

EFFECTS OF AQUEOUS EXTRACTS OF *NIGELLA SATIVA* AND *OCIMUM GRATISSIMUM* ON ELECTROLYTE, UREA, CREATININE OF WISTAR RAT.

ABSTRACT

Aim: Effects of aqueous extracts of *Nigella sativa* (black seed) and *Ocimum gratissimum* (scent leaf) on electrolytes, urea and creatinine of Wistar rats were investigated.

Materials and method: Twenty-five Wistar rats were used for the study, the rats were arranged into five groups with five rats in each of the groups. The rats had access to their normal feed but sucrose and margarine were used to induce hyperglycemia and hyperlipidemia respectively on the rats with the exception of the rats in the positive control group. The rats in the negative control were induced using the sucrose and margarine but were not treated using the aqueous extracts. The rats in the scent leaf group were treated with 2ml of scent leaf aqueous extract, while the rats in the black seed group were treated with 2ml of black seed aqueous extract. The rats in the black seed and scent leaf group were treated with 2ml of the combined aqueous extract

Results: The result showed that the extracts decreased the levels of the electrolytes in the rats in a time-dependent manner with the highest decrease obtained on the third week of treatment with the extracts. The sodium levels (mmol/ml) on the third week of treatment showed for the negative control (193.83 ± 2.96), scent leaf (125.80 ± 8.27), black seed (119.60 ± 6.24), black seed and scent leaf (110.93 ± 10.14) ($p < 0.05$). The decrease for potassium levels (mEq/l) on the third week of treatment, showed for the negative control (0.11 ± 0.05), scent leaf (0.09 ± 0.14), black seed (0.08 ± 0.10), black seed and scent leaf (0.06 ± 0.11). Furthermore, the extracts also had a decreasing effect on the urea and creatinine in a time-dependent manner with the highest decrease obtained on the third week of treatment ($p > 0.05$). The urea levels (mmol/ml) showed for the negative control (26.64 ± 0.33), scent leaf (16.73 ± 0.88), black seed (15.86 ± 2.31) and black seed and scent leaf (12.88 ± 1.98). The decrease for creatinine levels (mmol/l) showed for negative control (662.68 ± 18.00), scent leaf (287.10 ± 12.30), black seed (192.44 ± 10.44) and black seed and scent leaf (188.66 ± 10.88).

Conclusion: The extracts significantly decreased the elevated electrolytes, urea and creatinine levels and therefore scent leaf and black seed can be used to restore kidney function.

Keywords: Creatinine, Electrolytes, *Nigella Sativa*, *Ocimum gratissimum*, Urea

1.0 INTRODUCTION

The use of plants for the treatment of human illness is since time immemorial. *Nigella sativa* is an annual herbaceous plant which name comes from Latin word *nigellus*, meaning black and it is translated as 'seed of blessing' or 'miracle herb'. The seed is called black cumin or black seed in English. *Nigella Sativa* is a member of the Ranunculaceae family [1].

Black seed is used traditionally to support the immune system, aid digestion and the respiratory issues, black cumin has scientific support for its use in the treatment and prevention of numerous chronic diseases. Black seed has also been extensively studied for its biological activities and therapeutic potential and shown to possess wide activities such as being diuretic. It is also antihypertensive, antidiabetic, anticancer, and anti-inflammatory [2]. The seeds of *Nigella sativa* are widely used in the treatment of various diseases like bronchitis, asthma, diarrhoea, rheumatism and skin disorders. It is also used as a liver tonic, anti-diarrheal, appetite stimulant, emmenagogue, to increase milk production in nursing mothers. It is also used to fight parasitic infections, and to support the immune system [2].

Most of the therapeutic properties of this plant are due to the presence of thymoquinone (TQ) which is a major active chemical component of the essential oil. Black seeds are also used in the

food industry for flavouring and also as an additive in the bread and pickles because it has a very low level of toxicity [3]. The seed in the Mediterranean is believed to be used for Immune system support, well-being, digestive health, respiratory issues, kidney and liver support, plus heart health. In Asia and the Middle East, the black cumin has been used for long for treatment to stop vomiting [4].

