Original Article

Hormonal, Biochemical and Haematological Changes in Adult Albino Rats Treated with *Daucus Carota* Root Tuber Extract

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Abstract

The effects of *Daucus carota* tuber extract (DCTE) on the hormonal (testosterone and estrogen), biochemical, and hematological profile of adult male and female rats were investigated. Extract prepared from the carrots were subjected to an acute toxicity test. Two separate experiments were conducted, one for male rats and the other for females. In each experiment, 15 rats were divided into 3 groups of 5 rats each and were assigned, treatment groups. Group 1 was untreated and served as the control while groups 2 and 3 were treated with 500 and 1000 mg/kg body weight of DCTE. All treatments were through the oral routs and lasted for 28 days. At the end of the experimentation, the rats were sacrificed using mild ether soaked in cotton wool for sedation and cervical dislocation. Blood was collected through cardiac puncture for both hematology and serology. Results obtained showed an acute toxicity value greater than 5000 mg/kg body weight. Testosterone and estrogen concentrations were significantly higher in the DCTE treated male and female rats respectively when compared to control. Results of biochemical tests including total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, urea, creatinine, and triglycerides (TAG) showed no significant difference between DCTE treated rats and the control ($p>0.05$) but total cholesterol concentration was significantly reduced while high-density lipoprotein cholesterol (HDL-C) concentration increased following treatment ($p<0.05$). But, hematological evaluation of the treated rats did not significantly differ from control ($p>0.05$) except for red blood cells (RBCs) count and hematocrit values which were significantly improved ($p<0.05$) in both male and female albino rats.

Keywords: *Daucus carota*, albino rats, hormonal, biochemical and hematological changes

Introduction

Dacus carota (carrot) is a member of the Apiaceae family that is widely distributed and cultivated globally for its fleshy edible roots.[1, 2] In Nigeria, the plant is mainly grown in the north but consumed across the nation's six geopolitical zones. It is known as karasby the Hausas, karoti, or atoka by the Yorubas and karotu by the Igbos. In Nigeria, the carrot has become a readily available source of dietary carotenoids, phenolic compounds, polyacetylenes, and vitamins A, B, and C. [3-5] Extracts from carrot tuber are currently being used traditionally to manage diseases such as diabetes mellitus and all forms of pain. Aphrodisiac and diuretic functions have also been attributed to the extract. [6]

Extensive scientific work has been carried out on carrots leading to the identification and isolation of a large number of active compounds such as volatile oils, steroids, tri-terpenes, carbohydrates, glycosides, tannins, flavonoids, amino acids, carotene, hydro-carotene, carotenoids, and anthocyanins, which are responsible for its nutritional and pharmacological effects. [7, 8] Data available on carrot extract in other areas include anti-cancer effect, [9, 10] anti-carcinogenic effect, [11] immune system enhancer, [12] anti-diabetic effect, [13] hypolipidaemic effect, [14] antihypertensive activity, [15] hepatoprotective effect and wound healing activities. [16, 17]

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Hormones are chemical messengers synthesized by the body that acts by binding with high affinity to target cells within the same individual. The modern definition takes the view that a hormone is any substance that acts at the cellular level to initiate, stop, or modulate a cellular process. The site of action can be nearby or at a distant target. [18] Reproductive functions in both male and female animals can be assayed by the serum level of reproductive hormones. This is because reproductive cycles in females and spermatogenesis in males function primarily due to the interplay between these hormones and the organs of reproduction. [19]

Biochemical and hematological assessments are relevant tools for the evaluation of the physiological and pathological status of animals, as they provide the basis for disease diagnosis, making a prognosis, assessment of the efficacy and toxicity of treatment agents. [20] Consumed food including herbs and vegetables are known to affect the hematological and serum biochemical parameters in apparently healthy individuals and laboratory animals, [21] hence alterations in the values of these parameters may be markers for diseases or toxicity. [22, 23]

This study was therefore designed to evaluate the effect of *Daucus carota* tuber extract on the hormonal (testosterone and estrogen), biochemical and hematological profiles of adult male and female rats to provide further scientific and reliable data to support the traditionally acclaimed reproductive and hematological effects of carrot extract.

