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INVESTIGATING THE USE OF AQEUOUS EXTRACTS OF BLACK SEED (*NIGELLA SATIVA)* AND SCENT LEAF TEA (*OCIMUM GRATISSIMUM)* TO AMELIORATE THE SYMPTOMS OF CORONAVIRUS (COVID -19)

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ABSTRACT

Aim: The coronavirus disease COVID – 19 has turned into a global pandemic and efforts are out by scientists to help produce solutions to combat this disease especially in immune boosting. Plants and plant products provide a potential source of immune boosting medication. This study investigated the potential use of aqueous extracts of black seed *Nigella sativa and* scent leaf *Ocimum gratissimum* tea to ameliorate the symptoms of coronavirus COVID 19 using hyperlipidimic and hyperglycemic Wistar rats. COVID -19 disease has worse effects on people with underlying conditions like diabetes and heart condition. This informed the decision to check the effects of black seed and scent leaf on cholesterol, triglycerides, high density lipoproteins (HDL), Low density lipoproteins (LDL) and very low density lipoproteins (VLDL) using induced hyperlipidemic and hyperglycemic Wistar rats. **Materials and Method:** Twenty-five (25) Wistar rats were used for this research, five groups were created with five rats in each group. The rats were fed their normal rate feed but they gavaged on sucrose and margarine to induce hyperlipidemia and hyperglycemia on the rats with exception to the positive control. 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 results showed that the extracts decreased the levels of cholesterol, triglycerides, high density lipoproteins, low density lipoproteins and very low density lipoproteins in the rats in a time dependent manner with highest decrease obtained on the third week of treatment with the extracts. The cholesterol level decreased from 10.58±0.12mmol/l to 2.51±0.28mmol/l, while triglyceride level decreased from 5.69±0.03mmol/l to 1.19±0.04mmol/l. **Conclusion:** The results of the study indicate that the aqueous extract of *N. sativa* and *O. gratissimum* might be effective immune booster in the fight against coronavirus (COVID 19).

KEYWORDS: Black seed, Coronavirus, COVID - 19, Lipids, *Nigella sativa***,** *Ocimum gratissimum*, Scent leaf.

1.0 INTRODUCTION

In December 2019, the world woke up to the strange flu disease that was ravaging Wuhan city in Hubei China. It was a type of corona virus confirmed to be Severe Acute Respiratory Syndrome Coronavirus 2 (SARS – COV-2). By January 2020 the World Health Organisation named it COVID-19 and the disease was subsequently declared a global pandemic. Coronaviruses are a large family of viruses which may cause illness in animals or humans. In humans, several coronaviruses are known to cause respiratory infections ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS).[1] According to WHO (2020),[1] the most common symptoms of COVID-19 are fever, tiredness, and dry cough. Some patients may have aches and pains, nasal congestion, runny nose, sore throat or diarrhea. These symptoms are usually mild and begin gradually. Some people become infected but do not develop any symptoms and do not feel unwell. Most people (about 80%) recover from the disease without needing special treatment. As at the time of this study, there were more than 2076015 COVID -19 cases worldwide where more than 522881 were recovered cases and more than 138008 deaths,[2] There is currently no treatment or vaccine available to treat this disease as at yet. Since coronavirus disease COVID – 19 turned into a global pandemic, scientists all over the world have been making efforts to produce solutions to combat this disease especially in immune boosting. Plants and plant

products provide a potential source of immune boosting medication.

Nigella sativa (black seed, black caraway, also known as black cumin, nigella, and kalonji) is an annual flowering plant in the family Ranunculaceae, native to south and southwest Asia. The seeds of *Nigella sativa* are used as a spice in Indian and Middle Eastern cuisines. The black seeds taste like a combination of onions, black pepper, and oregano. They have a pungent, bitter taste and smell,[3] The dry-roasted seeds flavor curries, vegetables, and pulses. They can be used as a "pepper" in recipes with pod fruit, vegetables, salads, and poultry. In some cultures, the black seeds are used to flavor bread products, and are used as part of the spice mixture panch phoron (meaning a mixture of five spices) and alone in many recipes in Bengali cuisine and most recognizably in naan.[4] *Nigella sativa* is also used in Armenian string cheese, a braided string cheese called majdouleh or majdouli in the Middle East. The seeds of Nigella sativa, commonly known as black seed or black cumin, are used in folk (herbal) medicine all over the world for the treatment and prevention of a number of diseases and conditions that include asthma, diarrhea and dyslipidaemia. The black seed possess both pharmacological and toxicological properties and contain essential oils, proteins, alkaloids and saponin. Much of the biological activity of the black seed has been shown to be due to thymoquinone, the major component of the essential oil, which is also present in the fixed oil. The pharmacological actions of the crude extracts that have been reported include protection against nephrotoxicity and hepatotoxicity induced by either disease or chemicals. The seeds/oil have anti-inflammatory, analgesic, antipyretic, antimicrobial and antineoplastic activity. The oil decreases blood pressure and increases respiration. Treatment of rats with the seed extract for up to 12 weeks has been reported to induce changes in the haemogram that include an increase in both the packed cell volume (PCV) and haemoglobin (Hb), and a decrease in plasma concentrations of cholesterol, triglycerides and glucose. The seeds are characterized by a very low degree of toxicity. Two cases of contact dermatitis in two individuals have been reported following topical use. Administration of either the seed extract or its oil has been shown not to induce significant adverse effects on liver or kidney functions. It would appear that the beneficial effects of the use of the seeds and thymoquinone might be related to their cytoprotective and antioxidant actions, and to their effect on some mediators of inflammation.[5]