Scent leaf, known as *Ocimum gratissimum* is an aromatic perennial herb, with an erect stem, much branched, glabrous and woody at the base often with epidermic peeling in strips. *Ocimum gratissimum* is grown for the essential oil in its leaves and stems while eugenol and to a lesser extent thymol extracted from the oil substitutes from clove oil and thyme oil. The essential oil possesses antibacterial properties and is also an important insect repellent so also are the leaves when left dry and burnt [5].

The plant *Ocimum gratissimum* is one of those plants widely known and used for both medicinal and nutritional purposes. It is a perennial plant that is widely distributed in the tropics of Africa and Asia. It belongs to the family labiatae and it is the most abundant of the genus *Ocimum*. The common names of the plant are basil fever plant or tea bush and vernacular names include daidoyatogida (Hausa), nchanwu (Igbo), Tanmotswangiwawagi (Nupe) and Effinrin (Yoruba) [6].

Ocimum gratissimum plants are known to have common phytochemical compounds which are non-nutritive plants and used in traditional medicine for the treatment of several ailments and the extracts have been evaluated for their ability to stall the activities of organisms responsible for spoilage of fresh catfish (*Clarias gariepinus*) thereby extending its shelf-life [7].

Hyperlipidemia is considered one of the major risk factor causing cardiovascular diseases (CVDs). It is a medical condition characterized by an increase in one or more of the plasma lipids, including triglycerides, cholesterol, cholesterol esters, phospholipids and plasma lipoprotein along with reduced density lipoprotein levels. This elevation of plasma lipids is among the leading risk factors associated with cardiovascular diseases. CVDs account for one-third of total deaths around the world, it is believed that CVDs will turn out to be the main cause of death and disability worldwide by the year 2020 [8].

Hypercholesterolemia and hypertriglyceridemia are the main cause of atherosclerosis which is strongly related to ischemic heart disease (IHD) [9]. IHD and the high mortality rate has a strong relationship. Furthermore elevated plasma cholesterol level cause more than four million deaths in a year [10].

Mishra *et al*, [11] said hyperlipidemia can increase oxidative stress causing significant production of oxygen free radicals, which may lead to oxidative modifications in the initiation and progression of atherosclerosis and associated cardiovascular disease.

The main function of the kidney is the excretion of water-soluble waste products from our body, the kidney has various filtration, excretion and secretory functions. Kidney function test check that the kidney is working properly by measuring the levels of these parameters: urea, creatinine, electrolyte and certain dissolved salts. These parameters indicate the functionality of the kidney in a normal range. If high then the kidney is compromised. Hence, the major marker is creatinine. Blood urea nitrogen (BUN) provides a rough measurement of the glomerular filtration rate, the rate at which blood is filtered in the kidneys [12].

2.0 MATERIALS AND METHOD

2.1 LABORATORY ANIMAL

The experimental animals used were Wistar rats, 25 of the rats were purchased from animal holding unit of animal farm Choba, Department of Biochemistry University of Port Harcourt, Rivers State. The animals were put into different groups for acclimatization, this process took over a week.

2.2 SAMPLE COLLECTION

The black seed (*Nigella sativa*) was bought from Barki-dogo market in Kaduna State while the scent leaf (*Ocimum gratissimum*) was obtained from a compound around Choba market, Obi-Akpor Local Government Area, Rivers State and they were identified at Department of Plant Science and Biotechnology, Faculty of science, University of Port Harcourt Choba.

2.3 SAMPLE PREPARATION

Fifty grams of each of the samples; scent leaf (*Ocimum Gratissimum*) and black seed (*Nigella sativa*), was soaked in 500ml of distilled water. After the stock preparation using a syringe, 2ml of the aqueous extract solution was collected and administered to the animals once daily.

