Effect of Ficus Carica Fruit Versus Atorvastatin on High Fat Diet Induced Hepatic Steatosis

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Abstract

Objective: To evaluate the effect of Ficus carica fruit (fig) and atorvastatin on high fat diet induced hepatic steatosis histologically.

Material and Methods: During this experimental study forty male Sprague dawley rats were randomly divided into five equal groups and were acclimatized for a duration of one week. Throughout the course of 12 week, normal control (NC) group was provided normal rat chow. The remaining four groups were induced hyperlipidaemia in 4 weeks duration. These rats were fed with high fat diet containing 1.5gm cholesterol, 8ml coconut oil and 1gm sodium cholate added per 100gm of standard rat chow diet. Disease control group (DC) was offered high fat diet continuously during the 12 weeks study period. On completion of 4 weeks Ficus carica ethanolic extract (FCE) and pulp of Ficus carica fruit (FCP) and standard hyperlipidemic agent, atorvastatin (ATO) were incorporated to the diet of three experimental animal groups respectively for next 8 weeks. All the animals were sacrificed at the end and their liver parenchymal slides were subsequently analysed for histopathology.

Results: Histopathological evaluation using (NASH) scoring showed that both pulp and ethanolic extract of Ficus carica fruit induced significant decrease in the fatty change of liver parenchyma.

Conclusion: This experimental study concludes that pulp of Ficus carica fruit has evident anti hyperlipidemic activity by reducing hepatic steatosis.

Key words: Ficus carica, pulp, ethanolic extract, Hepatic steatosis.

How to cite: *Perveen F, Nzqvi F, Siddiqui AH, Irum Z, Khan FA, Yaqoob N. Effect of Ficus Carica Fruit Versus Atorvastatin on High Fat Diet Induced Hepatic Steatosis. Esculapio - JSIMS 2023;20(01): 77-81.*

DOI: https://doi.org/10.51273/esc24.251320115

Introduction

Hyperlipidemia, a familial ailment is revealed by the rise of one or more than one lipids in the blood plasma. Lipids are fatty, oily or waxy complexes which are vital to perform many critical functions of body and also act as integral part of building blocks for all living cells. Cholesterol, triglycerides, phospholipids, fatty acids are the main lipids in our body. The macromolecules that transport lipids are labelled as lipoproteins. The metabolic disorder which comprises of disproportionate rise of lipids in the body, especially rise of bad lipids i.e. LDL and reduced amounts of good lipids i.e. HDL is called Dyslipidaemia or hyperlipidaemia.[1] There is a strong assosiation between dyslipidaemia and sluggish life style, high caloric meals, absence of exercise, continious stress & nicotine smoking. Dyslipidemia is the culprit behind many life threatening diseases like hypertension, diabetes mellitus,[2] stroke, myocardial infarctions and arrythmias.[3] Drugs may be

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Submission Date: 21-10-2023
1st Revision Date: 11-01-2024
Acceptance Date: 09-03-2024

prescribed to individuals who are unable to manage their blood lipids even after life style modifications and improved exercise routine. Commonly prescribed drugs to treat dyslipidaemia include statins, fibrates, resins and Bile acid sequestrants.[4] In addition to these drugs many herbal remedies are also used to treat dyslipidaemias, like ginger juice, fenugreek seeds and leaves, turmeric, rosemary, Artichoke leaf extract, Yarrow etc.[5]

Fig (Ficus carica Linn) commonly available in Pakistan, embraces one of the biggest species of medicinal plants which include woody trees and shrubs. Recent research has shown that Ficus carica has proven hypoglycaemic, antipyretic, hepato-protective, antispasmodic & anti HSV potential.[6] It has also shown antimicrobial effects against Proteus Mirabillis & Bacillus subtilis. Ficus carica has established antioxidant and protective effect on ischemic myocardial tissue as well as antiplatelet effect.[7] Furthermore hepato-protective effect of Ficus Carica has been proven in hepatotoxicity induced by CCl4.[8]

We have planned this study to test the anti-hyperlipidemic activity of Ficus carica fruit on liver of high fat diet (HFD) rat by comparing histopathological evidence using NASH scoring. This will provide an alternate treatment of hyperlipidaemia in form of Ficus carica fruit which is cost effective as well as easy to take.

