Protective and curative effects of *Beta vulgaris* on dimethyl 2,2-dichlorovinyl phosphate-exposed albino rats [version 1; peer review: 1 approved with reservations]

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**Abstract**

**Background:** This study evaluated the effect of *Beta vulgaris* (beetroot) smoothie on some biochemical parameters on dimethyl 2,2-dichlorovinyl phosphate (DDVP, known as dichlorvos)-exposed albino Wistar rats.

**Methods:** A total of 30 rats of both sexes were grouped into five groups of six animals each. Group I served as the negative control and were not exposed to dichlorvos. Group II served as the positive control and were exposed to dichlorvos but received no smoothie. Group III received 500 mg/kg body weight beetroot smoothie and was not exposed to dichlorvos. Groups IV and V were exposed to dichlorvos but received beetroot before and after exposure, respectively. At the end of the 6-week experiment, the animals were euthanized, the blood samples collected for some biochemical assays while the organs (kidney and liver) were harvested and subjected to histopathological examination.

**Results:** From the biochemical assay, it was observed that the beetroot smoothies regulated and significantly reduced the elevated levels of AST, ALT, urea and creatinine observed in the animals that were exposed to dichlorvos. Additionally, the beetroot was able to regenerate the liver and kidney organs that were damaged on exposure to dichlorvos.

**Conclusion:** This study concluded that beetroot smoothie possesses hepatoprotective, hepato-curative as well as nephro-curative properties.

**Keywords**

Beta vulgaris, Dichlorvos, Biochemical assay, Histopathological studies, Hepatoprotective function
Introduction

Organophosphates are a group of pesticides and are massively utilized globally due to their numerous benefits in the health and agricultural sectors. According to Abdollahi et al. (2004), many severe environmental and health hazards have been associated with utilization of these compounds. To achieve a high yield agricultural produce, pesticides have long been employed as it gets rid of unwanted insects and disease vectors (Prakasam et al., 2001). According to Aly & El-Gendy (2000), the liver, kidney, nervous system, immune system and reproductive system are all affected when in contact or exposed to these compounds, indicating the toxicity potential of organophosphates, which varies depending on the class, biological activities and the different antagonistic effects they cause in living organisms, including man. Restricted-use pesticides (RUPs) can be acquired and used by certified applicators only. The recommended disposal methods for RUPs include alkaline hydrolysis, landfiling, and incineration (Das, 2013).

Pesticide poisoning is commonly caused by the action of pesticides on mostly the internal organs of the body. Some pesticides that are referred to as external irritants cause pesticide-related injuries. According to George & David (2004), the degree of action or effects of a pesticide could be mild or severe depending on the exposure limit or time and the type of pesticide used. These pesticides are extremely deleterious to humans, as droplets applied in the mouth or on the body can result in extreme harmful effects. Some pesticides are mildly poisonous and may trigger damaging effects when one is exposed to them in large quantity (Sarwar, 2015). Despite the various preventive measures to control the use of pesticides, the persistent use has caused serious complication to both humans and the ecosystem.

Dimethyl 2,2-dichlorovinyl phosphate (DDVP, known as dichlorvos), is an organophosphate compound used daily on a massive scale to kill insects. Dichlorvos is an organophosphorus compound, a pesticide used to kill household and public health insects that attack stored products. This compound has been used as a fumigant and in the production of pest strips (Hayes & Laws, 1990). It is widely obtainable as an aerosol and soluble concentrate. Snipper, Fly-Die, Fly-Fighter and Duravos are some of the common trade names of dichlorvos. According to Das (2013), this compound is classified as toxicity class I (highly toxic), because it may cause cancer. Thus, products containing dichlorvos must bear the signal words “Danger – Poison” as it is confirmed that this compound is a Restricted Use type.

In order for a pesticide to be listed for a particular use in Canada, a regulatory body known as the Pest Management Regulatory Agency (PMRA) studies the aspects of its projected use including the site for exposure and method of application (Harry & Brown, 1974). Other commonly fumigants used globally include methylbromide, hydrogen cyanide, iodofom, formaldehyde, chloropicrin, 1,3-dichloropropene, dazomet (methyl isothiocyanate precursor), phosphine, sulfuryl fluoride, etc. These fumigants are known to have a deleterious effect to human health.

