EFFECT OF BLACK SEED (*NIGELLA SATIVA*) AND UZIZA (*PIPER GUINEENSE*) LEAF ON THE HISTOLOGY OF THE LIVER OF WISTAR ALBINO RATS

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ABSTRACT

Aim: Certain plants such as black seed (*Nigella sativa*) and uziza leaf (*Piper* guineense) can be used to treat various illnesses which occur in man. This study was aimed at investigating the effects of aqueous extracts of both *Nigella sativa* and *Piper guineense* on the liver enzymes; alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP). Also the effects of *Nigella sativa* and *Piper guineense* extracts on the histology of the liver of Wistar rat was also studied.

Materials and methods: A total of twenty five Wistar rats were used for the study. The animals were grouped into five groups, each having five animals. They were induced with sucrose and margarine to cause high sugar levels and hyperlipidemia respectively except the positive control group which was fed normal feed. The groups were: the positive control group, the negative control group which were induced without treatment, the uziza leaf group which were induced and were treated with 2ml of aqueous extract of uziza leaf, the black seed group which were induced and were treated with 2ml of aqueous extract of black seed, and the black seed and uziza group which were induced and were treated with 2ml of aqueous extract of black seed and 2ml of aqueous extract of uziza leaf.

Results: The result showed that the extracts decreased the ALT and AST activities in the rats in a time dependent manner with highest decrease obtained on the third week of treatment with the extracts. The ALT activity (U/L) on the third week of treatment showed for the, negative control (64.48 ± 0.22), uziza leaf (28.82 ± 0.12), black seed (32.65 ± 0.02), black seed and uziza leaf (16.04 ± 0.02) ($p \leq 0.05$). The decrease in activity for AST levels (U/L) on the third week of treatment, showed for the negative control (58.00 ± 0.02), uziza leaf (11.00 ± 0.01), black seed (12.00 ± 0.02), black seed and uziza leaf (8.00 ± 0.02). Furthermore, the extracts also had a decreasing effect on the activity of the ALP in a time dependent manner with the highest decrease in activity obtained on the third week of treatment ($p \leq 0.05$). The ALP levels (U/L) showed for the negative control (80.22 ± 0.02), uziza leaf (30.42 ± 0.02), black seed (34.16 ± 0.04) and black seed and scent leaf (28.48 ± 0.01).

Conclusion: It can be concluded that both uziza leaf and black seed have hepatoprotective effect on the liver.

Keywords: Alanine amino transferase, Alkaline phosphatase, Aspartate amino transferase, Enzymes, Liver, *Nigella sativa, Piper guineense*

1.0 INTRODUCTION

Nigella sativa (black seed) is an annual flowering plant in the family Ranunculaceae, native to south and southwest Asia. It grows to 20-90 cm (7.9-11.8) tall, with finely divided, linear (but not thread-like) leaves. The flowers are delicate, and usually coloured plain blue and white with five to ten petals. The fruit is a large and inflated capsule composed of three to seven united follicles, each containing numerous seeds which are used as spice [1]. The seeds can be eaten whole or pressed to make an oil extract. The oil can be taken orally or topically and has even been given intravenously in animal experiments.

The seeds of *Nigella sativa* have been prized for their healing properties since time immemorial. It was found that black seed oil has an anti-inflammatory effect and that it could be useful to relieve the effects of arthritis. Within the ancient healing systems of the Persian Gulf, black seed was used to treat a host of illnesses such as cancer, diabetes mellitus, epilepsy, fever, cough, bronchitis, asthma, chronic headache, migraine, dizziness, chest congestion, dysmenorrhea, obesity, diabetes, paralysis, hemiplegia, back pain, infection, inflammation, rheumatism,

hypertension and gastrointestinal problems such as dyspepsia, flatulence, dysentery and diarrhoea [2].

While frequently referred to among English-speaking cultures as Roman coriander, black sesame, black cumin, black caraway and onion seed, it is known today as black seed, which is an accurate description of its physical appearance. The earliest record of its cultivation and use come from ancient Egypt. Black seed oil was found in Egyptian pharaoh Tutankhamun's tomb dating back to approximately 3,300 years ago [3].