Ocimum gratissimum commonly called Scent leaf and Ncahnwu in Igbo is an herbaceous plant which belongs to the Lamiaceae family. The plant is indigenous to tropical areas especially India and it is also in West Africa. In Nigeria, it is found in the Savannah and coastal areas. It is cultivated in Ceylon, South Sea Islands, and also within Nepal, Bengal, Chittagong and Deccan. *Ocimum gratissimum L*. (Family Lamiaceae) commonly called basil, is a culinary herb with pungent sweet smell. Propagation of basil is through seeds and also reliably from cuttings.[6] The foliage is commonly used fresh in cooked recipes or added at the last moment, as cooking quickly destroys the flavor. Studies have established that compounds in basil oil have potent antioxidant, anticancer, antiviral and antimicrobial properties.[7]

The essential oil of *Ocimum gratissimum* contains eugenol and shows some evidence of antibacterial activity. Leaf extract of *O. gratissimum* showed antidiabetic properties in alloxan-induced diabetic rats, also the leaf extract of the plant prevented diarrhea. *Ocimum gratissimum* has anti-fertility effects in male mice. *Ocimum gratissimum* ethanolic extracts showed a hepatoprotective effect in rats. Analysis of *Ocimum gratissimum* showed considerable antioxidant capacity upon several doses. *Ocimum gratissimum* extracts possess appreciable natural antioxidant potentials, thereby providing good justification for their increased domestication and consumption.[8]

Lipids have a wide variety of roles in biological systems. These roles are a consequence of their chemical and physical properties. Lipids also encompass molecules such as fatty acids and their derivatives (including tri-, di-, monoglycerides, and phospholipids), as well as other sterol-containing metabolites such as cholesterol. Although humans and other mammals use various biosynthetic pathways both to break down and to synthesize lipids, some essential lipids can't be made this way and must be obtained from the diet.[9]

Diabetes is an age long, serious metabolic disorder with complications that result in significant morbidity and mortality rate, increasing economic burden and disease condition among infected people. Chronic hyperglycemia during diabetes has shown to cause glycation of body protein, which in turn leads to secondary complications that affects the eyes, kidneys nerves and arteries. Diabetes mellitus has its other complications, especially diabetic acidosis due to excessive production of ketone bodies, low resistance of infections especially those involving extremites, increase in incidence of toxemia in pregnancy and cardio vascular disorder, disturbance in electrolyte balance, retinopathy and others causing boils, carbuncles, loss of weight, emaciation and weakness.[10]

A lot of research has been done and a lot of literature written on the healing properties and nature of black seed *Nigella sativa* and scent leaf *ocimum gratissium*, therefore the aim of this study is to determine the use of black seed (*Nigella sativa)* and scent leaf (*Ocimum gratissimum*) as potential remedies in the fight against coronavirus COVID 19 with particular emphasis on the effect of *Nigella sativa* and *Ocimum gratissium* on lipid parameters. Considering that COVID -19 has worse effects on people with underlying conditions like

diabetes and heart condition. This informed the decision to investigate the effects of black seed and scent leaf on cholesterol, triglycerides, HDL, LDL and VLDL using induced hyperlipidemic and hyperglycemic Wistar rats.

2.0 MATERIALS AND MEFTHOD

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, the extract was filtered and stored in an air tight container. Two ml of the aqueous extract solution was collected and administered to the animals once daily by gavaging method.

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 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 equal proportion of the scent leaf and black seed aqueous extracts.

