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# Potential Effect of Nigella Sativa Tablets on Fatty Liver Disease in Rats

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## Abstract

**Background:** Several studies have reported the incidence of fatty liver and metabolic syndrome are direct proportionality with age. The seeds of Nigella sativa and oil have been widely used to promote health and fight disease.

**Objective:** This prospective study was conducted to explore the effects of crushed seeds of Nigellasativa dietary supplementation on fatty liver disease in rats.

**Methods:** This study was conducted at the Department of Pharmacy, University of Malakand, Khyber Pakhtunkhwa, Pakistan on 16-18 months male Sprague dawley rats weighing 150-170gm. Animals were randomly assigned into three (03) groups; Group-I served as (Control group, C) received standard diet; Group-II served as (Liver Fatty group, F) that received diets having high % age of fructose (60% fructose w/w), Group-III (Fatty liver/Nigella sativa F/NS), fed fructose diet along with Nigella sativa tablets (1.6g/kg diet) to get daily intake of Nigellasativa at rate 170mg/kg body weight. The daily food intake and weekly body weight of the animals were recorded. After receiving respective drugs for 06 weeks following parameters such as Therapy; Body Mass index (BMI Body weight final), total cholesterol, liver weight, LDL-C, HDL-C, VLDL-C, serum glucose, Adiponectin,bilirubin, insulin, AST, ALT and TNF-, were measured. HOMA-R calculation was used to determine insulin resistance. Furthermore, kidneys, livers and brains Histopathological examination were also taken out.

**Results:** The weight of visceral body fat increased significantly in group II (F group) compared to group I (C group), but it dropped significantly in group III (F/NS group) compared to group II (F group). The serum level of glucose, HOMA-R, bilirubin, LDL-c, TNF-, vLDL-c, AST, ALT and insulin level significantly increased in both F and F/NS groups and a significant decrease in serum adeponectin level occurred as compared to C group. However, the serum levels of adeponectin increased in the F/NS group compared to the F group, and there was a substantial drop in glucose insulin, total cholesterol, HOMA-R, TNF-, LDL-c, vLDL-c, AST, ALT, and bilirubin. Histopathological examination of F group indicates vascular congestion in kidney and liver, renal tubular necrosis, hepatocytes andlocalized cerebral haemorrhage while the histology appearance in the F/NS group was nearly normal.

**Conclusions:** Animals receiving crushed tablets of Nigella sativa along with fructose diet produced significant attenuation in level of total cholesterol, serum glucose, insulin,TNF-, LDL-c, HOMA-R, AST, bilirubin and ALT and a significant rise in adiponectin level in fatty liver disease in old tested animals.

**Key words:** Adiponectin, dyslipidemia, fatty liver, visceral adiposity, metabolic syndrome

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## Introduction

The fatty liver along with metabolic syndrome is growinggloballyin several countries makingmajor social dilemma.1 Several studies have reported the incidence of fatty liver and metabolic syndrome are direct proportionality with age[2-3].The sensitivity to type II diabetes, cirrhosis, cardiovascular disease and NASH (non-alcoholic steatohepatitis) are more in individuals having liver fatty. It has been previously reported that the fatty liver/metabolic syndrome has six distinct components including prothrombotic, elevated blood pressure, atherogenic dyslipidemia, abdominal obesity, glucose intolerance, insulin resistance, and pro-inflammatory states, which are highly likely to lead to the development of

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various cardiovascular diseases (CVD). Any person who satisfies three or more of these requirements, investigation of fatty liver by performing different diagnostic test becomeessential.[4-5]

The seeds of Nigella sativa and oil have been widely used to promote health and fight disease since immortal times especially in Asia and Middle East. Recently, other therapies and natural products utilization is greatly increasing. Seed of Nigella sativa contains about 30% of fixed oil, 0.45% (w/w) of volatile oil which contains up to 24% thymoquinone and 46% monoterpenes such as α-pinene and p-cymene.6 Despite of traditional use, earlier studies carried out proved that Nigella sativa seeds has hypotensive, hepatoprotective and anti-diabetic effects.[7-8] The literature survey about Nigella sativa confirmed that no investigation on Nigella sativa tablets on fatty liver disease has been carried out until now.

Hence, present study was aimed on aged animals to determine Nigella sativa crushed tablets co feeding with high fructose diet could prevent criteria of fatty liver disease.