Many active compounds have been isolated, identified and reported so far in different varieties of black seeds. The most important active compounds are nigellone, thymoquinone, thymohydroquinone, dithymoquinone, thymol, [4]. Nigellone and thymoquinone were first discovered in black seed in 1985. Nigellone offers both anti-spasmodic and bronchodilating properties which contribute to black seed's potency against respiratory ailments. It also acts as antihistamine which helps to reduce the negative symptoms of allergy sufferers. Thymoquinone contains excellent anti-inflammatory and analgestic properties. It is also a strong oxidant and helps cleanse the body of toxins. It contains linoleic acid, oleic acid, palmitic acid, and trans-anethole, among other minor constituents. Black seed provides a rich supply of polyunsaturated fatty acids. These ingredients play a key role in daily health and wellness. They help to regulate the metabolism, carry toxins to the skin's surface for elimination, balance insulin levels, regulate cholesterol, improve body circulation and promote health liver function [5].

The majority of our health problems have the same causes-infection by micro-organisms such as bacteria, viruses, parasites and fungi. When using allopathic medicines each symptom is treated individually and usually with synthetic chemically manufactured medicines.

Black seed is found effective at treating the body as a whole and fights the actual cause of the symptoms. Black seed regulates too weak or too strong reactions of the immune system and is excellent for treating chronic, allergic and hormonal diseases [5]. It uses are many as well as its benefit. Black seed supports metabolism, improves digestion, and lowers blood sugar levels. It is used to dispel worms and parasites from intestinal track. It is useful in soothing bronchitis and coughs. It increases body tone, stimulates menstrual periods, increases the flow of breast milk, provides quick energy, increases sperm count, calms the nervous system, encourages hair growth and retards hair fall out, prevents skin wrinkling, and much more. Studies have shown that black seed oil contains antioxidants that protect the body from free radicals. Additionally black seed oil is a tremendous source of essential fatty acids [2].

Black seeds are now used as skin care naturals. Some use it for healthy hair and nails and to restore good health. Skincare Naturals said that black seed is a wonderful oil to use in a Gardener‟s Skin Balm due to its healing, pain relief and skin conditioning properties.

Black cumin seed oil inhibits cancer cell activity and can even kill some types of cancer cells. Scientific research has shown that black seed oil is an effective treatment for cancer in animal studies. Black seed oil and its extract have powerful benefits for various inflammatory diseases including various types of cancer. Black cumin seed oil and its extract thymoquinone have powerful benefits for various inflammatory diseases including liver cancer, melanoma skin cancer, pancreatic cancer, cervical cancer, breast cancer, bone cancer, stomach cancer, lymphoma, prostate cancer, colon cancer, and brain cancer [6]. Black cumin is a rich source of essential fatty acids – omega 3 and omega 6, the building blocks of cells, and used for relief of acne, psoriasis, eczema and pain.

Piper guineense is a spice plant from the family Piperaceae and from genus piper. It is a West African spice plant commonly called Ashanti pepper. It is known as uziza in Igbo and iyerein Yoruba. Other common names are Benin pepper, Guinea pepper and false cubeb [7, 8]. Spices generally are parts of various plants cultivated for their aromatic pungent or otherwise desirable substances. They consist of rhizomes, bulbs, flower bud, fruit, seed, and leaves. They usually are categorized into tiny wild fruits, nuts, herbs, and leafy vegetables. The plants that provide Ashanti pepper are vines that grow up to 20m tall climbing up bole of trees by means of adventitious roots. It is a perennial plant that is characterized by heart-shaped leaves and oval, petiole, alternate, 12cm long. The leaves which have a peppery taste, are pale greenish color when fresh and darker green when frozen or dried. The inflorescence is a pedicel flower spike between 3 and 6cm long and the peduncle 5mm long. Flowers are greenish yellow and arranged in a spiral along the spine [9]. The fruits of *Piper guineense* occur in clusters, small, reddish or reddish brown when ripe and black when dry [10]. The fruit is a drupe mesocarp or fleshy, oval, 5mm in diameter. It is a West African species of piper derived from its dried fruit known as West African pepper, kale, ashanti pepper, guinea pepper or benin pepper [10].

Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic disorders in which there are high blood sugar levels over a prolonged period [11]. Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced. According to American Diabetes Association, diabetes mellitus (DM) is a chronic metabolic disorder characterized by high levels of glucose in the blood due to the impaired secretion of insulin or insulin insensitivity.

Hyperglycemia and hyperlipidemia are two important characters of diabetes mellitus. Diabetic patients experience various vascular complications, such as atherosclerosis, diabetic nephropathy and neuropathy. It is now well established that the hyperlipidemia represents a major risk factor for the premature development of atherosclerosis and its cardiovascular complications [12]. Diabetes mellitus is linked with prejudice glucose metabolism that escorts to a rise in free radical production and augmentation in the lipoprotein and triglyceride levels. Many of the disorder have been characterized in hyperglycemic animals. Significant changes in lipid metabolism also crop up in diabetes [13].