The plasma levels of all the Lipids were determined using Mindray test kits.

2.5 Plasma Lipid Determination

Plasma total cholesterol estimation.

Cholesterol oxidase- peroxidase (CHOD-POD) method according to Roeschlau 1974,[11] was used to determine the level of total cholesterol in the samples.

Reaction Principle

Cholesterol ester + H_2O ↔ Cholesterol + Fatty acid

Cholesterol + O_2 ↔ Δ4-Cholestenone + H_2O_2

2 H_2O_2 + 4-Aminoantipyrine + Phenol ↔ Quinoneimine + 4H_2O

By the catalysis of cholesterrol esterase and cholesterol oxidase, Cholesterol ester is catalyzed to yield H_2O_2, which oxidizes 4- aminoantipyrine with phenol to form a colored dye of quinoneimine. The absorbance increase is directly proportional to the concentration of cholesterol.

Procedure

Two test tubes labeled 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 test sample. The contents of each tube were mixed thoroughly at 37℃. The absorbance was read 10 min. later.

Calculation

ΔA = [ΔA sample] - [ΔA blank]

Conc. of cholesterol = [change in absorbance of sample] – [change in absorbance of blank].

The result is expressed in mmol/L.

PLASMA TRIGLYCERIDES (TG) ESTIMATION

Glycerokinase Peroxidase- Peroxidase method according to Tietz 1975,[12] colorimetric method was used to determine the level of Triglyceride in the samples.

Reaction Principle

Triglycerides + 3H_2O ↔ Glycerol + fatty acid

Glycerol + ATP ↔ Glycerol-3-phosphate + ADP

Glycerol-3-phosphate + O_2 ↔ Dihydroxyacetone Phosphate + H_2O_2

H_2O_2 + 4-Aminoantipyrine + 4-Chlorophenol ↔ Quinoneimine + HCl + H_2O

Through a sequence of enzymatic catalysis steps by lipase, glycerol kinase and Dihydroxyacetone phosphate dehydrogenase, triglycerides is catalyzed to yield H_2O_2, which oxidize 4-aminoantipyrinel to yield a colored dye of quinoneimine. The absorbance increase is directly proportional to the concentration of triglycerides.

Procedure

Two test tubes labeled 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 test sample. The contents of each tube were mixed thoroughly at 37℃. The absorbance was read at a wavelength of 546 nm10 min. later.

Calculation

ΔA = [ΔA sample]- [ΔA blank]

Conc. of triglyceride = [change in absorbance of sample] – [change in absorbance of blank].
The result is expressed in mmol/L.

PLASMA HDL ESTIMATION
Method
The direct method of Tietz 1975,[12] was used to determine the level of high density lipoprotein – cholesterol in the samples.

Reaction Principle
1. LDL,VLDL, Chylomicrons ↔ Cholestenone + H_2O_2
   $2H_2O_2 \leftrightarrow 2H_2O + O_2$
2. HDL ↔Cholestenone + H_2O_2
   H_2O_2 + HDAOS + 4-aminoantipyrin ↔ Quinonimine

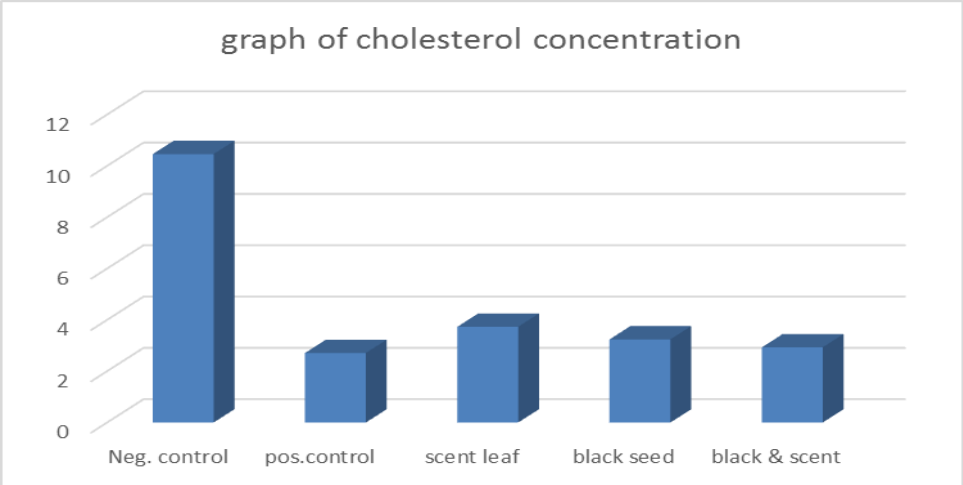
The System monitors the change in absorbance at 600 nm. This change in absorbance is directly proportional to the concentration of cholesterol in the sample and is used by the System to calculate and express the HDL-cholesterol concentration.

