

HEPATOPROTECTIVE ACTIVITY OF AQUEOUS SEED EXTRACT OF *NIGELLA SATIVA* AGAINST HIGHLY ACTIVE ANTIRETROVIRAL THERAPY INDUCED HEPATOTOXICITY IN RATS

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Abstract

Liver is a metabolically active organ responsible for many vital life functions. It performs many activities that are critical for survival. Due to its important activities, the liver is exposed to a number of insults and is one of the body's organs most subject to injury. In spite of tremendous advances in modern medicine, there are hardly any reliable drugs that protect the liver from damage and/or help in regeneration of hepatic cell. It is, therefore, necessary to search for effective and safe herbal drugs for the treatment of liver disease to replace currently used drugs of doubtful efficacy and safety.

The aim of this study is to investigate hepatoprotective activity of aqueous extract of *Nigella sativa* seed in highly active antiretroviral therapy (Lamivudine, Zidovudine and Efavirenz) administered rats. Sixty rats weighed between 150-200g were randomly divided into six groups and each group comprised of ten rats. Rats in group I were administered with distilled water. Rats in group II were administered with highly active antiretroviral therapy only. Rats in groups III - VI were administered 100, 200, 400 and 800 mg/kg *Nigella sativa* plus highly active antiretroviral therapy respectively.

The treatments were given orally for 28 consecutive days. On the 29th day, all rats were sacrificed under light diethyl ether anaesthesia; blood samples were collected for the assessment of biochemical parameters, while liver tissue was used for histopathological assessment. Serum levels of liver enzymes ALT, AST, ALP, and GGT were significantly ($p < 0.05$) increased and albumin concentration was significantly decreased in animals treated with highly active antiretroviral therapy as compared to the normal control. Histopathological observations also revealed severe damage in the structure of liver tissue in animals administered with highly active antiretroviral therapy. Treatment of highly active antiretroviral therapy exposed animals with *Nigella sativa* showed marked improvement in both biochemical and histopathological findings. Rise in liver enzymes was almost restored to normal in animals treated with *Nigella sativa*. *Nigella sativa* through its antioxidant activity effectively protects highly active antiretroviral therapy induced liver toxicity.

Keywords: HAART, *Nigella sativa*, Liver enzymes, hepatoprotective

Introduction

Liver is the largest organ in human body. It is a metabolically active organ responsible for many vital life functions. Liver plays a great role in carbohydrate, protein and fat metabolism, synthesis of bile components, detoxification of blood and storage of vitamins and minerals. It also performs many activities that are critical for survival such as synthesis of blood clotting factors, creation of proteins necessary for growth and metabolic processing of most drugs and toxins. It also has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction [1].

To maintain a healthy liver is a crucial factor for overall health and well-being, however; it is continuously and variedly exposed to environmental toxins and abused by poor drug habits and alcohol which can eventually lead to various liver ailments like hepatitis, cirrhosis and alcoholic liver disease [2].

Although antiretroviral drugs have significantly improved morbidity and mortality in HIV-infected patients, these benefits are compromised by numerous side effects, adverse clinical events and toxicities [3, 4]. Since the introduction of HAART, HIV infection has become a chronic disease. It has resulted in an increased prevalence and incidence of comorbidities among HIV-infected persons, requiring the use of more medications for longer periods. Therefore, it is not surprising that HAART-associated toxicity, especially liver toxicity, has perhaps become one of the main limitations to treatment [5, 6].

Almost all antiretroviral drugs belonging to all available classes are responsible for an intrinsic liver toxicity, which is increased by the combined use of at least three different antivirals, in the so-called Highly active antiretroviral therapy (HAART) [7, 8], despite the probability and extent of injury varies substantially with the individual agents [9].

In spite of tremendous advances in modern medicine, there are hardly any reliable drugs that protect the liver from damage and/or help in regeneration of hepatic cells. Conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. It is, therefore, necessary to search for effective and safe drugs for the treatment of liver disease to replace currently used drugs of

doubtful efficacy and safety [2].

Many folk remedies from plant origin were tested for their potential antioxidant and hepatoprotective liver damages [1]. Many active plant extracts are frequently utilized to treat a wide variety of clinical diseases including liver disease. *Aloe vera*, *Eclipta alba*, *Phyllanthus niruri*, *Solanum indicum* have been shown to possess anti-hepatotoxic properties. *Phyllanthus niruri* and *Maytenus emerginata* exhibit the excellent hepatoprotective properties as indicated by maximum prevention of increased serum biochemical parameters on paracetamol induced toxicity [10]. *Nigella sativa* (*N. sativa*) is a dicotyledon belonging to the botanical family of Ranunculaceae of herbaceous plants and known as black cummin seed. *Nigella sativa* is cultivated in many provinces of Ethiopia. It grows to a maximum height of 60cm, has blue flowers and finely divided foliage. Small caraway-type seeds are produced within the flowers [11, 12, 13]. The seeds of *N. sativa* are the source of the active ingredients of the plant [14]. *Nigella sativa* has been used for centuries as a spice, food preservative, preparation of candy and curative or medicinal remedy for various ailments, including infectious diseases [15, 16, 17].

Seed of the *N. sativa* has been used for medicinal purposes for centuries in Asia, Middle East, and Africa. It has been traditionally used as a natural remedy for a number of ailments that include headache, stomachache, asthma, chest congestion, hypertension, diabetes, inflammation, cough, bronchitis, fever, dizziness, and influenza and for general well-being [12]. *Nigella sativa* L. seeds were a good source of polyunsaturated fatty acids (PUFAs), phytosterols (PSs) and phospholipids (PhLs) for the human diet. These seeds could be used by the food industry for formulating functional foods enriched with PUFAs and PSs. In pharmaceutical applications, *N. sativa* conjugated sterols could be used as precursors for the hemisynthesis of many hydrosoluble steroids. Since, *N. sativa* L. seeds are a good source of PhLs and aroma compounds, and therefore, they could be utilized in biscuit manufacturing and in food flavoring [18].

