Research Article

Gender linked negative consequences of "*Nigella sativa*" seeds and "*Plantago ovata*" husk on fibrosis in the energy-rich diet (ERD) induced nonalcoholic fatty liver disease (NAFLD)

Afshan Syed Abbas*[1], Muddasir Hassan Abbasi[2], Muhammad Babar Khawar[3,4], Sheeba Batool[5], Faiza Jabeen[6], Asia Shad[1] and Nadeem Sheikh*[3]

[1]Department of Zoology, University of Education, Lower Mall Campus, Lahore, Pakistan.
[2]Institute of Pure & Applied Zoology, University of Okara, Okara-Punjab, Pakistan.
[3]State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China.
[4]Applied Molecular Biology and Biomedicine Lab, Department of Zoology, University of Narowal, Narowal, Pakistan.
[5]Cell & Molecular Biology Lab, Institute of Zoology, University of the Punjab, Q-A Campus, Lahore, 54590, Pakistan.
[6]Department of Zoology University of Education Bank Road campus Lahore
***Corresponding should be addressed to:**
1. Prof. Dr. Nadeem Sheikh, Institute of Zoology, University of the Punjab, Lahore, Pakistan. Email: s_nadeem77@yahoo.com, Ph: +92-322-4222036
2. Afshan Syed Abbas, Department of Zoology, University of Education, Lower Mall Campus, Lahore, Pakistan.
Email: afshan.syed@ue.edu.pk, Ph: +92-333-4771364

Abstract: Nonalcoholic fatty liver disease (NAFLD) is a developing liver problem mainly linked with the consumption of 'energy-rich-diet (ERD) in Asian countries including both females and males. NAFLD is associated with fibrosis in advanced stages. Medicinal herbs are being used to lessen the excessive fat of the body conventionally. Therefore, the current study was aimed to evaluate the lipid lowering factor against ERD induced fibrosis. 40 female (F) and 40 male (M) *Rattus norvegicus*, subdivided as four groups; 0, I, II and III according to their nutritional content. Group-0 received 100% rat chow and Group-I received ERD. Group-II and Group-III received ERD supplemented with 5% *Nigella sativa* seeds/*Plantago ovata* husk per kg ERD, respectively. Histopathological evaluation of the liver showed pericellular and portal fibrosis in both F-I and F-II. Radial fibrosis was detected in the M-II group, and peri lobular as well as bridging fibrosis between portal triads in the M-III and F-III groups. It is inferred that male livers were more susceptible to DRE-induced fibrosis. *N.sativa* seeds proved auspicious in minimizing fibrosis, whereas *P.ovata* pods caused advanced liver fibrosis with DRE.

Keywords: Fibrosis; Medicinal herbs; NAFLD; *N. sativa, P.ovata*)

Citation: Abbas A.F.; Abbasi M.H.; Khawar M.B.; Batool S.; Jabeen F.; Shad A.; Sheikh N.. Gender linked negative consequences of "Nigella sativa" seeds and "Plantago ovata" husk on fibrosis in the energy-rich diet (ERD) induced nonalcoholic fatty liver disease (NAFLD). *Pakistan Journal of Biochemistry and Biotechnology*, **2022**, 3 (2), x. https://doi.org/10.52700/pjbb.v3i2.161

Received: 04-10-2022
Accepted: 24-11-2022
Published: 31-12-2022

1. Introduction

NAFLD is a recurring hepatic disease in developed countries [1] (p. 05), that occurs as steatosis and nonalcoholic steatohepatitis [2]. In nonalcoholic steatohepatitis inflamed, ballooned hepatocytes lead to fibrotic and cirrhotic liver, and ultimately to hepatocellular carcinoma [3,4]. Usually, fibrosis initiates in zone 3 and may progressively develop into bridging and cellular fibrosis [5].

The history of medicinal use of plants is associated with human evolution [6]. Now a days, a lot of herbs such as black pepper, black seeds (Kalonji), cinnamon, Basil, Fennel, Apple Mint, Thyme and Golden Oregano are being used as medicinal herbs because of their salutary therapeutical prospective. The word "herb" has been

derived from the Latin word, "herba"meaning weed, grass or plant. Any part of the plant like root, fruit, stem, seed, bark, flower, stigma or a leaf can be taken as herb. There is evidence that the Vaid Indians, Chinese, Hakim Unani, and European and Mediterranean cultures have used herbs as a medicine for 4,000 years [10].