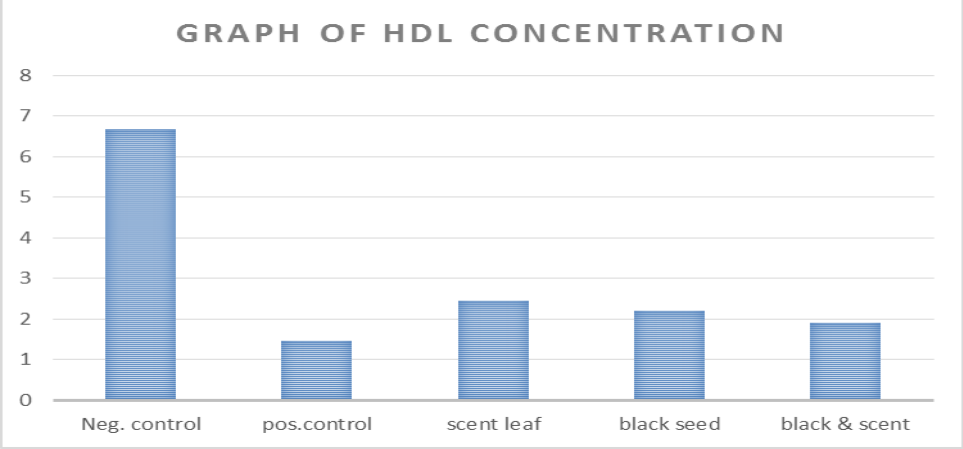
Procedure
Two test tubes labeled T1 (reagent blank) and T2 (test sample) were set up. T1 contained 900 µL of reagent (R1) and 12 µL of distilled water, while T2 contained 900 µL of reagent (R1) and 12 µL of test sample. The contents of each tube were mixed and incubated at 37℃ for 5 min. After incubating, 300 µL of the second reagent (R2) was added to both test tubes. The contents of each tube was incubated again for 5 minutes at 37^0C, the absorbance was read immediately.

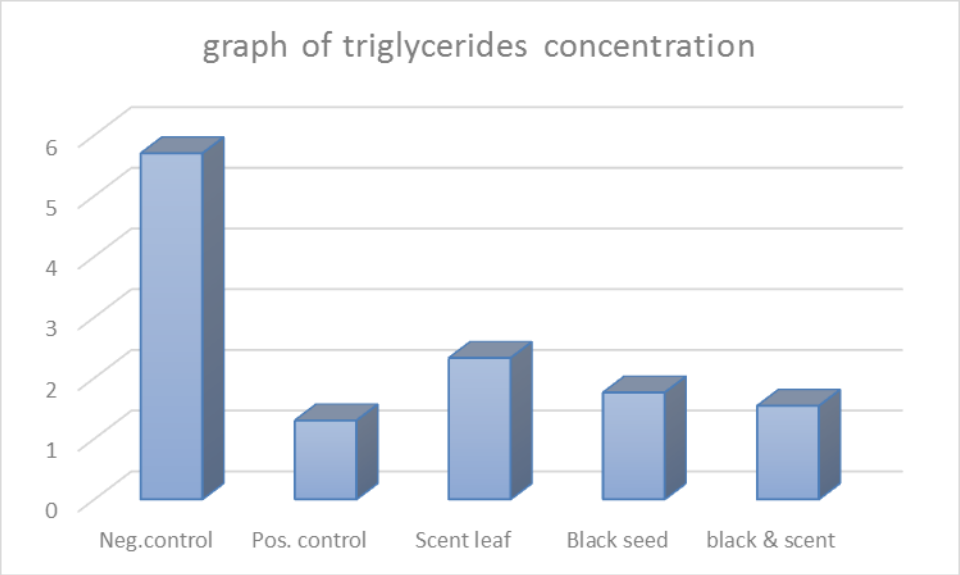
Calculation
ΔA = [ΔA sample] - [ΔA blank].
Conc. of HDL = [change in absorbance of sample] – [change in absorbance of blank].
The result is expressed in mmol/L.

