

Restorative Effect of Garlic on Fatty Liver

Aisha Qamar¹, Humera Waqar², Mohammad Saad Saeeduddin³

ABSTRACT:

Objective: To assess the restorative role of garlic on high-fat induced hepatocellular damage.

Materials and Methods: This experimental study was done at BMSI, JPMC from 1st - 30th October 2008 after obtaining ethical approval. Thirty adult albino rats, weighing 220 to 240 gram were divided into three groups according to dietary regimen. Group A served as control, group B received high-fat diet (20 mg butter in 100 gm of diet) and group C was given same quantity of butter as in group B along with 6% fresh crushed garlic in diet for 4 weeks. The rats were sacrificed, liver removed, weighed and processed for haematoxylin and eosin staining.

Results: There was significant increase in the body weight and absolute liver weight in group B animals receiving high-fat diet, both of which decreased significantly after the concomitant use of garlic in group C. Haematoxylin and eosin stained sections revealed shrunken portal triad, swollen hepatocytes with pyknotic nuclei in high-fat diet group as compared to control animals, while in group C hepatocytes were polygonal in shape with vesicular nuclei comparable to control group A.

Conclusion: Garlic has restorative role in high-fat induced hepatocellular damage. Fatty liver produced by high quantities of saturated fats, such as butter can be ameliorated by the use of garlic.

Keywords: High-fat diet, Hepatocellular damage, Fatty liver, Saturated fats, Garlic

INTRODUCTION:

Obesity is a major public health crisis worldwide, resulting from an energy imbalance that can be credited to little physical activity and excessive intake of foods rich in saturated fats. With the rising incidence of obesity, NAFLD (nonalcoholic fatty liver disease) is developing into a major health problem in modern society.¹ Currently, NAFLD is the most common cause of liver disease worldwide and the third most common indication for liver transplantation in North America.² Unhealthy dietary patterns and unbalanced nutrients are not only associated with the development and progression of fatty liver but is also a risk factor for metabolic syndrome.³ Patients with NAFLD often consume more saturated fatty acid (SFA), which has adverse effects on lipid and glucose homeostasis, which in turn aggravate the progression of metabolic syndrome and possibly NAFLD.^{4,5} Commercial butter contains high percentage of saturated fat. Fats rich in saturated fatty acids can result in the elevation of plasma total and lipoprotein cholesterol.⁶

Recent literatures suggest that some food supplements are also helpful for the treatment of NAFLD. Garlic is known for its herbal remedy for a long time. Studies

have shown extensive beneficial effects of garlic, which include hypocholesterolemic, hypoglycemic, antihypertensive, anticancer and antioxidant effects.⁷ Acetaminophen and carbon tetrachloride-induced acute liver injury in animal models have shown hepatoprotective effects of garlic extract.⁸ However, the protective effects of garlic in fatty liver is yet to be investigated. In this study role of fresh crushed garlic on high-fat induced hepatocellular damage is assessed in rat model.

MATERIALS AND METHODS:

This study was carried out after approval in the department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Center (JPMC), Karachi for 4 weeks from 1st -30th October 2008. The experimental animals used in this study were albino rats, 30 in number, with weights ranging from 220-240 gram. They were obtained from the animal house of BMSI. The animals were observed for one week before the beginning of the study, for the evaluation of their health status and the amount of food intake. The animals were divided into three groups according to the dietary scheme.

Group A (n=10) served as control. They were fed normal diet.⁹

Group B (n=10) was given high-fat diet (10 gm butter in 100 gm of diet).¹⁰

Group C (n=10) received same amount of high-fat diet as in group B, along with 6% fresh crushed garlic in the diet.¹¹

The animals were weighed and kept in cages. They were supplied calculated amount of food and water ad libitum under laboratory conditions of light, humidity and temperature. Animals were sacrificed at the end of study period that is after 4 weeks.

The animals were anaesthetized by ether and fixed to a dissecting board. A longitudinal midline incision was made in the abdomen. Liver was removed and its weight was recorded. It was washed with normal saline and fixed in buffered neutral formalin for 24 hours. Then it was processed in ascending grades of alcohol, embedded in paraffin¹² and 4 micron (4µm) thick sections were

✉ Dr. Aisha Qamar

Associate Professor
Department of Anatomy
Bahria University Medical and Dental College
Karachi
Email: asaeeuddin@yahoo.com

✉ Dr. Humera Waqar

Lecturer
Department of Anatomy
Bahria University Medical and Dental College
Karachi

✉ Mohammad Saad Saeeduddin

Final year MBBS Student
Dow University of Health Sciences
Karachi

Received: 26-08-2016

Revised: 30-09-2016

Accepted: 03-10-2016

cut on rotary microtome. Sections were stained with Haematoxylin and Eosin¹² to study general architecture of the liver. The results were evaluated by paired student “t” test. P-value was considered for significant differences.