## Material and Methods

Experimental Animals

This study was performed according to standard protocols approved by "animals bye-laws 2008 of University of Malakand (Scientific Procedures Issue-I) at Department of Pharmacy, University of Malakand. The rats that were used during this study were placed separately in wire made cages under normal controlled standard conditions of temperature (30.0±3.0 ˚C), light (12 hrs light/dark cycles) and relative humidity was (70±80 %). All the animals were fed with standard rat food (AIN-93 M diet), adult rodents formulated diet and ad labium on water. The animal studies were conducted in accordance with the protocols for the use and care of Laboratory animals. The approved protocols by Committee on Animal Ethics at University of Malakand under Animal Bye-Laws 2008 were followed.

Experiment design

Male Sprague Dawley rats having (16-18 months) were used that was purchased from NIH (National Institute of Health) Islamabad, Pakistan and were randomly divided into three groups:

Group-I: Served as Control group C fed standard rat diet

Group-II: Served as Fatty liver group F, Received h high fructose diet (60% pure fructose w/w added in diet)

Group-III:Served as Fatty liver and Nigella sativa group, F/NS, Fed high fructose diet as F group and supplementation with Nigella sativa that was crushed tablets (1.6 g/kg diet) in order to achieveNigella sativa daily intake of 170 mg/kg (b.wt). Crushed tablets of Nigella sativa mean daily intake per animals was measured according to the procedure use byBuriro and Tayyabi.e. 54±1.4 mg with some changes.[9]Nigella sativa Tablets (Kalonji) were purchased from market in Peshawar, Pakistan.

Food intake and body weight of the tested animals were measured daily followed weekly. After 6 weeks of receiving respective drugs, all the animals were put on fasting for a period of 12 hrs, weighed and anesthetized with diethyl ether to handle easily. The back and length of the tested animals was measured from anus to the tip of the nose while for measurement of BMI (body mass index) neck was extended after placing all the animals on dissecting table. After incision of abdominals, blood sample was collected from aorta and transferred into plastic tubes. For biochemical analysis, serum was separated by centrifuging the blood sample at 3000 rpm for 15 minutes and was stored at -80˚C. A digital balance was used to weigh excised visceral fat. Biochemical Assay of Serum Eliza kit EIA 2018 and Randox(DRG international Inc, USA) were used to determine the insulin and glucose levels. Total cholesterol (tc),HDL-c and LDL-c were estimatedwithBioMerieux kit, serum adiponectin and TNF-was determined with the help of ALPCO ELIZA kit. Diagnostic kits of QuimicaClinicaAplicada, Spain were used for Liver function tests. The equation used to determine the vLDL-c is presented below:

$$\mathbf{vLDL-c = Total\ cholestrol - (HDL-c + LDL-c)}$$

Histopathological Examination

Brain, liver, and kidney were placed in 10% solution of formalin for investigation of histopathalogical studies, dehydrated, cleared in xylol and finally embedded in paraffin. A paraffin part stained by eosin and hematoxylin (H&E) was chopped serially at 6 cm thickness for microscopic assessment. Histopathological examination was carried out by score system. Inflammatory cell infiltration, vascular degeneration, necrosis and congestion were used as a standard. Grading parameters were: 0=no abnormality, +=mild abnormality, ++=moderate abnormality, +++=sever abnormality.(10)

Statistical Analysis: All the data were analyzed by using SPSS version 15.Standard deviation and mean were used to express the data. Further analysis was performed to determine the differences between the groups using one-way ANOVA and LSD (Least Significance Difference). P<O.05) was considered statistically significant. Least square method was used for calculating correlations and lines of regression.

## Results

Results showed that 40% of animals in group F and 15% in group F/NS were died in the 6th week of the treatment. While, food intake, weight gain, body weight (initial, final), liver weight, BMI (body mass index) were not significantly (P>0.05) different in all three tested groups. Although, slight significant increase (P<0.05) in visceral fat weight were found group F as compared to group C and group F/NS attenuates slight significant (P<0.05) compared to F group came close to normal control values in both F and F/NS groups. Adiponectin level decreased significantly (P<0.05) to C group and F/NS group significantly increased (P<0.05) compared to group F. The serum glucose,

