Effect of Aqueous Seed Extract 0f Nigella Sativa on Some Haematological Values, Lipid Peroxidation And Osmotic Fragility Changes In Alloxan Induced Diabetic Rats.

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Abstract

This study was designed to investigate the effect of extracts of *Nigella sativa* on some haematological values, lipid peroxidation and osmotic fragility changes in alloxan-induced diabetic rats.

Eighteen albino rats were divided into 3 experimental groups of six rats each; control, diabetic untreated, diabetic treated with aqueous extract of *Nigella sativa.* At the end of experimental period (Four weeks) animals in all the groups were fasted for 12 hours and blood samples were taken for the determination of red blood cell count (RBC), packed cell volume (PCV), Haemoglobin concentration (Hb). In addition Malondialdehyde (MDA, marker of lipid peroxidation) and erythrocytes osmotic fragility test were carried out.

In diabetic untreated rats, the PCV, RBC, Hb. were decreased, but application of the extract significantly increased the values ($P<0.05$, n=6).

Similarly, treatment with aqueous extract of *Nigella sativa* decreased the elevated Malondialdehyde level ($P<0.05$, n=6) of diabetic rats. The results also showed that *Nigella sativa* extract significantly reduced the MCF_{50} from 0.68% to 0.50% ($P<0.05$ n=6). These findings suggest that *Nigella sativa* extract might improve the diabetic-induced disturbances of some haematological parameters by decreasing the elevated lipid peroxides and susceptibility of red blood cell to haemolysis.

Key words: *Nigella sativa, Lipid peroxidation, Osmotic fragility, Haematological parameters, Malondialdehyde.*

Introduction

Diabetes mellitus is a heterogeneous disease and is one of the most common metabolic disorders affecting approximately 5% of the World population[1]. The increasing prevalence of diabetes mellitus in the world is a cause for concern.

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This disease leads to abnormalities in carbohydrates, protein and lipid metabolism. Patients with diabetes have approximately 2 - 6 fold risk for all cardiovascular diseases[2,3,4] and the relative risk of death from all causes is increased by 75% [5,6].

Many modern medicines have their origin in plants that were often used in the treatment of illness and disease. In fact plants have formed the basis of sophisticated traditional medicine systems among which are Ayurvedic, Unani, Chinese, etc. These systems of medicine have given rise to some important drugs still in use today[7]. There is an increasing attempt to find out newer drugs that are more potent with fewer side effects compared with those in the market now. Similarly the World Health Organization (WHO) encourages scientific research into indigenous herbal medicine[8].

The seed of *Nigella sativa* plant, an annual herbaceous plant, has been used traditionally for centuries in the Middle East, North Africa, West Africa, Far East and Asia, as a natural remedy for a variety of diseases, like asthma, tumor, inflammation, and diabetes mellitus[9,10].

Major constituents of *Nigella sativa* are crystalline nigellone, the essential amino acids, linolenic acid, oleic acid, volatile oils, carotene, minerals such as Ca^{2+}, K^{+}, Fe^{2+}, etc. [11]. Because there are evidences that haematological parameters are altered in diabetes mellitus [12,13,14,15], this study was undertaken to determine whether or not *Nigella sativa* extract could restore changes in haematological values, lipid peroxidation and osmotic fragility due to diabetes.

Materials And Methods

Preparation of the plant extract

The aqueous extract of *Nigella Sativa* seed was prepared using the method described by Farida *et al* [16].The powdered seed (500g) of *Nigella sativa* was percolated in distilled water for 12 hours and filtered, the filtrate was subsequently evaporated in a regulated water bath at 70°C and later dried to constant weight in an oven at 38°C and yielded a blackish brown concentrates (37.8g).

Albino rats, weighing 150-200g, were used for this study. All the rats were kept at an average room temperature of 30°C in the animal

house of Physiology Department, College of Health Sciences, University of Ilorin. They were allowed free access to water and food. They were also maintained under standard photoperiodic condition of 12 hours of light alternating with 12 hours of darkness i. e. LD 12:12. Eighteen albino rats were divided into 3 experimental groups of six rats each; that is Group A- control, Group B – diabetic untreated and Group C – diabetic treated with aqueous extract of *Nigella sativa.*

Induction of diabetes by alloxan

Alloxan was administered intraperitoneally at 100mg/kg and was equivalent to 0.001 ml/g of rat to rats in group B and C. Rats in control group A received equal amount of normal saline. Diabetes was confirmed by glucose oxidase method using glucostix, when plasma glucose is ≥ 200mg/dl [17], 72 hours after alloxan administration. *Nigella sativa* extract was then administered orally at 180mg/Kg and this was equivalent to 0.036ml/g of rat, to rats in group C only for 4 weeks.