Seeds of the *N. sativa* contain 37% oil and 4.1 % ash (calcium salts), protein (16-19.9%), carbohydrates (33-34%), fibre (4.5-6.5%), saponins (0.013%), moisture (5-7%) [16, 19].

Phytochemical screening of the seeds of *N. sativa* have led to the discovery of many active principles of the *N. sativa* like: Nigellicine, nigellidine, nigellimine-N-oxide, thymoquinone, dithymoquinone, nigellone,

thymohydroquinone, thymol, arvacrol, oxy-coumarin, 6-methoxycoumarin, 7-hydroxycoumarin, alpha-hedrin, steryl-glucoside as well as rich amounts of flavinoids, tannins, essential fatty acids, essential amino acids, ascorbic acid, iron and calcium [18].

Nigella sativa has been extensively studied pharmacologically to justify its broad traditional therapeutic value, from which, it was found to have hepatoprotective and immunopotentiating properties. Previous study [12] investigated that aqueous extract have protected against Carbon tetra chloride induced acute hepatotoxicity through restoration of the anti-oxidative defense system and down-regulation of the pro-inflammatory pathway.

Other study revealed that the seeds, and the major active constituent thymoquinone, exhibited hepatoprotective effect against liver damage induced by carbon tetrachloride and tetra-butyl hydrogen peroxide [20].

The general objective of this study is to investigate the hepatoprotective activity of aqueous extract of *N. sativa* seed in highly active antiretroviral therapy administered rats.

Material and Methods

Plant Collection and Preparation of the Extracts

Seed of *N. sativa* was purchased from Goro district, Bale zone 530 kms southeast of Addis Ababa in September 2013. The taxonomic identity of the plant was verified at the Department of Biology, Addis Ababa University. Voucher specimen of the plant (k-001/2013) was kept at the national herbarium, Science faculty, Addis Ababa University.

The plant material was then carefully washed with distilled water to remove any extraneous materials, dried under shade at room temperature, grounded to a coarse powder using electronic grinder and the aqueous extract of the seeds of the plant was prepared by decoction as follows:

200g of the powdered seed of *N. sativa* was weighed by analytical balance. 1500mL of distilled water was added and boiled for 15 minutes with continuous stirring. After cooling, the solution was decanted and the supernatant solution was filtered with 0.1mm² mesh gauze. The filtrate was transferred into a petridish and was frozen in a deep freezer overnight. On the next day the freeze-dried extract was allowed to dry in a freeze-dryer (lyophilizer) under vacuum pressure at lower temperature (-40°C) and lower pressure (133x10⁻³

3mbar) for a week to obtain a freeze-dried product. After the extract was dried, it was collected in air-tight plastic containers, weighed, labeled and put in a desiccator for subsequent experiment [12, 21].

The weight of the dry extract was expressed as percentage of the total mass of dry plant powder to determine the percentage yield.

Phytochemical Screening Test

A preliminary qualitative phytochemical screening of the plant material was carried out employing the standard procedures [22, 23, 24, 25] to reveal the presence of saponins, flavonoids, alkaloids, tannins, phenols, and glycosides.

Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a white coloured precipitate indicates the presence of alkaloids.

Dragendorff's Test: Filtrates were treated with Dragendorff's reagent (solution of Potassium Bismuth Iodide). Formation of yellow orange precipitate indicates the presence of alkaloids.

Detection of tannins

About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Detection of saponins

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of flavonoids

Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of

flavonoids.

Test for cardiac glycosides (Keller-Killani test): Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlaid with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Preparation of Highly Active Anti-retroviral Therapy

The three antiretroviral drugs used for the study (Lamivudine, Zidovudine and Efavirenz) were obtained from department of pharmacy, Black Lion Hospital, Addis Ababa.

The drugs were combined at the doses of 26.46 mg/kg Lamivudine (3TC), 52.91 mg/kg Zidovudine (ZDV) and 52.91 mg/kg Efavirenz (EFV). The drugs were prepared by grinding the tablets into fine powder and dissolved in distilled water.

Extract preparation for the experiment

The graded concentrations of 100, 200, 400 and 800 mg/kg were prepared from *N. sativa* aqueous extract. HAART and *N. sativa* aqueous extract were mixed together before administration. Only fresh drugs (prepared daily) were used.

Experimental Animals preparation

The experimental animals used in this study were 60 Albino Wistar rats of both sexes, each weighing 150–200g and aged three months. All rats were maintained under the controlled conditions of temperature ($25 \pm 2^\circ\text{C}$), humidity, and light (12 hours of light and dark) in the Animal House of Ethiopian public health institute (EPHI).

The animals had free access to food and clean tap water. The animals were housed in standard environmental conditions in stainless steel cages. The rats were acclimatized for 7 days before the start of the experiment. During the acclimatization the animals fed with Standard pelleted rat chow and water *ad libitum*.

Ethical approval for this study was obtained from the Research Ethics Committee of the Department of Biochemistry, Addis Ababa University.

Animal grouping and Drug dose

A modified method [26] was used for this test. In

this study, 60 Albino rats were randomly allotted into one of the six experimental groups, and each group consisted of ten rats:

- Group I received only distilled water and served as a normal control (2mL).
- Group II received only HAART and served as a positive control (2mL.)
- Group III received combination of HAART and (100 mg/kg) *N. sativa* seed extract.
- Group IV received combination of HAART and (200 mg/kg) *N. sativa* seed extract.
- Group V received combination of HAART and (400 mg/kg) *N. sativa* seed extract.
- Group VI received combination of HAART and (800 mg/kg) *N. sativa* seed extract.

Animals were deprived of food before drug administration after which they were allowed access to food.

A volume of 2mL of each treatment was administered for each rat by oral intubation (blunt intragastric catheter or gavage) once a day in the morning at 9.00 a.m. for 28 consecutive days. The blunt intragastric catheter was cleaned, placed in an oven and sterilized after each administration to avoid any contamination. Toxicity signs and mortality were monitored daily.

Blood Sample Collection

At the end of the experiment, animals were fasted overnight and anesthetized with diethyl ether. Immediately each animal was placed in supine position on operating board. The extremities of the animals were stretched and fixed on a dissecting board. The abdominal cavity was opened and blood sample was withdrawn by cardiac puncture using sterile needle of 5ml syringe [27].