Alternative and traditional medicines have scores of advantages over the conventional medicines. Despite many conventional therapies present in the market to curtail the diabetes and its complications, traditional medicines such as Unani formulations has unambiguous advantage of being almost free from adverse effects. Diversity, flexibility, easy accessibility, broad continuing acceptance in developing countries and increasing popularity in developed countries, relative low cost, low levels of technological input, relative low side effects and growing economic importance are some of the positive features of traditional medicine [11]. Therefore searching herbal product with antidiabetic activity possessing fewer side effects receives considerable publicity and provides an opportunity to cure this disease. Plants play a major role in the discovery of new therapeutic agents and have received much attention as sources of biologically active substances including antioxidants, hypoglycemic and hypolipidemic agents.

Hyperlipidemia is a medical condition characterized by an increase in one or more of the plasma lipids, including triglycerides, cholesterol, cholesterol esters, phospholipids and or plasma lipoproteins including very low-density lipoprotein and low-density lipoprotein along with

reduced high-density lipoprotein levels. This elevation of plasma lipids is among the leading risk factors associated with cardiovascular diseases [14].

The pathophysiology of hyperlipidemia can be studied under the two basic classification of hyperlipidemia-primary and secondary hyperlipidemia. The pathophysiology of primary hyperlipidemia involve the idiopathic hyperchylomicronemia in which defect in lipid metabolism leads to hypertriglyceridemia and hyperchylomicronemia caused by a defect in lipoprotein lipase activity or the absence of the surface apoprotein CII31. In secondary hyperlipidemia, the postprandial absorption of chylomicrons from the gastrointestinal tract occurs 30-60 min after ingestion of a meal containing fat that may increase serum triglycerides for 3-10 hours [15].

The liver transaminases aspartate transaminase (AST or SGOT) and alanine transaminase (ALT or SGPT) are useful biomarkers of liver injury in a patient with some degree of intact liver function. Most liver diseases cause only mild symptoms initially, but these diseases must be detected early. Hepatic (liver) involvement in some diseases can be of crucial importance. This testing is performed on a patient's blood sample. Some tests are associated with functionality (e.g., albumin), some with cellular integrity (e.g., transaminase), and some with conditions linked to the biliary tract (gamma-glutamyl transferase and alkaline phosphatase). Several biochemical tests are useful in the evaluation and management of patients with hepatic dysfunction. These tests can be used to detect the presence of liver disease, distinguish among different types of liver disorders, gauge the extent of known liver damage, and follow the response to treatment. Some or all of these measurements are also carried out (usually about twice a year for routine cases) on

those individuals taking certain medications, such as anticonvulsants, to ensure the medications are not damaging the person's liver [16, 17, 18].

Alkaline phosphatase (ALP) is an enzyme in the cells lining the biliary ducts of the liver. It can also be found on the mucosal epithelium of the small intestine, proximal convoluted tubule of the kidneys, bone, liver, and placenta. It plays an important role in lipid transposition in small intestines and calcification of bones. 50% if all the serum ALP activities in blood are contributed by bone. Acute viral hepatitis usually has normal or increased ALP [17, 18].

2.0 MATERIALS AND METHODS

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 uziza leaf (*Piper guineense)* 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; uziza leaf (*Piper guineense*) 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 uziza 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 uziza 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 uziza 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 uziza leaf and black seed aqueous extracts.

2.4 Blood Collection

The animals, after being induced with sucrose and margarine for one month, were treated and sacrificed on a weekly basis. A dessicator with chloroform soaked cotton wool was used to weaken the rats before sacrificing them. The blood was collected with lithium heparin bottles. The liver was collected and put in a urine bottle containing formalin. The samples were taken to the laboratory for analysis.

2.5 ALKALINE PHOSPHATASE TEST.

The principle: Serum alkaline phosphate hydrolyzes a colourless substrate of phenolphthalein monophosphate giving rise to phosphorus acid and phenolphthalein which at alkaline PH values turns into pink colour that can be photometrically determined [19].

