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Original Reserach Article

Vernonia amygdalina, Ocimum gratissimum and *Talinum triangulare* Mitigates Kidney Toxicity in Male Wistar Rats Administered with Dichlorvos

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Abstract: This study aimed to evaluate the therapeutic potentials of extracts from medicinal plants (*Vernonia amygdalina, Ocimumgratissimum, Talinum triangulare*) against DDVP-induced renal toxicity in Wistar rats. A total of 50 male adult rats, each weighing approximately 272g, were divided into ten groups (n=5). The groups were given normal saline (normal control), 8.0mg/kg body weight DDVP (positive control), 8.0mg/kg body weight DDVP along with 200mg/kg and 400mg/kg body weight of the plant extracts respectively, and 20mg/kg and 40mg/kg body weight of vitamin C for 28 days. DDVP significantly ($P<0.05$) increased urea, creatinine, Na^+, K^+, Cl^-, and significantly ($P<0.05$) decreased $HCO3^-$ compared to the normal control. Treatment with the aqueous leaf extracts of *Vernonia amygdalina, Ocimumgratissimum,* and *Talinum triangulare* significantly corrected these biochemical imbalances when contrasted with the positive control. Histology of the kidney tissues supported these findings. In conclusion, the aqueous leaf extracts of *Vernonia amygdalina, Ocimumgratissimum, and Talinum triangulare* may have therapeutic potential against DDVP-induced renal toxicity in rats.

Keywords: DDVP, Renal Toxicity, *Vernonia amygdalina, Ocimumgratissimum, Talinum triangulare*, Urea, Creatine, Na^+, K^+, HCO_3^-,Cl^-.

INTRODUCTION

Africa is rich in herbs with both nutritional and medicinal benefits. In countries like Nigeria, over 80% of the population relies on these medicinal herbs to manage or cure various diseases (Fokunang *et al*., 2011; Salmeron *et al*., 2020). These herbs contain phytochemicals with therapeutic properties beneficial to humans and animals. For many years, these plants have been used in native medicine for treating diseases and as precursors for drug production (Evans, 2008; Sofowora, 2008; Salmeron *et al*., 2020).

*Vernonia amygdalina*is a small shrub from the daisy plant family that grows in tropical Africa and reaches heights of 2-5 meters (Ijeh *et al*., 2011). It is commonlyreffered to as bitter leaf due to its bitter taste that is as a result of a compound called sesquiterpene lactone. The bitterness is also linked to itscontent of anti-nutrients like tannins, glycosides, saponins, and alkaloids (Arhoghro *et al*., 2009). In Nigeria, the plant is generallyreferred to as ewuroin Yorubaland, onugbu in Igboland and shuwakain Hausaland (Kokwaro, 2009; Egedigwe, 2010; Appiah, 2018). *Vernonia amygdalina* is valued for its medicinal properties due to phytonutrients like flavonoids, terpenes, alkaloids, and phenolic acids, as well as vitamins such as vitamins C and E, which have antioxidant properties (Abdulmalik *et al*., 2020; Henrrietta *et al*., 2021; Edo *et al*., 2023).

Ocimumgratissimum, herbal plant that isnative to Nigeria and is used to prevent and treat various

diseasaes (Tanko *et al*., 2008; Akara *et al.*, 2021). It is one of the plants that belong to the family known asLamiaceaeand is native to Nigeria and parts of Africa and Asia. It is commonly found in farms and gardens and used as a spice in dishes like pepper soup, porridge yam, and stews (Onifade *et al*., 2023). In Nigeria, it is called Efirin (Yoruba), Ahuju or Nchanwu (Igbo), and GanyenKamsh or Doddoya or Daidoya (Hausa) (Achinewu *et al*., 1995; Effraim *et al*., 2003; Faluyi, 2020; Akara *et al*., 2021).

Talinum triangulare, known as water leaf, is an edible leafy vegetable from the portulacaceae family. Its highwater content (90.8g per 100g) gives it a sticky texture due to its pectin content, contributing dietary fiber (Fontem *et al*., 2010). It is tolerant to dry conditions and adapts to the crassulacean acid metabolism (CAM) pathway for efficient water and CO2 usage (Ezekwe *et al*., 2001). *Talinum triangulare* has been found to be rich in, omega-3 fatty acids, calcium, β-carotene, proteins, vitamins C and E, pectin, potassiumand dietary fiber, providing significant antioxidant benefits (Enete&Okon, 2010). Phytonutrient studies have confirmed its medicinal potential, with compounds like tannins, alkaloids, saponins, flavonoids, and carotenoids (Aja *et al*., 2010). These compounds make it useful for disease prevention and treatment.

Dichlorvos (DDVP) is a commonly used organophosphate insecticide effective in pest control across various environments. It works by inhibiting acetylcholinesterase, disrupting the nervous system of pests, leading to their death (Fu *et al*., 2022). However, it also poses significant risks to non-target species, including mammals (Binukumar *et al.*, 2010; Okoroiwu *et al*., 2018; Izah& Richard, 2020). DDVP is particularly toxic to the kidneys, which are crucial for detoxifying and excreting pesticides (Sobolev *et al*., 2022; Flora, 2016). While chronic exposure can result in long-term renal impairment through oxidative stress, inflammation, and fibrosis, acute exposure can have immediate nephrotoxic effects. This delineates the critical need for understanding the mechanisms and impacts of both acute and chronic exposures on renal health to develop effective preventive and therapeutic strategies (Sule *et al*., 2022).