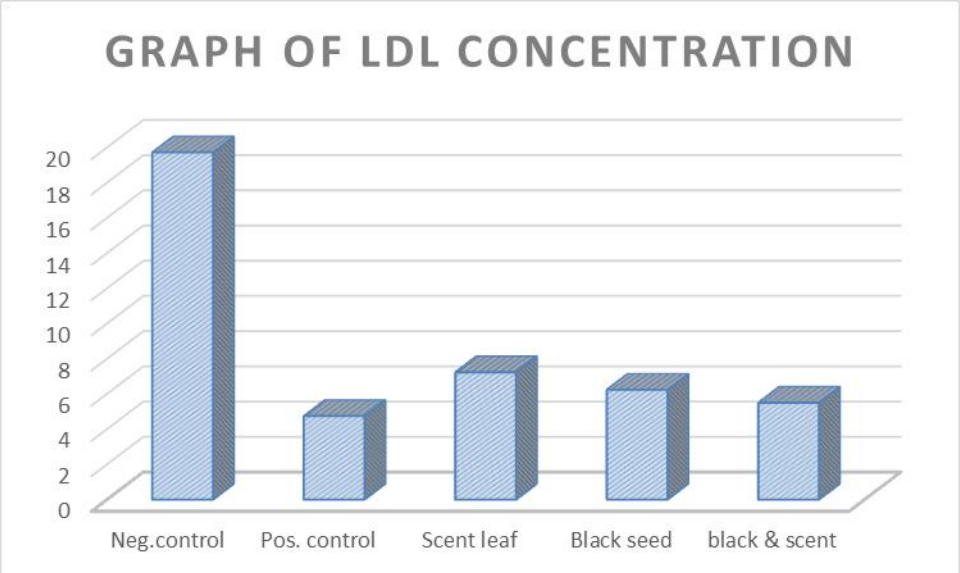
2.6 Statistical Analysis
Data analysis was performed using the Statistical package for the Social Sciences software (SPSS, version 11.0). Data is displayed in mean ± SD. The statistical method of one way analysis of variance (ANOVA) was used to compare the mean values obtained among different groups.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

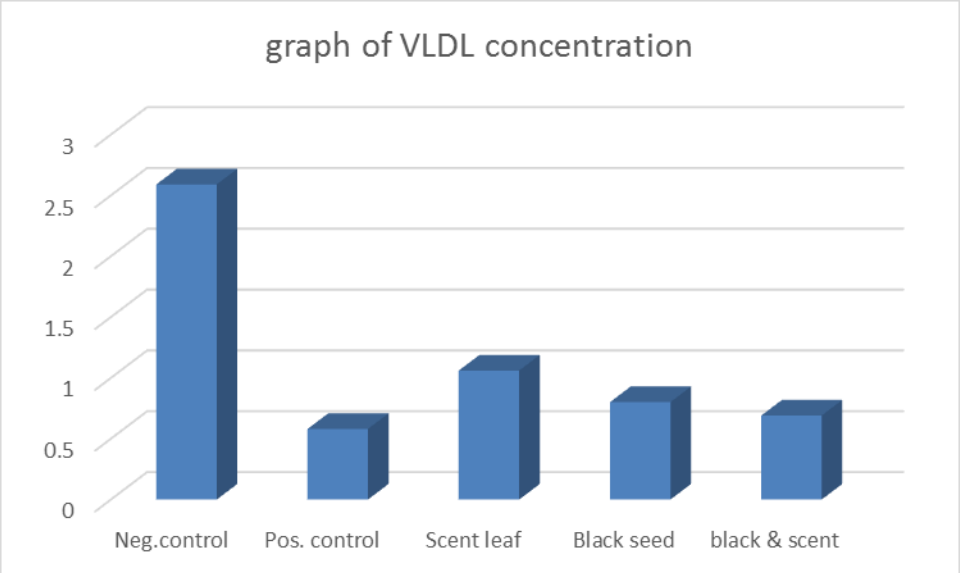
3.0 RESULTS






graph of triglycerides concentration
6
5
4
3
2
1
0
Neg.control
Pos. control
Scent leaf
Black seed
black & scent


GRAPH OF LDL CONCENTRATION
20
18
16
14
12
10
8
6
4
2
0
Neg.control
Pos. control
Scent leaf
Black seed
black & scent


graph of VLDL concentration
3
2.5
2
1.5
1
0.5
0
Neg.control
Pos. control
Scent leaf
Black seed
black & scent

4.0 DISCUSSION

The choice of this particular animal (Wistar albino rat) as experimental model was considered because of the following factors: a rat is defined as an animal with standardized characteristics; its small dimension allow an adequate keeping of several animals in a small spaces; lower cost; relative facility of handling; great resistance to infection; short life span; and high scale reproduction. In addition, the existing knowledge on the anatomy, physiology, behavior, and genetics of its several strains, allow the results obtained in experimental research to contribute for a better understanding of medical problems in humans. The rats used in this study were induced to have hyperglycemia and hyperlipidemia because it has been reported that corona virus disease is more serious on patients with underlying conditions like diabetes.