Assessment of Hepatoprotective Activity: In the present study the hepatoprotective activity was evaluated biochemically and histopathologically.

Biochemical Assay

Initially the blood samples were allowed to clot. Then the clotted blood was centrifuged (using Humax 4k bench top centrifuge) at 5000 revolution per minute (rpm) for 10 minutes to separate the serum. Serum marker enzymes of liver function: alanine aminotransferase (ALT) activity, aspartate aminotransferase (AST) activity, alkaline phosphatase (ALP) activity, total bilirubin and total albumin were assayed using Automated Clinical Chemistry Analyzer (Cobas Integra 400 plus).

Histopathology of the Liver

Animals of each group were sacrificed while under diethyl ether anesthesia. Animals lay up on a clean paper towel and had all four extremities pinned to thin corkboard. A vertical midline incision with scissors cut from the neck to pubis and opens the peritoneum. Then 3-4mm wide strips of tissue samples were randomly taken from right lobe of liver were cut lengthwise. These tissue samples were transferred by a blunt forceps to a test tube containing 10% buffered formalin that completely immerses the tissues for the purpose of fixation [28]. Following washing, tissues were dehydrated in a series of an increase graded ethanol (70%, 80%, 95% and 100%), cleared in xylene, and embedded in paraffin wax [28, 29]. The sections, which are 5-6 μ m thick, were then prepared using rotary microtome (Leica RM 2125 RTS, Singapore) and stained with hematoxylin and eosin dye for microscopic observation of histopathological changes in the liver [28, 30].

Using light microscope, sections of the liver were examined at different magnifications (x25, x40, and x100) objectives using MC 80 DX Microscope Camera (Carl Zeiss, Germany). Photomicrographs of the selected samples of the liver were taken with Fuji color C200 film under microscope camera [29].

Statistical Analysis

The data (expressed as mean \pm SEM) were analyzed by one way ANOVA followed by Tukey–Kramer post hoc test using SPSS software version 16.0 program. P values less than 0.05 were considered to be statistically significant.

Results

Percentage Yield from plant material

Percentage yield (%Yield) of the crude extract of *N. sativa* was calculated by the following formula.

$$\begin{aligned} \%Yield &= \frac{\text{weight of the aqueous extract obtained} \times 100}{\text{Weight of the powder used for extraction}} \\ &= \frac{29g \times 100\%}{200g} \\ &= 14.5\% \text{ (w/w)} \end{aligned}$$

Phytochemical screening Test

The result of phytochemical screening of powdered seed of *N. sativa* showed the presence of many secondary metabolites (Table 1). The result revealed the presence of saponins, flavonoids, alkaloids, tannins, phenols and glycosides.

Effects of *Nigella sativa* seed extract on the Biochemical parameters

The mean values of liver biomarkers (ALP, ALT, AST and GGT) increased significantly ($P < 0.05$) in HAART administered rats (group II) compared to rats in normal control group (Table 2). On the other hand, mean values of albumin concentration decreased significantly ($P < 0.05$) in rats treated with HAART when compared to the rats in normal control group (Table 2). Administration of *N. sativa* plus HAART (groups III – VI) significantly decreased ($P < 0.05$) the mean values of ALP, ALT, AST and GGT and significantly increased ($P < 0.05$) the mean value of albumin concentration when compared to positive control group (group II). Mean values of total Bilirubin has shown non-significant increase in HAART administered rats and decreased in *N. sativa* extract plus HAART administered rats (Table 2). Though there were no statistically significant differences among different doses of *N. sativa*, slight decrease in mean values of ALT, ALP, AST, and GGT was observed as the concentration increases from 100 to 800 mg/kg.

Histopathological examination of Liver

Histopathological examination of the liver sections under the light microscope revealed that liver sections from normal control rats (group I) showed normal hepatic cells with well-preserved cytoplasm, prominent nucleus, nucleolus, central vein and compact arrangement of hepatocytes (Figure 1). However; the histology of the liver in HAART treated rats (group II) showed wide spread inflammation, vascular congestion, dilated sinusoidal spaces and focal necrosis (Figure 2). The histological examination of the liver tissues in rats treated with *N. sativa* aqueous extract (groups III) showed improvement in the liver tissue. Minor vacuolation in the cytoplasm of the hepatocytes and minor focal hepatocellular necrosis was observed (Figure 3). The histological examination of the liver tissues in rats treated with *N. sativa* aqueous extract (groups IV) showed significant improvement in the liver tissue. Only minor distortion in architecture and vacuolation was observed (Figure 4). The histological examination of the liver tissues in rats treated with *N. sativa* aqueous extract (groups V) showed very minor change in cytoplasm of hepatocytes and small clear spaces in few hepatocytes were observed (Figure 5). The histological examination of the liver tissues in rats treated with *N. sativa* aqueous extract (groups

VI) showed very minor change in cytoplasm of hepatocytes; Sinusoids begin to appear; Normal architecture of the liver begins to regenerate (Figure 6).

Discussion

Because of its functional roles in the body, liver is the major target organ of toxicity. Injury to the liver may affect the integrity of hepatocytes leading to the release of liver enzymes such as ALP, ALT, AST, and GGT since these enzymes are confined to hepatocytes and released into the blood following liver injury. Hence, these enzymes are commonly used as markers of hepatic injuries [2, 31].

In the current study, damage of the liver caused by HAART was evident by the alteration in serum marker enzymes and albumin concentrations. Administration of HAART (Group II) significantly increased the serum levels of liver enzymes (AST, ALT, ALP, and GGT) and significantly decreased albumin concentration. This result indicates liver cell damage; leakage of enzymes from cells and loss of functional integrity of cell. This is in consistent with the previous work [3] that indicated oral administration of rats with antiretroviral drugs (Efavirenz, Abacavir and Lamivudine) caused significant liver damage.