From the past to the present day, man has consumed local herbs and vegetables (medicinal plants) to improve health, and to prevent, protect from and cure diseases. Beetroot is one of such plants that has been greatly consumed globally by man on a daily basis because of its desirable taste, nutritional value, low cost and little or no visible side effects. According to Clifford et al. (2015), the recent and frequent consumption of red beetroot (Beta vulgaris) is linked to its biological activity, medicinal and food value. Its medicinal potential includes treatment and control of pathological disorders which include oxidative stress, liver disease (Nifnali & Angelino, 2013; Vulić et al., 2014), hypertension, atherosclerosis (Gilchrist et al., 2014), inflammation, arthritis (Vidal et al., 2014), type 2 diabetes and dementia (Nifnali & Angelino, 2013), and cancer (Kapadia et al., 2003; Kapadia et al., 2014). Beetroot is frequently consumed as part of normal diet in many countries of the world, especially in Africa. The common food colouring agent E162 is a product of beetroot (Georgiev et al., 2010; Zielińska-Przyjemska et al., 2009). According to Wootton-Beard & Ryan (2011), phytochemicals like ascorbic acid, carotenoids, phenolic acids and flavonoids are found in beetroot. According to Vulić et al. (2014), the plant is among the few vegetables that contain betalains (Lee et al., 2005). Vulić et al. (2014) reported betalains to have antioxidant and anti-inflammatory capabilities in vitro and in vivo in a variety of animal models (Zielińska-Przyjemska et al., 2009). A study by Vidal et al. (2014) showed further backing for the anti-inflammatory ability of betalains. According to Ricciotti & FitzGerald (2011), betadin extracted from beetroot suppresses cyclooxygenase-2 (COX-2) synthesis, inhibited lipooxygenase, a catalytic enzyme vital for generating pro-inflammatory leukotriene molecules. Vidal et al. (2014) linked these effects that appear to be facilitated by inhibition of membrane binding activity. This proves that betalains target cell signalling pathways at the molecular level and acts in a similar fashion to selective COX-2 inhibitor drugs (Ricciotti & FitzGerald, 2011). According to Park et al. (2001), the vegetable plant (Beetroot) has shown its protective and healing potential against tumours and heart conditions. Chakole et al. (2001), reported beetroot as a powerful antioxidant, sex hormone enhancer and an aphrodisiac agent. Its juice is also administered as a natural remedy for sexual weakness and to remove kidney and bladder stones (Chakole et al., 2001). Beetroot leaves have the potential to induce wound healing within a short time (Singh et al., 2011).

This study assessed the curative and protective potentials of Beetroot using albino Wistar rats exposed to an organophosphate, dichlorvos. This was successfully carried out by accessing the effects of this plant on some biochemical parameters including alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total protein, total bilirubin, albumin, creatinine, urea and electrolytes (sodium, potassium and bicarbonate). The liver and kidney were also subjected to histopathological examination to assess the level of damage caused by dichlorvos as well as the protective and restoring ability of the plant smoothie on damaged organs.

Methods

Materials

The biochemical reagents and materials used in this research work were of analytical grade and include diethyl ether (Sigma chemicals Ltd.), formalin 10%, biochemical reagent kits (Randox) and rat chow (Top Feeds Ltd.).
Experimental animals
The animals used for this experiment were 30 50-days-old albino Wistar rats of equal sex, weighing between 120 and 160 g. They were purchased from and housed in the Pharmacology Department’s Animal House, University of Port Harcourt, Rivers state, Nigeria. Protocol for animal handling for this experiment was sought and obtained from the University of Port Harcourt Research Ethics Committee. Housing of animals was in Biochemistry Animal House of the University of Port Harcourt. Natural day/night cycle and temperature were maintained. Access to food and water (clean tap water) was ad libitum. Cages containing 6 animals per each were regularly cleaned and experiment lasted for 6 weeks.