Materials and Methods

Collection and identification of plant materials

Fresh carrot tubers were purchased from Orie-Ugba vegetable market, Umuahia, Umuahia North Local Government Area of Abia State, Nigeria and were taken to the Department of Forestry, College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike where the samples were identified as *Daucus carota* root tubers. A specimen sample was preserved in the herbarium of the Department of Zoology and Environmental Management, Michael Okpara University of Agriculture, Umudike with voucher number MOUAU/ZEB/20/005.

Preparation of plant extract

The tubers were washed, chopped into pieces, and dried in a hot air oven at 40^0C before been pulverized into powder for extraction. The extract was prepared from the pulverized sample by cold maceration following the method used by Orieke *et al*. [24] Briefly, 200 g of the powdered sample was added to 1 liter of ethanol in a container and was macerated intermittently within 24 hours. This was followed by filtration using a Whatman no.1 filter paper to obtain the extract in solution. This resulting solution was thereafter dried in a hot-air laboratory oven at temperature (40^0C) to obtain an oily red extract with characteristic aromatic smell. The extract so obtained weighed 8.03 g, represented a yield of 8.03% and it was referred to as *Daucus carota* tuber extract (DCTE).

Acute toxicity study

Thirty albino rats (120-140 g) assigned to 6 groups of 5 rats each were used for acute toxicity evaluation of the extract according to the method used by Enegide *et al*., [25] but with little modification. Each group was assigned a single oral dose of the extract such that Group 1= 500 mg/kg, Group 2 = 1000 mg/kg, Group 3 = 2000 mg/kg, Group 4 = 3000 mg/kg, Group 5 = 4000 mg/kg and Group 6 = 5000 mg/kg body weight. After treatments, the rats were returned to their respective cages and allowed free access to feed and water. The number of deaths recorded in each group within 24 hours and a further 7 days was noted and was used to evaluate the LD_{50}. The LD_{50} value of the extract was then calculated using Karber's arithmetic formula expressed as:

$$LD_{50} = LD_{100} - \frac{\sum(Dd \times Md)}{N}$$

Where:
LD_{100} = Dose that killed all animals in a group
LD_{50} = Dose that killed 50% of a given population
$\sum$ (Dd × Md): Sum of all products of dose difference and mean death
N = Number of animals in each group.

Animals and study design

A total of 30 adult male and female albino rats (120 to 180g) obtained from the laboratory animal house of the Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture, Umudike were used. The animals were housed in aluminum cages, allowed access to feed and water *ad libitum,* and acclimatized for two weeks before the commencement of the experiment. All animal experiments were carried out in compliance with international standards for care and use of laboratory animals. The animals were assigned to 3 groups of ten rats each such that group 1 rats were administered 0.2 ml distilled water and served as the control group while groups 2 and 3 were administered 500 and 1000 mg/kg body weight DCTE respectively. Treatment lasted for 28 days and was via the oral route. Body weights of the rats were taken on days one and twenty-eight of treatment before all animals were sacrificed by sedation using mild ether and cervical dislocation. Blood was collected through the cardiac puncture into EDTA and plain bottles for hematological and serological analysis respectively.

Determination of serum testosterone concentration

Serum testosterone concentration was determined by chemiluminescence immunoassay techniques using test kits and following protocols prescribed by the producer Autobio Diagnostics Co., Ltd. Zhengzhou, China. [26]

Serum biochemical analysis

Concentrations of serum biochemical parameters including total protein, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total bilirubin, urea, creatinine, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and very-low-density lipoprotein cholesterol (VLDL-C) were determined using their respective Randoxcommercial test kits following standard protocols and techniques described by Ugbogu *et al*. [27]

Hematological study

Hematological parameters including red blood cell (RBC) count, packed cell volume (PCV), hemoglobin content (HC), white blood cell (WBC) count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelets (PLT) count were determined at once for each blood sample using an automated hematology analyzer model BC-2800, Mindray company, India following standard protocols outlined by the producer.

