

HEPATOPROTECTIVE EFFECT OF NIGELLA SATIVA SEED IN STREPTOZOTOCINE INDUCED DIABETIC ALBINO RATS: HISTOLOGICAL OBSERVATIONS

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ABSTRACT

Background: Diabetes mellitus is associated with biochemical, physiological and pathological alterations in the liver. Liver damage is associated with the formation of oxygen derived free radicals. Reactive oxygen species as well as nitrogen species are responsible for nuclear DNA fragmentation and cell death. The aim of this study was to investigate the hepatoprotective effects of Nigella sativa seed.

Materials and Methods: This work was conducted as part of Ph.D work under Department of Anatomy, Shri BM patil Medical College, BLDE University, Bijapur. University ethical committee and Institution Animal Ethical committee are approved the work according to CPCSEA (Govt of India) Rules. 18 rats were selected for this study and divided in to 3 groups each contains 6 rats, one group served as normal control, one group served as Streptozotocin (50mg/kg BW) induced diabetic control rats and one group served as diabetic rats treated with the nigella sativa seed powder(300mg/Kg body weight).

Results: Histopathological observations of present study showed that nigella sativa treatment has proved its hepatoprotective effect in streptozotocin induced diabetic rats. The liver enzyme parameters significant change also supported histopathological findings.

Conclusion: The results of present study concluding that Nigella sativa seed powder having hepatoprotective effect in streptozotocine induced diabetic rats.

KEY WORDS: Hepatocytes, Nigella Sativa, Antioxidant, Nigella sativa, Thymoquinone.

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INTRODUCTION

Streptozotocin is a naturally occurring nitrosourea. It is widely used to induce insulin dependent diabetes mellitus in experimental animals because of its toxic effects on islet cells. The diabetogenic action of streptozotocin is the direct result of irreversible damage to the pancreatic cells resulting in degranulation and loss of capacity to secrete insulin. Streptozotocin has various biological actions, including the production of acute and chronic cellular injury, carcinogenesis, teratogenesis and mutagenesis. streptozotocin is a nitrosourea compound which generally shares similar fate of disposition with other nitrosoureas and is a drug of choice in islet cell carcinoma and malignant carcinoid tumors [1]. It has been suggested that increased oxidative stress is a widely accepted participant in the development of diabetic complications. It is clearly described that diabetes mellitus is attended by an increased production of free radicals. The majority of these free radicals and toxic molecules are derived from oxygen. The principal free radicals are superoxide, hydroxyl and peroxy radicals, all of which may play a role in DNA damage, glycation, and protein modification reactions, and in lipid oxidative modification in diabetes [2]. So, the antioxidant defenses are impaired in diabetes mellitus.

Nigella sativa is an annual herbaceous plant that belongs to the family of Ranunculaceae. It contains both fixed and essential oils, proteins, alkaloids and saponin. The seeds of Nigella sativa have been used traditionally for centuries in the Middle East, Northern Africa and South Asia for the treatment of various diseases. Black seed is a complex substance of more than 100 compounds, some of which have not yet been identified or studied. Thymoquinone was identified as the main component (up to 50%) besides it contains p-cymene (40%), α -pinene (up to 15%), dithymoquinone and thymohydroquinone. Furthermore, the essential oil contains significant (10%) amounts of fatty acid ethyl esters. On storage, thymoquinone yields dithymoquinone and higher oligo condensation products – nigellone[3,4]. Thymoquinone, the major component of the seed, is the biologically active gradient of this plant [5,6,7].

Nigella sativa extract and its active principle like thymoquinone have been shown to possess protective effect against hematological, hepatic and renal toxicities induced by anticancer drugs. The mechanism of the hepatoprotective action of thymoquinone is not certain, but may be related to the preservation of intracellular glutathione, the depletion of which by oxidative stress is known to increase the susceptibility of cells to irreversible injury [8]. The present study is studied to find out the hepatoprotective effect on Nigella sativa seed powder in streptozotocine induced diabetic rats.

MATERIALS AND METHODS

Study design: This work was conducted as part of Ph.D work under Department of Anatomy, Shri BM patil Medical College, BLDE University, Bijapur. University ethical committee and Institution Animal Ethical committee are approved the work according to CPCSEA Rules. 18 rats were selected for this study and divided into 3 groups each contains 6 rats, one group served as normal control rats, one group served as streptozotocin induced diabetic control rats and one group as streptozotocin induced diabetic rats treated with nigella sativa seed powder, at the end of 45th day blood was collated and measured Total protein, ALT, AST, ALP and liver tissue was collected and preserved in 10% formalin and proceeded for further tissue process.