Collection of plant materials
Fresh samples of red beetroot were purchased from Choba Market, along East-West Road, Choba, Rivers state, Nigeria.

Preparation and administration of Beetroot smoothie
The root of the Beetroot was used for this study. Plant smoothies were prepared using the method of Ejere et al. (2013). The fresh experimental plants were washed thoroughly under running tap water, cut into small pieces, blended without adding water and stored at -4°C until usage. Beetroot smoothie were administered orally at the doses of 500 mg kg⁻¹ (Kujawska et al., 2009).

Administration of DDVP to the animals
The mode of administration of DVVP to the experimental animals was through inhalation. Cotton wool soaked in 15 ml DVVP was placed in a container and was kept inside poorly ventilated cages. The container prevents the rats from ingesting the cotton wool soaked in DVVP. During the night, the animals inhaled the pesticide for 4 hours daily throughout the exposure period as stipulated in the groups defined under ‘Experimental Design’.

Experimental design
The 30 albino Wistar rats used for this study were grouped into five groups comprising of six animals (3 males and 3 females selected at random) per group. All animals received distilled water and rat chow alongside their specific treatments. At the end of the experimental period, the animals were sacrificed by cervical dislocation of the neck. Each group consisted of n=6 animals in a group and was as follows: Group I: Negative control. Rat chow + clean water only.

Group II: Positive control. Exposed to DDVP throughout the experimental period.

Group III: Beetroot control – administered 500 mg/kg/body weight beetroot smoothie.

Group IV: Beetroot before DDVP - administered 500 mg/kg/body weight beetroot smoothie for 3 weeks before exposure to DDVP till the end of the experiment.

Group V: DDVP before Beetroot - exposed to DDVP for 3 weeks before receiving 500 mg/kg/body weight beetroot smoothie till the end of the experiment.

Determination of renal markers
The concentrations of the electrolytes Na⁺, K⁺ and HCO₃⁻ were assayed using an electrolyte auto-analyzer (Medica EasyLyte). The concentrations of Na⁺ and K⁺ were determined by direct measurement procedures described by Walker et al. (1971), while that of HCO₃⁻ was determined by manometric method of Rogers et al. (1976). Randox test kits (Randox Laboratories) were used to determine the levels of urea (Cat. No. UR220) and creatinine (Cat. No. CR510) in the samples.

Assay of studied hepatic markers
The plasma activities of alanine transaminase, aspartate transaminase and alkaline Phosphatase were assayed using Reitman and Frankel method, as reported by Chuku et al. (2012). Brom cresol green method was used to determine the level of plasma albumin in the samples according to the method of Doumas et al. (1971). The biuret method was used to determine the level of total protein in the samples according to the method of Flack and Woollen, as described by Tietz (2005). Randox test kit was used to determine the level of total bilirubin in the samples.

Method for histopathological staining
Tissue sections were put into processing/embedding cassette and transferred into the tissue processor for a 24-h cycle of automatic processing. They were embedded using the moulds, cassette and molten paraffin wax and trimmed, picked, floated out in a warm water bath, labelled and dried in a hot plate. The slides were de-waxed in xylene two times and dehydrated through descending grades of alcohol from absolute alcohol down to water. The sections were stained in haematoxylin solution for 20 min, washed in water and briefly differentiated in 1% acid-alcohol. After further washing with tap water for 8 min, counterstaining in 1% eosin for 5 min followed. They were washed in water and dehydrated in ascending grades of alcohol, starting from 70% alcohol to absolute alcohol. Clearing in xylene preceded covering with a DPX mount and coverslip, labelling and examination under the microscope.

Statistical analysis
All replicate data were subjected to statistical analysis. Values were reported as mean ± standard error of mean (SEM). Using SPSS v20, One-way ANOVA was used to test for differences between treatment groups. Post hoc analysis was performed using Duncan’s multiple range test. The results were considered significant at p-values less than or equal to 0.05, that is, at 95% confidence level.