Statistical analysis

Statistical analysis was done using a one-way analysis of variance (ANOVA) while results were presented as means ± standard error of the mean (SEM). Significant differences were assessed at a 95% level of significance between control and DCRE treated groups. P values less than 0.05 were considered significant. The computer software package, SPSS version 21 was employed

Results

Acute toxicity study

No mortality was observed in any of the treatment groups assigned graded doses of DCTE within the acute toxicity study period of seven days, even at the highest dose of 5000 mg/kg body weight administered. The animals in all groups remained physically agile and had stable disposition through-out the period, indicating an acute value greater than 5000 mg/kg body weight (Table 1).

Effects of DCTE on the hormone profile of the treated albino rats

In the male rats, testosterone concentration was significantly increased in the 1000 mg/kg body weight treated rats when compared with control ($p<0.05$), while for the female rats all dose treatments with DCTE significantly increased estrogen concentration when compared with control ($p<0.05$). Increase in the dose of DCTE did not significantly increase estrogen profile (Table 2)

Effects of DCTE on some serum biochemical parameters in Wistar rats

Results here showed that of all the serum biochemical tests carried out on the male and female rats treated with DCTE, only the lipid profile parameters were significantly altered when compared with control ($p<0.05$). The treated rats had significantly lowered total concentration values with significantly higher HDL values ($p<0.05$). The values of triglycerides, liver function, and renal function parameters were all not significantly altered when compared with control (Tables 3a and 3b).

Effect of DCTE on hematological values of albino rats

Hematological parameters including RBC, PCV, Hb, platelets, MCV, MCH, and MCHC were not significantly altered in the male rats following treatment with DCTE when compared with control ($p>0.05$), even though slight increase in RBC, PCV and Hb values were observed. However, the number of white blood cells was significantly higher in the DCTE treated male rats when compared to the control ($p<0.05$). A similar pattern of results was obtained for the female rats treated with DCTE as RBC and PCV values were significantly increased ($p<0.05$). MCHC values were however significantly lower than control in the female rats treated with a higher dose of DCTE. The results on the effects of DCTE on hematology of the male and female rats are presented in tables 4a and 4b respectively.

Discussion

Daucus corata (Carrot) is widely consumed by both humans and animals because of its health benefits. It ranks tenth in nutritional value among various fruits and vegetables. [28] Carrots contain pro-vitamin A carotene which maintains good eye health. Carrot is a good source of dietary fiber and the trace mineral molybdenum, rarely found in many vegetables. Several studies have also been reported on the medicinal and health benefit of *Daucus corata* such as antioxidant activity because of the presence of carotenoids and polyphenols contained in it, [10] antidiabetic activity, [13] as an immune enhancer. [27]

The result of the hormonal assay showed that the plant extract has a better effect on the female hormone (estradiol) receptor than on the male hormone (testosterone) receptor even at the least dose used in this study. The effect in the male hormone profile is dose-dependent. An increase in dose led to a significant increase in testosterone profile while in the female, an increase in dose did not lead to a significant increase in estradiol profile. Hence, it is suggested that *Daucus corata* could be used to improve sexual behavior, enhance semen quality, and boost libido in healthy males, and for estrous synchronization in female animals. This is in line with the study of Chennai, *et al*, [29] who reported that *Daucus corata* has an aphrodisiac effect with an enhanced libido, in addition to the aromatic, stimulant, diuretic and abortifacient effects.

The serum biochemistry revealed that male and female rats treated with carrot extract had significantly lowered total cholesterol and triglycerides concentrations with increased

HDL values, suggesting that the extract may have cardio-protective effects. Similar results were obtained in a study carried out on carrot fed rats, where the conclusion was that the consumption of carrots may be associated with a lower risk of heart attacks. [30] This effect of the extract may be due to its reported carotene, vitamin E, and polyphenol contents. [14] These substances are also known to exert a cardioprotective effect via the antioxidant pathway. [30]