Method and Material

Total 40 healthy male Sprague dawley rats were procured and were retained for 1 weeks in the Post Graduate Medical Institute, Lahore animal facility for acclimatization. The rats were provided a room temperature of 25±5°C, with 12 hours of light dark cycle. All the rats were given free access to rat chow diet and tap water. They were then divided into five group by simple random sampling technique. During the course of 12 week, normal control (NC) group was provided normal rat chow. The remaining four groups were induced hyperlipidemia in 4 weeks duration. These 4 groups were given high fat diet (HFD) containing. 1.5gm cholesterol, 8ml coconut oil and 1gm sodium cholate added per 100gm of standard rat chow diet.[9-11] Disease control group (DC) was offered high fat diet throughout the research period of 12 weeks. On completion of 4 weeks Ficus carica ethanolic extract (FCE) and pulp of Ficus carica fruit (FCP) and standard hyperlipidemic agent, 30mg/kg atorvastatin (ATO) were incorporated to the diet of three experimental animal groups respectively for next 8 weeks.[12]

Ficus carica plant was purchased locally and was identified by Botany department of GC University, Lahore. To prepare ethanolic extract of Ficus carica fruit, we soaked 100 gm of Ficus carica in a solution of 80% ethanol, 13 in 1: 10 ratio, producing a 40% yield of extract. The ethanolic extract of Ficus carica fruit was kept at 4ºC. The ethanolic extract of Ficus carica was dispensed daily by oral route in morning at a dose of 500mg/kg to HFD + FCE group for next 8 weeks. Bits of Ficus carica pulp were dispensed to HFD+FCP group in amount of 1250 mg/kg as daily morning oral dose.[11,14] Similarly, HFD+ATO group was dispensed a single oral dose of 30mg/kg atorvastatin in morning for next 8 weeks.[15] Afterwards, on completion of 12 weeks, all the animals were sacrificed and liver histopathology was performed.

Histopathology of samples were performed at Pathology department of PGMI Lahore. An adequate portion of liver was excised and the liver sample was processed in an automatic tissue processor. Afterwards, the sample was embedded in paraffin and with the aid of microtome, approximately 5 micrometre thick sections of liver parenchyma were sliced. These cut sections were then positioned on the glass slides and subsequently stained with eosin and haematoxylin. Under light microscope, each slide was carefully reviewed from one side to other with help of pathologist, to grade the non-alcoholic hepatic steatosis.

Hepatic steatosis was graded by using Non-Alcoholic Steatosis Hepatitis, Clinical Research Network (NASH CRN) scoring system.[16] This scoring system categorised the tissue according to involvement of parenchyma into four grades. Grade 1 indicated Parenchymal involvement 0-5% by steatosis, Grade 2 indicated parenchymal involvement 2:5-33% by steatosis, Grade 3 indicated parenchymal involvement 33-66% by steatosis and Grade 4 indicated parenchymal involvement above 66% by steatosis.[17]

Results

Hepatic steatosis was graded by using Non-Alcoholic Steatosis Hepatitis, Clinical Research Network (NASH CRN) scoring system. Under light microscope, the prepared slides of liver were examined histologically. The fatty change in liver parenchyma in all groups were compared by applying Kruskal Walli's test. This test revealed statistically highly significant difference in fatty change among different groups with p value 0.001. (Table 1) In group wise comparison using Mann Whitney

U test, the disease control and experimental groups had highly significant histopathological changes as compared to NC group. The change in experimental groups was significantly lower than DC group. However, the experimental groups had no significant difference among themselves. (Table 2)