AST and ALT are elevated in nearly all liver diseases, but are particularly high in conditions that cause extensive cell necrosis, such as severe viral hepatitis, toxic injury, and prolonged circulatory collapse. ALT is a metabolic enzyme expressed primarily in the liver. Increase in serum ALT activity is typically associated with hepatocellular membrane damage and leakage of enzyme from hepatocytes [31]. Damage to the liver causes the release of ALT enzyme into the blood. Elevation of ALT levels is an indication of liver damage and has been associated with liver injury. The increase in serum ALP activity is also associated with a pathological damage occurrence in the liver [32].

When damage to heart or liver cells occurs, intracellular enzymes, such as AST, are released into the peripheral blood. Since AST is located in the parenchymal hepatic cells and heart muscle, this enzyme is used to assess damage to these areas. Increases in AST can be seen in hepatitis, liver necrosis, cirrhosis, and liver metastasis [10]. Gamma Glutamyl Transferase (GGT) is also widely used to assess liver function. Some drugs, and also alcohol, induce the liver to produce more of this

enzyme [33].

Total bilirubin results are comprised of the conjugated and unconjugated forms of bilirubin. Hyperbilirubinemia can occur in three areas as bilirubin is addressed by the body. In the prehepatic phase, increased bilirubin levels are caused by an increase in heme degradation and hemolysis. In the hepatic phase, increase levels are due to defective transport to the liver or defective conjugation by the liver. In the post hepatic phase, increase levels are due to defects transporting the conjugated bilirubin and bile out of the liver. Therefore, total bilirubin measurements are used to diagnose and treat liver, hemolytic, hematological, and gallbladder obstructive disorders [34].

Albumin is a small protein which accounts for nearly 50% of the total plasma protein. Albumin is primarily synthesized by the hepatic parenchymal cells. Albumin's primary function is the maintenance of colloidal osmotic pressure in the vascular and extravascular areas of the body and preventing edema. Additionally, albumin is a carrier transport protein. Hypoalbuminemia occurs in GI malabsorption, glomerulonephritis, nephritic syndrome, cirrhosis, severe burns, neoplasms, and autoimmune diseases. Low albumin levels indicate poor liver function and contribute to peripheral edema and ascites sometimes seen in very late stage liver disease. Albumin levels are usually normal in chronic liver disease until significant liver damage is present [35].

In this study, results of histopathological studies also provided supportive evidence for biochemical analysis. Examination of liver sections of rats received HAART revealed disruption of the normal structural organization of the hepatic lobules and loss of the characteristic cord-like arrangement of the normal liver cells. Many hepatic cells were damaged and lost their characteristic appearance while others showed marked cytoplasmic vacuolization. The architecture of the liver elicited severe hepatic injury as evidenced by the observation of pathological changes in the architecture of the liver viz. focal necrosis, and degenerative changes in the hepatocytes. These pathological changes correlated well with the altered enzyme activities. This is in agreement with the observations noted by previous work [36]. According to this study a significant increase in the activities of serum enzymes with in eighteen hours of exposure of the rats to single dose of D-Galactosamine Lipopolysaccharide (D-GaIN/LPS)

induced hepatotoxicity in rats, indicating the severity of hepatocellular injury. Rats given D-GalN/LPS elicited severe hepatic injury as confirmed by the observation of pathological changes like: infiltration of inflammatory cells, kupffer cell hyperplasia, neutrophil accumulation and focal necrosis.

Liver cells or hepatocytes are grouped in interconnected plates and constitute two-thirds of the mass of the liver [37]. Hepatocytes, with their high degree of metabolic activities, are readily disturbed by toxins, especially drugs or alcohol, and may demonstrate the histological cell responses known as cloudy swelling, fatty changes and necrosis. With more severe metabolic disruption, the hepatocytes undergo hydropic degeneration and become swollen and vacuolated. The fatty changes due to metabolic injury to hepatocytes are also manifested by large cytoplasmic vacuoles within some hepatocytes, which usually displace nucleus to one side [38].

Viral hepatitis, toxins, drugs, and systemic infections are the most important groups of conditions causing acute liver inflammation. Acute inflammation of the liver parenchyma is usually marked by focal accumulations of inflammatory cells in the site of necrotic hepatocytes. Liver sections may appear with vacuolated hepatocytes, dilated sinusoids and increased number of kupffer cells [39]. Certain liver diseases cause obliteration of the normal sinusoidal arrangement that causes impairment of the liver function [40].

Many folk remedies from plant origin were tested for their potential antioxidant and hepatoprotective liver damages [1]. Many active plant extracts are frequently utilized to treat a wide variety of clinical diseases including liver disease [10].

In this study, treatment with aqueous extract of *N. sativa* seeds significantly lowered the values of liver enzymes elevated by HAART and restored the damaged hepatocellular architecture. The decreased level of liver biomarkers and restoration of hepatocytes in *N. sativa* treated group leads to the inference that *N. sativa* seed aqueous extract counteracts the abnormal increase in serum enzymes and repair the hepatic tissue damage induced by HAART. These findings are in accordance with the finding of the previous study [30], where the aqueous extract of *N. sativa* seed reported as an effective protector against carbon tetra chloride induced liver damage which was evidenced by

decreasing elevated levels of liver enzymes and restoration of hepatocellular architecture. Similar works were also reported by other researchers [12, 41, 42]. The mean values of ALT, ALP, AST and GGT decreases as the concentration of *N. sativa* extract increases (Figures 7-10 respectively) which was supported by histopathological investigations (Figures 1-4 respectively) provided that hepatoprotective activity of aqueous extract of *N. sativa* seed was enhanced with concentration and best activity was achieved at the dose of 800mg/kg. It is chiefly the plant based preparations which are employed for the treatment of liver disorders. A number of scientific reports indicated that certain flavonoids, triterpenoids and steroids have protective effect on liver due to its antioxidant properties [10].

Flavonoids have been reported to exhibit antioxidant, anti-inflammatory, and hepatoprotective activities. Furthermore, condensed tannins have been suggested to possess free radical scavenging, antioxidant, anti-inflammatory, and hepatoprotective activities, while saponins have been reported also to exhibit hepatoprotective activity via modulation of its antioxidant and anti-inflammatory activities. Hepatoprotective activity of medicinal plants may also be due to synergistic action of flavonoids, condensed tannins, and saponins [28].