2.4 EXPERIMENTAL ANIMAL AND DESIGN

The rats were grouped into 5 groups with 5 rats in each group.

GROUP 1: this group served as the positive control with a mean weight of 150g. This group was fed with normal feed (ad libitum) without treatment with scent leaf and black seed extracts.

GROUP 2: this group served as the negative control, it had 5 rats fed with normal feed (ad libitum) & distilled water but was induced with sucrose and margarine without treatment with either black seed or scent leaf extract.

GROUP 3: this group contained 5 rats fed with normal feed (ad libitum) & distilled water, was induced with sucrose and margarine but treated with aqueous extract of black seed.

GROUP 4: this group contained 5 rats fed with normal feed (ad libitum) & distilled water was induced with sucrose and margarine but treated with aqueous extract of scent leaf.

GROUP 5: this group contained 5 rats fed with normal feed (ad libitum) & distilled water was induced with sucrose and margarine but treated with an equal proportion of the scent leaf and black seed aqueous extracts.

2.5 BLOOD COLLECTION

The animals after being induced with sucrose and margarine for one month were treated and sacrificed on a weekly basis on the kidney electrolytes, urea and creatinine were determined. The treatment lasted for one month.

2.6 Determination of blood sodium

Sodium levels were determined by colourimetric test. Magnesium-uranyl acetate method.

The Principle of this method is that after the precipitation of sodium magnesium uranyl acetate, in the supernatant form with uranyl ions in solution with thioglycolic acid a yellow-brown coloured complex is formed. The optical density difference between the reagent blank (without precipitation of sodium) and the result of the analysis is proportional to the sodium concentration [13]. Reagent A kit contained uranyl acetate (19mM) and magnesium acetate

(140mM) while reagent B kit contained ammonium thioglycolate (550mM), ammonia (550mM) and the standard aqueous solution of sodium equivalent 150mmol. 2.00ml of reagent A was mixed with 0.02 ml of the sample. For the standard, 2.00 ml of reagent A and 0.02 ml of the standard were mixed. The mixtures were let to stand for 5 minutes, they were then shaken thoroughly for 30 seconds. The mixtures were allowed to stand for 30 minutes. They were centrifuged at 2,000rpm for 5 minutes. The supernatant was then separated. 0.05ml of the clear supernatant was mixed with 2.00ml of reagent B. For the blank, 0.05 ml of reagent A and 2.00 ml of reagent B were mixed, while the standard tube contained 0.05 ml of supernatant and 2.00ml of reagent B. The absorbance of the mixtures was read after 10 minutes at 405nm with spectronic – 20 spectrophotometer.

Calculations: $\frac{\text{Blank O.D} - \text{Sample O.D}}{\text{Blank O.D} - \text{Standard O.D}} \times 150 = \text{mmol/L}$

Blank O.D – Standard O.D

2.7 Determination of potassium

Potassium levels were determined by colorimetric endpoint method [14]. One millilitre of reagent was mixed with 0.1ml of sample except for the controls, which had no samples. The blank tube contained 1.0ml of reagent while the standard tube contained 1.0ml of reagent and 0.1ml of standard. The mixtures were incubated at 25°C for 3mins. The absorbance was read against reagent blank at 500nm with Spectronic -20 spectrophotometers.

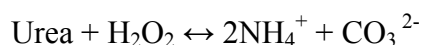
Calculations: $\frac{\Delta A_{\text{unknown}}}{\Delta A_{\text{standard}}} \times C_{\text{standard}} = \text{potassium concentration mEq/L}$

ΔA standard

2.8 Determination of blood urea

Urease-glutamate dehydrogenase -UV method according to Berthelot's method [15] was used to determine the level of Urea in the samples. Mindray test kits were used for the analysis.

