidative and detoxifying capabilities of these extracts and Vitamin C (Gershenzon et al., 2022).

These findings are also in concordance with earlier studies by Ngatu et al., (2020), who found that water extracts from Vernonia amygdalina exhibited both antioxidant and hepatoprotective properties; Chiu et al., (2014), who discovered that leaf extracts from Ocimum gratissimum decreased liver damage and elevated antioxidative enzyme activities in serum; and Liang et al., (2011), who discovered that polysaccharides from Talinum triangulare had both hepatoprotective and antioxidant properties. The rich phytochemical content of Vernonia amygdalina, Ocimum gratissimum, and Talinum triangulare-which comprises flavonoids, saponins, and polyphenols-is responsible for the hepatoprotective effects seen in this study (Olusola et al., 2023; Faluyi, 2020; Aja et al., 2010). Strong antioxidant qualities have been observed for these substances, which scavenge free radicals and strengthen the body's natural antioxidant defenses.

Vitamin C, a well-known antioxidant, further supports this action by directly neutralizing reactive oxygen species and regenerating other antioxidants within the body. The combined effect of these treatments likely involves the stabilization of hepatocyte membranes, reduction in lipid peroxidation, and improvement in overall liver function.

Results of histological examinations corroborate these findings. Photomicrographs obtained reveal the attenuation of liver damage by *Vernonia amygdalina, Ocimum gratissimum Talinum triangulare* leaf extracts.

# CONCLUSION

In summary, the aqueous leaf extracts of Vernonia amygdalina and Ocimum gratissimum, as well as Talinum triangulare caused a significant (p<0.05) reduction in DDVP-induced liver toxicity in albino rats indicated by the decrease in the liver marker enzymes upon treatment with the plants. The observed dosedependent reductions in serum ALT, AST, ALP, and GGT activities underscore the therapeutic potential of these natural products in mitigating chemical-induced hepatic damage. These findings suggest that these plant extracts could be explored further as natural hepatoprotective agents, potentially offering a safer alternative to synthetic drugs in the management of liver disorders. Future studies should aim to isolate specific active compounds and elucidate their precise mechanisms of action in hepatoprotection.

Conflict of Interest: There is no conflict of interest.

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