



Journal Homepage: - www.journalijar.com
**INTERNATIONAL JOURNAL OF
 ADVANCED RESEARCH (IJAR)**

Article DOI: 10.21474/IJAR01/3130
 DOI URL: <http://dx.doi.org/10.21474/IJAR01/3130>



RESEARCH ARTICLE

HEPATOPROTECTIVE EFFECT OF *NIGELLA SATIVA* ON DIABETIC MICE.

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Manuscript Info

Manuscript History

Received: 13 December 2016
 Final Accepted: 16 January 2017
 Published: February 2017

Key words:-

Alloxan, Glucose, Liver, *Nigella sativa*,

Abstract

Diabetes mellitus is a major health problem for the people of the world. Diabetes is a chronic metabolic disorder resulting from a variable interaction of hereditary and environmental factors and it is characterized by abnormal insulin secretion or insulin receptor affecting β cells of pancreas. It is associated with a number of chronic complications including nephropathy, neuropathy, retinopathy and cardiovascular diseases. Diabetes mellitus affects a large number of people throughout the world and India also. Experts estimate that diabetic population will grow from 195 to 360 million by 2030 almost 4.5 percentage of the global population. Present study included histological and biochemical parameters of mice. Three groups of mice were prepared for comparative study on control, diabetic, and *N. sativa*. Diabetic models were prepared in mice by intraperitoneal administration of single dose of alloxan@120mg/kg b.w. Alcoholic extract of *Nigella sativa* was administered @100 mg/kg b.w/day for four and eight weeks. In diabetic group of mice glucose, creatinine, urea and SGPT were increased. Effective restoration was observed in glucose, SGPT, urea and creatinine of *N. sativa* administered diabetic group of mice. Liver also shows effective restoration in *N. sativa* administered group of mice. Thus, it is concluded from study that alcoholic extract of *N. sativa* restores glucose level to normal. *Nigella sativa* acts effectively on diabetes mice on biochemical and histological parameters.

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Introduction:-

Incidence of diabetes is increasing worldwide at an alarming rate. Diabetes is on a rapid rise in developing nations¹. People suffering with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030². The past two decades have seen an explosive increase in the number of people diagnosed with diabetes worldwide³. The World Health Organization has predicted that the diabetic patients will occur in the developing countries and increase 42% in the developed countries and 170% increase in the developing countries. The countries with the largest number of diabetes patients are India, China and United States⁴.

India has the largest number of people with diabetes in the world. Diabetes is a group of common metabolic disorders that share the phenotype of hyperglycemia⁵. Diabetic hyperglycaemia indicate that plasma levels of urea

and creatinine which are considered as perfect markers of renal dysfunction⁶. Adults with DM have cardiovascular disease death rates that are 2–3 times higher than adults without DM⁷.

Medicinal plants used in India has been practiced for more than 5,000 years. Medicinal plants used for prevention of disease, rejuvenation of our body systems, and extension of life span through lifestyle and natural therapies⁸. According to the WHO, over 80% of the world's population relies on traditional forms of medicine, largely plant based to meet primary health care needs.

Nigella sativa has been used for medicinal purposes for centuries *N. sativa* traditionally used for a variety of conditions and treatments related to respiratory health, stomach and intestinal health, kidney and liver function, circulatory and immune system⁹. *Nigella sativa* possess biological, pharmacological and biochemical actions, including antibacterial bronchodilator and anti-parasitic¹⁰. *Nigella sativa* oil has the ability to protect testis against oxidative stress possibly through antioxidant effects of its bioactive compounds¹¹. After the treatment of *Nigella* which decreases the elevated lipid peroxidation, liver enzyme levels and increases the reduced antioxidant enzyme levels¹². *Nigella* oils has gastro protective activity against gastric mucosal injury¹³.

Present study is designed to evaluate effect of *Nigella sativa* on histological and biochemical parameters of liver in diabetic mice.

Materials and Methods:-

Animals: - The mice (*Mus musculus*) were reared in animal house. The mice were selected for the study was 12 weeks old with 30 ± 2 gm body weight (b.w). The mice were housed at controlled environmental conditions $22 \pm 2^\circ\text{C}$, relative humidity $50 \pm 10\%$, and 12h dark-light cycle. All experimental were conducted as per the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Chemicals: - Alloxan, purchased by Loba chem Pvt. Ltd., Mumbai was utilized for the experimental design.