Reaction Principle



Urea is hydrolyzed by urease, and one of the products, ammonia, oxidises NADH to NAD^+ catalysed by glutamate dehydrogenase (GLDH). The absorbance decrease is directly proportional to the concentration of urea.

Procedure

Two test tubes labelled T1 (reagent blank) and T2 (test sample) were set up. T1 contained 1000 μL of reagent (R1) and 10 μL of distilled water, while T2 contained 1000 μL of reagent (R1) and 10 μL of the test sample. The contents of each tube were mixed and incubated at 37°C for 2 min. After incubating, 250 μL of the second reagent (R2) was added to both test tubes. The contents of each tube were incubated again for 30 seconds at 37°C , the absorbance was read after 2 minutes at a wavelength of 546 nm.

Calculation

$$\Delta A = [\Delta A \text{ sample}] - [\Delta A \text{ blank}]$$

$$\text{Conc. of urea} = [\text{change in absorbance of sample}] - [\text{change in absorbance of blank}].$$

The result is expressed in mmol/L.

2.9 Determination of blood creatinine

Modified Jaffé method according to Bartels and Bohmer [16] was used to determine the level of creatinine in the samples. Mindray test kits were used for the analysis.

Reaction Principle



At an alkaline solution, creatinine combines with picric acid to form an orange-red coloured complex. The absorbance increase is directly proportional to the concentration of creatinine.

Procedure

Two test tubes labelled T1 (reagent blank) and T2 (test sample) were set up. T1 contained 180 μL of reagent (R1) and 18 μL of distilled water, while T2 contained 180 μL of reagent (R1) and 18 μL of the test sample. The contents of each tube were mixed thoroughly at 37°C for 1 min. After incubating, 180 μL of the second reagent (R 2) was added to both test tubes. The content of the tube was mixed thoroughly, incubated at 37°C for 30 seconds and the absorbance read at 492 nm wavelength 2 minutes later.

Calculation

$$\Delta A = [\Delta A \text{ sample}] - [\Delta A \text{ blank}]$$

$$\text{Conc. of creatinine} = [\text{change in absorbance of sample}] - [\text{change in absorbance of blank}].$$

The result is expressed in mmol/L.

2.10 STATISTICAL ANALYSIS

Data analysis was performed using the Statistical Package for the Social Sciences software (SPSS, version 11.0). Data is displayed in mean $\pm$ SD. The statistical method of one-way analysis of variance (ANOVA) was used to compare the mean values obtained among different groups. Differences were considered significant whenever the p-value is $p \leq 0.05$.

3: RESULTS

The results of this study are shown below.

Table 1: Effect of first, second and third-week oral administration of scent leaf and black seed on sodium levels (Na) of Wistar rat.

(Mmol/l)			
Treatment	Week 1	Week 2	Week 3
Negative control	191.53 ± 2.15 ^a	193.85 ± 2.86 ^b	193.83 ± 2.96 ^a
Positive control	107.30 ± 4.08 ^b	107.50 ± 2.97 ^b	107.25 ± 2.60 ^b
Scent leaf	128.59 ± 7.85 ^c	126.73 ± 7.55 ^c	125.80 ± 8.27 ^c
Black Seed	122.27 ± 6.67 ^a	120.85 ± 4.85 ^b	119.60 ± 6.24 ^a
Scent leaf and black seed	112.64 ± 7.16 ^d	111.18 ± 1.18 ^d	110.93 ± 10.14 ^d

Results are the means of three determinations ± standard deviation.

^{abcd} Different letters in a given row denote significant difference, p<0.05.

Table 2: Effect of first, second and third-week oral administration of scent leaf and black seed on potassium levels (K) of Wistar rat.