After the third week of treatment, the cholesterol result showed that the combined mixture of *Nigella sativa* and *Ocimum gratissimum* showed the most ameliorating potency (2.51mmol/l) followed by black seed only (2.52mmol/l) and lastly scent leaf (2.82mmol/l). These values are quite low compared to the induced (diseased) rat which shows a higher level of cholesterol 10.58mmol/l.

After the third week of treatment, the triglyceride result showed that the combined mixture of *Nigella sativa* and *Ocimum gratissimum* showed the most ameliorating potency (1.19mmol/l) followed by black seed only (1.24mmol/l) and lastly scent leaf (1.72mmol/l). These values are quite low compared to the induced (diseased) rat which shows a higher level of triglyceride (5.69mmol/l).

After the third week of treatment, the combined mixture of *Nigella sativa* and *Ocimum gratissimum* showed the most ameliorating potency for HDL (1.31mmol/l) followed by black seed only (1.33mmol/l) and lastly scent leaf (1.60mmol/l). These values are quite low compared to the induced (diseased) rat which shows a higher level of high density lipoprotein (HDL) (6.79mmol/l).

After the third week of treatment, the combined mixture of nigella sativa and ocimum gratissimum showed the most ameliorating potency for LDL (4.34mmol/l) followed by black seed only (4.40mmol/l) and lastly scent leaf (5.20mmol/l). These values are quite low compared to the induced (diseased) rat which shows a higher level of low density lipoprotein (LDL) (19.96mmol/l).

After the third week of treatment, the combined mixture of nigella sativa and ocimum gratissimum showed the most ameliorating potency for VLDL (0.53mmol/l) followed by black seed only (0.56mmol/l) and lastly scent leaf (0.78mmol/l). These values are quite low compared to the induced (diseased) rat which shows a higher level of very low density lipoprotein (VLDL) (2.58mmol/l).

Nigella sativa contains high levels of vitamin C. Research suggest that taking black seed extract by mouth improves coughing, wheezing and lung function in people with asthma. However, black seed may not be as effective as the drugs theophylline or salbutamol. Black seeds contain a cocktail of essential and health-promoting compounds. The natural ingredients present in black cumin oil have many benefits for asthma patients; they can boost the immunity, reduce inflammation and ease the airways to bring relief to those with asthma.[13] Scent leaves can be used for the prevention and treatment of malaria, catarrh, cough and fever,[14,15] postulated that scent leaf is a very good source of vitamin A and vitamin C and this makes scent leaf a very good immune booster. Constituents of *Ocimum gratissimum* consist of aromatic and volatile oil, linolenic acid, oleic acid, alkaloid, flavonoid, saponin and cardiac glycosides.[16] It is commonly used in the treatment of upper respiratory tract infection, diarrhea, fever, conjunctivitis,[17] and traditional treatment of diabetes mellitus.[18] Coronavirus (COVID 19) has been reported to have some of the above mentioned symptoms and from this research study, black seed and scent leaf have been shown to ameliorate these symptoms hence the study shows that aqueous extracts of black seed and scent leaf can be used as one of the solutions in the fight against corona virus (COVID 19). Okoye and Igwilo (2019).[19] and Okoye and Benjamin (2019).[20] stipulated that black seed is good for renal function. In their research on the effects of black seed and uziza leaf on electrolytes, urea and creatinine of Wistar rats, they found out that black seed extracts significantly decreased elevated levels of electrolytes, urea and creatinine with and no side effects observed from the dosage used for the research. They concluded that black seed and uziza leaf apart from their nutritional benefits can serve as traditional medicine used in treating ailments relating to the kidney. Similarly Okoye and Ikiriko (2019).[21] reported that black seed and uziza leaf (*Piper guineense*) had a protective effect on the liver. They opined that both uziza leaf and black seed have hepatoprotective effect on the liver. The anti-oxidant and anti-inflammatory effects of black seed are the main features involved in the reduction of high lipid levels and elevated liver functions. Furthermore, they also be reported that the use of both black seed and uziza leaf had a very high reducing effect on elevated liver function levels. It was recommended that the combination of black seed *(N. sativa)* and uziza leaf (*P. guineense*) should be used by diabetics and hyperlipidemia patients to effectively reduce high sugar levels and high lipid levels as these medicinal plants have been seen to be non-toxic to the human system. Also, black seed *(N. sativa)* and uziza leaf *(P. guineense*) have hepatoprotective effects hence patients with liver damage or liver injury are recommended to take these medicinal plants.[21] There have been cases of COVID - 19 patients who successfully managed their symptoms

