

Influence of *Daucus carota* on the Hepatic and Renal Biomarkers of Dichlorvos-Exposed Albino Rats

Nwaichi EO*, Essien EB and Ibeh UC

Department of Biochemistry, University of Port Harcourt, Rivers State, Nigeria *Corresponding Author: Nwaichi EO, Department of Biochemistry, University of Port Harcourt, Rivers State, Nigeria. Received: July 09, 2019; Published: September 09, 2019

Abstract

Aim: This study was premeditated to evaluate the effect of *Daucus carota* (carrot) smoothie on some biochemical assays in dichlor-vos-exposed albino wistar rats.

Methodology: Twenty (30) rats of both sexes were grouped into five comprising six (6) animals each. Group I served as the negative control and were not exposed to DDVP. Group II served as the positive control and were exposed to DDVP. Group III received 300 mg/kg/body weight carrot smoothie throughout the period of the experiment and were not exposed to DDVP. Groups IV and V were exposed to DDVP but received carrot smoothie before and after the exposure respectively. At the termination of the 6-weeks period of 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 carrot smoothie significantly reduced the levels of AST, ALT, urea and creatinine in study samples. It was also observed that administered carrot smoothie increased total protein concentration of the blood. The histopathology examination showed that the carrot smoothie was able to regenerate the liver and kidney organs that were damaged by dichlorvos exposure.

Conclusion: This study concluded that carrot tubers possess hepatoprotective, hepatocurative and nephro-curative properties and could be explored in nutrition and health.

Keywords: Daucus carota; Dichlorvos; Biochemical Indices; Histopathology; Hepatocurative

Introduction

To achieve a high yield agricultural produce, pesticides has long been employed as it gets rid of unwanted insects and disease vectors [1]. This group of chemicals commonly known as organophosphate compounds are massively utilized globally due to its numerous benefits to human in the health and agricultural sector. According to Abdollahi, *et al.* [2] so many severe environmental and health hazards has been associated to the utilization of these compounds. Aly and El-Gendy [3] has reported the effects of exposure to these compounds to the liver, kidney, nervous, immune and reproductive system. Thus, indicating the toxicity potential of organophosphate compounds which differs according to the class, biological activities and the different antagonistic effects they cause in living organisms which includes man. Dichlorvos, this study pesticide is an organophosphate compound used daily on a massive scale to get rid of insects. Recently, the consumption and use of medicinal plants to combat various diseases has been on the increase globally because of their natural origin and less significant side effects. In the time past and now, man has been consuming local herbs and vegetables (medicinal plants) as to

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improve human health, which includes prevention, protection and cure of disease. Daucus carota (carrot), given its reported nutritional and medicinal value are not exempted from such plants highly consumed by man. This is due to the presence of some bioactive substances with pharmacological properties found in them [4]. Carrot has been greatly consumed by humans globally on a daily basis due to its desirable taste, nutrients, low cost and little or no side effect. It belongs to the family Apiaceae and is planted globally as a valuable vegetable. According to Jasicka-Misiak., et al. [5] different parts of this plant has shown antagonistic effect over broad spectrum aliments which includes kidney dysfunction, asthma, worm infections and inflammation. Balasubramaniam., et al. [6] clearly stated the protective effects of carrot extract on myocardial infarction and lindane-induced hepatotoxicity. According to John and Spence [7] the plant contains several secondary metabolites such as steroids, tannins, flavonoids, carotene and volatile oils. Also contained in carrots are carotenoids and anthocyanins, which are very important antioxidant, and can further act as an anti-carcinogen and immunoenhancer. Its medicinal value is also linked to the high pro-vitamin A content [8]. Horbowicz., et al. [9] reported the presence of kaempferol, quercetin and luteolin in Daucus carota extract. Gonçalves., et al. [10] also stated the existence of chlorogenic acid, hydroxycinnamic acid and its derivatives in different carrot tissues. Carrot has been confirmed to be a very good source of certain nutrients such as dietary fiber, magnesium (Mg) and some trace elements like manganese (Mn) and molybdenum (Mo) which is hardly contained in many vegetables. Zaini., et al. [11] reported the anti-carcinogenic affinity of carrot extracts on myeloid and lymphoid leukemia cell lines. Carrot is also known to have high content of β-carotene which has the affinity to restore vision. Thus, high consumption of carrots should be encouraged for clear, efficient and adequate visual purpose. According to Wu., et al. [12] smokers that consume carrots frequently stand the chance of minor threat of lung cancer and β -carotene rich diet has the tendency to inhibit the progress of prostate cancer. Dias [13] also reported the presence of pyridoxine (Vit. B_c) in carrot. This vitamin inhibits the development of homocysteine and thus, decreases the risk of heart diseases; as toughening of arteries due to the build-up of fatty plaques is associated with high levels of homocysteine. Bishayee., et al. [14] stated that carrot extract has the tendency of protecting the liver from chemical-induced acute injury. Despite the various preventive measures to control the use of pesticides, the persistent use has caused serious complication to both humans and the ecosystem. Thus, it becomes a major concern to researchers, and hence this study assesses the curative and protective potentials of carrot in exposed albino Wistar rats exposed to the organophosphate.