Results
Biochemical tests
Biochemical tests such as alanine transferase, aspartate transferase, alkaline phosphatase, total protein, total bilirubin, albumin, creatinine, urea, and electrolytes (sodium, potassium and bicarbonate) were carried out to determine their activities or concentrations in the various blood samples from test and control animals across all groups. The obtained results are presented in Table 1 and Table 2 while histopathology photomicrographs are presented in Plates 1 to 10.
Table 1. Effect of beetroot smoothie on the liver biomarkers of the experimental animals.

<table>
<thead>
<tr>
<th>Groups</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/I)</td>
<td>64.33±52.78abc</td>
<td>99.33±22.94c</td>
<td>60.67±34.02abc</td>
<td>41.00±10.15abc</td>
<td>35.67±11.68abc</td>
</tr>
<tr>
<td>ALT (U/I)</td>
<td>12.33±2.52abc</td>
<td>47.67±4.73c</td>
<td>14.67±5.86c</td>
<td>10.00±2.00abc</td>
<td>9.33±2.31abc</td>
</tr>
<tr>
<td>ALP (U/I)</td>
<td>73.00±20.95abc</td>
<td>170.67±5.511c</td>
<td>47.33±6.43c</td>
<td>62.67±32.25abc</td>
<td>93.67±12.90abc</td>
</tr>
<tr>
<td>Total Protein (g/l)</td>
<td>74.00±12.00abc</td>
<td>57.00±14.80a</td>
<td>69.00±1.73bc</td>
<td>83.67±6.35abc</td>
<td>75.00±1.73abc</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>33.67±2.31a</td>
<td>33.33±2.52a</td>
<td>35.00±2.65a</td>
<td>34.33±3.06a</td>
<td>33.33±4.16a</td>
</tr>
<tr>
<td>Total Bil. (µmol/l)</td>
<td>17.83±3.73a</td>
<td>15.43±5.61a</td>
<td>17.63±4.10a</td>
<td>12.37±1.10a</td>
<td>16.03±2.14a</td>
</tr>
</tbody>
</table>

Values are expressed in Mean ± SEM
Groups with different superscript(s) are significantly different at p≤0.05

Group I = Negative control; Group II = Positive control; Group III = Beetroots only; Group IV = Beetroots before DDVP; and Group V = DDVP before beetroots.

Table 2. Effect of beetroot smoothie on the renal function of the experimental animals.

<table>
<thead>
<tr>
<th>Groups</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mmol/l)</td>
<td>5.37±4.31a</td>
<td>7.77±1.63a</td>
<td>6.07±1.27a</td>
<td>4.50±0.95a</td>
<td>5.07±2.76a</td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>90.33±61.16b</td>
<td>453.33±102.63b</td>
<td>89.33±48.34ab</td>
<td>92.53±61.21b</td>
<td>40.00±20.00b</td>
</tr>
<tr>
<td>Na+ (mmol/l)</td>
<td>108.67±14.84b</td>
<td>89.33±8.96ab</td>
<td>64.67±12.10a</td>
<td>81.67±16.86ab</td>
<td>86.00±18.19b</td>
</tr>
<tr>
<td>K+ (mmol/l)</td>
<td>5.01±1.78abc</td>
<td>7.37±1.07abc</td>
<td>5.13±0.72a</td>
<td>5.18±0.44ab</td>
<td>5.00±0.75ab</td>
</tr>
<tr>
<td>HCO3- (mmol/l)</td>
<td>28.33±1.15b</td>
<td>29.33±2.31ab</td>
<td>28.63±1.15a</td>
<td>24.67±1.15a</td>
<td>27.33±1.15ab</td>
</tr>
</tbody>
</table>

Values are expressed in Mean ± SEM
Groups with different superscript(s) are significantly different at p≤0.05

Group I = Negative control; Group II = Positive control; Group III = Beetroots only; Group IV = Beetroots before DDVP; and Group V = DDVP before beetroots.