with black seed oil, carrots and honey.[22] Thus proving that black seed can be successfully used in the management of COVID 19. This study showed that scent leaf performed as well as black seed in the biochemical analysis assayed and has also showed to produce no adverse effects on the body. Scent leaf is already being widely used in cooking staple foods. Different chemical components in *Ocimum* species have been found to have antihyperglycemic effects. Eugenol has been found to lower the blood glucose level.[23] A diabetic study was done in vivo showing that eugenol can reduce blood glucose levels by 38% by inhibiting α-glucosidase while insulin and glycated hemoglobin levels were the same. The polyphenols, caffeic acid, p-coumaric acid of aqueous extracts of *O. sanctum* leaves show anti-diabetic effect.

Recently, *O. gratissimum* hypoglycaemic have been reported to be one of the most hypoglycaemic plant agents at ameliorating lipid metabolism abnormalities and reduction of total cholesterol in normal rats fed with *O. gratissimum* supplemented diet for six months.[24,25,26,27] Prolonged oral administration of aqueous leaf extract of *O. gratissimum* may reduce the plasma lipid imbalances associated with diabetes mellitus which support its traditional use in the treatment of diabetes and cardiovascular diseases though the precise mechanism and site of action require further elucidation.[26,28]

The oils of *Ocimum gratissimum* are also used in the treatment of many ailments, including upper respiratory tract infections, diarrhea, headache, fever, eye problems, skin diseases, and pneumonia.[29,30,31] So the authors are proposing that by extrapolation *Ocimum gratissimum* should also be used in the management of coronavirus COVID – 19 symptoms. Hence there is no cure nor vaccine at present to treat this disease, every little helps and every option should be given a chance especially for asymptomatic patients. Medical practitioners should be consulted at all times in the treatment and management of COVID -19.

5.0 CONCLUSION

In conclusion, the aqueous extracts of *Nigella sativa* and *Ocimum gratissimum* plants have shown to be potential remedies in the fight against coronavirus (COVID 19) and clinical trials are recommended. The seeds of *Nigella sativa* and the leaves of *Ocimum gratissimum* can therefore serve as supplements and their nutritional properties harnessed in the management of the symptoms of COVID – 19. *Nigella sativa seeds* and *Ocimum gratissimum* leaves are safe for human and animal consumption and pose no toxic effect to the liver and kidney. Therefore, aqueous extracts of black seeds and scent leaves are good remedies and have shown good potential in the fight against COVID - 19 as there is no vaccine or treatment for coronavirus (COVID 19) yet.

Competing Interests
Authors have declared that no competing interests exist.

6.0 Ethical approval

This research work was carried out with the approval of the University of Port Harcourt research ethics committee.

REFERENCE

1. World Health Organisation https://www.who.int/ news-room/q-a-detail/q-a-coronaviruses, 2020.
2. Coronavirus Resource Center John Hopkins University. Coronavirus COVID -19 global cases by the Center for Systems Science and Engineers (CSSE), 2020.
3. Bharat BA. Molecular Targets and Therapeutic Uses of Spices. Google Books, 2009; 259.
4. Bramen L. "Nigella Seeds: What the Heck Do I Do with Those?", 2011.
5. Ali BH, Blunden G. Pharmacological and toxicological properties of Nigella sativa. Phytother Res., 2003; 17(4): 299 – 305.
6. Calixto JB. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicine (phytotherapeutic agents). Braz J Med Biol Res., 2000; 33: 179-189.
7. Brain KR, Turner TD. The Practical Evaluation of Phytopharmaceuticals. Wright- Scientica, Bristol. 1975; 57-58.
8. Ekaiko MU, Ndulaka JC1, Ogbonna CR, Asiegbu EI. Anti-Diabetic Effect of Occimum Gratissimum on Blood Glucose Level in Alloxan Induced Diabetic. J of Pharm and Biol Sci., 2016; 11(2): 12-14.
9. Michelle A, Hopkins J, McLaughlin CW, Johnson S, Warner MQ, LaHart D, Wright JD Human Biology and Health. Englewood Cliffs, New Jersey, USA: Prentice Hall, 1993.
10. Genuth S, Alberti KG, Bennett P, Buse J, Defronzo R, Kahn R. Follow-up report on the diagnosis of diabetes mellitus. Diabetes Care, 2003; 26(11): 3160–7.
11. Roeschlau P, Bernt E, Gruber W. Enzymatic determination of total cholesterol in serum. Z Klin Chem Klin Biochem, 1974; 12(5): 226.
12. Tietz NW. Clinical guide to laboratory tests, 3rd edn. WB Saunders Co. Philadelphia, PA, 1995; 874.
13. Wasway Y. Black seed oil for asthma + 3 practical ways to use it. Nature's Blend, 2019; 1.
14. Famodun O. 11 Amazing health benefits of scent leaf. Pharm News, 2019; 1.
15. Awe S, Okeke OL, Usman AA. Comparism of proximate, mineral and vitamin composition of selected fruits and vegetable. FUTA J of Res in Sci., 2018; 14(1): 1-8.
16. Abdel-Barry JA, Al-Hakiem MH. Acute intraperitoneal and oral toxicity of the leaf glycosidic extract of Trigonella foenum-graecum in mice. J. Ethnopharma Apr., 2000; 70(1): 65-68.