Therefore; the hepatoprotective activity exhibited by *N. sativa* extract might be due to the anti-oxidative nature of the plant. The preliminary phytochemical analysis of the extracts in this study has also confirmed the presence of components such as flavonoids, tannins, saponins and phenolic compounds, which have been known for its antioxidant and hepatoprotective activities.

Conclusion

This study demonstrated that aqueous extract of *N. sativa* seed can protect against HAART induced acute hepatotoxicity through restoration of the anti-oxidative defense system and down-regulation of the pro-inflammatory pathway. Possible mechanism of hepatoprotective activity of *N. sativa* may be due to its free radical scavenging and antioxidant activity as the result of the presence of flavonoids, tannins and phenolic compounds in the extracts.

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References

- Ahsan R, Islam KM, Musaddik A, et al. Hepatoprotective Activity of Methanol Extract of Some Medicinal Plants against Carbon Tetrachloride Induced Hepatotoxicity in Albino Rats. *Global Journal of Pharmacology* 2009; 3(3):116-122
- Panda BB, Gaur K, Nema RK, et al. Hepatoprotective Activity of *Jatropha Gossypifolia* Against Carbon Tetrachloride Induced Hepatic Injury in Rats. *Asian Journal of Pharmaceutical and Clinical Research* 2009; 2(1):50-54
- Kayode AA, Kayode OT, Aroyeun OA, et al. Hematologic and Hepatic Enzyme Alterations Associated with Acute Administration of Antiretroviral Drugs. *Journal of Pharmacology and Toxicology* 2011; 6(3):293-302
- Barrose E. Interaction of Traditional Remedies against HIV, Nutrients and ARVs. *Recent Translational Research in HIV/AIDS* 2011; 1:111-126
- Aranzabal L, Casado JL, Moya J, et al. Antiretroviral Therapy-Associated Hepatotoxicity in Patients with HIV and Hepatitis C Virus Coinfection. *Clinical Infectious Diseases* 2005; 40:588-93
- Setzer B, Lebrecht D, Walker UA. Pyrimidine Nucleoside Depletion Sensitizes to the Mitochondrial Hepatotoxicity of the Reverse Transcriptase Inhibitor Stavudine. *The American Journal of Pathology* 2008; 172(3): 681-690
- Labarga P, Soriano V, Vispo ME, et al. Hepatotoxicity of Antiretroviral Drugs Is Reduced after Successful Treatment of Chronic Hepatitis C in HIV-Infected Patients. *The Journal of Infectious Diseases* 2007; 196:670-676
- Manfredi R, Calza L, Chiodo F. Prospective, Open-Label Comparative Study of Liver Toxicity in an Unselected Population of HIV infected patients treated for the first time with Efavirenz or Nevirapine. *HIV Clinical Trials* 2005; 6(6):302-311
- Ocama P, Katwera M, Piloya T, et al. The spectrum of liver diseases in HIV infected individuals at an HIV treatment clinic in Kampala, Uganda. *African Health Sciences* 2008; 8(1): 8-12
- Parmar SR, Vashrambhai PH, Kalia K. Hepatoprotective Activity of some plants extract against Paracetamol induced Hepatotoxicity in Rats. *Journal of Herbal Medicine and Toxicology* 2010; 4(2): 101-106
- Gall A, Shenkute Z. Ethiopian Traditional and Herbal Medications and their Interactions with Conventional Drugs. *Journal of ethnic medicine* 2009; 1:1-4
- Michel CG, El-Dine NS, Fahmy SM, et al. Phytochemical and biological investigation of the extracts of *N. sativa* L. seed waste. *Drug Testing and Analysis* 2010; 1:1-10
- Roshan S, Abdullah K, Tazneem B, et al. To study the effect of *N. sativa* on various biochemical parameters on stress induced in albino rats. *International Journal of Pharmacy and Pharmaceutical Sciences* 2010; 2(4):185-189
- Salem ML. Immunomodulatory and therapeutic properties of the *N. sativa* L. seed. *International Immunopharmacology* 2005; 5:1749-1770
- Tonkal A. In Vitro Antitrichomonal Effect of *N. sativa* Aqueous Extract and Wheat Germ Agglutinin. *Journal of King Abdul-Aziz University: Medical Science* 2009; 16(12):17-34
- Raval BP, Shah TG, Patel JD, et al. Potent anticancer activity of *N. sativa* Seeds. *Archives of Applied Science Research* 2010; 2(1):52-56
- Ali BH, Blunden G. Pharmacological and Toxicological Properties of *N. sativa*. *Phytotherapy Research* 2003; 17:299-305
- Paarakh PM. *Nigella sativa* Linn. – A Comprehensive review. *Indian Journal of Natural Products and Resources* 2010; 1(4):409-429
- El-Tahir KE, Bakeet DM. The Black Seed *N. sativa* Linnaeus - A Mine for Multi Cures: A Plea for Urgent Clinical Evaluation of its Volatile Oil. *Journal of T U Medical Science* 2006; 1(1):1-19
- Mahmoud MR, El-Abhar HS, Saleh S. The effect of *Nigella sativa* oil against the liver damage induced by *Schistosoma mansoni* infection in mice. *Journal of Ethnopharmacology* 2002; 79:1-11
- Dehkordi FR, Kamkhah AF. Antihypertensive effect of *N. sativa* seed extract in patients with mild hypertension. *Fundamental & Clinical Pharmacology* 2008; 22:447-452
- Khan FA, Hussain I, Farooq Sh, et al. Phytochemical Screening of Some Pakistanian Medicinal Plants. *Middle-East Journal of Scientific Research* 2011; 8(3): 575-578
- Tiwari P, Kumar B, Kaur M, et al. Phytochemical screening and Extraction: A Review. *Internationale Pharmaceutica Scientia, an International Peer Reviewed Journal* 2011; 1(1):98-106
- Savithamma N, Linga Rao M, Suvrulatha D. Screening of Medicinal Plants for Secondary Metabolites. *Middle-East Journal of Scientific Research* 2011; 8(3): 579-584
- Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology* 2005; 4(7):685-688
- Peters W, Robinson BL. The Chemotherapy of rodent malarial. Studies on *Puronaridine* and other manich base antimalarials. *Annals of tropical and medical parasitology* 1992; 86:455-465
- Gabriel O, Harrision N, Okey O, et al. Changes in Lipid and Hematological profile of aqueous ethanolic extract of *Alstonia boonei* in rats. *The internet journal of Hematology* 2008; 4:1-5
- Yahya F, Mamat SS, Kamarolzaman MFF, et al. Hepatoprotective Activity of Methanolic Extract of *Bauhinia purpurea* Leaves against Paracetamol-Induced Hepatic Damage in Rats. *Evidence-Based Complementary and Alternative Medicine* 2013; 1:1-11
- Adjene JO, Igbigbi PS. Effects of chronic administration of efavirenz on DNA of the intracranial visual relay centers of adult Wistar rats. *Biology and Medicine* 2012; 4(1):20-26
- Essawy AE, Abdel-Moneim AM, Khayyat LI, et al. *N. sativa* seeds protect against hepatotoxicity and dyslipidemia induced by carbon tetrachloride in mice. *Journal of Applied Pharmaceutical Science* 2012; 2(10):021-025
- Gaskill C, Miller L, Matton J, et al. Liver Histopathology and Serum ALT and ALP activities in Epileptic Dogs receiving phenobarbital. *Journal of Veterinary pathology* 2005; 87:155-161
- Haldar P, Gupta M, Mazumder U, et al. Hepatoprotective effect of *Wedelia calendulacea* against Thioacetamide induced liver damage in rats. *Journal of Pharmacology* 2007; 3:414-42136. Gani AMS, John SA. Evaluation of