Two (2) ml of the blood was collected and put in a lithium heparin bottle. It was spun for 5 minutes in order to separate the various constituents of the blood. Two test tubes were labeled, the first one was labeled as 'standard' and the second one was labeled as the 'test'. Using a pipette, 1000µl of alkaline phosphatase water was collected and put in each test tube. A drop of alkaline phosphatase substrate was added to each test tube and was allowed to stand for 5 minutes. Using a pipette, 5000µl (5ml) of alkaline phosphatase colour developer was put into each test tube. The absorbance was read with a UV/ visible spectrophotometer under wavelength 540-550nm. The concentration was calculated using:

$$\frac{absorbance\ of\ sample\ \times 30}{absorbance\ of\ standard}$$

2.6 ASPARTATE TRANSAMINASE (AST) TEST:

Working Principle

Oxoglutarate + L – aspartate GOT = L – glutamate + oxaloacetate.

AST is measured by monitoring the concentration of oxaloacetate hydrazine formed with 2,4-dinitrophenylhydrazine (the endpoint colour change is brown) [20].

Two (2) ml of the blood sample was collected and poured in a lithium heparin bottle and the blood sample was spun. Two test tubes were labeled, the first test tube was labeled as 'sample' and the second test tube was labeled as 'blank'. Using a pipette, 500µl of reagent 1 (R1) was put into both test tubes, then 100µl of the sample was added into the test tube labeled as sample. Into 'blank' test tube, 100µl of distilled water was added. They were both incubated for 30 minutes. 500µl of reagent 2 (R2) was added to both test tubes and then the test tubes were incubated for 20minutes. 5000µl of sodium hydroxide (NaOH) was added to each test tube. The absorbance was read using UV/ visible spectrophotometer under 546nm wave length. The machine was zeroed with the blank.

The activity of the AST in the serum/plasma was obtained from the table

Absorbance	**µ/l**	**Absorbance**	**µ/l**
0.020	7	0.100	36
0.030	10	0.110	41
0.040	13	0.120	47
0.050	16	0.130	52
0.060	19	0.140	53

0.070	23	0.150	67
0.080	27	0.160	76
0.090	3	0.170	89

2.7 ALANINE TRANSAMINASE TEST:

Working Principle

Oxoglutarate + L- alamine GPT L- gluyamate of pyruvate

Alanine transaminase is measured by monitoring the concentration of pyruvate hydrazine formed with 2,4-dinitrophyenylhydrazene [20].

Two (2) ml of the blood sample was collected and poured in a lithium heparin bottle. The blood sample was spun. Two test tubes were labeled, the first test tube as the sample test tube and the second test tube as the blank. 500µl of reagent 1(R1) was put in each test tube using a pipette, 100µl of sample was then added to the sample test tube and 100µl of distilled water was added to the test tube labeled as blank. Both test tubes were incubated for 30 minutes and then 500µl of reagent 2(R2) was added to each test tube. The test tubes were incubated for 20 minutes and then 500µl of sodium hydroxide (NaOH) was added.

The activity of ALT in the serum was obtained from the table below

Absorbance	µl	Absorbance	µl
0.025	4	0.275	48

0.050	8	0.300	52
0.075	12	0.325	57
0.100	17	0.350	62
0.125	21	0.350	67
0.150	25	0.400	72
0.175	29	0.425	77
0.200	34	0.450	83
0.225	39	0.475	88
0.250	43	0.500	94

2.8 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 $p \leq 0.05$.

3.0 RESULTS

The results of the study are as shown below.

Table 1: LIVER FUNCTION TEST RESULT

WEEK ONE

Sample	ALT (U/L)	AST (U/L)	ALP (U/L)
Negative Control	54.19±0.07[a]	42.00±0.70 [a]	77.36±0.10 [a]
Positive Control	23.47±0.01 [a]	17.00±1.16 [a]	43.79±0.02 [a]
Uziza leaf Only	42.26±0.03 [a]	18.00±0.04 [a]	45.08±0.02 [a]
Black Seed Only	44.38±0.01 [a]	14.00±0.01[a]	52.06±0.02 [a]
Black Seed and Uziza leaf	37.18±0.07 [a]	12.00±0.05 [a]	38.43±0.02 [a]

Values represents mean ± Standard Error of Mean (SEM). Means in the same column with same superscript alphabet are significantly different at P≤0.05.