Given the critical functions of the kidneys and the severe impact of damage to kidneytissues, it is essential to explore protective strategies against DDVP-induced nephrotoxicity. Recent studies have focused on using natural antioxidants and plant extracts to mitigate oxidative stress and restore normal kidney function. This work's main aims was toascertain the nephroprotective capabilities of aqueous leaf extracts of *Vernonia amygdalina, Ocimumgratissimum, Talinum triangulare,* and Vitamin C in rats exposed to DDVP. By investigating these potential treatments, we hope to identify effective methods for preventing or reducing kidney damage caused by organophosphate toxicity.

MATERIALS AND METHOD

Plant Materials and Preparation of Extracts

Vernonia amygdalina, *Ocimumgratissimum*, and *Talinum triangulare*leaves were gathered from Amassoma, Southern Ijaw Local Government Area of Bayelsa State, Nigeria. Professor Kola Ajibesin from the Department of Pharmacognosy, Faculty of Pharmacy, Niger Delta University, identified the plants.

After being cut off from their stalks, they were cleaned with clean water and then left to air dry, with occasional turning to prevent fungal growth. Talinum triangulare leaves took 33 days to dry completely, while Vernonia amygdalina and Ocimumgratissimum leaves dried fully in just 13 days. The dry leaves were ground into powder using an electric blending machine. For extraction, 250g of the ground material per plant was soaked in one liter of distilled water for three days, with occasional stirring. The mixtures were filtered through sterile cheesecloths and the filtrates were concentrated into thick pastes using a rotary evaporator, dried usinga freeze-drier and then stored in the refrigerator until needed

Experimental Animals

Fifty male adult Wistar rats, averaging 272g in weight were obtained from the animal care unit of University of Port Harcourt, Rivers State, Nigeria. They were kept for two weeks for acclimatization, in well-ventilated cages at the animal house of the Department of Pharmacology, Faculty of Basic Clinical Sciences, College of Health Sciences, Niger Delta University. They were fed standard feed and had unlimited supply of drinking water. All animal care and use guidelines were strictly followed.

Chemicals and Reagents

Commercial-grade dichlorvos (DDVP, 100% solution with 100g per liter of 2,2-dichlorovinyl-dimethyl phosphate) sold as Sniper, was obtained from an agrochemical store in Amassoma, Bayelsa State, Nigeria. Biochemical kits for urea, creatinine, sodium, potassium, chloride and bicarbonate assays were obtained from purchased from Randox™ Laboratories Ltd, Crumlin, Co, Antrim, United Kingdom.

Experimental Procedure

Fiftyadult-male Wistar rats were randomly shared into 10 groups of 5 and given the following treatments for 28 days:

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

5. **Group 5:** 8.0mg/kg body weight DDVP + 200mg/kg body weight *Ocimumgratissimum* extract.
6. **Group 6:** 8.0mg/kg body weight DDVP + 400mg/kg body weight *Ocimumgratissimum* extract.
7. **Group 7:** 8.0mg/kg body weight DDVP + 200mg/kg body weight *Talinum triangulare* extract.
8. **Group 8:** 8.0mg/kg body weight DDVP + 400mg/kg body weight *Talinum triangulare* extract.
9. **Group 9 (Standard Control 1):** 8.0mg/kg body weight DDVP + 20mg/kg body weight Vitamin C.
10. **Group 10 (Standard Control 2):** 8.0mg/kg body weight DDVP + 40mg/kg body weight Vitamin C.

All treatments were administered orally. The doses used were derived from a previous study determining the lethal dose (LD50) of the three plants.

Periodic collection of blood samplesfrom the sub-mandibular veins of the rats on days 0, 1, 7, 14, and 21 to evaluate biochemical parameters was done. Day 28 saw the sacrifice of every rat following a chloroform anesthesia. Portions of the kidneys were taken for histological studies and blood was obtained via heart puncture for biochemical examination.

Biochemical Assays:

Kidney biomarkers were assayed following the instructions in the Randox brand biochemical kit leaflets.

Statistics

Data was analyzed using one-way analysis of variance ANOVA and further confirmed using Turkey's test with Statistical Package for the Social Sciences (SPSS) Version 20, IBM, USA. Values are presented as mean ± standard error of the mean (Mean+S.E.M) P-values less than 0.05 ($P < 0.05$) were regarded as significantly significant.

RESULTS

The data on serum urea levels (mg/dl) for control rats and those exposed to DDVP and treated with aqueous leaf extracts of Vernonia amygdalina (V.A.), Ocimumgratissimum (O.G.), Talinum triangulare (T.T.), and Vitamin C over 28 days are shown in Fig. 1. The results revealed that exposure to 8.0 mg/kg body weight DDVP (positive control) resulted in to a significant ($P<0.05$) increase in serum urea levels in the experimental rats when contrasted with the normal control group. However, treatment with 200 mg/kg and 400 mg/kg body weight of V.A., O.G., and T.T. extracts, as well as 20 mg/kg and 40 mg/kg body weight of Vitamin C, gave rise to a significant, dose-dependent reduction in serum urea levels when contrastedwith the positive control group (8.0 mg/kg body weight DDVP only).