Furthermore, changes in hematological values have been used to access the toxicity potentials of administered plant extracts and may be a means of assessing the nutritional, physiological, and pathological status of an animal. [23, 31] The role of nutrition in blood-boosting is increasingly being recognized as studies have revealed that different diets could exert diverse effects on hematological indices of animals and humans. [32-34] The insignificant changes in the values of these parameters observed across the treatment groups in the male and female rats further attests to the non-toxic potential of DCTE. However, the increase in the values of RBCs and PCV in the test rats further suggests that DCTE may contain substances that support erythropoiesis. Vitamins A and B have been reported to be part of the nutritional materials in carrots and may have been responsible for the rise in RBC and PCV values. [12, 28] This increase observed in the number of white blood cells following treatment suggests possible improvement strength of the body due to the effect of the carotenoid in the extract. A similar study had reported a significant increase in lymphocytes, eosinophils, and monocytes counts in albino rats treated carrot extract. [12]

Conclusion

Based on the findings in this study it can be concluded that *Daucus carota* has no deleterious effect in the blood profile of the experimental rats irrespective of the gender. However, results obtained showed that *Daucus carota* could be used in animal breeding as an aphrodisiac to enhance sexual behavior, and by extrapolation improve semen quality of male animals, and also as an estrous synchronizing agent because of the increased estradiol concentration recorded in this study. Increased estradiol concentration halts the synthesis of the gonadotropin-releasing hormone due to its negative feedback effect on the hypothalamus, which also affects the secretion of pituitary reproductive gonad tropic hormones (FSH and LH) negatively. The decreased secretion of these hormones negatively affects ovarian activity. As a tool in estrous synchronization, it could be suggested that the females whose estrus are to be synchronized should be treated with *Daucus carota* extract for a period not exceeding 28 days. Withdrawal of treatment would lead to estrous synchronization. More work should be done to ascertain the exact number of days the animal should be treated and how long it takes for the animal treated to come to estrus.

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The manuscript has been read and approved by all authors and the requirement for authorship has been met by all authors, even as manuscript represents honest and real data. All authors were directly involved in the research and data reporting in areas such as concept design, content definition, literature search, data collection, and analysis. They collect read and approved of the final manuscript.

Conflict of interest:

None declared.

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Table 1: Result of acute toxicity evaluation of DCTE

Dose (mg/kg)	Number of Deaths	Percentage mortality	Dose Difference (DD)	Mean Death (MD)	DD X MD
500	0	0	500	0.00	0.00
1000	0	0	1000	0.00	0.00
2000	0	0	1000	0.00	0.00
3000	0	0	1000	0.00	0.00
4000	0	0	1000	0.00	0.00
5000	0	0	…..	…..	…..

LD_{50}>5000 mg/kg body weight

Table 2: Effect of DCTE on testosterone and estrogen levels in albino rats

Parameters (ng/ml)	Control	500 mg/kg DCTE	1000 mg/kg DCTE
Testosterone in males	13.92±0.31	14.49±0.26	16.29±0.53*
Estrogen in females	56.41±1.38	63.37±1.23*	63.88±1.42*

Values represent the mean ± SEM for N =5. Values in the same row marked * are significantly different from control (p< 0.05).

Table 3a: Effect of DCTE on some serum biochemical parameters of male albino rats

Parameters	Control	500 mg/kg DCTE	1000 mg/kg DCTE
Total protein (g/dL)	7.34±0.18	7.32±0.21	7.54±0.27
AST (IU/L)	26.80±1.31	26.00±1.30	27.60±1.36
ALT (IU/L)	14.60±0.74	15.40±0.74	15.60±0.67
ALP (IU/L)	57.40±2.48	59.40±0.50	58.20±2.05
Bilirubin (mg/dL)	0.55±0.04	0.53±0.33	0.54±0.02
Urea (mg/dL)	15.00±0.49	15.26±0.20	14.50±0.28
Creatinine (mg/dL)	0.69±0.01	0.70±0.01	0.67±0.02
Cholesterol (mg/dL)	104.22±1.38	100.94±1.41*	97.28±1.06*
HDL (mg/dL)	56.14±0.96	61.26±1.00*	63.74±1.19*
TAG (mg/dL)	125.40±1.20	124.02±1.03	123.44±2.89