Medicinal plant used: - Alcoholic seed extract of *Nigella sativa* is orally administered to diabetic group of mice. Fresh seed of *Nigella sativa* was purchased from herbal store in Patna, India.

Study groups & sampling:- The control group of six mice received distilled water orally. The 'treatment' groups (n=6) received alloxan 120 mg/kg b.w by intra-peritoneal method for diabetic model preparation. *Nigella sativa* (100 mg/kg/b.w/day) administered to diabetic mice orally through Gavage method. Mice were sacrificed after the scheduled treatment. Serum was collected for SGPT, glucose, creatinine and urea estimation. The Liver from all the mice were removed and washed three times in isotonic saline (0.85 v/w %) and fixed in neutral formalin for Light Microscope (LM) study.

Results:-

Fasting level of glucose was observed in every group of mice. Level of glucose in control group was 99.00 ± 2.30 mg/dl. In diabetic group it was 201.3 ± 12.55 mg/dl. While it was 135.0 ± 3.46 mg/dl and 106.3 ± 2.18 mg/dl in *Nigella sativa* 4 weeks and 8 weeks administered group of mice (Graph: I).

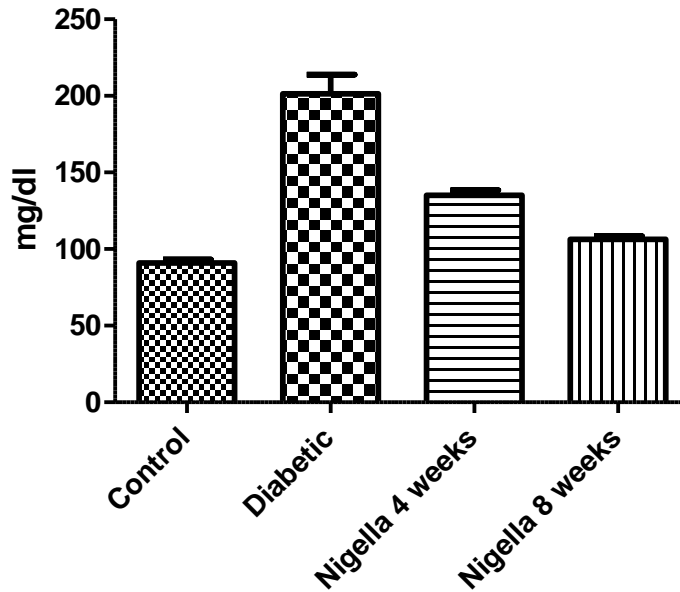
Serum Glutamate-Pyruvate Transaminase (S.G.P.T) level in control group of animal was 22.00 ± 3.21 U/ml. Diabetic group of mice S.G.P.T level was 312.3 ± 4.09 U/ml. While it was 255.0 ± 3.78 and 192.3 ± 7.44 U/ml in *Nigella sativa* four weeks and eight weeks administered group of mice (Graph: II).

Level of Creatinine in control group of mice was 0.74 ± 0.06 mg%. In group of diabetic mice creatinine level was 1.97 ± 0.04 mg%. Creatinine level was 1.71 ± 0.01 and 1.42 ± 0.02 mg% in *Nigella sativa* four weeks and eight weeks administered group of mice (Graph: III).

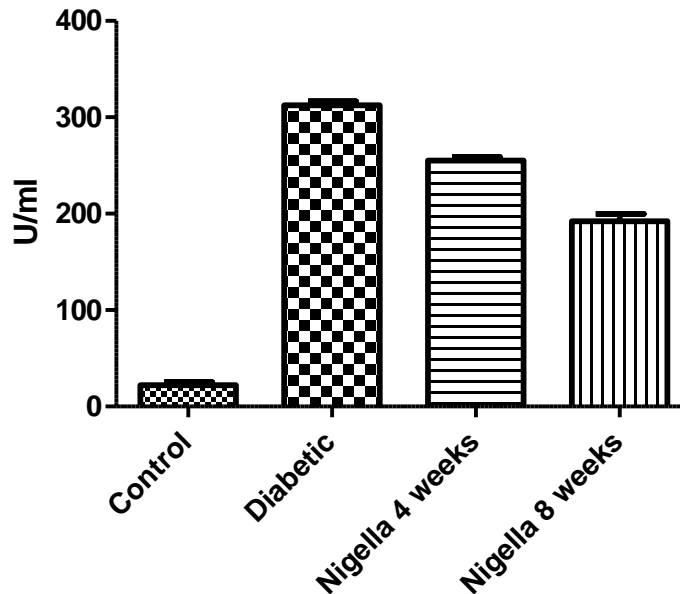
Urea value in control group of mice was 20.33 ± 2.33 mg/dl. While in diabetic group of mice was 46.73 ± 0.079 mg/dl. Urea level was 39.37 ± 0.33 and 32.40 ± 1.29 mg/dl in *Nigella sativa* four weeks and eight weeks administered group of mice (Graph: IV).