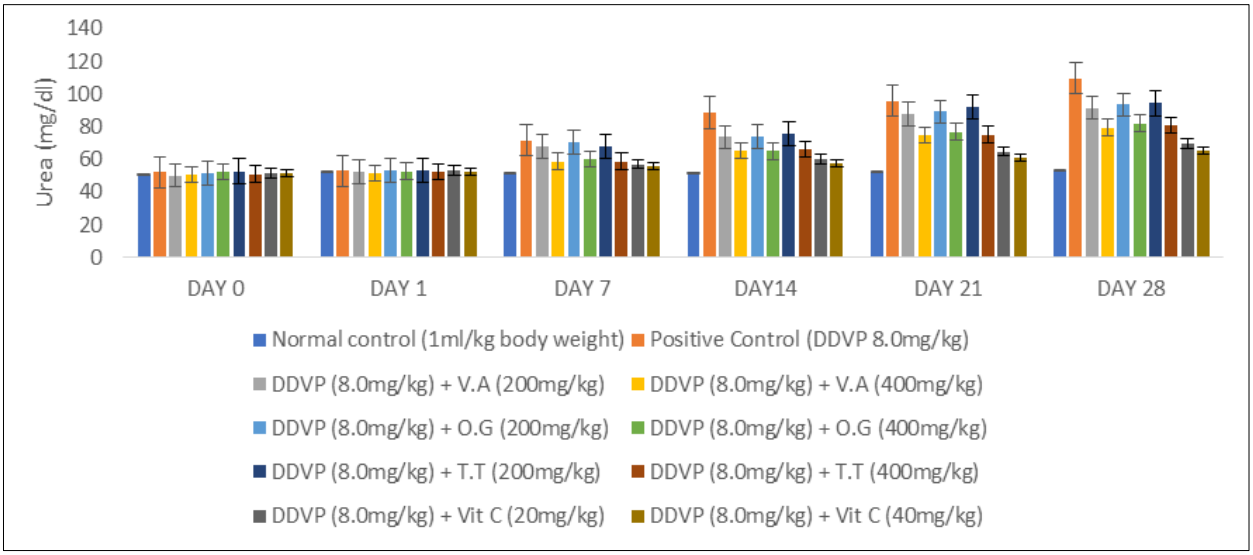


Fig. 1: Illustrates the comparison of urea levels between the normal control group, the positive control group (8.0 mg/kg body weight DDVP only), and DDVP-exposed rats (8.0 mg/kg body weight DDVP) treated with aqueous leaf extracts of Vernonia amygdalina, Ocimumgratissimum, Talinum triangulare, and Vitamin C for 28 days. The data show statistically significant differences ($P<0.05$) and are expressed as Mean ± SEM.

The data on serum creatinine levels (mg/dl) for control rats and those exposed to DDVP and treated with aqueous leaf extracts of Vernonia amygdalina (V.A.), Ocimumgratissimum (O.G.), Talinum triangulare (T.T.), and Vitamin C over 28 days are shown in Fig. 2. The results revealed that exposure to 8.0 mg/kg body weight DDVP (positive control) resulted in a significant ($P<0.05$) increase in serum creatinine levels in the experimental rats when contrasted with the normal control group. However, treatment with 200 mg/kg and 400 mg/kg body weight of V.A., O.G., and T.T. extracts, as well as 20 mg/kg and 40 mg/kg body weight of

Vitamin C, gave rise to a significant, dose-dependent reduction in serum creatinine levels when contrasted with the positive control group (8.0 mg/kg body weight DDVP only).