Nigella. sativa (Black seed/Kalonji) is one of the auspicious sanative herbs having flush religious and historic heritage [7]. *N. sativa* belongs to Ranunculaceae family and is a dicotyledon. It is a wondrous herb with rich medicinal properties, especially as a hepatoprotective herb due to the antitoxic and antioxidant properties of *N. sativa* seeds and oil [2,8,9,10].

The substantial health benefits of a high fiber diet have been reported previously [11]. *Plantago ovata* belongs to family plantaginaceae.The bark of *P. ovata*, commonly known as ispagol, is water soluble and swells and becomes slime when wet. Humans cannot digest it and is generally consumed as a source of dietary fiber. Several recent studies have demonstrated their promising part in hypolipidemic and hypoglycemic effects in patients with type 2 diabetes, hypercholesterolemic and allergic individuals, and experimental models [11,12,13].

Therefore, the present study was performed to scrutinize the gender-based impact of *P. ovata* husk and *N. sativa* seeds against NAFLD linked fibrosis in rats.

2. Materials and Methods

The Four months old rats were divided into two groups depending upon the gender: males (M) and females (F) group having body weight (200 ± 15g). All the groups were provided with different diet formulations for the period of fifteen weeks given in the Table 1.

Table 1. This is showing gender and diet formulation among the groups (0, I, II, III, IV) of rats (males and females).

Sr. No.	**Gender/Diet formulations**	**Groups**			
1.	Males (200 ± 15g)	**M-0**	**M-I**	**M-II**	**M-III**
	Diet	100% rat chow	ERD	ERD + 5% *N. sativa* seeds/ kg	ERD + 5% *P.ovata* husk/ kg
2.	Females (200 ± 15g)	F-0	F-I	F-II	F-III
	Diet	100% rat chow	ERD	ERD + 5% *N. sativa* seeds/ kg	ERD + 5% *P. ovata* husk/ kg

'ERD" previously reported by Nader ali et al., (2001) [30]. Its composition was modified in G-II as 34% Rat chow, 33% Sucrose, 20% tea whitener and 13% water. The experiment was designed under the instructions of the ethical committee of University of Education, Lahore (UE/16-10-17/7232).

After fasting overnight, the livers of rats were relieved and settled in 10% formalin. The 4 μm sections were then embedded in paraffin, deparaffinized and rehydrated with graded alcohol in phosphate buffered saline (PBS). The Masson trichrome staining method was used to stain collagen fibers from liver tissue.

3. Results

The results of histological examination showed that the portal and pericellular fibrosis were obvious in the M-I group and the F-I group. Likewise, compared with the F-II group, the M-II group developed radiating fibrosis

in the portal area, whereas in the M-III and F-III groups, perilobular fibrosis and bridging between the portal triads were obvious. (Figure 1 & 2).

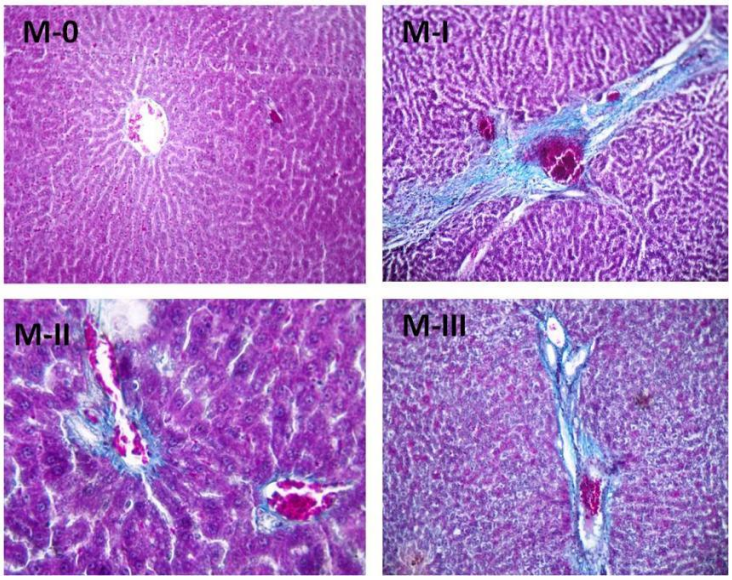

Figure 1: Liver sections of male groups M-0 (Control), M-I (ERD), M-II (ERD+ *N. sativa* seeds), and M-III (ERD= *P. ovata* husk) stained with Masson's trichrome. The blue colour indicates fibrosis.