17. Al-Logmani A, Zari T. Long-term effects of Nigella sativa L. oil on some physiological parameters in normal and streptozotocin-induced diabetic rats. J DiabMellit, 2011; 1: 46-53.
18. Bnouham M, Ziyyat A, Mekhfi H, Tahri A, Legssyer A. Medicinal plants with potential antidiabetic activity – a review of ten years of herbal medicine research (1990–2000) Int. J. Diabetes Metabol, 2006; 14: 1–25.
19. Okoye NF, Igwilo EI. Effects of Black Seed (Nigella sativa) and Uziza Leaf (Piper guineense) on Electrolytes, Urea and Creatinine of Wistar Albino Rats. Inter J of Biochem Res & Rev., 2019; 26(2): 1-10.
20. Okoye NF, Benjamin P. Effects of Aqueous Extracts of Nigella sativa and Ocimum gratissimum on Electrolyte, Urea, Creatinine of Wistar Rat. J of Appl Life Sci Inter, 2019; 21(3): 1-8.
21. Okoye NF, Ikiriko FI. Effect of Black Seed (Nigella sativa) and Uziza (Piper guineense) Leaf on the Histology of the Liver of Wistar Albino Rats. Biot J Inter., 2019; 23(2): 1-11.
22. Oke J. I fought COVID-19 with carrots, black seed oil, honey, vitamin C. Daily Trust., 2020.
23. Prakash P, Gupta N. Therapeutic Uses of Ocimum sanctum Linn (Tulsi) With a Note on Eugenol and Its Pharmacological Actions: A Short Review. Indian J of physiol and pharmacol, 2005; 49(2): 125-131.
24. Aguiyi JC, Obi CI, Gyang SS. Igweh AC. Hypoglycaemic activity of Ocimum gratissimum in rats. Fitoter, 2000; 71(4): 444-60.
25. Mohammed A, Tanko Y, Okasha MA, Magaji RA, Yaro AH. Effects of aqueous leaves extract of Ocimum gratissimum on blood glucose levels of streptozocin- African induced diabetic wistar rats. Afri J of Biotech, 2007; 6(18): 2087-90.
26. Egesie UG, Adelaiye AB, Ibu JO, Egesie OJ. Safety and hypoglycaemic properties of aqueous leaf extract of Ocimum gratissimum in streptozotocin induced diabetic rats. Niger J Physiol Sci., 2006; 21(1-2): 31-5.
27. Thompson Coon JS, Ernst E. Herbs for serum cholesterol reduction: A systematic view. . Fam Pract. 2003; 52: 468-478.
28. Iweala EEJ, Obidoa O. Studies on some biochemical and histological changes associated with long term consumption of leaves of Ocimum gratissimum L. in male rats. Am J Food Technol, 2010; 5(6): 376-384.
29. Ofem OE, Ani EJ, Eno AE. Effect of aqueous leaves extract of *Ocimum gratissimum* on hematological parameters in rats. Internatioal Journal of Applied Basic Medical Research, 2012; 2(1): 38- 42.
30. Ilori M, Sheteolu AO, Omonibgehin EA, Adeneye AA. Antibacterial activity of *Ocimum gratissimum* (Lamiaceae) J Diarrh Dis Res., 1996; 14: 283–5.
31. Onajobi FD. Smooth muscle contracting lipid soluble principles in chromatographic fractions of *Ocimum gratissimum*. J Ethnopharmacol, 1986; 18: 3–11.