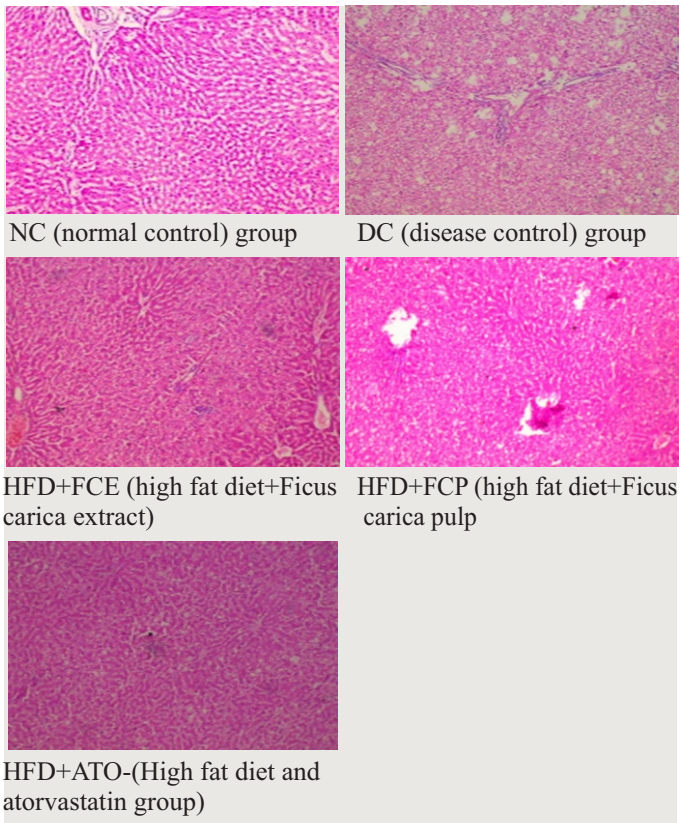

Fig-1: *Hematoxylin and eosin stained section of rat liver showing fat deposition (magnification 10x)*

Table 1: *Comparison of hepatic steatosis among different groups by Kruskal Walli's test*

Groups	N	Mean Rank	Chi square	p value
NC	8	4.5	29.55	0.00
DC	8	34.5		
HFD+FCE	8	21.06		
HFD+FCP	8	18.56		
HFD+ATO	8	23.88		

Discussion

Hepatic steatosis is a disease state in which there is accumulation of triglycerides in more than 5% of the hepatic cells when liver is examined histologically.[18] Hepatic Steatosis is well known as fatty liver disease or adipose liver disease. Hepatic steatosis is alarming as it may advance to dreadful outcomes related to liver

Table 2: *Pairwise comparison of hepatic steatosis among various groups using Mann Whitney U test*

(I) GROUP	(J) GROUP	Z	p value
Normal control	Disease Control	-3.87	<0.00
	HFD+FCE	-3.66	<0.00
	HFD+FCP	-3.66	<0.00
	HFD+ATO	-3.65	<0.00
Disease Control	HFD+FCE	-3.30	0.001
	HFD+FCP	-3.28	0.001
	HFD+ATO	-2.95	0.003
HFD+FCE	HFD+FCP	-0.81	0.4
	HFD+ATO	-0.79	0.4
HFD+FCP	HFD+ATO	-1.43	0.152

such as hepatic dysfunction and insufficiency and hepatic carcinoma[19]. There may be added extra-hepatic clinical manifestations like type 2 diabetes mellitus, chronic renal disease, cardiovascular disease, and extra-hepatic neoplasms in this disease. In order to prevent the advancement of fatty liver disease and its complications, one must address the related risk factors linked to this condition, such as obesity, excessive alcohol consumption, diabetes mellitus, and metabolic syndrome.[18] The most common cause of hepatic steatosis is hyperlipidemia. Hyperlipidemia can cause hepatic steatosis which needs to be treated by different allopathic and herbal medicines.[20] Various plant seeds and fruits have been investigated to impart their anti hyperlipidemic effects and there role in prevention of hepatic steatosis is being evaluated.