RESULTS:

The mean values of initial and final body weight in control group A were 234.1± 2.12gm and 248.8± 2.41gm respectively. The mean values of initial and final body weight in high-fat diet treated group B were 234.0±1.58gm and 265.2±0.71gm respectively. The data showed a significant increase (P<0.01) in the final body weight of control and group B animals when compared to initial body weight (Table 1). The mean values of initial and final body weight in high-fat with garlic treated group C were 234.0±3.20gm and 235.0±3.50gm respectively. There was non-significant increase in final body weight in group C when compared to initial body weight in the same group and a significant decrease (P<0.05) in the final body weight in group C when compared with high-fat diet treated group B (Table 1). The mean values of absolute liver weight in control group A, high-fat diet treated group B and high-fat with garlic treated group C were 5.32±0.23gm, 8.22±0.35gm and 5.80±0.11gm respectively. This data showed that there was highly significant increase (P<0.001) in absolute liver weight in group B when compared to control group A. Compared to high-fat diet treated group B, there was a significant decrease (P<0.005) in group C animals (Table 2).

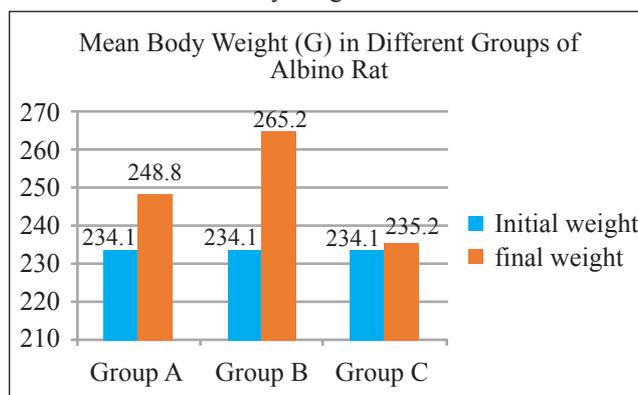
The Haematoxylin and Eosin stained sections in control group A showed normal and intact architecture of hepatic lobules. Portal areas were normal, with few mononuclear cells in the area of portal triad, which consisted of normally arranged portal vein, hepatic artery and bile duct. Hepatocytes were rounded to polygonal in shape, with uniform eosinophilic cytoplasm and identifiable boundaries. Nuclei were centrally placed, round in shape, with fine, regular and even distribution of chromatin. Few binucleate cells were also seen. Sinusoids showed variation in caliber, with clear boundaries and distinct endothelial lining. Fixed monocytes (Kupffer cells) were present in the lining of sinusoids (Figure 1).

Haematoxylin and Eosin stained sections in high-fat diet treated group B showed that normal architecture of hepatic lobules was altered. Portal areas were shrunken due to ballooning of hepatocytes. The hepatocytes appeared empty due to dissolution of lipid granules, with shrunken and pyknotic nuclei. Some cells appeared empty due to absence of nuclei. Their cytoplasm showed mild granularity and small vacuoles due to accumulation of fat globules. The nuclei showed disrupted chromatin pattern. Binucleate cells were also frequent. The sinusoids were irregular in shape, at some places they were very narrow, and at other places they appeared dilated. The monocyte infiltration was increased (Figure 2).

The hepatic lobular architecture in high-fat diet with garlic treated group C revealed disruption in Haematoxylin and Eosin stained tissue sections. Portal triads

were dilated, with dilated portal vein and normal lymphocytic infiltration. Hepatocytes were normal in size similar to control, although some cells still showed empty spaces due to presence of lipid granules. Most of the hepatocyte nuclei had normal chromatin pattern with distinct nucleoli. Binucleate cells were frequent. Kupffer cells were prominent (Figure 3).