In control group of mice, well-organized hepatic cells were observed. Distinct cytoplasmic and nuclear material was also observed. Central vein was well organized with its intact cell wall (Figure: 1). Liver of diabetic mice shows vacuolization. Clustered and multilobed nuclei were observed. Degenerated cytoplasmic materials were observed. Clustered nuclei were also shows on periphery on central vein (Figure: 2). Diabetic mice followed by four weeks of *Nigella sativa* administration shows vacuolization in hepatic cells. Clustered nuclei were shows in hepatic cells. Many vacuolated spaces were observed with degenerated central vein (Figure:3). Diabetic mice followed by 8 weeks of *Nigella sativa* administration shows restoration in nuclear material were observed to least extend. Restored cytoplasm was observed in liver cells. Least vacuolization were also observed (Figure: 4).

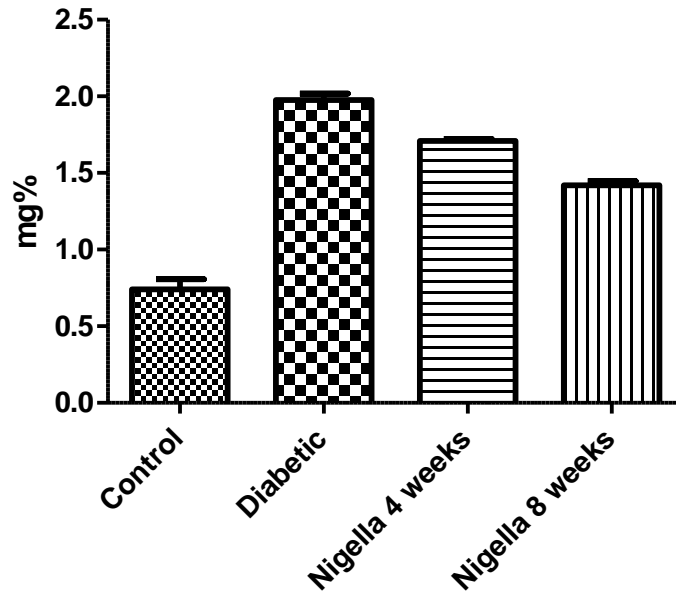
Graph - I: Glucose level in serum of mice