insulin and HOMA-R significantly increased (P<0.05)in group F and F/NS while compared to group C and in the significantly decreased (P<0.05) in group F/NS when matched to group F.Significant increase (P<0.05) in LDL-c, vLDL-, TNF-, TC (total cholesterol),AST,ALT and bilirubin in the F and F/NS groups compared to the C group. There was significant (P<0.05) decrease in total cholesterol (TC) and LDL-c was found in F/NS group compared to F group while HDL-c there was no significant difference among the three groups (Table 1).
In group F and F/NS, visceral fat significantly correlated positively with blood glucose, HOMA-R, insulin, TC, LDL-c, vLDL-c, ALT, AST, TNF-, and bilirubin but negatively with serum adiponectin as presented in table 3.
Furthermore, central veins and blood sinusoids congestion, hepatocytes necrosis in the form of Pyknosis and hepatocyte vacillations were noted during histopathalogical examination of liver of group F as showed in Figure 1 A, B, C. However, there was less changes were observed in the livers of group F/NS group compared to group F. Table 2 displays the results of the histology analysis. Neuronal pyknosis, focal cerebral haemorrhage, and focal gliosis were observed in histopathological analyses of the brains of F group rats, while pyknosis of certain neurons and neuronophagia of pyknotic neurons were observed in F/NS group animals (Fig 2 A, B, C).
Histopathological studies of the kidneys of F group rats showed congestion of renal blood vessels and necrobiotic alterations in the epithelial lining of renal tubules, whereas the kidneys of F/NS group animals were determined to be less widespread (Fig 3 A, B, C).

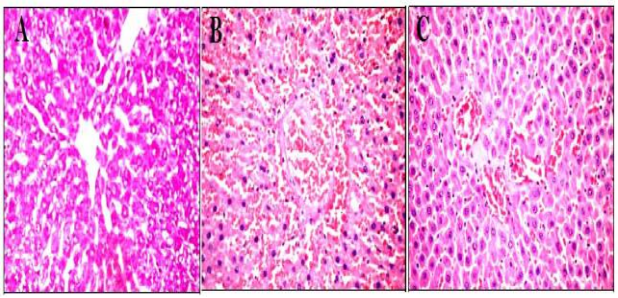


Fig-1 (A) Under microscopic examination, the liver of the C rat displayed a typical and healthy histological appearance. Conversely, the liver of the F rat demonstrated distinct signs, including central vein and blood sinusoidal congestion, along with hepatocyte necrosis characterized by pyknotic nuclei and vacillations. In contrast, the livers of the F/NS group exhibited a nearly normal histological presentation, approaching a healthier appearance under 400 x magnifications with H&E staining.

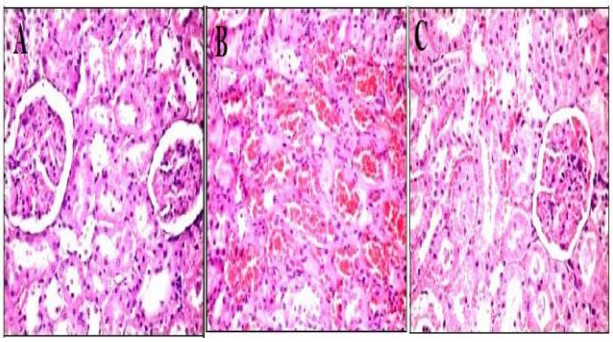


Figure 2 (A) illustrates the microscopic analysis of th brain tissue from the C rat, displaying a typical and healthy histological appearance. In contrast, the brain tissue of the F rats (B) exhibited signs of focal gliosis, neuronal pyknosis, and focal cerebral hemorrhage. Remarkably, the brain tissue of the F/NS rats (C) displayed neuronal pyknosis, albeit to a lesser extent, under 400 x magnifications with H&E staining.

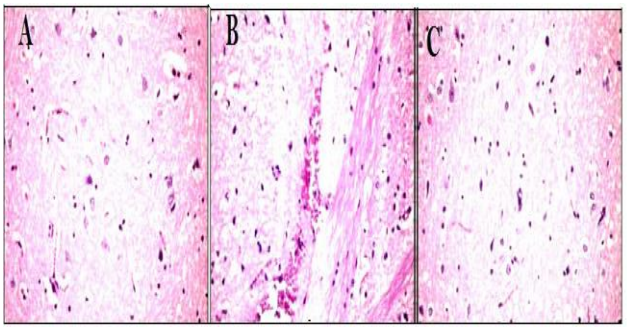


Fig-3 (A) under microscopic analysis, the kidney tissue from the C rat (Figure A) depicted a typical and healthy histological presentation of renal corpuscles and tubules. Conversely, the kidney tissues of the F rats (Figure B) exhibited evident signs of renal blood vessel congestion and tubular cell necrosis. Notably, the kidney tissues from the F/NS group (Figure C) displayed reduced vascular congestion and lesser necrosis of tubular cells under 400 x magnifications with H&E staining.).