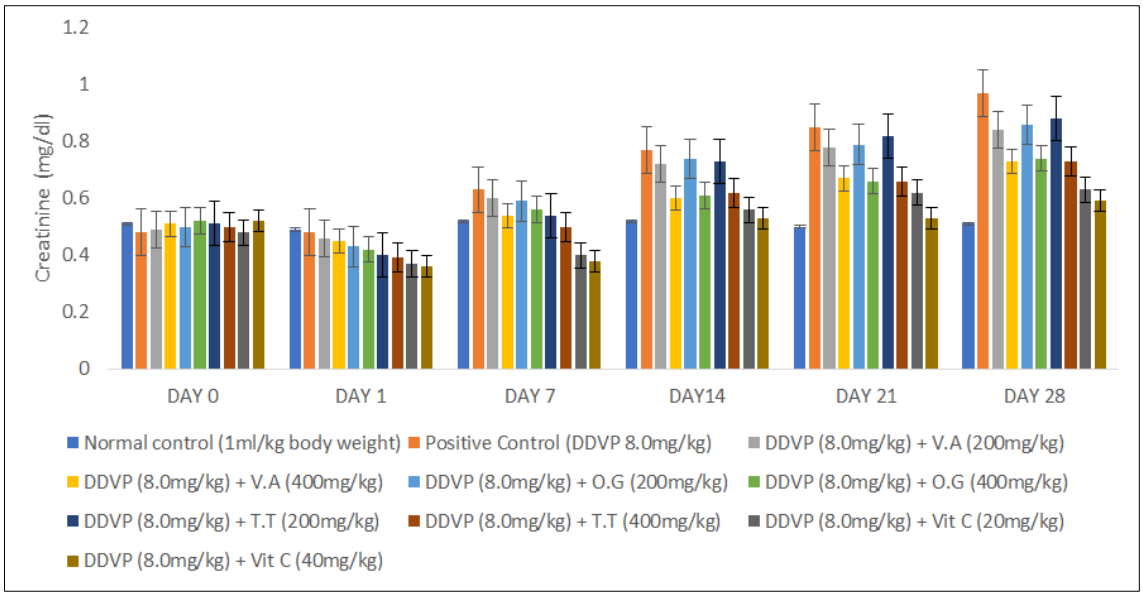


Fig. 2: Illustrates the comparison of creatinine levels between the normal control group, the positive control group (8.0 mg/kg body weight DDVP only), and DDVP-exposed rats (8.0 mg/kg body weight DDVP) treated with aqueous leaf extracts of Vernonia amygdalina, Ocimumgratissimum, Talinum triangulare, and Vitamin C for 28 days. The data show statistically significant differences (P<0.05) and are expressed as Mean ± SEM.

The data on serum sodium levels (mEq/L) for control rats and those exposed to DDVP and treated with aqueous leaf extracts of Vernonia amygdalina (V.A.), Ocimumgratissimum (O.G.), Talinum triangulare (T.T.), and Vitamin C over 28 days are shown in Fig. 3.

The results revealed that exposure to 8.0 mg/kg body weight DDVP (positive control) significantly (P<0.05) increased serum sodium level in the experimental rats when contrasted the normal control group. However, treatment with 200 mg/kg and 400 mg/kg body weight of V.A., O.G., and T.T. extracts, as well as 20 mg/kg and 40 mg/kg body weight of Vitamin C, gave rise to a significant, dose-dependent reduction in serum sodium levels when contrasted to the positive control group (8.0 mg/kg body weight DDVP only).

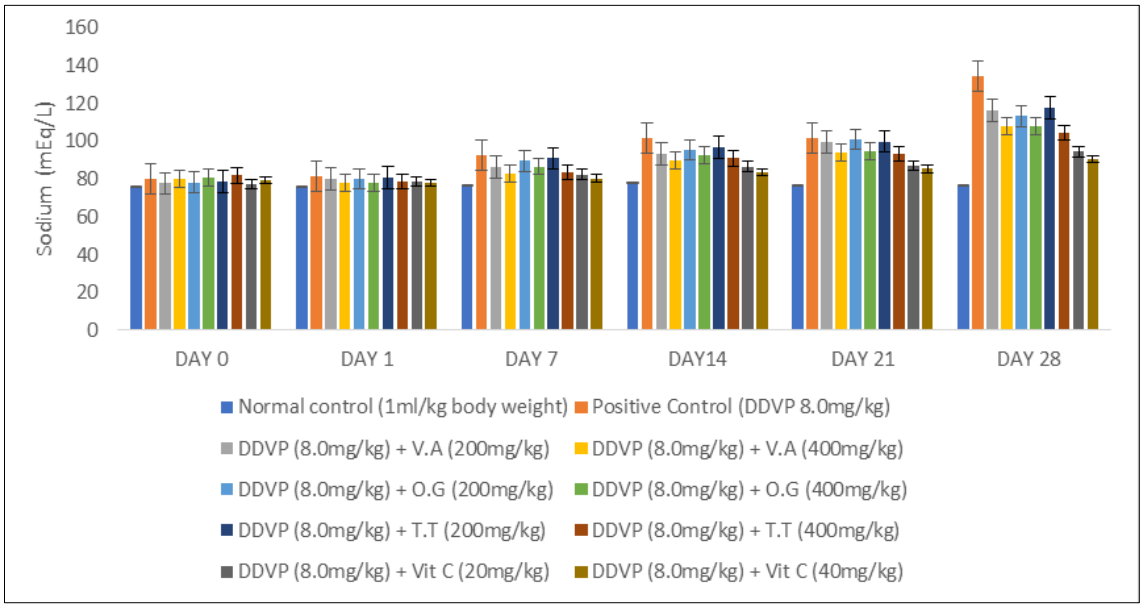


Fig. 3: illustrates the comparison of sodium levels between the normal control group, the positive control group (8.0 mg/kg body weight DDVP only), and DDVP-exposed rats (8.0 mg/kg body weight DDVP) treated with aqueous leaf extracts of Vernonia amygdalina, Ocimumgratissimum, Talinum triangulare, and Vitamin C for 28 days. The data show statistically significant differences (P<0.05) and are expressed as Mean ± SEM.

The data on serum potassium levels (mEq/L) for control rats and those exposed to DDVP and treated with aqueous leaf extracts of Vernonia amygdalina (V.A.), Ocimumgratissimum (O.G.), Talinum triangulare (T.T.), and Vitamin C over 28 days are shown in Fig. 4.

The results revealed that exposure to 8.0 mg/kg body weight DDVP (positive control) resulted to a significant ($P<0.05$) increase serum potassium levels in the experimental rats contrasted with the normal control group. However, treatment with 200 mg/kg and 400 mg/kg body weight of V.A., O.G., and T.T. extracts, as well as 20 mg/kg and 40 mg/kg body weight of Vitamin C, gave rise to a significant, dose-dependent reduction in serum potassium levels when contrasted with the positive control group (8.0 mg/kg body weight DDVP only).