- Hepatoprotective effect of *N. sativa* L. *International Journal of Pharmacy and Pharmaceutical Sciences* 2013; 5(4):428-430
33. Sule OJ, Godwin J, Nnoku IA. Biochemical Investigation of Hepatotoxic effects of Antiretroviral Drugs on Wistar Albino Rats, *Journal of Physiology and Pharmacology Advances* 2012; 2(4): 171-175
34. Tietz N.W., editor. Text book of Clinical Chemistry. 3rd edition, Philadelphia: WB Saunders 2001
35. Rosalki SB, McIntyre N. Biochemical investigations in the management of liver disease. In: Oxford textbook of clinical hepatology, 2nd edition. New York; Oxford university press 1999; 503-521.
36. Gani AMS, John SA. Evaluation of Hepatoprotective effect of *N. sativa* L. *International Journal of Pharmacy and Pharmaceutical Sciences* 2013; 5(4):428-430
37. Junqueira L, Carneiro J. Basic histology: Text in Atlas. Eleventh edition, MC Graw-Hill, Medical Publishing Division, USA, 2005; 223-389
38. Burkitt H, Stevens A, Lowe J, et al. Wheater's Basic Histopathology. Third edition. Churchill Livingstone, U.K. 1996; 154-180.
39. Ebaid H, Dkhil M, Danfour M, et al. Piroxicam-induced hepatic and renal histopathological changes in mice. *Libyan Journal of Medicine* 2007; 2:1-13
40. Young B, Lowe J, Steven A, et al. Wheater's Functional Histology: A text and Color Atlas, fifth edition, Churchill Livingstone, Elsevier, 2006; 288-327
41. Türkdogan MK, Ozbek H, Yener Z, et al. The Role of *Urtica dioica* and *N. sativa* in the Prevention of Carbon Tetra chloride induced Hepatotoxicity in Rats. *Phytotherapy Research* 2003; 17:942-946
42. Yesmin F, Rahman Z, Dewan JF, et al. Hepato-protective role of the aqueous and n-hexane extracts of *N. sativa* in experimental liver damage in rats. *Asian journal of pharmaceutical and clinical research* 2013; 6(3): 205-209

Table 1: Phytochemical screening of the seed of *N. sativa*

Test	Color observed	Result
Alkaloids (Dragendroff's)	Yellow orange ppt*	Positive
Alkaloids (mayer's reagent)	White ppt*	Positive
Cardiac glycosides	Reddish brown	Positive
Anthranides	Yellow	Negative
Polyphenols	Green blue	Positive
Tannins	Blue black	Positive
Chromophores	Brown	Negative
Flavonoids	Yellow ppt*	Positive
Carotenoids	White	Negative
Saponins	Honey comb forth (foam)	Positive

Positive: secondary metabolite present;

Negative: secondary metabolite absent;

*ppt: precipitate

Table 2: Comparison of the Mean \pm SEM of the Biochemical parameters

Dose	ALP (U/L)	ALT (U/L)	AST (U/L)	GGT (U/L)	Albumin	Total
					(g/dl)	Bilirubin (mg/dl)
Normal control	117.00 \pm 14.92 ^a	84.75 \pm 13.42 ^a	286.00 \pm 28.62 ^a	4.20 \pm 0.48 ^a	4.68 \pm 0.08 ^a	0.03 \pm 0.00
Positive control	265.75 \pm 6.52	166.75 \pm 13.92	472.50 \pm 22.40	12.35 \pm 2.53	3.32 \pm 0.10	0.27 \pm 0.14
100mg/kg <i>N. sativa</i> + HAART	164.00 \pm 8.54*	99.33 \pm 9.26*	311.67 \pm 35.59*	5.90 \pm 0.30*	4.53 \pm 0.03*	0.08 \pm 0.02
200mg/kg <i>N. sativa</i> + HAART	161.67 \pm 23.15*	98.00 \pm 9.29*	298.20 \pm 37.24*	5.84 \pm 0.69*	4.50 \pm 0.07*	0.06 \pm 0.00
400mg/kg <i>N. sativa</i> + HAART	147.60 \pm 16.60*	92.60 \pm 2.98*	262.00 \pm 12.10*	5.37 \pm 0.69*	4.33 \pm 0.03*	0.04 \pm 0.01
800mg/kg <i>N. sativa</i> + HAART	143.00 \pm 12.49*	87.67 \pm 12.55*	226.67 \pm 8.45*	5.07 \pm 0.88*	4.44 \pm 0.15*	0.08 \pm 0.01

* The mean difference of treated groups is significant at the 0.05 level when compared to positive control.