Table: 1
Mean body weight of animals



*Mean ±SEM

Table: 2
Mean absolute weight of liver (G) in different groups of Albino rat

Groups	Treatment Given	Absolute weight (G) of liver at the time of sacrifice
A (n=10)	ND	5.32±0.23
B (n=10)	High SFD	8.22±0.35
C (n=10)	High SFD + Garlic	5.80±0.11

*Mean ±SEM

Figure: 1

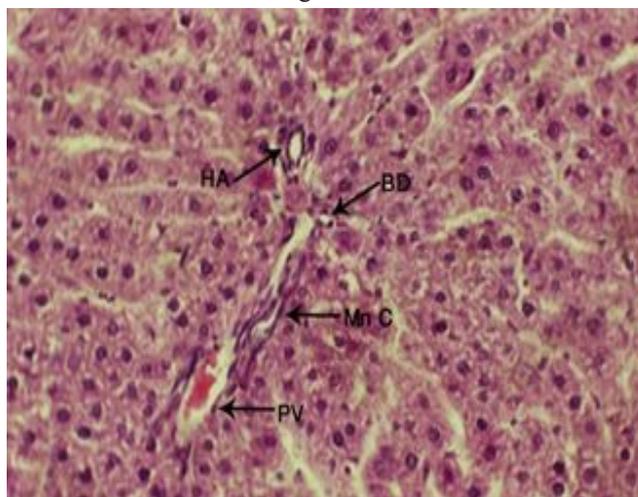


Figure-1: H and E stained, 4 µm thick section of control rat liver showing portal triad, with portal vein (PV), hepatic artery (HA), bile duct (BD) and mononuclear cells (Mn C) (Photomicrograph x400).

Figure: 2



Figure-2: H and E stained, 4 μm thick section from high fat diet treated rat liver showing shrunken portal triad due to ballooned hepatocytes (BaH) and increased mononuclear infiltration (Mn C) (Photomicrograph x400).

Figure: 3

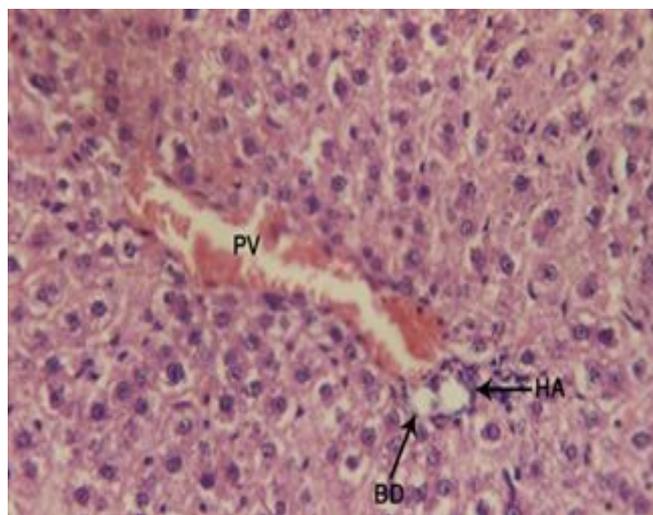


Figure-3: H and E stained, 4 μm thick section from high fat diet with garlic treated rat liver showing dilated portal triad, with dilated portal vein (PV), hepatic artery (HA), bile duct (BD) and normal mononuclear infiltration (Mn C) (Photomicrograph x400).

DISCUSSION:

Nutrients in the diet regulate cellular functions at different levels. Recent research has shown that the total amount of fat in the diet is not really significant; what really matters is the type of the fat and the total calories in the diet.^{13,14} Various studies have revealed that diet high in saturated fatty acids, such as butter increases the level of total cholesterol, low density lipoprotein and triglycerides.¹⁵

The worldwide epidemic of obesity has brought extensive consciousness about associated abnormalities, such as non-alcoholic fatty liver disease,¹⁶ the pathogenesis of which is directly related to the high intake of fats, leading to the accumulation of lipids, primarily triglycerides into the liver.^{17,18} With the increasing prevalence of

nonalcoholic fatty liver disease, researchers have become more focused to investigate the efficacy of everyday natural foods for their protective effect on liver injury. Of these, garlic has been used for a long time for its cholesterol lowering effect,¹⁹ so it was used in this study to assess its protective role on high-fat diet induced liver injury.