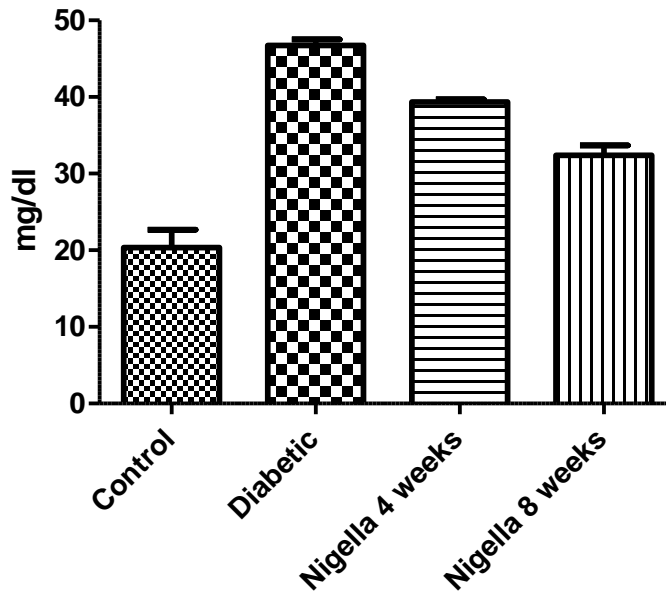
Graph - II: SGPT level in serum of mice



Graph - III: Creatinine level in serum of mice



Graph - IV: Urea level in serum of mice



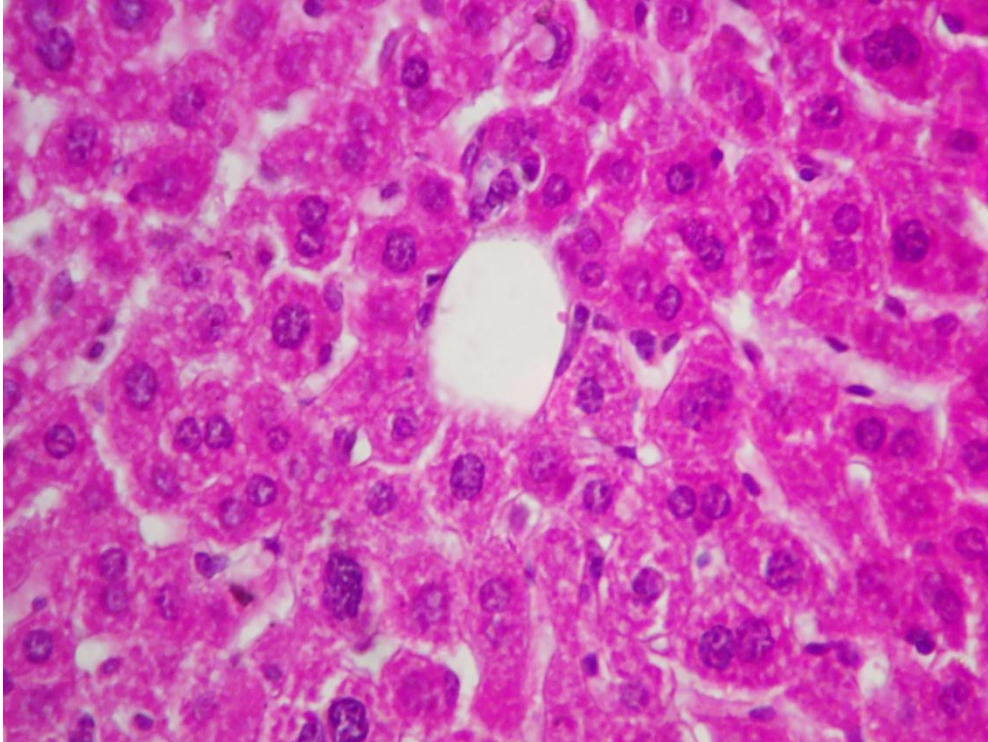


Figure 1:- In control group of mice well organized hepatic cells were observed. Distinct cytoplasmic and nuclear material was also observed. Central vein was well organized with its intact cell wall. X 600.