Materials and 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 feed (Top Feed Ltd.).

Experimental animals

The animals used for this experiment were thirty (30) albino wistar rats weighing between 120 - 160g. They were purchased from and housed in the Pharmacology Department Animal House, University of Port Harcourt, Rivers state, Nigeria.

Collection of plant materials

Fresh samples of carrot were purchased from Choba market, along East-West Road, Choba, Rivers state, Nigeria.

Preparation and administration of beetroot smoothie

The carrot tuber was used for this study. Smoothie was prepared using the method of Ejere., *et al* [15]. The fresh carrots were washed thoroughly under running tap water, cut into small pieces, blended without adding water and stored at -4°C until usage. Carrot smoothie was administered orally at the doses of 300 mgkg⁻¹ [16].

Administration of dichlorvos (DDVP) to the animals

The mode of administration of dichlorvos to the experimental animals was through inhalation. Cotton wool soaked in dichlorvos was placed in a container and was kept inside poorly ventilated cages. The container prevents the rats from ingesting the cotton wool soaked

in dichlorvos. The animals were allowed to inhale the pesticide for 4 hours daily throughout the exposure period as stipulated in the groups.

Experimental design

The thirty (30) albino Wistar rats used for this study were grouped into five groups comprising of six (6) animals per group. All the animals received distilled water and rat chow alongside their specific treatments. The grouping is as follows:

- 1. Negative control:Rat chow + clean water only
- 2. Positive control: Exposed to DDVP throughout the experimental period
- 3. Carrot control: Fed 300 mg/kg/body weight carrot smoothie
- 4. Carrot before DDVP: Fed 300 mg/kg/body weight carrot smoothie for 3 weeks before exposure to DDVP till the end of the experiment.
- 5. DDVP before Carrot: Exposed to DDVP for 3 weeks before receiving 300mg/kg/body weight carrot smoothie till the end of the experiment.

Statistical analysis

All data were subjected to statistical analysis. Values were reported as Mean \pm standard error of mean (SEM) while one way ANOVA was used to test for differences between treatment groups. The results were considered significant at p-values of less than 0.05, that is, at 95% confidence level (p < 0.05).

Results

Biochemical tests such as alanine transferase (ALT), aspartate transferase (AST), alkaline phosphatase (ALP), total protein, total bilirubin, albumin, creatinine, urea, and electrolytes (sodium, potassium and bicarbonate) were assayed for activities and amounts in various blood samples. The results obtained are presented in table 1 and 2 while the histopathology results are presented in figure 1 and 2.

Histopathology results

The following plates show the histopathological results obtained for the liver (Figure 1a-1e) and kidney (Figure 2a-2e) of the experimental animals in typical photomicrographs.

Groups	I	II	III	IV	V
AST (U/I)	$64.33 \pm 52.78^{a,b,c}$	99.33 ± 22.94°	$72.67 \pm 9.24^{\rm b}$	23.67 ± 4.16^{a}	38.33 ± 1.53 ^{a,b}
ALT (U/I)	$12.33 \pm 2.52^{a,b}$	47.67 ± 4.73°	$11.00 \pm 1.73^{a,b}$	8.33 ± 1.53ª	$11.33 \pm 1.53^{a,b}$
ALP (U/I)	$73.00 \pm 20.95^{a,b}$	170.67 ± 5.51°	43.33 ± 3.21 ^a	125.67 ± 31.97°	78.33 ± 17.56 ^{a,b}
Total Protein (g/l)	$74.00 \pm 12.00^{a,b}$	57.00 ± 14.80^{a}	58.67 ± 4.73 ^a	73.67 ± 11.24 ^{a,b}	$74.33 \pm 2.31^{a,b}$
Albumin (g/l)	33.67 ± 2.31ª	33.33 ± 2.52ª	36.00 1.00ª	$35.00 \pm 2.00^{\circ}$	34.33 ± 1.53^{a}
Total Bil. (μmol/l)	17.83 ± 3.73^{a}	15.43 ± 5.61^{a}	11.73 ± 3.86^{a}	11.73 ± 2.80^{a}	17.30 ± 1.04^{a}

Table 1: Effect of carrot smoothie on the liver biomarkers of the experimental animals.