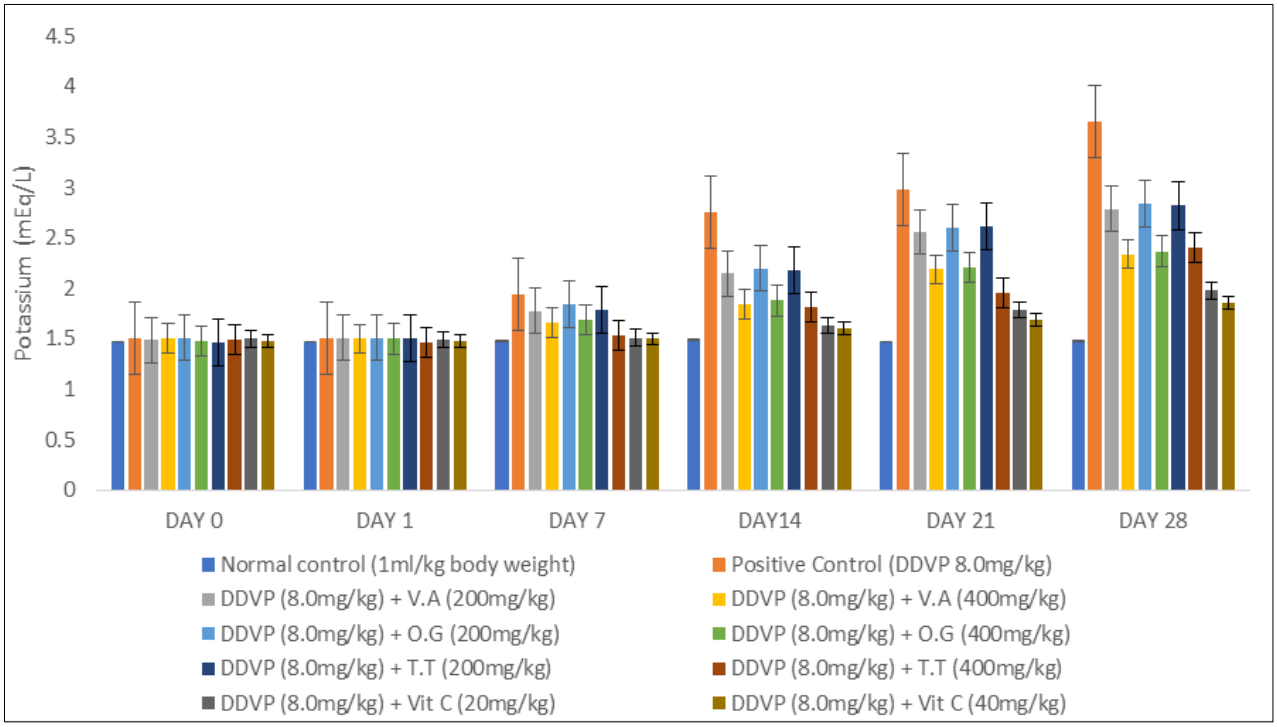


Fig. 4: illustrates the comparison of potassium levels between the normal control group, the positive control group (8.0 mg/kg body weight DDVP only), and DDVP-exposed rats (8.0 mg/kg body weight DDVP) treated with aqueous leaf extracts of Vernonia amygdalina, Ocimumgratissimum, Talinum triangulare, and Vitamin C for 28 days. The data show statistically significant differences ($P<0.05$) and are expressed as Mean ± SEM

The data on serum chloride levels (mEq/L) for control rats and those exposed to DDVP and treated with aqueous leaf extracts of Vernonia amygdalina (V.A.), Ocimumgratissimum (O.G.), Talinum triangulare (T.T.), and Vitamin C over 28 days are shown in Fig. 5.

The results revealed that exposure to 8.0 mg/kg body weight DDVP (positive control) significantly ($P<0.05$) increased serum chloride levels in the experimental rats compared to the normal control group. However, treatment with 200 mg/kg and 400 mg/kg body weight of V.A., O.G., and T.T. extracts, as well as 20 mg/kg and 40 mg/kg body weight of Vitamin C, gave rise to a significant, dose-dependent reduction in serum chloride levels when compared to the positive control group (8.0 mg/kg body weight DDVP only).