Table 2: WEEK TWO

Sample	ALT (U/L)	AST (U/L)	ALP (U/L)
Negative Control	57.25±0.01 [a]	46.00±0.55 [a]	79.55±0.02 [a]
Positive Control	24.27±0.01 [a]	17.05±0.03 [a]	43.61±0.01 [a]
Uziza leaf Only	36.22±0.01 [a]	18.00±0.01 [a]	42.16±0.01 [a]
Black Seed Only	39.96±0.03 [a]	14.00±0.03 [a]	43.12±0.02 [a]
Black Seed and Uziza leaf	24.62±0.01 [a]	10.00±0.03 [a]	41.05±0.02 [a]

Values represent mean ± Standard Error of Mean (SEM). Means in the same column with same superscript alphabet are significantly different at P≤0.05.

Table 3: WEEK THREE

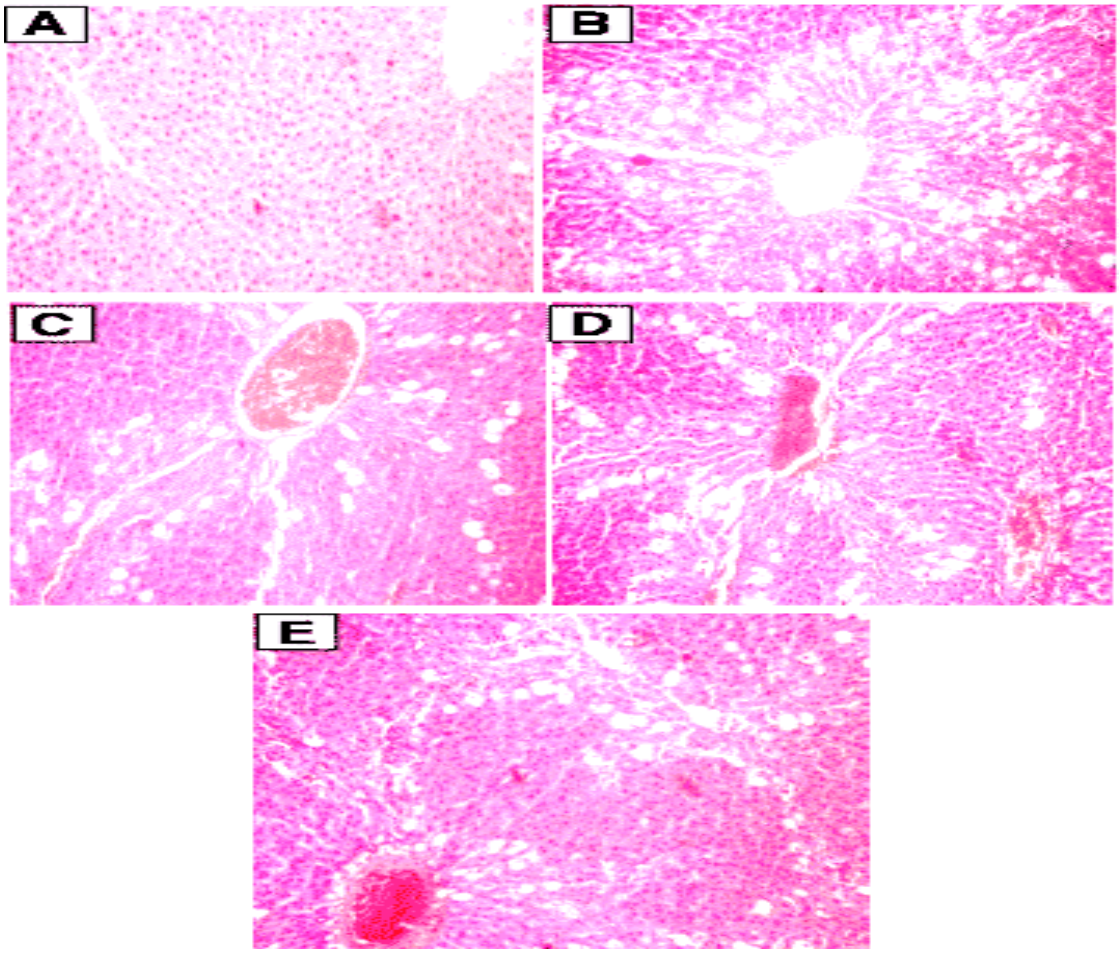
Sample	ALT (U/L)	AST (U/L)	ALP (U/L)

Negative Control	64.48±0.22 [a]	58.00±0.02 [a]	80.22±0.02 [a]
Positive Control	23.67±0.11 [a]	13.00±0.04 [a]	45.36±0.17 [a]
Uziza leaf	28.82±0.12 [a]	11.00±0.01 [a]	30.42±0.02 [a]
Black Seed	32.65±0.02 [a]	12.00±0.02 [a]	34.16±0.04 [a]
Black seed and uziza leaf	16.04±0.02 [a]	8.00±0.02 [a]	28.48±0.01 [a]

Values represent mean ± Standard Error of Mean (SEM). Means in the same column with same superscript alphabet are significantly different at $P \leq 0.05$.