Table I: Changes in various clinical and biochemical parameters among the three groups

| Parameters | Group-I (C-Group n=20) | Group-II (F group n=14) | Group-III (F/NS group n=18) | P Value |
|---|---|---|---|---|
| Initial body weight (g) | 296.7±3.8 | 297.3±6.8 | 306.2±3.6 | NS |
| Final body weight (g) | 357.9±5.1 | 365.3±7.7 | 368.6±1.9 | NS |
| Weight gain (g) | 61.3±2.4 | 69.2±4.1 | 63.4±2.8 | NS |
| Food intake (g) | 30.8±0.7 | 32.3±0.7 | 32.8±0.7 | NS |
| BMI (kg/m2) | 8.9±0.2 | 8.6±0.4 | 8.5±0.2 | NS |
| Liver weight (g/100gm B.W) | 3.89±0.23 | 3.92±0.20 | 3.50±0.22 | NS |
| Visceral fat (g) | 9.7±0.3 | 25.7±2.2 | 12.9±0.4 | <0.001 |
| Adiponectin (ng/ml) | 1.07±0.05 | 0.5±0.03 | 0.6±0.02 | <0.001 |

| | | | | |
|---|---|---|---|---|
| Serum glucose (mg/ml) | 87.9±0.9 | 139.2±4.5 | 107.8±1.7 | <0.001 |
| Serum insulin (U/ml) | 12.3±0.2 | 30.9±0.4 | 15.3±0.3 | <0.001 |
| HOMA-R | 2.7±0.05 | 10.6±0.3 | 3.8±0.09 | <0.001 |
| TC (mg/dl) | 84.7±0.9 | 219.5±2.2 | 121.6±1.9 | <0.001 |
| HDL-c (mg/dl) | 34.8±0.4 | 36.3±0.6 | 36.2±0.8 | NS |
| LDL-c(mg/dl) | 34.2±0.5 | 155.8±1.5 | 59.9±1.3 | <0.001 |
| vLDL-c(mg/dl) | 15.9±0.8 | 29.5±2.5 | 27.7±1.3 | <0.001 |
| TNF- (pg/ml) | 19.4±0.612 | 32.1±0.671 | 25.1±0.593 | <0.001 |
| ALT (U/L) | 53.7±0.989 | 86.5±1.727 | 74.8±0.749 | <0.001 |
| AST (U/L) | 42.5±0.764 | 83.7±1.453 | 63.3±1.706 | <0.001 |
| T-Bilirubin (mg/dl) | 0.351±0.008 | 0.499±0.007 | 0.432±0.008 | <0.001 |
| D-bilirubin (mg/dl) | 0.151±0.006 | 0.244±0.01 | 0.203±0.005 | <0.001 |

Table2:Semi quantitative score of Histopathological findings

| Group | Hydropic degeneration | Steotosis | Inflammatory cell infiltration | Congestion | Necrosis |
|---|---|---|---|---|---|
| Group-I (n=20) | 0 | 0 | 0 | 0 | 0 |
| Group-II (n=14) | ++ | +++ | +++ | +++ | +++ |
| Group-III (n=18) | + | ++ | + | + | + |

Damage grade are as follows; 0(absent) + (mild) ++(moderate) +++ severe

**Table III: Correlations of visceral fat weight versus serum level of different biochemical parameter in F and F/NS groups**

| Parameter | F group n=14 | F/NS group n=18 |
|---|---|---|
| Glucose (mg/dl | R 0.80<br>P <0.001 | R 0.72<br>P <0.001 |
| Insulin ((U/ml) | R 0.88<br>P <0.001 | R 0.89<br>P <0.001 |
| HOMA-R | R 0.86<br>P <0.001 | R 0.84<br>P <0.001 |
| TC (mg/dl) | R 0.88<br>P <0.001 | R 0.8<br>P <0.001 |
| LDL-c(mg/dl) | R 0.83<br>P <0.001 | R 0.69<br>P <0.001 |
| vLDL-c(mg/dl) | R 0.96<br>P <0.001 | R 0.48<br>P <0.005 |
| Adiponectin (ng/ml) | R -0.78<br>P <0.001 | R -0.78<br>P <0.001 |
| TNF- α (pg/ml) | R 0.94<br>P <0.001 | R 0.52<br>P <0.001 |
| ALT (U/L) | R 0.86<br>P <0.001 | R 0.76<br>P <0.001 |
| AST (U/L) | R 0.88<br>P <0.001 | R 0.79<br>P <0.001 |
| T-Bilirubin (mg/dl) | R 0.80<br>P <0.001 | R 0.72<br>P <0.001 |
| D-bilirubin (mg/dl) | R 0.82 | R 0.73 |
| | P <0.001 | P <0.001 |