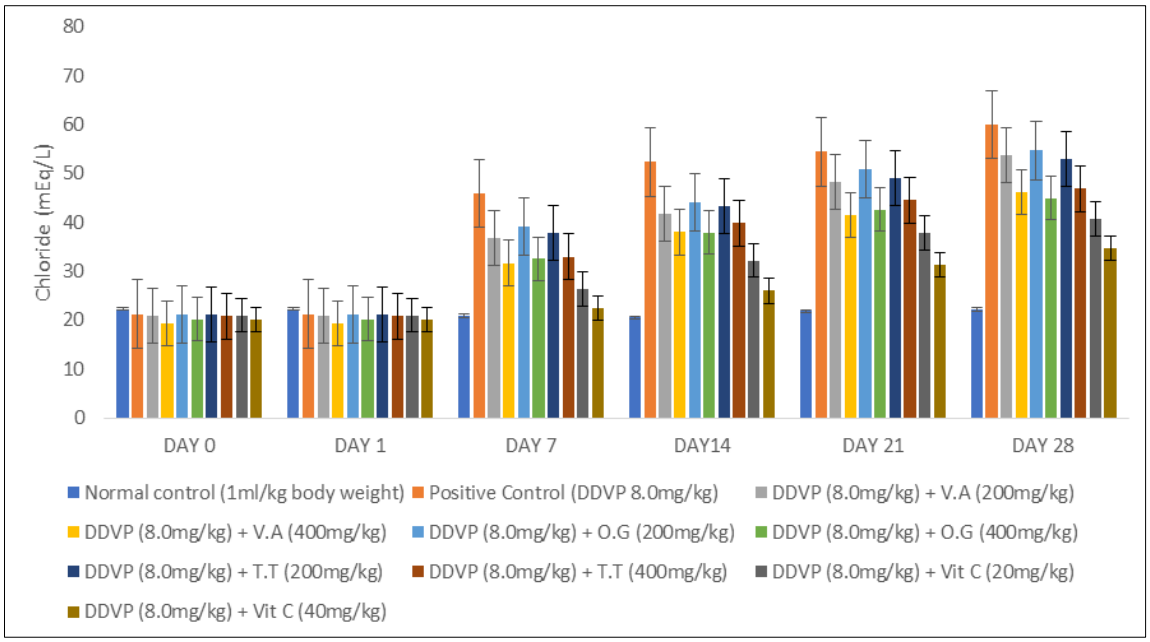


Fig. 5: illustrates the comparison of chloride levels between the normal control group, the positive control group (8.0 mg/kg body weight DDVP only), and DDVP-exposed rats (8.0 mg/kg body weight DDVP) treated with aqueous leaf extracts of Vernonia amygdalina, Ocimumgratissimum, Talinum triangulare, and Vitamin C for 28 days. The data show statistically significant differences (P<0.05) and are expressed as Mean ± SEM.

The data on serum bicarbonate levels (mmol/L) for control rats and those exposed to DDVP and treated with aqueous leaf extracts of Vernonia amygdalina (V.A.), Ocimumgratissimum (O.G.), Talinum triangulare (T.T.), and Vitamin C over 28 days are shown in Fig. 6.

The results revealed that exposure to 8.0 mg/kg body weight DDVP (positive control) resulted in a significantly (P<0.05) reduced serum bicarbonate levels in the experimental rats when contrasted with the normal control group. However, treatment with 200 mg/kg and 400 mg/kg body weight of V.A., O.G., and T.T. extracts, as well as 20 mg/kg and 40 mg/kg body weight of Vitamin C, gave rise to a significant, dose-dependent increase in serum bicarbonate levels when contrasted with the positive control group (8.0 mg/kg body weight DDVP only).