At the end of experimental period, rats were fasted for 12 hours and were sacrificed. Blood was collected by cardiac puncture and transferred into an heparinized bottle for osmotic fragility test and EDTA bottle for MDA and haematological analysis.

Determination of haematological parameters

All haematoogical parameters were determined by an automated haematological analyzer, Symex KY-21 (Symex corporation, Japan) using whole blood sample.

Determination of erythrocytes osmotic fragility.

Osmotic fragility of red blood cell was determined according to the method of Dacie and Lewis[18]. A 10% buffered NaCl solution was prepared by dissolving 90g of NaCl, 2.43g sodium dihydrogen phosphate and 13.6g disodium hydrogen phosphate in distilled water. The solution was then made up to 1 liter with distilled water as the stock saline solution. From the stock saline solution, a 1% buffered saline solution was then prepared and its pH adjusted to 7.4 using a pH meter (model 7050, Electronic Instrument Ltd Chertsey, Surrey, UK). Lower dilutions of NaCl solution ranging from 0.1-0.9% were prepared in a centrifuge tube and the 10th tube contained only distilled water. The dilution of NaCl solution was used to assess the susceptibility of erythrocytes to haemolysis (osmotic fragility) as follows; 5ml of each concentration of NaCl was put in a centrifuge tube (9 in all) and 5 ml of distilled water was put in the 10th tube. To each of the centrifuge tube was added 0.02 ml of blood, the content was thoroughly mixed by gentle inversion and was allowed to stand at room temperature for 30 min. Thereafter the tubes were centrifuged for 10 min. at 2000 rpm. The supernatant from each test tube was carefully decanted into freshly labeled tubes and the optical density of the supernatant was read on a spectronic 20 colorimeter (spectronic 20; Baush and Lomb) at a wavelength of 540 nm, using the supernatant from 0.9% NaCl solution as blank. The optical density of the solution in the tube with distilled water to which erythrocytes had been added was taken as 100% haemolysis. An erythrocyte fragilligram was then obtained by plotting the mean percentage haemolysis against the concentration of NaCl solutions.

Estimation of Malondialdehyde.

Plasma MDA was measured by a thiobarbituric acid assay procedure [19] which was calibrated using 1,1,3,3 tetraethoxypropane (Sigma chemical, St Louis, Mo USA) as a standard. Results were expressed as nanomoles of MDA per milliliter of serum.

Statistical Analysis.

The mean values of the measured variables were determined and the standard deviation, standard error of mean (SEM) were also calculated. The test for significance was done using ANOVA and Duncan's new multiple range test (DMRT)[20]. All the results were expressed as mean ± SEM at a level of significance for comparison set at $P < 0.05$

Results

The results of this study are shown in tabular and graphic forms. Table 1 shows the comparative values of some haematological parameters of the control and treatment groups. *Nigella sativa* treatment significantly increased the RBC, PCV and Hb. concentration values in diabetic treated rats, compared with the untreated diabetic group.

The mean of the erythrocyte osmotic fragility at different saline concentration and the cumulative osmotic fragiligram for each group is shown in fig.1. Osmotic fragility decreased as the concentration of NaCl increases with a fall being more pronounced between saline concentrations of 0.5% to 0.8%. The osmotic fragiligram of diabetic untreated group is significantly shifted to the right due to greater haemolysis, however there

is no significant difference (P< 0.05) in osmotic fragiligram between the control and diabetic treated group. The median mean corpuscular fragility (MCF_{50}) in the diabetic untreated group is 0.68%, this is significantly higher(P< 0.05) than the one observed in the control and *Nigella sativa* treated diabetic group which are 0.48% and 0.50% respectively.

There was a significant decrease (P< 0.05) in MDA level in the *Nigella sativa* treated diabetic rats when compared with diabetic untreated group. MDA level in the untreated group increased significantly (P< 0.05) compared with the control and the diabetic treated group, but there is no significant difference (P< 0.05) between the control and the diabetic treated group.

Discussion

In the present study some haematological parameters were analyzed and the results showed that red blood cell, packed cell volume and haemoglobin concentration value were decreased in diabetic rats. *Nigella sativa* treatment increased the reduced RBC, PCV and Hb concentration values in diabetic untreated rats. This is consistent with previous report that *Nigella sativa* significantly increase red blood cell count, packed cell volume and haemoglobin concentration values in diabetic rats [17]. The increase in erythrocytes level might have alternatively led to the increase in packed cell volume, and haemoglobin concentration, since there is a direct relationship between erythrocyte count, packed cell volume and haemoglobin concentration [21, 22]. The increase in blood indices could also be related to the chemical composition of the seed extract of *Nigella sativa,* which include 15 amino acids, protein, iron, calcium sodium and potassium etc [23]. Most of these constituents are well known haematological factors that have a direct influence (increase) on the production of blood from the bone marrow [24].