Ficus carica herb has many therapeutic uses. Ficus carica leaves have demonstrated hypolipidemic effect in rats in recent studies.[21] We compared ethanolic extract of Ficus carica fruit, the pulp of Ficus carica fruit with standard drug atorvastatin. Preparation of ethanolic extract is a lengthy and cumbersome process, whereas Ficus carica fruit is easily available, edible and its use is simpler than extract. In our research, we compared the effect of extract as well as pulp of Ficus carica fruit with atorvastatin, a standard drug to treat hyperlipidemia. Hyperlipidemia was induced by the use of 1.5gm cholesterol, 8ml coconut oil and 1gm sodium cholate in all the animal groups except normal control. After sacrificing the animal, liver samples were obtained to evaluate them histopathologically, and hepatic steatosis was graded in the light of NASH scale.[16] The results revealed that normal control (NC) had normal liver parenchyma without signs of added fatty infiltration. In contrast, the disease control (DC) group displayed highest grade of hepatic steatosis in the liver parenchyma. Afterwards,

the experimental groups were evaluated. It was quite obvious that the experimental groups demonstrated considerably less hepatic steatosis in contrast to disease control group. Unfortunately, the normal parenchymal lobular configuration of liver was not restored in experimental groups. Similar studies on activity of Ficus carica Linn was demonstrated using Lead acetate induced hepatotoxicity.[22] Another related research was performed to evaluate the hepato-protective effects of Ficus carica in Non-alcoholic fatty liver disease (NAFLD). The results were astonishing with chronic treatment of 16 weeks as it reduced the liver enzymes including ALP, AST and ALT.

Regarding comparison between the experimental groups, histological evaluation and NASH scoring revealed that pulp of Ficus carica fruit was most effective in reducing hepatic steatosis as compared to ethanolic extract of Ficus carica fruit and Atorvastatin. Ficus carica fruit pulp is beneficial as it is more convenient to take orally and has no lengthy preparation like ethanolic extract. Research conducted on phytochemical analysis of Ficus carica fruit shows that it contains phenols, organic acids, flavonoids, fibre, phytosterols, anthocyanin pigments, coumarins and certain volatile compounds e.g. aliphatic alcohols and hydrocarbons. These high contents of polyphenols, condensed tannins and flavonoids are responsible for anti-oxidant and antimicrobial effects of Ficus carica fruit and are the probable reason of its preventive effect on hepatic steatosis in our research.[23] Consequently, it is concluded that pulp of Ficus carica fruit reduces development of hepatic steatosis and it opens avenues for more advanced research to evaluate the molecular mechanism of this effect.

Conclusion

This experimental study concludes that pulp of Ficus carica fruit has beneficial role in preventing development and progression of diet induced hepatic steatosis.