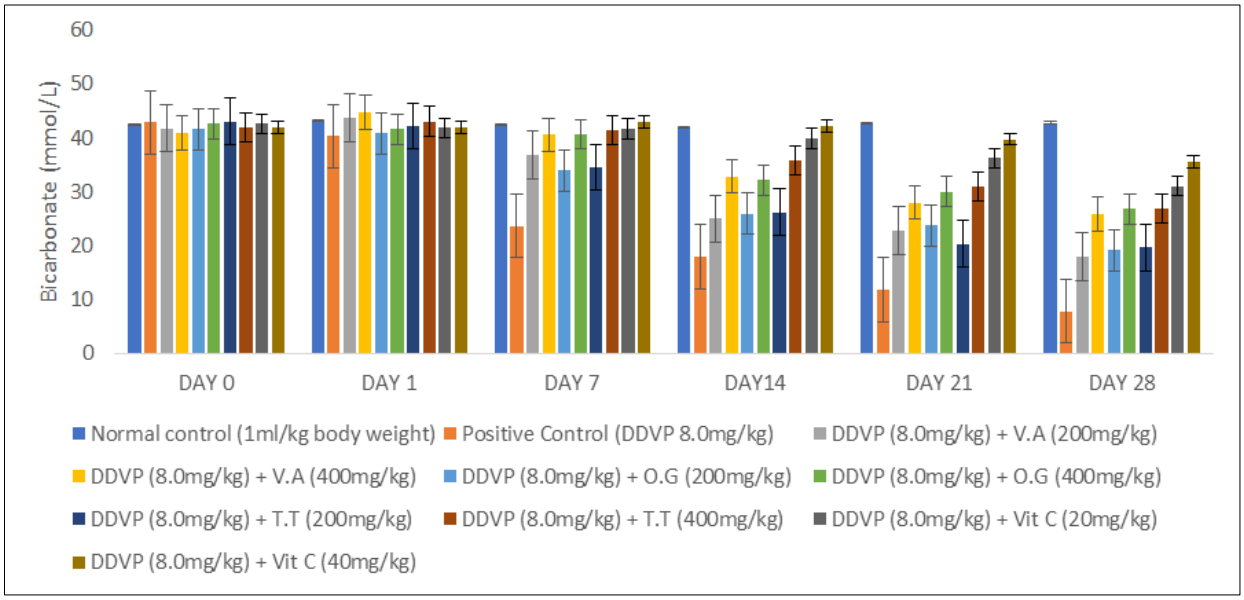
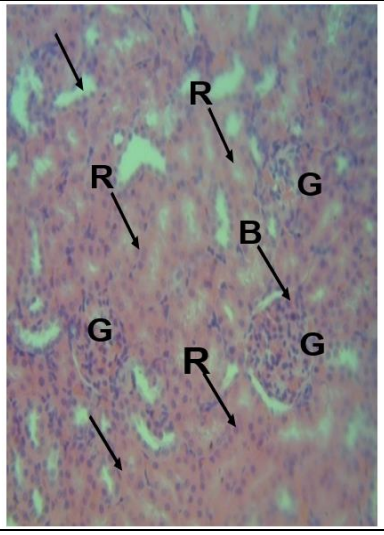
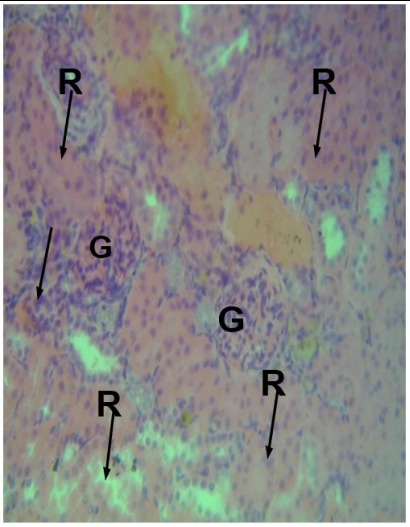
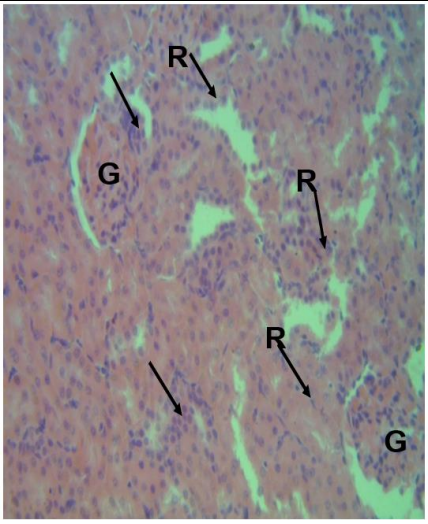
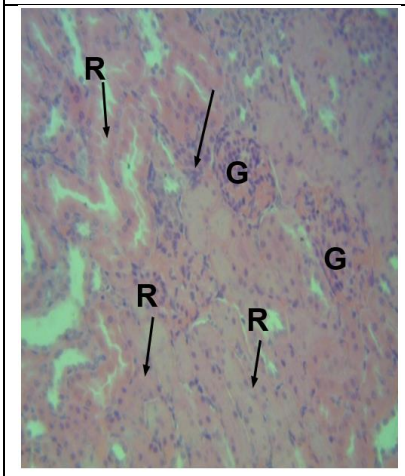
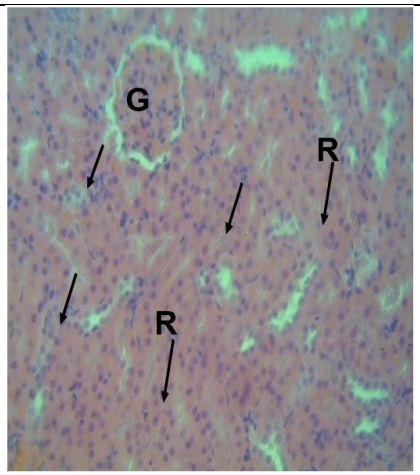
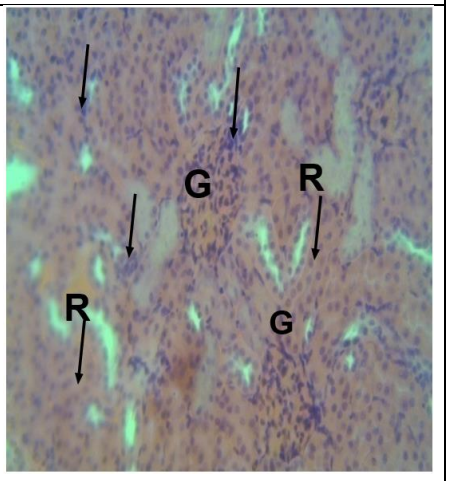


Fig. 6: illustrates the comparison of bicarbonate levels between the normal control group, the positive control group (8.0 mg/kg body weight DDVP only), and DDVP-exposed rats (8.0 mg/kg body weight DDVP) treated with aqueous leaf extracts of Vernonia amygdalina, Ocimumgratissimum, Talinum triangulare, and Vitamin C for 28 days. The data show statistically significant differences (P<0.05) and are expressed as Mean ± SEM.

Results of Kidney Histopathology

Plate 1	**Plate 2**	**Plate 3**
		
Normal Control: **Diagnosis:** Kidney tissue appears normal.	**Positive Control (8.0mg/kg DDVP only):** **Diagnosis:** Inflammation and distortion of the kidney tissue	**8.0mg/kg DDVP + V.A (400mg/kg):** **Diagnosis:** mild inflammation of the kidney tissue.
Plate 4	**Plate 5**	**Plate 6**
		
8.0mg/kg DDVP + O.G (400mg/kg): **Diagnosis:** Mild degeneration of the glomerulus and renal distortion.	**8.0mg/kg DDVP + T.T (400mg/kg):** **Diagnosis:** Kidney tissue appears moderately normal	**8.0mg/kg DDVP+ Vit C (40mg/kg):** **Diagnosis:** Mild degeneration of the glomerulus.

DISCUSSION

Dichlorvos (DDVP) is a commonly used pesticide effective in controlling pests. However, it poses serious health risks due to its high toxicity, affecting the kidneys, liver, and nervous system. Exposure to DDVP can lead to severe health adverse health conditions, including kidney damage and electrolyte imbalances. Therefore, it is important to find ways to protect against DDVP's harmful effects (Adeoye *et al*., 2023).