HISTOPATHOLOGY RESULTS

Plate 1: WEEK ONE



a- Positive control

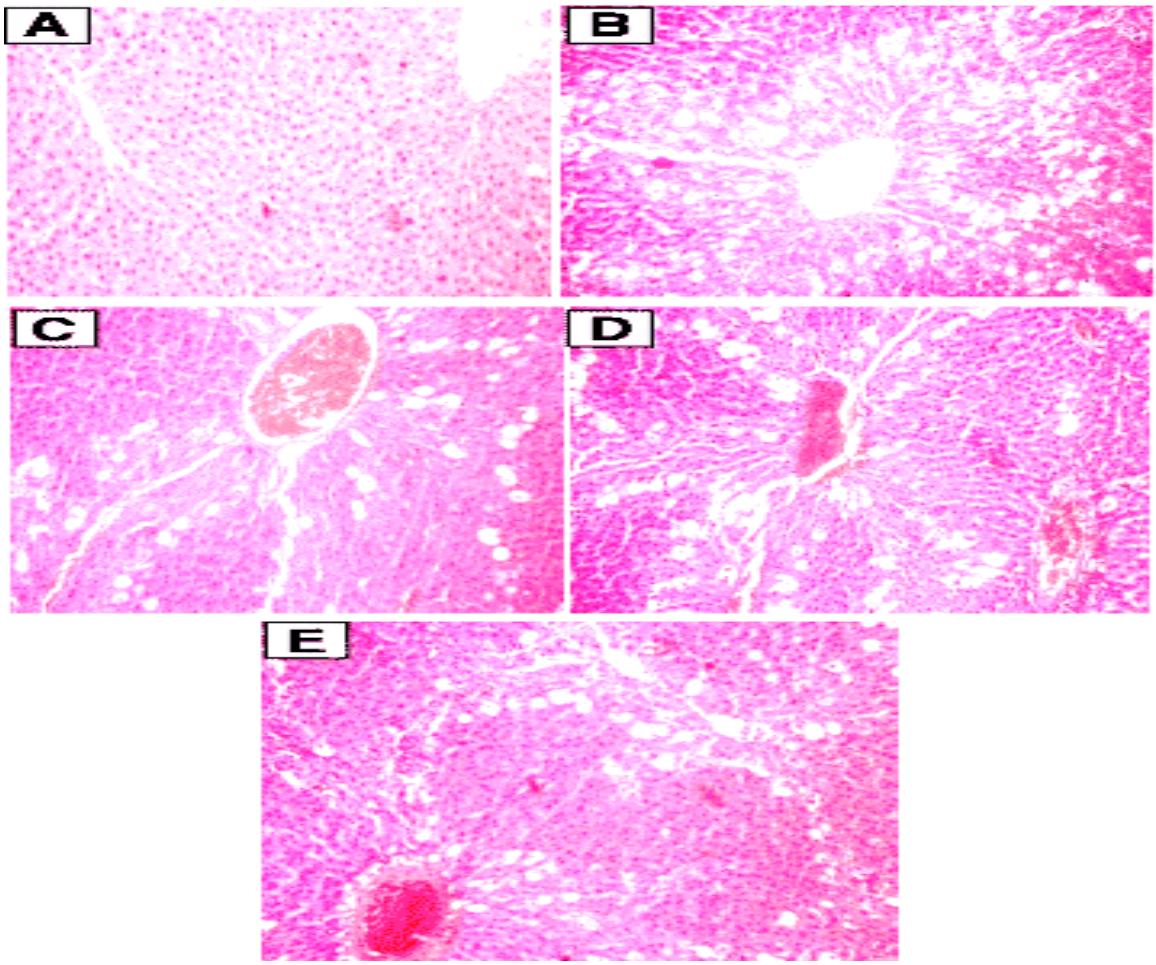
b- Negative control

c- Uziza leaf

d- Black seed

e- Black seed and uziza leaf

Plate 2; WEEK TWO



f- Positive control

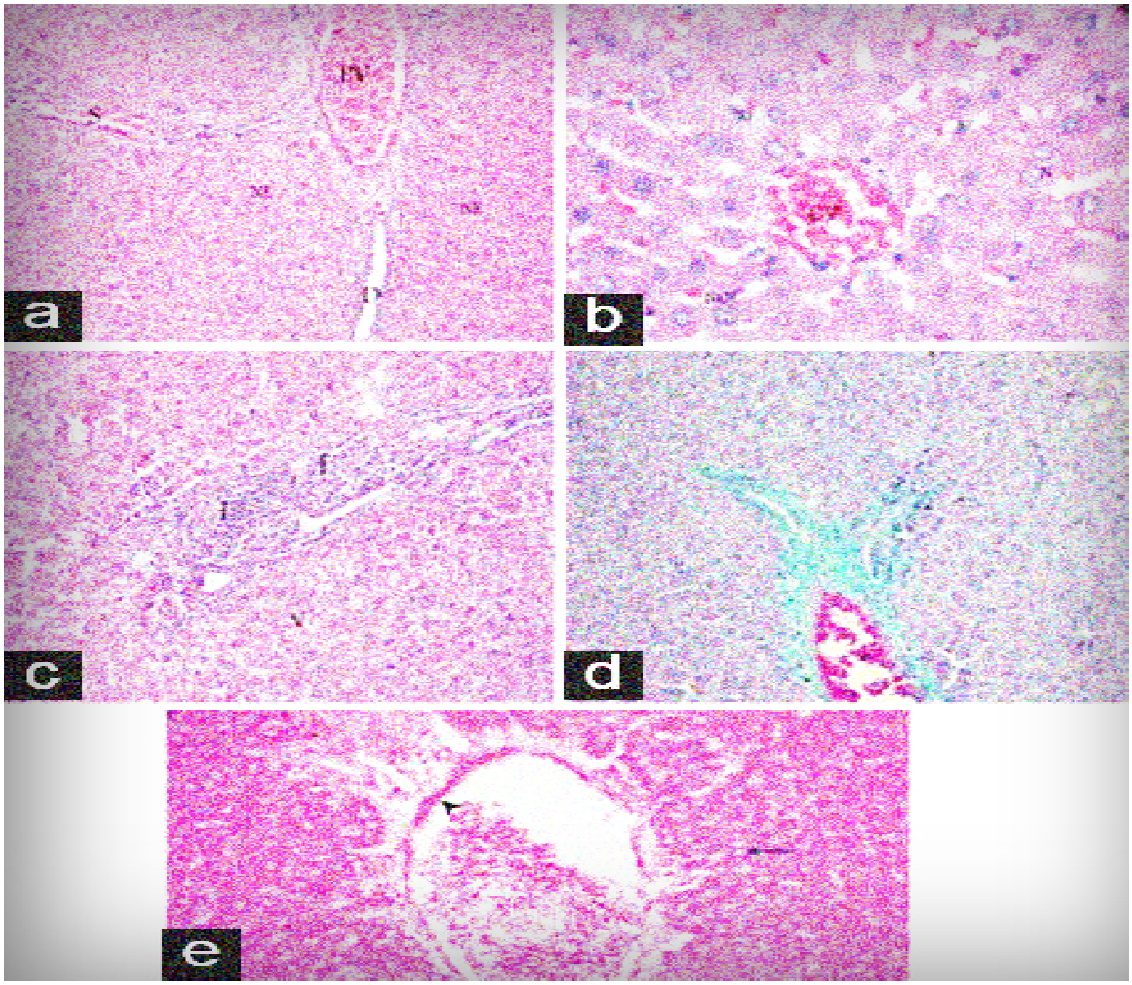
g- Negative control

h- Uziza leaf

i- Black seed

j- Black seed and uziza leaf

Plate 3: WEEK THREE



a- Positive control

b- Negative control

c- Uziza leaf only

d- Black seed only

e- Black seed and uziza leaf

4.0 DISCUSSION

This study investigated the effect of black seed *(Nigella Sativa)* and uziza *(Piper guineense)* on the liver enzymes which are used to assess the hepatocellular integrity of liver tissue. From the pilot study, the blood glucose levels were lower in the animals that were induced than the non-induced. High sugar levels have been known to be associated with hyperlipidemia and in turn affect the liver. Hyperlipidemia caused increase in the weights of the animals that were induced. This observation agrees with the research done by Nwozo *et al* [21]. From the biochemical study, it can be deduced that the activity levels of the liver enzymes, that is Alanine Transaminase, Alkaline Phosphatase and Aspartate Transaminase were relatively normal in the positive control. Compared to the positive control, the negative control had relatively high activity levels of the liver enzymes which increased as the weeks went by except for Alkaline Phophatase which reduced in the third week. At the end of 3 weeks, the liver enzyme values for the positive control were 64.48±0.22 U/L, 58.00±0.02 U/L, and 80.22±0.02 U/L for Alanine Transaminase, Aspartate Transaminase and Alkaline Phosphatase respectively were elevated compared to the first week.