Histopathology results

The histopathological results of the liver (Figure 1a–e) and kidney (Figures 2a–e) are shown using photomicrographs. Figure 1a is the section of the liver from group I animals showing normal lobular architecture. A liver photomicrograph of Group II animals showed congested central vein with increased inflammatory cells as shown in Figure 1b. Group III animals showed congested central vein with normal liver cells as given in Figure 1c. From Figure 1d, distorted architecture with hyperchromatic nuclei in some of the hepatocytes was seen in group IV animals. Distorted liver sections of group V animals (Figure 1e) showed hepatic fat accumulation with distinct histologic features.

Figure 2a shows a kidney section from a Group I animal, showing normal renal tubules and glomeruli containing the Bowman’s capsule. Group II animals (Figure 2b) showed distorted renal tubules and glomeruli with obliterated capsular spaces. For group III animals, kidney sections revealed normal renal tubules and glomeruli containing Bowman’s capsule (Figure 2c). For group IV animals, as shown in Figure 2d, distorted kidney sections revealed renal tubules and glomeruli with obliterated capsular spaces. Also, group V animals showed renal tubules and glomeruli with obliterated capsular spaces in the distorted kidney section (Figure 2e).

Discussion

The pesticide dichlorvos, widely used in most homes in Africa against household pests affects some organs in the body such as the liver and kidney. According to Sheiner et al. (2003), these organs are affected by direct or indirect exposure to dichlorvos. Thus, dichlorvos toxicity on these organs were investigated by assessing the liver enzyme activities and renal function tests which serves as the biomarkers for the liver and kidney function respectively. According to Ozer et al. (2008), liver enzymes, ALT and AST, are the common liver damage biomarkers (Nwaichi et al., 2017) because these enzymes are readily released into the extracellular space by the hepatocytes. Thus, the high levels of AST and ALT observed in this study (Table 1) indicate that the organophosphate compound dichlorvos is hepatotoxic. This hepatotoxic affinity of dichlorvos to the liver and its enzymes have been earlier reported by Celik et al. (2009) and Garba et al. (2013), who posited that dichlorvos causes liver damage in rats. According to Edelstein (2008), the elevated levels of creatinine and urea (outside the recommended ranges of 60–110 mmol/L and 2.5–7.1 mmol/L, respectively) in the blood (Mayo Clinic Laboratories, 2019) samples from dichlorvos-exposed population used to diagnose kidney injury and the tremendous rise in the concentration of other kidney biomarkers in this study is good enough to categorically state that dichlorvos...
Figure 1. Histopathological staining of the liver. (a) Liver photomicrograph of Group I animals showing the central vein, sinusoids & normal hepatocytes. (b) Liver photomicrograph of Group II animals showing congested central vein with increased inflammatory cells. (c) Distorted liver photomicrograph of Group III animals showing congested central vein (CV) with normal hepatocytes. (d) Distorted liver photomicrograph of Group IV animals showing microvesicular steatosis. (e) Distorted liver photomicrograph of Group V animals showing microvesicular steatosis.

Figure 2. Histopathological staining of the kidney. (a) Kidney photomicrograph of Group I animals showing normal renal tubules & glomeruli containing Bowman’s capsule. (b) Kidney photomicrograph of Group II animals showing distorted renal tubules (DRT) & glomeruli with obliterated capsular spaces. (c) Kidney photomicrograph of Group III animals showing normal renal tubules & glomeruli containing Bowman’s capsule. (d) Distorted kidney photomicrograph of Group IV animals showing renal tubules & glomeruli with obliterated capsular spaces. (e) Distorted kidney photomicrograph of Group V animals showing renal tubules & glomeruli with obliterated capsular spaces.
is possibly nephrotoxic. Higher potassium levels, above the 6.0 mmol/L obtained for the animals exposed to dichlorvos could be potentially dangerous, as reported by Mayo Clinic Laboratory, (2019).

The toxicity of this organophosphate compound did not spare the organs (liver and kidney) under examination, as shown by the histopathological results. According to Abdelhalim & Jarrar (2012), dichlorvos-induced histological alterations in the liver of rats might be an indication of injured hepatocytes due to the toxicity of the agent. Sharma & Singh (2012) linked hepatocytes degeneration and destruction to the generation of reactive oxygen species (ROS) generated by dichlorvos. Histopathological examination of the kidney attacked by dichlorvos (in the positive control animals) revealed many alterations ranging from distorted renal tubules (tubular degeneration) and glomeruli with obliterated capsular spaces (atrophy of glomeruli) to congestion of renal blood vessels. This was not different from the reports of Elhalwagy et al. (2008); Kalender et al. (2007); Sulak et al. (2005) and Mohnsen (2001) that pesticides cause various histopathological changes in kidney tissues of experimental animals.