*r is Correlation coefficient P is significance at 0.05 level*

## Discussion

Obesity has caused a raise in non-alcoholic fatty liver disease (NAFLD). Due to stationary life style and excessive intake of fructose in foods has increased metabolic syndrome and NAFLD worldwide. The F group rats fed with high fructose diet for 06 weeks developed 03 criteria of fatty liver / metabolic syndrome as the current investigation revealed. The F group rats developed visceral adiposity, atherogenic dyslipidemia in the form of elevated TC, LDL-c vLDL-c and insulin resistance. In F group compared to F/NS group the higher death ratio suggests fatal complication development at termination period of the study.

The histopathological examination of the vital organ of the tested animals e.g. livers along with brains and kidneys showed vascular congestion, cellular degeneration, necrosis and cerebral hemorrhage in group F group that may be the responsible factor of higher death ratio. Visceral adiposity was developed significantly in group F that received high fructose feeding in 6th weeks of the study without showing any significant changes in final body weight suggest that fatty liver and its complications are more associated with adiposity not with obesity[10].The intake of food was not changed significantly in three (03) tested groups indicating that if the amount of fructose in normal ingested food increased then the chances of development of liver to become fatty might progress even with ingestion of normal energy and food intake. The development of hepatic insulin resistance and hypertriglyceridemiamay be reason of fructose induced visceral adiposity.[12] The high level of inflammatory mediators like IL-6 and TNF- caused by excess visceral adipositywaspreviously reported. Our investigation demonstrated a positive and significant relationship between the prevalence of insulin resistance and dyslipidemia and visceral fat weight, as well as a negative relationship between serum adiponectin.[13-14] Because there was no discernible variation in BMI between group F and group C, the contribution of total body fat to hypoadeponectinemia was ruled out. The increased visceral fat decreases serum adiponectin that may strip the animal from natural anti-oxidant anti-inflammatory, cardio protective and hepatoprotective potentialthat might rationalize the vascular microscopic and cellular changes observed in livers, kidney and brains of F group.[15-17] In current study, the involvement of visceral adiposity to insulin resistance demonstrates significant positive relationship among visceral adiposity and HOMA-R same as previously documented in human and animal models of metabolic syndrome. On the other hand, this correlation not completely clarified the expected mechanisms included cytolysis of visceral deposits and enhanced non-esterifies fatty acids influx into the portal vein of the blood to the liver and hypoadeponectinemia.[18]In present study showed that hypercholesterolemia found in treated group F rats may be due

to elevated LDL-c and vLDL-c rather than HDL-c that might remains consistent according to the standard ATP-III criteria of metabolic syndrome. This poor lipid profile may possibly due to insulin resistance that has resulting increased vLDL-c and LDL-c formation by the liver and diminished discharge from the circulation.[19] The displayed results showed that Nigella sativa tablets supplementation with high fructose diet in F/NS rats decrease hyperlipidemia, insulin resistance, hypoadeponectinemia, visceral fat and normalize the histopathological architecture of the brains, livers and kidneys observed in F group. These results showed similarity to previously published studies on human. The Nigella sativa seeds were reported to have potential ofanti-inflammatory, antioxidant, hypoglycemic and hypolipidemic activities. The Nigella sativa cholesterol lowering effect was earlier reported owing to either stimulating bile acid excretion or cholesterol synthesis inhibition by 3-HMG co-enzyme down regulations. The decrease level of LDL-c was reported outstanding to LDL receptor gene up regulation.[20-23] Nigella crushed tablets supplementation in diet showed significant improvement in TNF-□ and liver dysfunction by reducing the activity of ALT, AST and the level of direct bilirubin and total bilirubin thus reducing the progression of fatty liver disease.[24]

## Conclusion

It is concluded that animals' groups that received high amount of fructose diet along with normal diets for 6 weeks showed development of fatty liver disease with cellular and vascular degenerative changes. Co-feeding Nigella sativa tablets alongside a high fructose-enriched diet appears to confer safety from various metabolic issues, including insulin resistance, visceral adiposity, hyperlipidemia, inflammation, and correction of liver function tests in cases of fatty liver disease. These findings suggest potential benefits, particularly for elderly patients who may not adhere to or respond well to other prophylactic or therapeutic interventions.

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