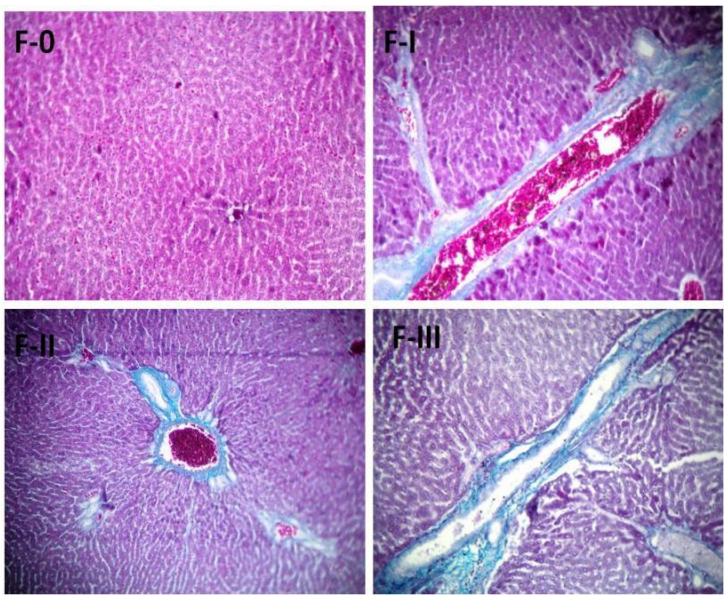

Figure 2: Liver sections of female groups F-0 (Control), F-I (ERD), F-II (ERD+ *N. sativa* seeds), and F-III (ERD= *P. ovata* husk) stained with Masson's trichrome. The blue colour indicates fibrosis.

4. Discussion

Inflammation is often referred to as a trigger for fibrogenesis [14]. Obese individuals who develop nonalcoholic steatohepatitis often have periportal fibrosis [15]. Sinusoidal and pericellular fibrosis [16,17,18] (p. 06), is most commonly associated with steatohepatitis. As the disease progresses, "periportal and perilobular fibrosis" can progress to cirrhosis [18]. In the present study, histopathology of group-I in males and females treated with ERD showed bridging fibrosis between portal triads, in group II supplemented with *N. sativa* seeds. Male rats showed radiating fibrosis in the portal region, while females in this group only showed periportal fibrosis. In portal fibrosis, changes in fat content

may be mild to moderate and irreversible, while other histological changes may be reversed with treatment [19]. As seen in Group II, given N. sativa seeds, all histological variations were reversed, with the exception of fibrosis, especially in females (F-II).

In the group supplemented with P. ovata hulls, there were M-I, II, and F-III perilobular and periportal fibrosis with enlarged portal triad. In addition, the male M-III group also showed pericellular fibrosis, possibly because increasing estrogen concentrations have been reported to decrease fibrosis. In general, the mortality rate from chronic liver disease is twice as high in men as in women [20]. Sex steroids modify the immune system at various levels and alter the cytokine environment [21,22]. Similarly, some studies reported that commonly fewer females while more males were prone to establish NALFD [23,24,25]. In postmenopausal women not receiving hormone therapy NAFLD is common. It recommends that estrogen plays a crucial role in NAFLD and fibrosis [26]. Hepatic fibrosis and cirrhosis are also linked with lipid peroxidation and reactive oxygen species as free radicals initiate damage to hepatocytes. These effects are partly checked by antioxidants [27,28]. *N. sativa* seeds have antioxidant potential [29].

5. Conclusions

It is concluded that the livers of males are more susceptible to ERD induced fibrosis. When ERD was supplemented with *N. sativa* seeds, it was helpful to minimize fibrosis. On the other hand, *P. ovata* husks supplementation stimulated advanced liver fibrosis. However, further studies are needed to understand the molecular mechanism involved.