From this study, beetroot has shown its potential in the reduction and/or control of stress response and also played enormous role in the protection of the liver and kidney organs damaged/affected by dichlorvos exposure, as shown by the histopathology results, and may require more time for adequate recovery or protection. This ability of beetroot plant to regulate the activities of these enzymes can be attributed to the presence of bioactive compositions (Vali et al., 2007) such as the phenols, flavonoids, terpenoids and saponins. Also, according to Vali et al. (2007), beetroot contains the bioactive agent betaine, which supports healthy liver functioning and flavonoids, which have been shown to have antibacterial, anti-inflammatory, anti-allergic, antiviral and antineoplastic (Mishra et al., 2009) properties. Reports have also shown that saponins possess tumour-inhibiting activity in vivo (Akindahunsi & Salawu, 2005). Thus, its presence can control human cardiovascular disease and reduce blood cholesterol. There was also a significant reduction in the observed levels of urea and creatinine in the rats exposed to dichlorvos (Table 2) from 7.77 and 453.33 to 4.5 and 92.53 mmol/L, respectively, for animals administered beetroot smoothie prior to dichlorvos exposure. This, again, could be attributable to the bioactive compounds (phytochemicals) present in the plants.

Conclusion
This study reveals that dichlorvos exposure is deleterious to the organs studied (liver and kidney), evident in the altered biomarkers and histopathological microphotographs of the organs. The efficacy of the beetroot smoothies in lowering elevated levels of liver enzymes (ALT and AST), urea and creatinine was observed in both subjects treated with beetroot pre- and post-dichlorvos exposure. Thus, beetroot potentially possesses both protective and curative properties and as well as anti-nephrotoxicity potential. The beetroot plant smoothie also showed their regenerative potential in recuperating the damaged organs. Thus, the study has shown that beetroot has the ability to regulate and reduce impaired cell integrity expressed in hepatic and renal markers in individuals exposed to dichlorvos and could be explored in the treatment of hepatic and renal conditions as well as in the protection of the liver and kidney from some oxidative stress.

Data availability
Underlying data

This project contains the following underlying data:

- Chinonso Raw Data.docx (uncropped microscopy images taken during the present study).
- MY RAW DATA Nonso.xlsx (the biochemical parameters for each rat assessed in this study).

Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain dedication).

Grant information
E.O.N. is an affiliate of the African Academy of Sciences. The authors declare that no grants were involved in supporting this work.

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Denis Zofou

Medical Research and Applied Biochemistry Laboratory (Drug Discovery and Development Research Unit), University of Buea, Buea, Cameroon

Nwaichi et al. submitted a manuscript where they explored the protective and curative potentials of the roots of Beta vulgaris to prevent and cure exposure to dichlorvos exposure in Wistar rats. It was a great pleasure reviewing this work, which is very relevant, considering the enduring burden of dichlorvos and other organophosphate residues in farmers and consumers in Africa. The work is well written, and the objectives are clearly presented. The methodology employed also reflects the results presented. However, the following issues need to be addressed before the manuscript can be considered for final publication.

1. **Study design:** In order to achieve the set objectives, the authors work with five experimental groups: Group I not exposed to dichlorvos, not treated (named by authors as “Negative Control”); Group II exposed to dichlorvos but received no smoothie (termed as “Positive control”); Group III not exposed and treated with 500 mg/kg beetroot smoothie; Groups IV treated before exposure to dichlorvos; and Group V receiving treatment after exposure.

   Though this design allows observing the effect of smoothie on animals both exposed or not, I might suggest the naming of the groups be readjusted. Group ‘not exposed and not treated’ is more of a “normal” or “reference group” than “Negative control”. Group II is a typical “Negative Control”. Group III, IV and V are test groups with different specific objectives.