The animals which were treated with Uziza leaf *(Piper guineense*) alone had higher activity of the liver enzymes in the first week compared to the animals in the second and third weeks. Uziza leaf extract reduced the liver enzymes considerably at the end of the third week with values 28.82±0.12 U/L, 11.00±0.01 U/L and 30.42±0.02 U/L for Alanine Transaminase, Aspartate Transaminase and Alkaline Phosphatase respectively compared to the negative control values. This observation agrees with the research which states that uziza leaf exerts a hypolipidemic effect, reverse the elevations witnessed in serum amino transferase activities and lipid peroxidation levels in hyperlipidemic rats [21].

The animals which were treated with black seed *(Nigella Sativa*) only had 32.65±0.02 U/L, 12.00±0.002 U/L and 34.16±0.04 U/L for Alanine Transaminase, Aspartate Transaminase and Alkaline Phosphatase respectively by the third week. These values were considerably lower than the values obtained after the first and second weeks of treatment and the values obtained in the negative control.

The animals which were treated with both aqueous extracts of both black seed (*Nigella Sativa*) and uziza leaf (*Piper guineense*) in the same proportion were seen to decrease the activity of the liver enzymes further than those treated with black seed alone and uziza alone at the end of the 3 weeks. When also compared to the values of the activity of the liver enzymes in the negative control, it was seen that this group had very high decrease in activity of the liver enzymes. The values obtained in this group by the third week were; 16.04±0.02 U/L, 8.00±0.02 U/L and 28.48±0.01 U/L for Alanine Transaminase, Aspartate Transaminase and Alkaline Phosphatase respectively were relatively lower than the activity levels of the positive control by the third week. This shows that the combination of both black seed (*Nigella Sativa*) and uziza leaf (*Piper guineense)* in the same proportion has a high hepatoprotective effect.

The histological results of this study showed no visible lesions in the positive control group, that is the positive control group had normal liver. The negative control however showed an enlarged sinusoid which is a very important cell in the liver. Enlargement and inflammation of the sinusoid indicates a reduction in the normal function of the liver and the inability of the liver to conduct its functions properly [8, 22, 23].

The livers of the animals treated with uziza leaf (Piper guineense) showed reduction in the inflammation of the sinusoid and restoration of the normal hepatic architecture.

The livers of the animals treated with black seed *(Nigella Sativa)* also showed reduction in the inflammation of the sinusoid and restoration of the normal hepatic structure [13, 24]. This is as a result of the anti-inflammatory effect of *Nigella Sativa*. This agrees with the research done by Abdelrazek *et al* [11] which showed the ability of *Nigella Sativa* in the reduction of inflammation in the liver of Wistar rats.

The animals which were treated with both aqueous extracts of black seed *(Nigella Sativa*) and uziza leaf *(Piper guneense*) however showed a great deal of reduction of the sinusoids and restoration of the normal hepatic architecture. The histopathology results obtained here were almost similar with the histopathology result which was seen in the positive control, hence the combination of *Nigella Sativa* and *Piper guineense* had a greater hepatoprotective effect than black seed (Nigella Sativa) only and uziza leaf (Piper guineense) only.

It can be concluded 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. It can also be said that uziza leaf has a greater effect in the treatment of hyperlipidemia and the reduction of elevated liver function levels than black seed.

Furthermore, it can also be concluded that the use of both black seed and uziza leaf has a very high reducing effect on elevated liver enzyme levels and as such, is more effective in the protection of the liver.

It is highly recommended that the combination of black seed *(Nigella Sativa)* and uziza leaf (*Piper 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 (Nigella Sativa) and uziza leaf (Piper guineense) have hepatoprotective effects hence patients with liver damage or liver injury are recommended to take these medicinal plants.

Competing Interests

Authors have declared that no competing interests exist.

6. ETHICAL APPROVAL:

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

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