The high-fat diet treated group B animals gained significant body weight in comparison to control animals. This was because of the intake of more total calories as well as more calories from the fat as compared to the control, leading to enhanced fat deposition in liver and subcutaneous adipose tissue of body, as suggested by another study²⁰ that intake of more calories is associated with obesity. Studies have^{21,22} observed significant body weight gain after feeding high-palm oil diet as compared to standard diet. The high-fat diet with garlic treated group C animals showed a significant decrease in body weight as compared to group B animals. This was due to the fact that garlic inhibits synthesis of fats in the body by inhibiting related enzymes in the liver. This was also observed by Bari,²³ who found that garlic supplementation in diet decreased hepatic activities of lipogenic enzymes such as malic enzyme, fatty acid synthase, glucose-6-phosphate dehydrogenase and 3-hydroxy-3-methyl-glutaryl-Co A reductase (HMG Co A reductase).

The increase in absolute liver weight in high-fat diet treated group B animals was due to the fatty change observed in these animals as compared to control, causing hypertrophy of hepatocytes due to the accumulation of fat globules, as also seen by Bari²³ in high-fat diet treated mice.

The significant decrease in absolute liver weight of liver in group C animals as compared to group B was due to the protection provided by garlic administration. These findings were similar to another study²⁴ who also observed significant reduction in liver weight in alcohol fed rats after simultaneous feeding of garlic protein (500mg/kg body weight/day for 45 days) as compared to alcohol fed controls. They described that this effect was due to reduced activity of HMG Co A reductase in liver and increased hepatic degradation of cholesterol to bile acids.

The histopathological findings of the present study revealed ballooned hepatocytes, along with disruption of hepatic lobular architecture in high-fat diet treated group B animals. This fatty change was due to intake of excessive fat into the diet, leading to excess deposition of triglycerides in the hepatocytes. Excess fats also caused damage to cell membranes by lipid peroxidation, leading to dilatation of veins. Two other studies^{25,26} have reported abundant mononuclear inflammatory cells in H and E stained sections in high fat diet treated group and pronounced vacuolated hepatocytes with focal necrosis in periportal areas.

The observations of present study in group C revealed protective effect of garlic as it restored architecture of liver tissue to a great extent. Portal areas were dilated but mononuclear infiltration was normal. Some

hepatocytes showed empty vacuoles, depiction presence of lipid with shrunken nuclei. The size of hepatocytes significantly decreased similar to control. These findings were in conformity with the study of Xiao, who had used S-allylmercaptocysteine, a water-soluble compound of aged garlic in high-fat diet treated rats. Garlic reduces the crucial events which contribute to the liver injury such as oxidative stress, inflammation, and necrosis.²⁷

CONCLUSION:

Garlic has restorative role in high-fat induced hepatocellular damage. Fatty liver produced by high quantities of saturated fats, such as butter can be ameliorated by the use of garlic.

REFERENCES:

1. Fan JG, Jia JD, Li YM. Guidelines for the diagnosis and management of nonalcoholic fatty liver disease: update. *Chinese Journal of Hepatology* 2010; 18:163-6.
2. Chalasani N, Younossi Z, Lavine JE. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Disease Gastroenterology. *Gastroenterology* 2012; 142: 1592-609.
3. Carthy EM, Rinella ME. The role of diet and nutrient composition in nonalcoholic Fatty liver disease. *J. Acad. Nutr. Diet.* 2012; 112: 401-9.
4. Mouzaki M, Allard JP. The role of nutrients in the development, progression, and treatment of nonalcoholic fatty liver disease. *J. Clin. Gastroenterol.* 2012; 46: 457-67.
5. Carvalhana S, Machado MV, Cortez-Pinto H. Improving dietary patterns in patients with nonalcoholic fatty liver disease. *Curr Opin Clin Nutr Metab. Care* 2012; 15: 468-73.
6. Hartvigsen K, Binder CJ, Hansen LF. A diet-induced hypercholesterolemic murine model to study atherogenesis without obesity and metabolic syndrome. *Arteriosclerosis, Thrombosis and Vascular Biology.* 2007; 27: 878-85.
7. Sobenin IA, Andrianova IV, Fomchenkov IV, Gorchakova TV, Orekhov AN. Time-released garlic powder tablets lower systolic and diastolic blood pressure in men with mild and moderate arterial hypertension. *Hypertens Res* 2009;32:433-7.
8. Xiao J, Liang EC, Ling MT. S-allylmercaptocysteine reduces carbon tetrachloride-induced hepatic oxidative stress and necroinflammation via nuclear factor kappa B-dependent pathways in mice. *Eur J Nutr* 2012; 51: 323-33.
9. Mahan LK, Arlin MT. Krause's Food, Nutrition and Diet Therapy. 8th ed. Philadelphia: W.B. Saunders Company, 1992 .p.726-7.
10. Woods SC, Seeley RJ, Rushing PA, D'Alessio D, Tso P. Controlled High-fat Diet Induces an Obese Syndrome in Rats. *J Nutr* 2003; 133: 1081-7.
11. Qureshi AA, Abuirmeileh N, Din ZZ. Inhibition of cholesterol and fatty acid biosynthesis in liver enzymes and chicken hepatocytes by polar fractions of garlic. *Lipids* 1983; 18: 343-8.
12. Bancroft JD, Gamble M. Theory and practical of Histological Techniques. 6th ed. Edinburgh: Elsevier Churchill Livingstone, 2008.p. 25-7, 134-5, 177-8.
13. Adams LA, Sanderson S, Lindor KD, Anguld P. The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with subsequent liver biopsies. *Journal of Hepatology*, 2005; 42 (1): 132-8.
14. Elsayed HH, Elrahman MK, Emara AH, El-Hafez AA. Compare Effect of fatty acid Composition (Olive oil, Coconut Oil and Butter) on Adipose Liver tissue, and Serum Lipid Profile in Albino Rats. *IOSR Journal of Biotechnology and Biochemistry*, 2015; 1(3): 28-38.
15. Yassin ME, Haggag E, Elsanhoty RM, Ramadan MF. Impact of dietary oils and fats on lipid peroxidation in liver and blood of albino rats. *Asian Pacific Journal of Tropical Biomedicine*, 2014; 4(1): 52-8.
16. Brunt EM. Pathology of fatty liver disease. *Modern Pathology*, 2007; 20: 40-8.
17. Bloomgarden ZT. Relationship between insulin resistance and nonalcoholic fatty liver disease, and aspects of insulin resistance in children and adolescence. *Diabetes Care*, 2007; 30 (6): 1663-9.
18. Aller R, Izoala O, Fuente B, Luis D. Mediterranean diet is associated with liver histology in patients with non-alcoholic fatty liver disease. *Nutr Hosp*, 2015; 32 (6): 2518-24.
19. Qamar A, Siddiqui A, Kumar H. Fresh garlic amelioration of high-fat-diet induced fatty liver in albino rats. *Journal of Pakistan Medical Association*, 2015; 65: 1102-7.
20. Fauci AS, Kasper DL, Longo DL, Braunwald E, Hauser SL, Jameson JL, Loscalzo J: Harrison's Principles of Internal Medicine. 17th ed., New York, McGraw-Hill Companies, 2008 .p.1982-83.
21. Jen KC, Buisson A, Pellizzon M, Ordiz F, Ana LS, Brown J. Differential effects of fatty acids and exercise on body weight regulation and metabolism in female wistar rats. *Experimental Biology and Medicine*, 2003; 228: 843-9.
22. Yeh Y, Liu L. Cholesterol-lowering effects of garlic extracts and organosulfur compounds: human and animal studies. *Journal of Nutrition*; 2001:131: 9895-938.
23. Bari O, Neuschwander-Tetri BA, Liu M, Portincasa P, Wang DQH. Ezetimibe: Its novel effects on the prevention and the treatment of cholesterol gallstones and nonalcoholic fatty liver disease. *Journal of Lipids*, 2012; Article ID 302847, 16 pages.
24. Rajasree CR, Rajmohan T, Augusti KT. Antiatherogenic and antiperoxidative effects of garlic and soy proteins in alcohol fed rats. *Indian Journal of Experimental Biology* 2008; 47: 1169-75.
25. Lieber CS, Leo MA, Mak KM, Xu Y, Cao Q, Ren C et al. Model of Non-alcoholic Steatohepatitis. *American Journal of Clinical Nutrition* 2004; 79: 502-9.
26. Das D, Mukherjee M, Das AS, Mukherjee S, Mitra C. Aqueous extract of black tea (*Camellia sinensis*) prevents chronic ethanol toxicity. *Current Science*, 2005; 88: 952-61
27. Xiao J, Guo R, Fung M, Liang EC, Chang RCC, Ching Y et al. Garlic-Derived S-Allylmercaptocysteine Ameliorates Nonalcoholic Fatty Liver Disease in a Rat Model through Inhibition of Apoptosis and Enhancing Autophagy. *Evidence-Based Complementary and Alternative Medicine* 2013; article ID 642920: 11 pages.