(mEq/l)			
Treatment	Week 1	Week 2	Week 3
Negative control	0.08 ± 0.09^a	0.09 ± 0.07^a	0.11 ± 0.05^a
Positive control	0.69 ± 0.05^a	0.07 ± 0.04^a	0.07 ± 0.11^a
Scent leaf	0.07 ± 0.56^b	0.08 ± 0.60^c	0.09 ± 0.14^c
Black Seed	0.06 ± 0.44^b	0.07 ± 0.33^c	0.08 ± 0.10^d
Scent leaf and black seed	0.58 ± 0.26^b	0.55 ± 0.27^c	0.06 ± 0.11^d

Results are means of three determinations $\pm$ standard deviation.

^{abcd} Different letters in a given row denote significant difference, $p < 0.05$.

Table 3: Effect of first, second and third-week oral administration of scent leaf and black seed on urea concentration on Wistar rat.

(Mmol/l)			
Treatment	Week 1	Week 2	Week 3
Negative control	26.88 ± 0.31 ^a	26.90 ± 0.08 ^a	26.64 ± 0.33 ^a
Positive control	14.24 ± 0.40 ^b	14.28 ± 0.30 ^b	14.18 ± 1.30 ^b
Scent leaf	19.46 ± 1.43 ^a	18.34 ± 0.77 ^b	16.73 ± 0.88 ^c
Black seed	17.87 ± 4.26 ^a	16.18 ± 0.98 ^b	15.86 ± 2.31 ^c
Scent leaf and black seed	15.24 ± 2.35 ^a	14.00 ± 0.88 ^b	12.88 ± 1.98 ^d

Results are the means of three determinations ± standard deviation.

^{abcd} Different letters in a given row denote significant difference, $p < 0.05$.

Table 4: Effect of first, second and third week oral administration of scent leaf and black seed on Creatinine concentration of Wistar rat.

(Mmol/l)			
Treatment	Week 1	Week 2	Week 3
Negative control	625.37 ± 18.50 ^a	640.99 ± 18.24 ^a	662.68 ± 18.00 ^a
Positive control	134.46 ± 7.82 ^a	132.67 ± 7.55 ^b	134.40 ± 7.80 ^b
Scent leaf	298.46 ± 12.98 ^a	291.86 ± 12.88 ^b	287.10 ± 12.30 ^c
Black Seed	199.58 ± 10.64 ^a	196.56 ± 10.68 ^b	192.44 ± 10.44 ^c
Scent leaf and black seed	190.90 ± 10.48 ^a	189.96 ± 10.54 ^b	188.66 ± 10.88 ^d

Results are means of three determinations ± standard deviation.

^{abcd} Different letters in a given row denote significant difference, $p < 0.05$.

4.0 DISCUSSION

This study investigated the effect of aqueous extracts of *Nigella Sativa* and *Ocimum gratissimum* on electrolytes (sodium and potassium), urea and creatinine.

The results showed that the extracts decreased the levels of the electrolytes in the rat's plasma in a time-dependent manner with the highest decrease obtained on the third week of treatment with the extracts. The sodium levels (mmol/l) on the third week of treatment showed for the negative control (193.83 ± 2.96), scent leaf (125.80 ± 8.27), black seed (119.60 ± 6.24), black seed and

scent leaf (110.93 ± 10.14) ($p < 0.05$). The decrease for potassium levels (mEq/l) on the third week of treatment, showed for the negative control (0.11 ± 0.05), scent leaf (0.09 ± 0.14), black seed (0.08 ± 0.10), black seed and scent leaf (0.06 ± 0.11). Furthermore, the extracts also had a decreasing effect on the urea and creatinine in a time-dependent manner with the highest decrease obtained on the third week of treatment ($p > 0.05$). The urea levels (mmol/l) showed for the negative control (26.64 ± 0.33), scent leaf (16.73 ± 0.88), black seed (15.86 ± 2.31) and black seed and scent leaf (12.88 ± 1.98). The decrease for creatinine levels (mmol/l) showed for negative control (662.68 ± 18.00), scent leaf (287.10 ± 12.30), black seed (192.44 ± 10.44) and black seed and scent leaf (188.66 ± 10.88).