Author Contributions:

Conceptualization, Afshan Syed Abbas and Nadeem Sheikh; methodology; Sheeba Batool and Faiza Jabeen; investigation, Mudassir Hassan Abbassi, writing- original draft preparation; Asia Shad, writing review and editing Babar Khawar. visualization, Sheeba Batool; supervision, Afsha Syed Abbas; project administration, Nadeem Sheikh.; funding acquisition, Afshan Syed Abbas. All authors have read and agreed to the published version of the manuscript.

Funding: The credit behind this research goes to Higher Education Commission (HEC) of Pakistan for providing funds to authors (Grant number:074-3594-BM4-217).

Acknowledgment: We acknowledge HEC of Pakistan and university of the Punjab for the support of this research work.

Conflicts of Interest: The authors declare no conflict of interest.

This section is not mandatory but can be added to the manuscript if the discussion is unusually long or complex.

References

1. Cohen, J.C.; Horton, J.D.; & Hobbs, H.H. Human fatty liver disease. old questions and new insights. *Science,* **2011**, *332 (6037),* 1519-1523.
2. Camargo, F.N.; Matos, S.L.; Araujo, L.C.C.; Carvalho, C.R.O.; Amaral, A.G.; Camporez, J.P.; Camargo, F.N.; et al. *Curr Issues Mol Biol*, **2022**, *8;44(10),* 4692-4703. doi: 10.3390/cimb44100320. PMID: 36286035.
3. Crabb, D.W.; Galli, A.; Fischer, M.; & You, M. Molecular mechanisms of alcoholic fatty liver: role of peroxisome proliferator-activated receptor alpha. *Alcohol*, **2004,** *34(1):* 35-38.

4. Harris, S.E.; Poolman, T.M.; Arvaniti, A.; Cox, R.D.; Gathercole, L.L.; Tomlinson, J.W.; Harris, S.E.; et al. *Am J Physiol Gastrointest Liver Physiol,* **2020**, *319(3),* G345-G360. doi: 10.1152/ajpgi.00055.
5. Lequoy, M.; Gigante, E.; Couty, J.P.; Desbois-Mouthon, C. Hepatocellular carcinoma in the context of non-alcoholic steatohepatitis (NASH): Recent advances in the pathogenic mechanisms. *Horm Mol. Biol Clin. Investig.,* **2020**, *41*.
6. Dattner, A.M. From medical herbalism to phytotherapy in dermatology: Back to future. *Dermatol Ther*, **2003**, *16,* 106-13.
7. Khan, H.; Saeedi, M.; Nabavi, S.M.; Mubarak, M.S.; & Bishayee, A. Glycosides from medicinal plants as potential anticancer agents: emerging trends towards future drugs. *Current Medicinal Chemistry,* **2019,** *26,* 2389–2406.
8. Ali, B.; Blunden, G. Pharmacological and toxicological properties of *Nigella sativa*. *Phytother. Res*, **2003**, *17*, 299–305. 10.1002/ptr.1309.
9. Al-Khalifa, K.S.; AlSheikh, R.; Al-Hariri, M.T.; El-Sayyad, H.; Alqurashi, M.S.; Ali, S.; Bugshan, A.S.; Al-Khalifa, K.S.; et al. *Molecules.* **2021,** *26(21),* 6451. doi: 10.3390/molecules26216451. Molecules. PMID: 34770860.
10. Yang, G.; Su, F.; Chen, M. **Origin and prospect of homology medicine and food.** *Modern Chinese Medicine,* **2021**; *23 (11),* 1851-1856.
11. Galisteo, M.; Sanchez, M.; Vera, R.; Gonzalez, M.; Anguera A.; et al. Duarte, J.; &. Zarzuelo, A. A diet supplemented with husks of Plantago ovata reduces the development of endothelial dysfunction, hypertension, and obesity by affecting adiponectin and TNF-alpha in obese Zucker rats. *J. Nutr,* **2005**; *135*, 2399-2404
12. Naiki-Ito, A.; Kato, H.; Naiki, T.; Yeewa, R.; Aoyama, Y.; Nagayasu, Y.; Suzuki, S.; Inaguma, S.; Takahashi, S. A novel model of non-alcoholic steatohepatitis with fibrosis and carcinogenesis in connexin 32 dominant-negative transgenic rats. *Arch. Toxicol,* **2020**, *94*, 4085–4097.
13. Anderson, M.; Clements, A. Resolving environmental disputes: a statistical method for choosing among competing cluster models. *Ecol Appl,* **2000**, *10(5)*,1341–1355.
14. Cortez-Pinto, H.; Baptista, A.; Camilo, M.E.; Demoura, M.C. Hepatic stellate cell activation occurs in nonalcoholic steatohepatitis. *Hepato-Gastroenterol.,* **2001**, *48*, 87-90.
15. Ratziu, V.; Giral, P.; Charlotte, F.; et al. Liver fibrosis in overweight patients. *Gastroenterology,* **2000,** *118*, 1117-1123.
16. Brunt, E.M. Nonalcoholic steatohepatitis: Definition and pathology. *Semin Liver Dis*., **2001**, *21*, 3-16.
17. Brunt, E.M. Nonalcoholic steatohepatitis. *Semin Liver*, **2004**, *24*, 3-20.
18. Brunt, E.M., Tiniakos, D., G. Pathology of steatohepatitis. Best Pract Res Clin *Gastroenterol.*, **2002**, *16,* 691-707.
19. Dixon, J.B.; Bhathal, P.S.; Hughes, N.R.; O'Brien, P.E. Nonalcoholic fatty liver disease: improvement in liver histological analysis with weight loss. *Hepatology,* **2004**, *39,* 1647–1654.
20. Guy, J.; Peters, M.G. Liver disease in women: the influence of gender on epidemiology, natural history, and patient outcomes. *Gastroenterol Hepatol (N Y),* **2013,** *9(10),* 633-9.
21. Donaldson, P.T. Genetics of liver disease: immunogenetics and disease pathogenesis. *Gut*., **2004**, *53(4)*, 599-608.