Plant material: Nigella sativa seeds were grinded into fine powder with piston and mortar with help of Bapuji pharmacy college, Davangere. Nigella sativa powder administered orally according to study of M. Murugesan [9].

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RESULTS

Histopathological observation

Normal control rats: Section studied under H&E stain showed normal parenchymal tissue which is composed of numerous hexagonal to pyramidal lobules. Each lobule consists of central vein from which the hepatic plates radiate outwards the portal areas, 3 to 5 portal triads are located at the periphery of the lobule, containing branches of bile duct, portal vein and hepatic artery and occasional mononuclear cells. Cords of hepatocytes and blood containing sinusoids radiate from central vein to the peripheral portal triads. The sinusoids are lined by both endothelial cells and Kuepfer's cells both of which have inconspicuous flattened nuclei and ill-defined cytoplasmic margins. The central vein are lined by endothelial cells surrounded by ring of collage fibres. The hepatocytes are polygonal in shape with well-defined borders. The nucleus is single, round and has a fine chromatin pattern with 1 to 2 clearly defined amphiphilic prominent nucleoli. The cytoplasm is eosinophilic and finely granular (Figure 1).

Diabetic control rats: Section studied under H&E stain showed the distortion in the arrangement of cells around the central vein, periportal fatty infiltration (fatty steatosis) with focal necrosis of hepatocytes, hydropic changes and aggregation and infiltration of lymphocytes between hepatocytes (Figure 2).

Diabetic rats treated with nigella sativa seed powder: Section studied under H&E stain showed the normal cellular arrangement around the central vein and reduced necrosis changes near to normal architecture, also observed that some places the blood vessels has come near to normal condition (Figure 3).

Serum parameters: The liver enzyme parameters and total proteins levels of serum are supporting histological observation. The levels of total protein was decreased significantly in diabetic induced rats compared

to normal control rats, after treatment of 45 days with nigella sativa seed powder the total protein levels were normalised. The serum levels of AST, ALT, ALP were increased significantly compared to normal control rats, after treatment of 45 days with nigella sativa seed powder the levels were normalised ($p < 0.05$).

Fig. 1: Histology of Liver – Normal control rats (Showing the normal architecture with central vein, hepatocytes and sinusoids).

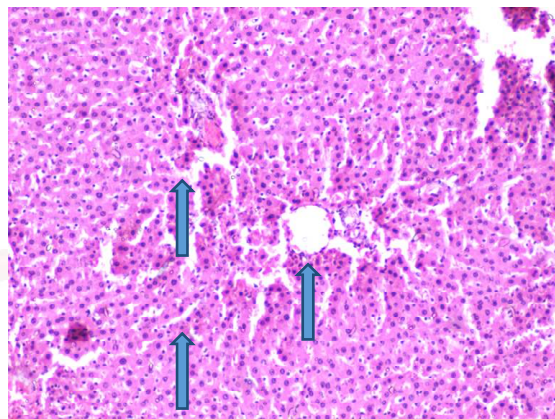


Fig. 2: Histology of Liver - Diabetic control rats (showing periportal fatty infiltration (fatty steatosis) with focal necrosis of hepatocytes).

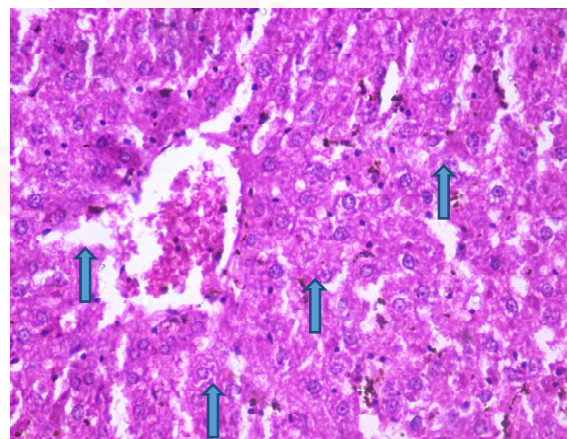
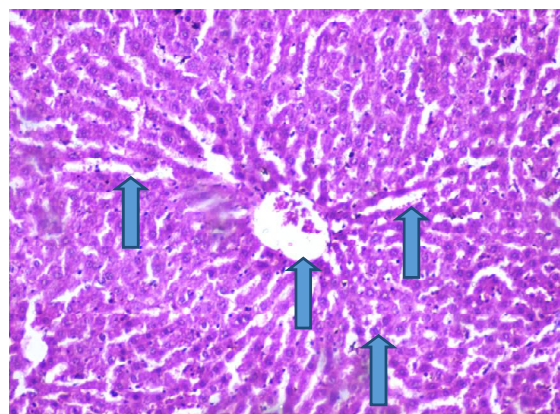