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 = C only; Group IV = C b4 DDVP; Group V = DDVP b4 C.

(N/B: C = Carrot, b4 = before).

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Groups	I	II	III	IV	V
Urea (mmol/l)	5.37 ± 4.31ª	7.27 ± 1.63^{a}	4.87 ± 1.03^{a}	$4.60 \pm 1.85^{\circ}$	4.00 ± 2.10^{a}
Creatinine (µmol/l)	90.33 ± 61.16 ^{a,b}	453.33±102.63°	148.00±67.67 ^{a,b}	$173.33 \pm 144.68^{a,b}$	$120.00 \pm 87.18^{a,b}$
Na⁺ (mmol/l)	108.67 ± 14.84 ^b	89.33 ± 8.96 ^{a,b}	82.67 ± 22.74 ^{a,b}	99.33 ± 2.52 ^b	106.00 ± 1.73^{b}
K⁺ (mmol/l)	$6.30 \pm 1.78^{a,b,c}$	$7.37 \pm 1.07^{b,c}$	$7.97 \pm 0.25^{b,c}$	5.33 ± 1.22^{a}	$6.40 \pm 0.72^{a,b,c}$
HCO ₃ ⁻ (mmol/l)	29.33 ± 1.15^{b}	27.33 ± 2.31 ^{a,b}	$28.00 \pm 3.46^{a,b}$	$26.00 \pm 0.00^{a,b}$	$25.33 \pm 4.16^{a,b}$

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

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 = C only; Group IV = C b4 DDVP; Group V = DDVP b4 C. (N/B: C = Carrot, b4 = before).

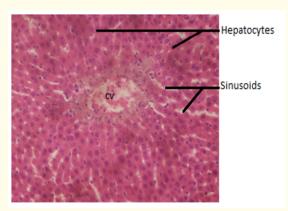


Figure 1a: Liver photomicrograph of Group I animals showing the central vein, sinusoids and normal hepatocytes.

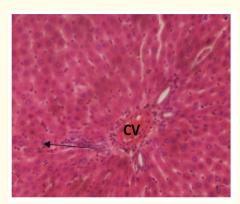


Figure 1b: Liver photomicrograph of Group II animals showing congested central vein with increased inflammatory cells.

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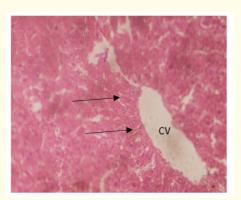


Figure 1c: Normal liver photomicrograph of Group III animals showing cords of hepatocytes radiating away from the CV.

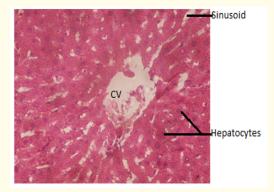


Figure 1d: Normal liver photomicrograph of Group IV animals showing the central vein (CV), hepatic sinusoids and cords of hepatocytes.

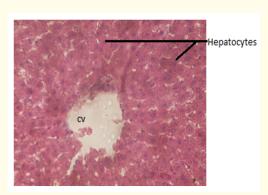


Figure 1e: Normal liver photomicrograph of Group V animals showing the central vein (CV) and cords of hepatocytes.

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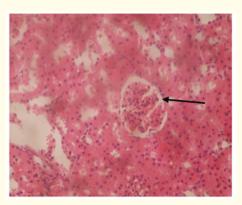


Figure 2a: Kidney photomicrograph of Group I animals showing normal renal tubules and glomeruli containing bowman's capsule.

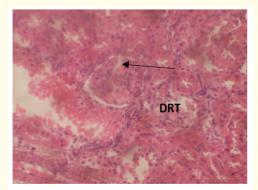


Figure 2b: Kidney photomicrograph of Group II animals showing distorted renal tubules (DRT) & glomeruli with obliterated capsular spaces.

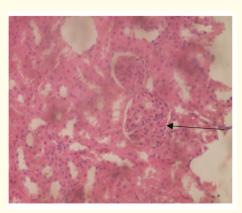


Figure 2c: Normal kidney photomicrograph of Group III animals showing renal tubules and glomeruli.

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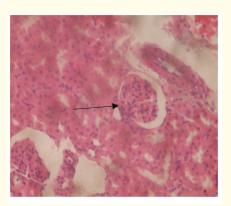


Figure 2d: Normal kidney photomicrograph of Group IV animals showing glomeruli containing bowman's capsule.