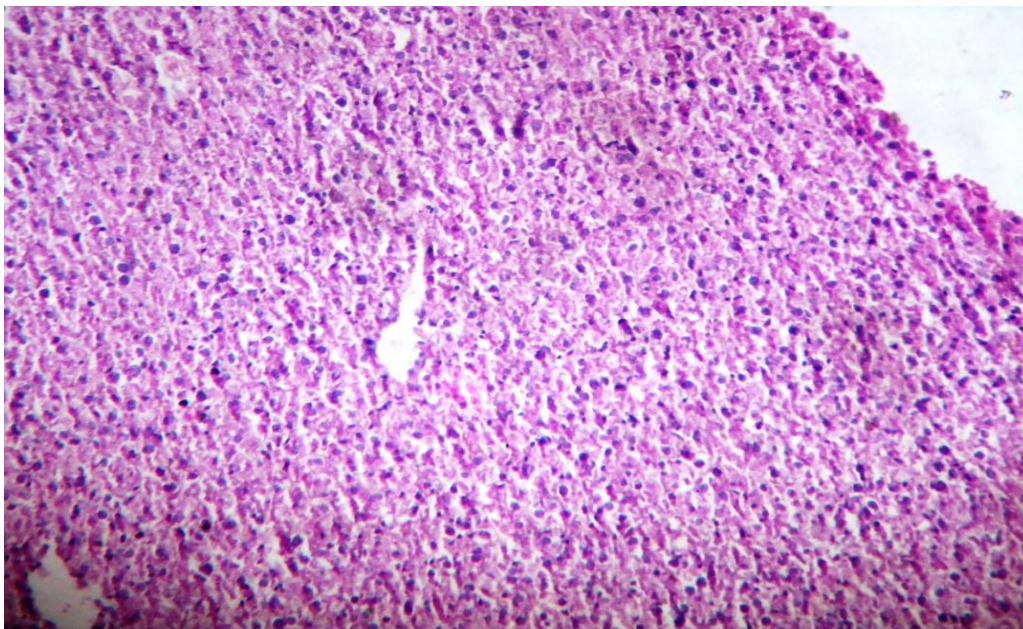


Figure 2:- Liver of diabetic mice shows frequent vacuolization. Clustered and multilobed nuclei were observed. Degenerated cytoplasmic materials and Clustered nuclei were also observed on periphery on central vein. X 200.

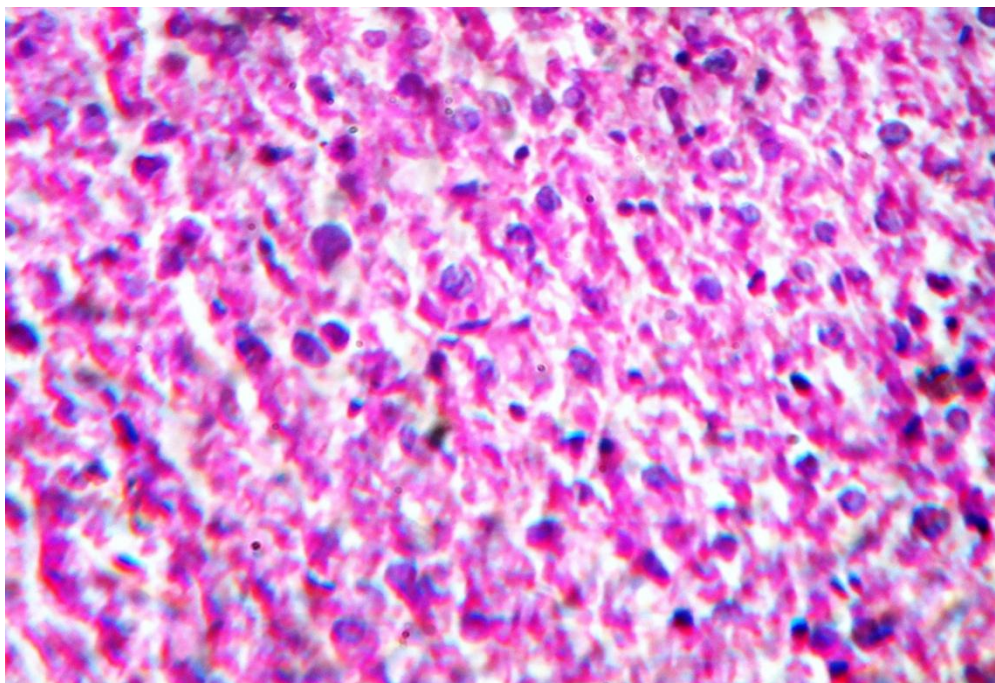


Figure 3:- -Liver of diabetic mice followed by four weeks shows degenerated hepatic cell. Many vacuolated spaces were observed degenerated cytoplasm. Fragmented nuclear materials were observed. Vacuolization were also observed nuclear material of liver cells. X 600.