Fig. 3: Histology of Liver – Diabetic rats treated with nigella sativa seed powder (normal cellular arrangement around the central vein, reduced necrosis changes, normal appearing hepatocytes and sinusoids).



DISCUSSION

In our present study H&E stained section of liver of normal rats showed normal histological architecture of liver with normal arrangement of hepatocytes, hepatic sinusoids around central vein and normal appearance of portal triad. In streptozotocine induced diabetic rats observed destruction arrangement of hepatocytes around the central vein and monocyte infiltration observed. In treated induced diabetic rats with nigella sativa seed powder observed reduced destruction of cells and reduced monocyte infiltration. There were no much earlier histopathological studies on hepatoprotective role in streptozotocine induced diabetic rats but other chemically damaged liver injuries treated with nigella sativa are studied. In Samad A et al [10] study studied on the effect of nigella sativa on gluconeogenesis and liver glucose production helps to clarify part of the hypoglycemic mechanism since hepatic glucose production through gluconeogenesis is known to contribute to hyperglycemia in diabetic patients. In their study research on isolated hepatic cells showed a significant decrease in glucose production from gluconeogenic elements like glycerol, alanine and lactate in nigella sativa treated animals as compared to the untreated animals. This significant decrease in liver glucose output and ameliorative effect on regeneration of pancreatic islets suggests that the observed antidiabetic action of nigella sativa is at least partially mediated through an effect on hepatic gluconeogenesis. Meral I et al [11] study was carried out to investigate the nigella sativa effect on the lipid peroxidation, increase the anti-oxidant defence system and also prevent the lipid-peroxidation-induced liver damage in experimentally induced diabetic rabbits. In this study treatment with nigella sativa decreased the elevated glucose and MDA concentrations, increased the lowered GSH and ceruloplasmin concentrations, and prevented lipid-peroxidation-induced liver damage in diabetic rabbits. In their study it was concluded that nigella sativa might be used in diabetic patients to prevent lipid peroxidation, increase anti-oxidant defence system activity and also prevent liver damage. In study of Manik

S et al [12] hepatic injury was produced in albino rats by administration of Paracetamol, hepatotoxicity was revealed by biochemical and histopathological findings. The biochemical and histological results of this study proved that nigella sativa possess potential to protect the liver tissue against oxidative damages and could be used as protective drug against Paracetamol induced liver damages. In study of Daba MH et al [13] performed clinical and experimental investigations have showed that nigella sativa has a protective effect against oxidative damage in isolated rat hepatocytes. El-Dakhkhany et al [14] study has reported the protective effect of nigella sativa against CCL4 and D-galactosamine induced hepatic toxicity in rats. In study of Mansour et al [15] investigated the effect of nigella sativa by intraperitoneal route on carbon tetrachloride-induced hepatotoxicity in mice, results of this study indicated that thymoquinone (major active biochemical components of nigella sativa) may play an important role as antioxidant and may efficiently act as a protective agent against chemically-induced hepatic damage. Turkdogan et al [16] study observed that nigella sativa has a significant hepatoprotective effect in CCL4-administrated rabbits and that hepatocellular degenerative and necrotic changes are slight without advanced fibrosis and cirrhotic process in nigella sativa treated group. In study of Al-Ghamdi [17] studied protective effect of nigella sativa seeds against carbon tetrachloride-induced liver damage. Animals treated with CCL4 showed remarkable centrilobular fatty changes and this effect was significantly decreased in animals pretreated with nigella sativa. The results of previous studies are consistent with our results, after comparing our histopathological findings in treated rats with nigella sativa, concluded that toxic histopathological changes can be prevented using nigella sativa.

Conflicts of Interests: None

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