Values represent the mean ± SEM for N =5. Values in the same row marked * are significantly different from control (p< 0.05). AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase, HDL, High-density lipoprotein cholesterol; TAG, Triglycerides

Table 3b: Effect of DCTE on some serum biochemical parameters of female albino rats

Parameters	Control	500 mg/kg DCTE	1000 mg/kg DCTE
Total protein (g/dL)	7.31±0.18	7.32±0.22	7.15±0.18
AST (IU/L)	27.20±0.73	28.00±1.30	29.00±1.94
ALT (IU/L)	17.80±1.01	16.60±3.96	18.40±1.02
ALP (IU/L)	60.60±0.74	59.40±1.36	57.80±2.43
Bilirubin (mg/dL)	0.54±0.01	0.57±0.03	0.58±0.01
Urea (mg/dL)	15.35±0.21	14.63±0.54	15.52±0.53
Creatinine (mg/dL)	0.76±0.03	0.75±0.02	0.78±0.01
Cholesterol (mg/dL)	118.68±0.83	110.80±0.97*	113.20±0.72*
HDL (mg/dL)	60.02±0.46	64.70±0.67*	66.32±0.85*

TAG (mg/dL)	126.26±0.69	126.28±1.84	125.44±1.62

Values represent the mean ± SEM for N =5. Values in the same row marked * are significantly different from control (p< 0.05). AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase, HDL, High-density lipoprotein cholesterol; TAG, Triglycerides.

Table 4a: Effect of DCTE on the hematological values of male albino rats

Parameters	Control	500 mg/kg DCTE	1000 mg/kg DCTE
Red Blood Cell ($\times10^{12}$/l)	6.90±0.18	7.23±0.12	7.21±0.45
Pack Cell Volume (%)	44.20±0.37	45.00±0.31	44.80±0.37
Haemoglobin (g/dl)	14.30±0.21	14.84±0.31	14.96±0.21
White Blood Cell ($\times10^{3}$/L)	8.86±0.24	9.32±0.28*	10.38±0.29*
Platelets ($\times10^{9}$/l)	530.40±12.31	521.00±9.55	527.60±16.30
MCV (fl)	64.12±1.21	62.21±0.69	62.13±0.36
MCH (pg)	20.72±0.27	20.49±0.15	20.74±0.17
MCHC (g/dl)	32.34±0.25	32.96±0.49	33.38±0.30

Values represent the mean ± SEM for N =5. Values in the same row marked * are significantly different from control (p<0.05). PCV, Packed Cell Volume; Hb, Haemoglobin; RBC, Red Blood Cells; MCH, Mean Corpuscular Haemoglobin; MCHC, Mean Corpuscular Haemoglobin Volume; WBC, White Blood Cell.

Table 4b: Effect of DCTE on the hematological values of female albino rats

Parameters	Control	500 mg/kg DCTE	1000 mg/kg DCTE
Red Blood Cell ($\times10^{12}$/l)	6.74±0.15	7.01±0.06*	7.08±0.08*
Pack Cell Volume (%)	41.80±0.08	43.60±0.50*	44.40±0.40*
Haemoglobin (g/dl)	14.18±0.10	14.40±0.20	14.12±0.13
White Blood Cell ($\times10^{3}$/L)	8.70±0.23	9.14±0.36	9.27±0.12
Platelets ($\times10^{9}$/l)	436.40±16.62	396.60±38.52	414.80±11.41
MCV (fl)	61.98±0.99	62.13±0.86	62.66±0.75
MCH (pg)	21.05±0.46	20.52±0.32	19.92±0.21*
MCHC (g/dl)	33.95±0.54	33.02±0.10	31.80±0.32*

Values represent the mean ± SEM for N =5. Values in the same row marked * are significantly different from control (p><0.05). PCV, Packed Cell Volume; Hb, Haemoglobin; RBC, Red Blood Cells; MCH, Mean Corpuscular Haemoglobin; MCHC, Mean Corpuscular Haemoglobin Volume; WBC, White Blood Cell.