   We also notice there was no “Positive Control” product used as a reference drug in this study.

2. **Authors’ contributions:** There are three authors of which two are said to have contributed in “Supervision” and the third contributed through fund raising, among other tasks. I propose the authors revise this section to clearly indicate those of the authors who carried out bench work.
3. **Collection of plant materials:** The material was purchased from a market. However, there is need to indicate whether the plant was properly identified by a botanist, since there is no assurance the sellers or the person who purchased the plant material have the required ability to clearly identify the plant and distinguish it from the closest species.

4. **Administration of DDVP to the animals:** For how long were the animals submitted to inhalation of the DDVP?

5. **Choice of extract dosage:** The authors used a single dose of 500 mg/kg of smoothie. While they didn’t provide any scientific data justifying why they selected this specific dosage, it would have been more consistent to test at least three different dosages of this extract, in order to confirm the plant effect through dose-response correlation analysis, and equally select the best dosage.

6. **Discussion:** I suggest the discussion be extended to suggest ideas on mechanism of action of this plant extract, based on its phytochemical composition.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Pharmacology / Drug discovery

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.
Eucharia Nwaichi, University of Port Harcourt, Port Harcourt, Nigeria

Study design: In order to achieve the set objectives, the authors work with five experimental groups: Group I not exposed to dichlorvos, not treated (named by authors as “Negative Control”); Group II exposed to dichlorvos but received no smoothie (termed as “Positive control”); Group III not exposed and treated with 500 mg/kg beetroot smoothie; Groups IV treated before exposure to dichlorvos; and Group V receiving treatment after exposure.

**Question:** Though this design allows observing the effect of smoothie on animals both exposed or not, I might suggest the naming of the groups be readjusted. Group ‘not exposed and not treated’ is more of a “normal” or “reference group” than “Negative control”. Group II is a typical “Negative Control”. Group III, IV and V are test groups with different specific objectives.

**Response:** Thank you so much for finding time to make great contributions to our work.

**Response:** Responding to your concerns, negative control is the opposite of the positive control, in which a known response is expected hence Group I is a negative control in this set up.

**Question:** We also notice there was no “Positive Control” product used as a reference drug in this study.

**Response:** On the other hand, a positive control is a group in an experiment that receives a treatment with a known result, and Group II contains dichlorvos with known result and therefore is appropriately labelled. It gives us something to compare with the test groups.

**Question:** Authors’ contributions: There are three authors of which two are said to have contributed in “Supervision” and the third contributed through fund raising, among other tasks. I propose the authors revise this section to clearly indicate those of the authors who carried out bench work.

**Response:** Thanks for the observation. We have a joint supervision style for Graduate Programs in my institution and EON and EBE were supervisors hence we wish to retain status quo.

**Question:** Collection of plant materials: The material was purchased from a market. However, there is need to indicate whether the plant was properly identified by a botanist, since there is no assurance the sellers or the person who purchased the plant material have the required ability to clearly identify the plant and distinguish it from the closest species.

**Response:** Thank you. The plant was duly identified by a botanist in the Department of Plant Science and Biotechnology of the University of Port Harcourt.

**Question:** Administration of DDVP to the animals: For how long were the animals submitted to inhalation of the DDVP?

**Response:** The animals were allowed to inhale the pesticide for 4 hours daily throughout the exposure period.

**Question:** Choice of extract dosage: The authors used a single dose of 500 mg/kg of smoothie. While they didn’t provide any scientific data justifying why they selected this specific dosage, it would have been more consistent to test at least three different dosages of this extract, in order to confirm the plant effect through dose-response correlation analysis, and equally select the best dosage.

**Response:** Kujawska et al. (2009)’s method was used and reported.
**Question**: Discussion: I suggest the discussion be extended to suggest ideas on mechanism of action of this plant extract, based on its phytochemical composition.

**Response**: Thanks a lot. Discussion was kept around the scope of the work and that excludes mechanism of action.

**Competing Interests**: There are no competing interests.