^a The mean difference of normal control group is significant at the 0.05 level when compared to positive control.

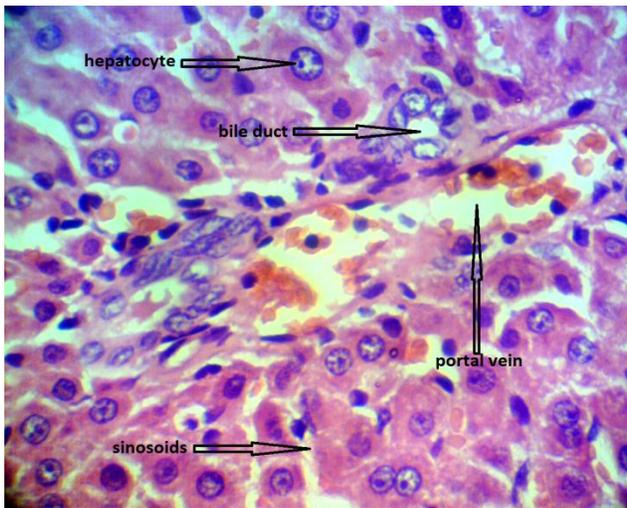


Figure 1: Photomicrograph of liver sections of the rats in normal control group: exclusively normal architecture and normal morphology.

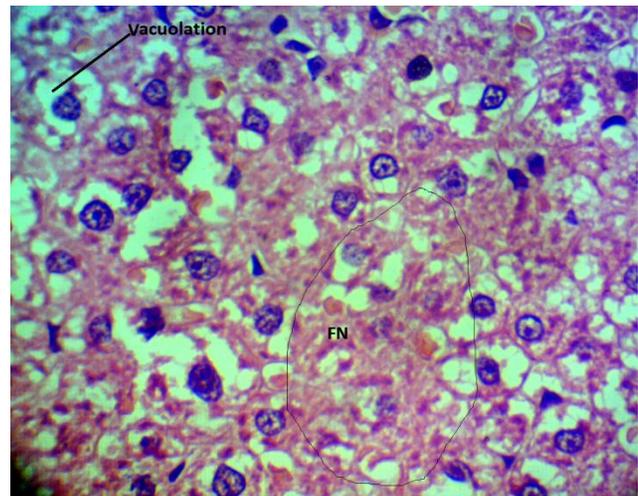


Figure 2: Photomicrograph of liver sections of the rats in group II (HAART treated group). Normal architecture is distorted; sinusoidal space is not clear. Focal necrosis (FN) is observed.

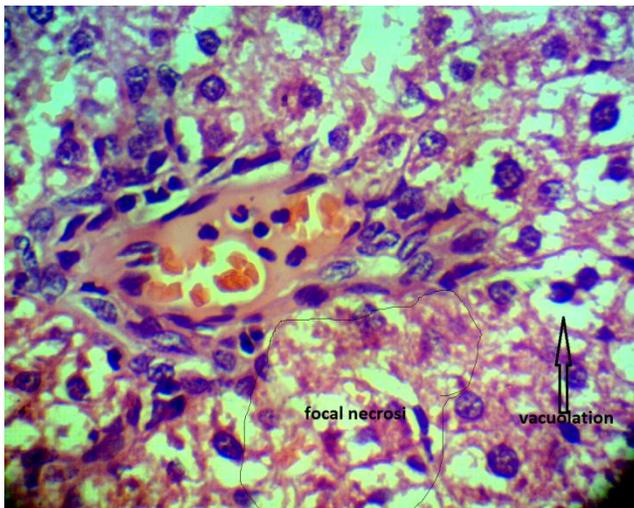


Figure 3: Photomicrograph of liver sections of the rats in group III (100mg/kg *N. sativa* extract + HAART treated)

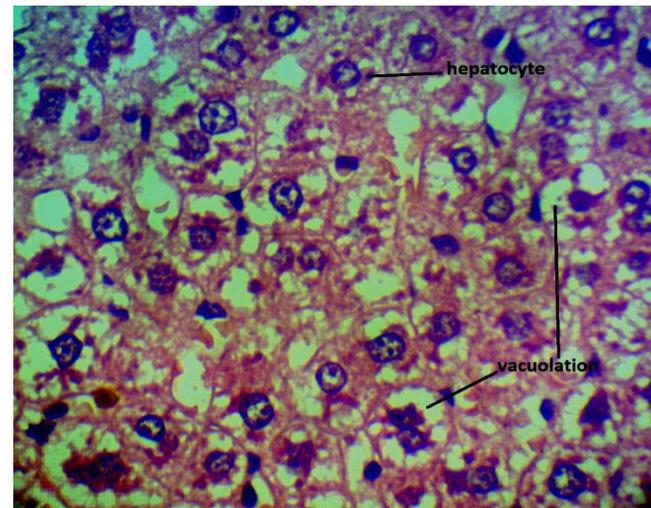


Figure 4: Photomicrograph of liver sections of the rats in group IV (200mg/kg *N. sativa* extract + HAART treated).

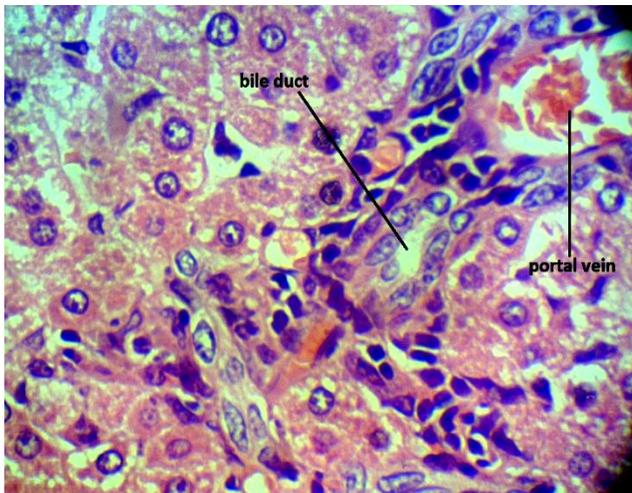


Figure 5: Photomicrograph of liver sections of the rats in group V (400mg/kg *N. sativa* extract + HAART treated).