In this study the physical integrity of the erythrocytes was investigated in order to give an insight into the mechanism of increasing red blood cell count, packed cell volume and haemoglobin concentration. The result also shows that there is a right shift of erythrocyte osmotic fragility curve in diabetic untreated group which is indicated by a high mean corpuscular fragility (MCF_{50}) of 0.68%. *Nigella sativa* treatment reduced significantly the shift of the curve to the right with a reduced MCF_{50} to 0.50%.

There was a significant increase in the level of MDA in the diabetic rats when compared with the control. *Nigella sativa* treatment led to reduction in malondialdehyde MDA level in diabetic treated group. The increase in serum MDA level in diabetic untreated group is a confirmation of previous report by Nwajo and Oberley [25, 26], demonstrating that serum lipid peroxide level increased in diabetic rats. Therefore elevated serum lipid peroxide level in alloxan induced diabetic rats recorded in this work could, in part, result from non enzymatic glycosylation which is known to occur during diabetes [27,28].

It has been suggested that occurrence of anaemia in diabetes mellitus is due to increased non-enzymatic glycosylation of red blood cell membrane proteins, which correlates with hyperglycemia [29]. Oxidation of these glycosylated membrane protein and hyperglycemia in diabetes mellitus cause an increase in the production of lipid peroxide causing haemolysis of RBC. Thus increased RBC count, packed cell volume and haemoglobin concentration of *Nigella sativa* treated rats could be due to reduction of lipid peroxide level in RBC membrane. hence to a decreased susceptibility of RBC to haemolysis.

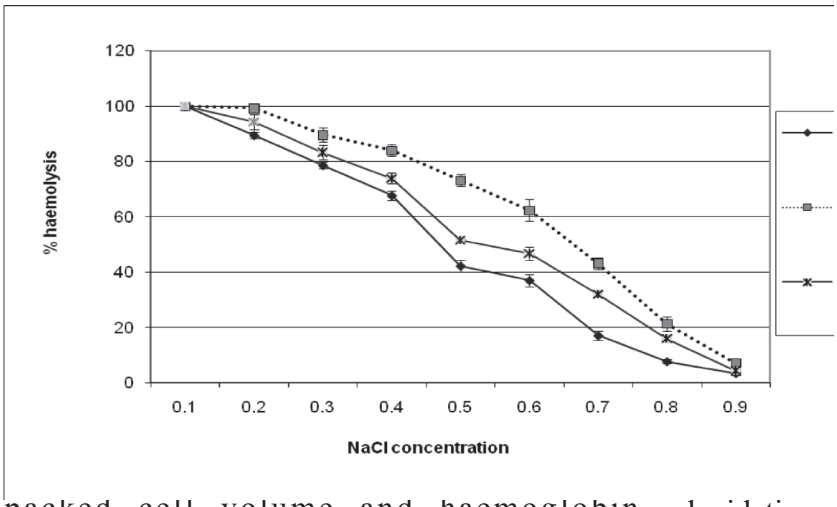
From this study, it could be deduced that aqueous seed extract of *Nigella sativa* stabilizes the red cell membrane by decreasing the level of peroxidation, thereby reducing haemolysis and prolonged life span of red blood cells. This

Table 1 Effect of *Nigella sativa* on haematological values and Lipid peroxidation in alloxan induced diabetic rats.

	controls	diabetics without treatment	diabetics treated with aqueous nigella sativa extract
RBC ($X10^6\mu L$)	6.73 ± 0.28^a	6.05 ± 0.12^b	7.19 ± 0.18^a
PCV (%)	40.20±0.96a	35.80 ± 1.02^b	$42.80\pm0\text{-}58^a$
HbConc (g/dl)	13.18±0.12a	11.50 ± 0.38^b	14.30 ± 0.28^a
MDA($X10^{-9}$/L	1.36 ± 0.53a	2.67 ± 0.96^b	2.33 ± 0.66^a

Note: values are expressed as mean ± SEM, number of rats in each group= 6, different superscripts on mean ±SEM along the same row indicate P<0.05.
MDA= Malondialdehyde, RBC= Red Blood Cell Count, PCV = Packed Cell Volume, HbCon = Haemoglobin Concentration,

Fig.1 Effect of aqueous extract if *Nigella sativa* on osmotic fragility in rats



packed cell volume and haemoglobin concentration found in diabetic treated rats, but it is not known whether the extract itself has a direct stimulatory effect on erythropoietin. Therefore, further studies is required to measure plasma erythropoietin, serum folic acid, cobalt, interleukins and calcium levels to elucidate other mechanism of action of *Nigella sativa* extract on the haematological indices.

It can be concluded that *Nigella sativa* treatment would reduce the disturbances of some haematological parameters in alloxan induced diabetic rats.

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