Plants have been in usefrom ancient times for their healing properties and are increasingly studied in modern research for their health benefits (Mahomoodally, 2013). Vernonia *amygdalina (bitter leaf), Ocimumgratissimum* (scent leaf), and *Talinum triangulare* (water leaf) contain beneficial compounds like antioxidants and flavonoids. These compounds are known for their protective effects against various toxins (Jongrungraungchok *et al*., 2023). Traditionally, these plants have been used to treat many ailments and have shown promise in reducing the harmful effects of toxins owing to their anti-inflammatory and antioxidant properties (Jongrungraungchok *et al*., 2023).

The effects of DDVP exposure and how aqueous extracts from Vernonia *amygdalina, Ocimum gratissimum*, *Talinum triangulare*, and Vitamin C can protect against DDVP's harmful effects on serum biochemical markers in rats over 28 days was studied in this work. The results provided significant insights into

how these treatments affect kidney function indicators altered by DDVP toxicity.

DDVP exposure (Positive control group) at 8.0 mg/kg body weight significantly ($p<0.05$) increased serum urea levels, indicating kidney damage when contrasted with the normal control group. However, treatments with Vernonia *amygdalina*, *Ocimumgratissimum*, *Talinum triangulare* extracts, and Vitamin C showed a significant and dose-dependent reduction serum urea levels when contrasted with the positive control group, suggesting these treatments help protect against DDVP-induced kidney damage (Fig. 1). Similarly, DDVP exposure increased serum creatinine levels also reflecting kidney damage. Treatments with *Vernonia amygdalina*, *Ocimumgratissimum*, *Talinum triangulare* extracts, and Vitamin C effectively lowered creatinine levels, demonstrating their potential in reducing DDVP-induced kidney damage (Fig. 2). These results align with Adebayo *et al.*, (2016) and Nwankwo *et al*., 2019 who previously revealed that there was significant nephrotoxicity in rats exposed to DDVP, marked by increased serum urea and creatinine levels. Findings also agree with Sharma *et al.*, (2023) who showed that treatment with antioxidant compounds improved biochemical indices of renal dysfunction.

Exposure to DDVP caused a significantly increased serum sodium levels in the positive control group, indicating an electrolyte imbalance. Treatments with the plant extracts and Vitamin C, however, significantly decreased sodium levels, suggesting they help maintain electrolyte balance and reduce DDVP-induced hypernatremia (Fig. 3). Results also showed that DDVP exposure significantly increased serum potassium levels, leading to hyperkalemia and associated health risks. Plant extracts and Vitamin C significantly reduced potassium levels, indicating their potential to protect against DDVP-induced potassium regulation issues (Fig. 4). There was a significantly increased serum chloride levels upon exposure to DDVP indicating an electrolyte imbalance. Treatments with Vernonia *amygdalina*, *Ocimumgratissimum*, *Talinum triangulare* extracts, and Vitamin C caused a significant dose-dependent decrease in chloride levels, helping to restore normal chloride levels and counteracting DDVP effects (Fig.5) DDVP exposure significantly decreased serum bicarbonate levels, indicating metabolic acidosis. Treatments with the plant extracts and Vitamin C significantly increased bicarbonate levels in a dose-dependent fashion, suggesting their effectiveness in mitigating DDVP-induced acidosis and promoting acid-base balance (Fig. 6). These findings are in line with previous studies (Nwankwo *et al*., 2019; Akara *et al*., 2021; Adeoye *et al.*, 2024).

The toxic effects of DDVP are thought to be related to oxidative stress. The plant extracts' protective effects may be due to their antioxidant properties. For example, *Vernonia amygdalina* contains compounds like flavonoids, terpenes, alkaloids, and phenolic acids. It has also been found to contain an appreciable amount of vitamins C and E, which are known to scavenge free radicals and protect against oxidative damage (Abdulmalik *et al*., 2020; Henrrietta *et al*., 2021; Bisi, 2023; Edo *et al.*, 2023). Other studies also highlight *Vernonia amygdalina's* antioxidant and anti-inflammatory properties due to its high concentrations of carotenoids, polyphenols, flavonoids, and tannins (Cho *et al*., 2020; Fawwaz *et al*., 2020; Oyesola *et al*., 2022; Ijeh *et al*., 2011; Eziuche *et al*., 2021).

CONCLUSION

The results from this study demonstrated that DDVP exposure adversely affects kidney function and electrolyte balance in rats, as evidenced by elevated levels of urea, creatinine, sodium, potassium, and chloride, and decreased bicarbonate levels. However, aqueous leaf extracts of *Vernonia amygdalina*, *Ocimumgratissimum*, as well aqueous stem bark extract of *Talinum triangulare*, and Vitamin C showed significant protective effects, restoring the biochemical parameters towards normal levels in a dose-dependent manner. These findings suggest that these plant extracts and Vitamin C could be potential therapeutic agents for managing DDVP-induced nephrotoxicity and related metabolic disturbances. More studies are recommended to elucidate the specific mechanisms and to explore the clinical relevance of these findings.

Conflict of Interest: We hereby affirm thatthere is no conflict of interest in this study.

Acknowledgement: Rebecca Eferebo for her invaluable laboratory assistance.

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