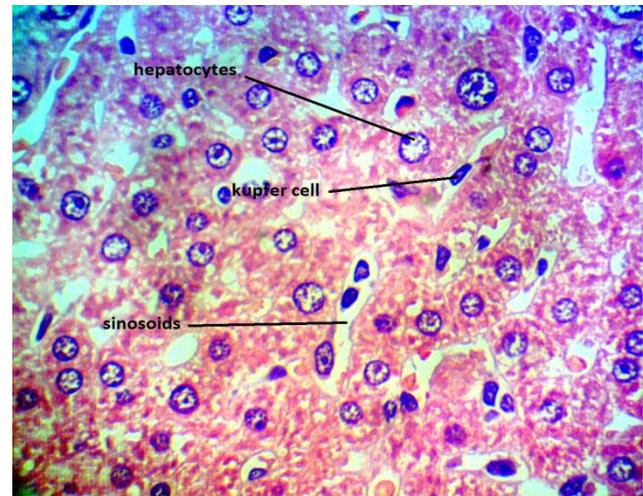


Figure 6: Photomicrograph of liver sections of the rats in group VI (800mg/kg *N. sativa* extract + HAART treated).

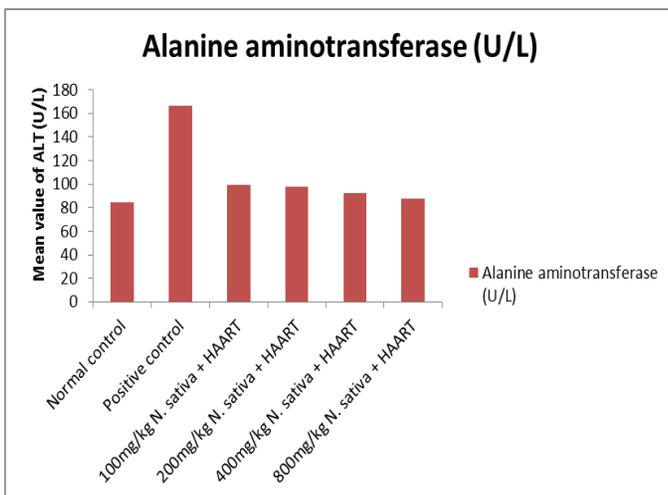


Figure 7: Effect of *N. sativa* seed extract on the ALT value of HAART induced rats

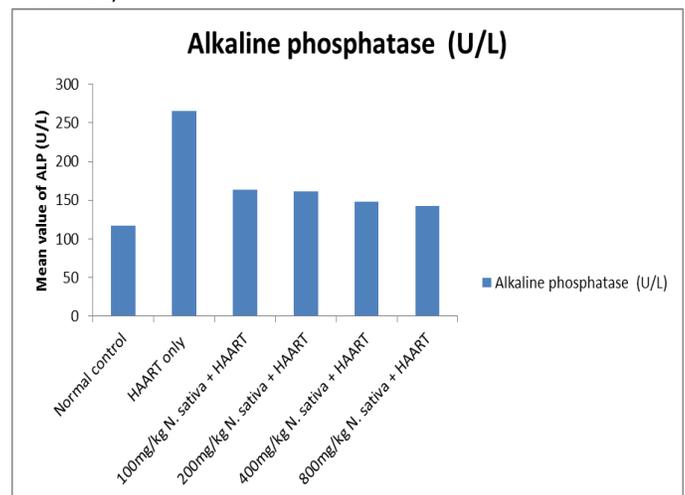


Figure 8: Effect of *N. sativa* seed extract on the ALP value of HAART induced rats

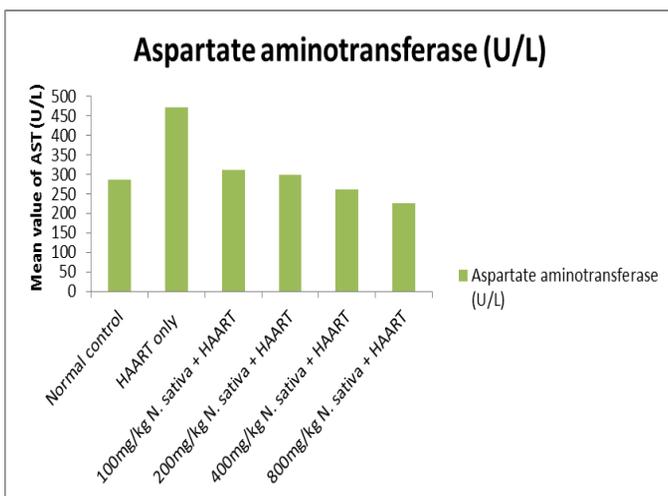


Figure 9: Effect of *N. sativa* seed extract on the AST value of HAART induced rats

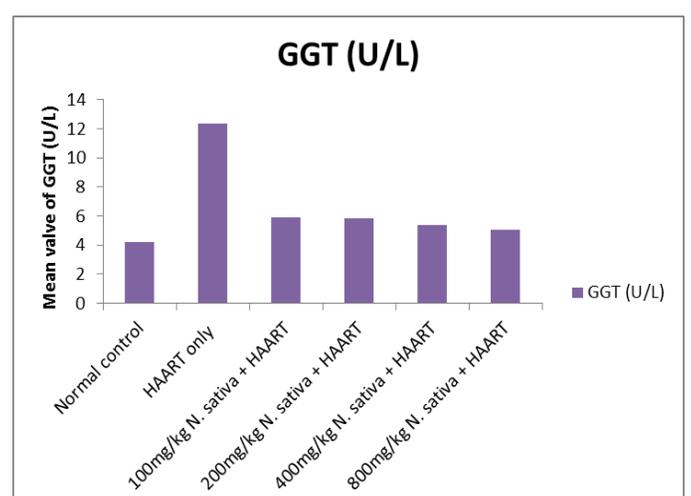


Figure 10: Effect of *N. sativa* seed extract on the GGT value of HAART induced rats