Conflict of Interest: *None*

Source of Funding: *None*

References

1. Zou Y, Zhong L, Hu C, Zhong M, Peng N, Sheng G. LDL/HDL cholesterol ratio is associated with new-onset NAFLD in Chinese non-obese people with normal lipids: a 5-year longitudinal cohort study. Lipids in Health and Disease. 2021;20(1):1-10.
2. Lu S-X, Wu T-W, Chou C-L, Cheng C-F, Wang L-Y. Combined effects of hypertension, hyperlipidemia, and diabetes mellitus on the presence and severity of carotid atherosclerosis in community-dwelling elders: a community-based study. Journal of the Chinese Medical Association. 2023;86(2):220-226.
3. Pappan N, Rehman A. Dyslipidemia. StatPearls [Internet]. StatPearls Publishing; 2022.
4. Abdul-Rahman T, Bukhari SMA, Herrera EC, et al. Lipid lowering therapy: an era beyond statins. Current problems in cardiology. 2022;47(12):101342.
5. Kang Y-M, Kim Y-J, Kim K. Significance of traditional herbal medicine for dyslipidemia. American Journal of Translational Research. 2023;15(8):5373.
6. Rasool IFu, Aziz A, Khalid W, et al. Industrial application and health prospective of fig (Ficus carica) by-products. Molecules. 2023;28(3):960.
7. Li Z, Yang Y, Liu M, et al. A comprehensive review on phytochemistry, bioactivities, toxicity studies, and clinical studies on Ficus carica Linn. leaves. Biomedicine & Pharmacotherapy. 2021;137:111393.
8. Aghel N, Kalatari H, Rezazadeh S. Hepatoprotective effect of Ficus Carica Leaf extract on mice intoxicated with carbon tetrachloride. Iran J Pharm Res. 2011; 10 (1):63-68.
9. Balasubramanian MN, Muralidharan P, Balamurugan G. Anti hyperlipidemic activity of Pedalium murex (Linn.) fruits on high fat diet fed rats. 2008;
10. Hussian M, Arain AQ, Chiragh S. Pioglitazone improves serum lipid profile in diet induced hyperlipidaemic non diabetic rats. Journal of Pakistan Medical Association. 2016;66(10):1286-1290.
11. Parveen F, Khan FA, Irum Z, Siddiqui AH, Bhatti M, Iqbal R. Anti Dyslipidemic Effect of FICUS Carica Fruit (Fig) on Lipid Profile of Diet Induced Hyperlipidemic Rats. Pak J Med Health Sci. 2021;15(7):1709-12.
12. Eissa AA, Abdelateef AS, Elkotby HM. Potential effect of curcumin versus atorvastatin on hepatic and metabolic changes in rat model of metabolic syndrome. International Journal of Medical Arts. 2021; 3(3): 1507- 1515.
13. Maurya A, Priyadarshini E, Rajamani P. Evaluation of Antioxidant Capacity and Antiproliferative Activity of Fruit Extract of Dry Figs (Ficus carica L.) on MDA MB-468 Cell Line. 2021.
14. Aghel N, Kalantari H, Rezazadeh S. Hepatoprotective effect of Ficus carica leaf extract on mice intoxicated with carbon tetrachloride. Iranian journal of pharmaceutical research: IJPR. 2011;10(1):63.
15. Ji G, Zhao X, Leng L, Liu P, Jiang Z. Comparison of

dietary control and atorvastatin on high fat diet induced hepatic steatosis and hyperlipidemia in rats. Lipids in health and disease. 2011;10:1-10.

16. Segura-Azuara NdlÁ, Varela-Chinchilla CD, Trinidad-Calderón PA. MAFLD/NAFLD biopsy-free scoring systems for hepatic steatosis, NASH, and fibrosis diagnosis. Frontiers in medicine. 2022;8:774079.
17. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. The American journal of gastroenterology. 1999; 94(9): 2467-2474.
18. Ballestri S, Mantovani A, Byrne C, Lonardo A, Targher G. Diagnostic accuracy of ultrasonography for the detection of hepatic steatosis: an updated meta-analysis of observational studies. Metabolism and Target Organ Damage. 2021;1(7)
19. Batool S, Morton Cuthrell K, Tzenios N, Shehryar Z. Hepatocellular Carcinoma in Non-alcoholic Fatty Liver Disease: Emerging Burden. International Research Journal of Oncology. 2022;6(4):93-104.
20. Feng K, Zhu X, Liu G, et al. Dietary citrus peel essential oil ameliorates hypercholesterolemia and hepatic steatosis by modulating lipid and cholesterol homeostasis. Food & function. 2020;11(8):7217-7230.
21. Alalwani AD, Hummdi LA, Qahl SH. Effect of nano extracts of olea europaea leaves, Ficus carica and liraglutide in lipidemic liver of type 2 diabetic rat model. Saudi Journal of Biological Sciences. 2022; 29(7): 103333.
22. Aziz FM. Protective effects of latex of Ficus carica L. against lead acetate-induced hepatotoxicity in rats. Jordan Journal of Biological Sciences. 2012; 147(619): 1-7.
23. Aljane F, Neily M, Msaddak A. Phytochemical characteristics and antioxidant activity of several fig (Ficus carica L.) ecotypes. Italian Journal of Food Science. 2020;32(4)

Authors Contribution

FP: Conceptualization of Project
FN: Data Collection
AHS: Literature Search
ZI: Statistical Analysis
FAK: Drafting, Revision
NY: Writing of Manuscript