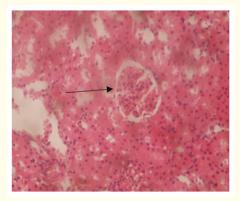


Figure 2e: Normal kidney photomicrograph of Group V animals showing glomeruli containing bowman's capsule

Discussion

To achieve a high agricultural produce by getting rid of unwanted insects and disease vectors, the application of pesticides becomes non-negotiable [1]. Pesticides are group of chemicals commonly known as organophosphate and they are massively utilized globally due to its vital need in agricultural sector. According to Abdollahi., *et al.* [2], so many severe environmental and health hazards has been associated to the utilization of these compounds. Aly and El-Gendy [3], stated the negative effect of this compound on the liver, kidney, nervous system, immune system and reproductive system. Thus, indicating the toxicity potential of organophosphates which varies depending on the class, biological activities and the different antagonistic effects they cause in living organisms which includes man. The negative impact of dichlorvos toxicity was assessed in this study by analysing the liver enzymes and renal function tests which serves as the biomarkers for the liver and kidney damage respectively. It was observed that exposure to dichlorvos led to significant deterioration of the liver and kidney organs. Thus, verifying earlier studies that indicated the hepatotoxic nature of organophosphate compounds. This hepatotoxic affinity of dichlorvos to the liver and its enzymes was also reported by Celik., *et al.* [17] and Garba., *et al* [18]. From the histopathological examination carried out in this study, it was observed that the organophosphate compound (dichlorvos) did not spare neither the liver nor the kidney. Its negative impact on the kidney was measured by the concentration of creatinine and urea in the blood. The tremendous

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rise in the concentration of these renal biomarkers was good enough to categorically state that the pesticide (dichlorvos) is possibly nephrotoxic. According to Sharma and Singh [19] the degeneration and destruction observed in the hepatic and renal tissues can be associated to the generation of reactive oxygen species (ROS) initiated by dichlorvos. The histopathological examination of the kidney attacked by dichlorvos revealed many alterations ranging from tubular degeneration and atrophy of the glomeruli to congestion of renal blood vessels. This was in agreement with the report of Elhalwagy, et al. [20] that pesticides cause various histopathological changes in kidney tissues of experimental animals [21]. It was observed from this study that Daucus carota (carrot) showed its potential in the reduction of elevated liver enzymes (Table 1), and renal biomarkers (Table 2), and also played enormous role in the regeneration of the liver and kidney tissues damaged by dichlorvos exposure as shown by the photomicrographs of sections of liver and kidney (Figure 1 and 2). This ability of carrot tubers to regulate these markers can be credited to the presence of flavonoids, kaempferol, quercetin and luteolin in carrot [9]. Other bioactive compounds found in carrot are high pro-vitamin A, carotenoids and anthocyanins, which are the major antioxidant pigments found in carrots [8]. The widely used orange carrot are also known to possess high amount of α - and β -carotene and is a rich source of pro-vitamin A. All these bioactive agents are known to support healthy liver function, and have also shown to possess antibacterial, anti-inflammatory, anti-allergic, antiviral, and antineoplastic properties [22]. Reports have also shown that carrot possess falcarinol which is one of the most bioactive phytochemical that has exhibited tumor inhibiting activity on experimental animals [23]. The efficacy of the carrot smoothie in increasing total protein, lowering elevated liver enzymes (ALT and AST), urea and creatinine was observed. Carrot is known as a good source of dietary fiber and trace mineral molybdenum, rarely found in many vegetables. Molybdenum aids in metabolism of fats and carbohydrates, and absorption of iron. It is also a good source of magnesium and manganese which are required for bone formation, synthesis of new cells, activating B vitamins, relaxing nerves and muscles, blood clotting, and energy production. Thus, its presence has the tendency to control human cardiovascular disease, reduce blood cholesterol and reduce free radical generation.

Conclusion

This study revealed the deleterious effect of dichlorvos exposure to some organs (liver and kidney) of the living system. This was confirmed by the assay of some biomarkers and histopathological examination of the organs. The carrot plant smoothie also showed its regenerative potential in recuperating the damaged organs. Thus, carrot possess both hepatoprotective and hepatocurative properties as well as anti-nephrotoxic potential which is dependent on its bioactive compounds. This study has shown that carrot has the ability to regulate and reduce elevated liver enzymes and renal biomarkers in individuals that are in constant exposure to the household pesticide, dichlorvos and attendant benefits could be optimised.

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