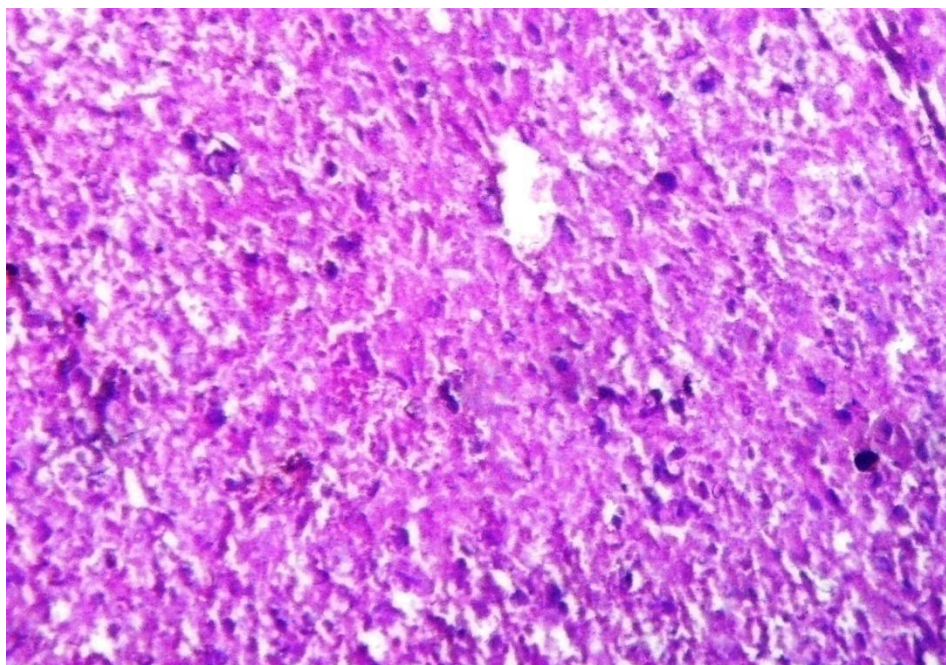


Figure 4:- Liver of diabetic mice followed by eight weeks of *N.sativa* administration shows many vacuolated spaces with degenerated nuclei of hepatic cells. Multilobed and vacuolated nuclear materials were observed. Central vein was also observed in degenerated condition. X 200.

Discussions:-

Diabetic hyperglycemias induce certain biochemical parameter such as increased blood urea production in diabetes by enhancing catabolism of liver & plasma protein. Recent scientific investigations have also confirmed the efficacy of plant preparations, few of which are most effective against diabetes¹⁴. In diabetes, Oxidative stress was thought to be a result of free radicals generated during autoxidation of glucose¹⁵. In diabetes blood urea level increases & reason behind this is due to increased catabolism of both liver & plasma proteins. This elevation of plasma level of urea & creatinine is marker of renal dysfunction¹⁶. In our study we have observed in urea and creatinine level about threefold increases in diabetic group of mice.

Another study based on carbon tetrachloride induced rats has shown that CCl₄ treatment increased the lipid peroxidation and liver enzymes, and decreased the antioxidant enzyme levels and furthermore, *Nigella sativa* treatment decreased the elevated lipid peroxidation, liver enzyme levels and increased the reduced antioxidant enzyme levels¹⁷. In present study S.G.P.T increases 13 folds in diabetic group of mice. Hepatic cells were observed in degenerated condition with elongated and fragmented nuclear material. Many vacuolated spaces were also observed in cytoplasm of hepatic cells in diabetic group of mice.

NS alcoholic extract maintained the levels of AST, ALT and ALP close to normal against D -Galactosamine induced toxicity¹⁸. In present study *Nigella sativa* causes effective restoration in urea and creatinine level. S.G.P.T. level was restored effectively in *N. sativa* administered group of mice.

Nigella sativa causes effective restoration in malathion induced increases in AST, ALT, and lipid peroxidation. *Nigella sativa* also improvement role in liver function tests, lipid peroxidation, and antioxidant enzymes alteration induced by malathion¹⁹. *Nigella sativa* administered group of mice shows effective restoration in SGPT level up to normal. NS showed hepatoprotective effects against isoniazid induced hepatotoxicity in rabbits and there are no histopathological or biological abnormalities were observed²⁰. *Nigella sativa* is efficient cytoprotective against CCl₄-induced hepatotoxicity, possibly via inhibition of the production of oxygen free radicals that cause lipid peroxidation²¹. In present study *Nigella sativa* causes restoration in hepatic cells. Central veins and sinusoids were also restored effectively. Restoration is observed in both cytoplasmic and nuclear material.

Conclusions:-

It is concluded from study that *N. sativa* acts effectively on SGPT level. Urea, uric acid and creatinine level were almost normal after *N. sativa* administration. It maintains normal morphology of hepatic cells with least vacuolization. *Nigella sativa* is effective cytoprotective agent on hepatocyte of diabetic mice as well as maintains biochemical parameters to normal level. It is evident from study that *Nigella sativa* acts well against diabetes.

Acknowledgement:-

The authors are thankful to Mahavir Cancer Institute and Research Centre for providing infrastructural facility during this work. We are also thankful to our entire research team who provided us every support during this study.

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