22. Whitacre, C.C. Sex differences in autoimmune disease. *Nat Immunol.,* **2001**, *2(9),* 777-780.
23. Adams, L.A.; Lymp, J.F.; St-Sauver, J.; et al. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology,* **2005**, *129 (1),* 113-121.
24. Admas, L.A.; Angulo, P. Recent concepts in non-alcoholic fatty liver disease. *Diabet Med.,* **2005,** *22,* 1129-1133.
25. Vernon, G.; Baranova, A.; Younossi, Z.M. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther,* **2001**, *34(3),* 274-285.
26. Gutierrez-Grobe, Y.; Ponciano-Rodriguez, G.; Ramos, M.H.; Uribe, M.; Mendez-Sanchez, N. Prevalence of nonalcoholic fatty liver disease in premenopausal, postmenopausal and polycystic ovary syndrome women. The role of estrogens. *Ann Hepatol.,* **2010,** *9(4),* 402-409.
27. Parola, M.; Leonarduzzi, G.; Biasi, F.; Albano, E.; Biocca, M.E.; et al. Vitamin E dietary supplementation protects against carbon tetrachloride-induced chronic liver damage and cirrhosis. *Hepatology,* **1992**, *16,* 1014-1021.
28. Kanter, M.; Coskun, O.; Budancamanac, M. Hepatoprotective effects of Nigella sativa L and Urtica dioica L on lipid peroxidation, antioxidant enzyme systems and liver enzymes in carbon tetrachloride-treated rats. *World J. Gastroenterol,* **2005**, *11,* 6684- 6688.
29. Abbas, A.S.; Abbasi, M.H.; Ihtazaz, A.; Malik, Sheikh, N. Non-Alcoholic Fatty Liver Disease and associated changes in serum hepcidin, iron, ferritin-R levels and total iron binding capacity in weaning wistar rats (Rattus norvegicus). *The Journal of Animal & Plant Sciences,* **2014,** *2,* 418-424.
30. Naderali, E.K.; Brown, M.J.; Pickavance, L.C.; Wilding, J.P.; Doyle, P.J.; et al. Dietary obesity in the rat induces endothelial dysfunction without causing insulin resistance: a possible role for triacylglycerols. *Clin.Sci.(Lond),* **2001**, *101(5),* 499-506 available from: PM:11672455.