The result showed a significant depletion of the kidney that led to an increase in the concentration of the plasma sodium ($P < 0.05$). Electrolyte disorders are usually multifactorial in nature. Various pathophysiological factors, such as nutritional status, gastrointestinal absorption capacity, coexistent acid-base abnormalities, pharmacological agents, others comorbid diseases (mainly renal disease/ or acute illness, alone or in combination) play a key role in the function of the kidney.

Serum urea is a byproduct from protein breakdown, about 90% of urea produced in the body is excreted through the kidney [17]. Research of Weber *et al*, [18], revealed that the urea accumulation in the blood of rats was significantly reduced when treated with a high dose of *Nigella Sativa*. Also, Almdal *et al*, [19] indicated renal diseases and malfunction as a severe complication of diabetes in rats which were induced with sucrose. The significant high levels of serum urea are common biomarkers for the predictions of renal dysfunction, due to the fact that they are elevated; *Ocimum gratissimum* was able to significantly reduce the serum level of urea and hence the kidney possess improved capacity to eliminate this waste product from the blood.

The research of Treasure, [20] revealed creatinine is commonly measured as an index of glomerular function. Kidney damage can be caused by creatinine accumulation in the blood. Hence, a high level of blood creatinine will indicate kidney damage. This study revealed a significant reduction ($P < 0.05$) in serum creatinine in the rats treated with a high dose of *Nigella Sativa*.

The results of the present study showed that the supplementation of *Nigella sativa* and *Ocimum gratissimum* to the diets of the rats for three weeks did not change the biochemical parameters of the kidney but regulated it to the standard range of the kidney function. In accordance with our findings, it was previously proved that oral administration of aqueous extract of *Nigella sativa* seeds showed no significant changes in kidney function [21]. Another study also failed to show any toxicity for *Nigella sativa* fixed oil in mice [22]. Our study showed that oral administration of *N. sativa* has no toxicity by the different *Nigella sativa* doses used. These results are in agreement with previous data reporting that *Nigella sativa* has a wide margin of safety [23, 24].

The biological activity of *Nigella Sativa* is related to the composition of its essential oil, which contains 30 to 48%. Thymoquinone (TQ), 7 to 15% P-cymene, 6 to 12% carvacrol, 2 to 7%, 4-terpineol, 1 to 4% TAnethole and 1 to 8% sesquiterpene (Burits et al,2000). The effect of TQ on the neuropathy and oxidative stress induced by Doxorubicin in rats was elevated and triglycerides total cholesterol and lipid peroxides in the kidneys of TQ treated rats decreased compared with Doxorubicin alone [25]).

Additionally, with TQ a complete reversal of the gentamicin-induced increase in blood urea, creatinine and lipid peroxides was observed. TQ supplementation prevented gentamicin-induced injury in the kidney [26]. *Nigella sativa* treatment also showed a reduction in total cholesterol, triglyceride and low-density lipoprotein and an increase of high-density lipoprotein ratio in

patients with diabetes. In addition, *Nigella sativa* was associated with improvement of glycemic status and lipid profile in diabetes models [27].

Ocimum gratissimum support the findings that most hypoglycemic plants have potentials of ameliorating diabetic lipid metabolism anomalies. This cholesterol-lowering effect was earlier reported by some researchers when used as a supplementary diet in normal rats for six months. The oral intake for 4 weeks reduced the lipid imbalances associated with diabetes mellitus in Wistar rats. The plant has a hypoglycemic effect and may be safely taken orally [28].

5.0 CONCLUSION

In conclusion, the results show the absence of the toxic effect of *Nigella Sativa* and *Ocimum gratissimum* on rat kidney. This aqueous extract of black seed and scent leaf have the ability to regulate, reverse renal injury and kidney disorders. The Black seed and scent leaf is a safe and effective herb that can be used by almost anyone. In general, the aqueous extract is not associated with serious side effects no irritations are caused when the right dose is correctly applied.

Ethical Approval:

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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