ORIGINAL ARTICLE

Effects of Ginger Extract on Hyperlipidemic Diet Induced Non Acholic Fatty Liver Disease

Hafsa Nisar, Rehana Rana, Shazia Imran

ABSTRACT

Objective: To determine the effects of ginger extract on the histomorphological changes of fatty liver of hyperlipidemic diet induced NAFLD.

Study Design: It was randomized control trial.

Place and Duration of Study: The study was carried out from October 2012 to March 2013, in the department of Anatomy, Islamic International Medical College, Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad.

Materials and Methods: A total of 35 male albino mice were used and divided into 3 groups. The control group (C) was fed on normal laboratory diet, while the remaining two groups, fatty group (FG), ginger group (GN), were fed on hyperlipidemic diet for twelve weeks to induce hyperlipidemia/nonalcoholic fatty liver. Then GN group with induced hyperlipidemia/ fatty liver was administered normal laboratory diet with ginger extract as a drink in replacement of water for another twelve weeks.

Results: Total body weight was reduced as compared to their initial body weights. The histological examinations of this study revealed reverse fatty (steatotic) changes and showed marked reduction in number of fat globules, ballooning degeneration, glycogenated nuclei.

Conclusion: This research shows that ginger extract has marked antihepatotoxic effects. Ginger extract ameliorates high fat induced fatty liver disease.

Key words: *Nonalcoholic fatty liver disease, Hyperlipidemia, Steatotic, Glycogenated nucleiand ginger.*

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a common histopathological condition characterized by significant deposition of lipids mainly triglycerides in the hepatocytes of the liver parenchyma.[1] Histologically it exists as simple steatosis occupying >5% of hepatocytes in the absence of significant inflammation and hepatocellular damage and sometimes fibrosis.[2] It resembles alcohol-induced liver injury histologically, but by definition it occurs in patients with little or no history of alcohol consumption. NAFLD is the most common chronic liver disease in USA and considered to be increasing in Asia Pacific region including South Asia.[3] NAFLD affects approximately 15-40% of general population and its prevalence is increasing worldwide. The prevalence increases to 50-75% in obese individuals.[4] The community prevalence of NAFLD in South Asia and South East Asia ranges from 5- 30%.[5] Recently a hospital based study in Pakistan had shown a frequency of approximately 14% however, there is no community based study from Pakistan to the best of our knowledge.[6] NAFLD is emerging as one of the most common causes of chronic liver disease.[7] Primary NAFLD is related to insulin resistance and thus frequently occurs as part of the metabolic changes that accompany obesity, diabetes, and hyperlipidemias.[7] This research was done among study groups with an aim to see the reverse histomorphological effects of ginger extract on non-alcoholic fatty liver disease. Ginger is a popular spice. For centuries it's been used as a medicinal plant. It has been discovered to possess many pharmacological activities, such as antioxidant, anti-inflammatory, anti-arthritic, anti-migraine, anti-thrombotic, anti-inflammatory, hypolipidemic, hypocholesterolaemic and anti-nausea properties making it a useful medication for a variety of disorders. The predominant pungent constituents of ginger are gingerols and shogaols which are responsible for many of its medicinal properties.[8] It has also been reported that ginger decreases the level of cholesterol and improves high-fat diet, fructose, cholesterol, or streptozocin-induced lipid derangements in rodents.[9]

Correspondence:
Dr. Hafsa Nisar
Department of Anatomy
Islamic International Medical College, Rawalpindi
E-mail: hafsanisar@gmail.com

Materials and Methods

This study was randomized control trial and was approved by the Institutional Review Committee of Riphah International University before its

commencement. Thirty-five adult male BALB/c mice having weight of 35-50g and age between 10-12 weeks were obtained from animal house of NIH, Islamabad where they were kept under standard laboratory conditions. Mice were randomly divided into 3 groups. The control group C (n = 10) was fed a standard pellet diet with tap water to drink. The fatty group FG (n = 10) was fed a hyperlipidemic diet for 12 twelve weeks to induce non-alcoholic fatty liver disease. This diet consisted of standard pellets supplemented with 4% cholesterol powder and 40% butter with tap water to drink. The ginger treated group GN (n = 15) was fed on hyperlipidemic diet for 12 twelve weeks, after which this group was given standard diet and their drink was substituted with ginger extract for a period of another twelve weeks. Ginger extract was prepared by soaking fifteen grams of ginger rhizome slices in 500 ml of boiling water for 30 min and were then filtered.[1] Mice were weighed at zero and twelve weeks after establishing fatty liver. Then again after further twelve weeks before sacrificing ginger treated group. Animals of group C and group FG were sacrificed at the end of 12 weeks while group GN were sacrificed at the end of 24 weeks. Animals were anaesthetized. They were dissected and liver was removed and preserved in containers containing 10% formalin. Tissue processing and embedding was done in paraffin. Slides were prepared and stained with haematoxylin and eosin. Special staining was done with Masson Trichrome for the demonstration of fibrosis. Microscopic study was done under 40X objective. Slides were studied for the histopathological criteria of diagnosis of NAFLD, which were macrovesicular fatty change in hepatocytes with displacement of nucleus to the periphery of the cell. Additional presence of features like ballooning degeneration, glycogenated nuclei, inflammatory infiltrates predominantly periportal or perivenular and fibrosis were also observed. All measurements were taken by using an ocular square reticule micrometer fitted into the eyepiece of the microscope. Statistical analysis was done in SPSS version 20.0. Results were compared by applying t-test and ANOVA. A p–value of <0.05 was considered as statistically significant.

Results

Body weight and histological analysis of all experimental animals were done. The mean initial and final body weights of animals in control group were 50.10g (SD ± 5.26) and 52.90g (SD ± 5.47) in control group respectively. In control group, there was significant increase in weight ($p < 0.001$). (Table I)

The mean initial and final body weights of animals in FG group were 50.50g (SD± 5.72) and 54.40g (SD ± 5.48) respectively. It was significantly increased ($p < 0.001$).The mean initial and final body weights of animals in group GN were 48g (SD ± 2.78) and 40.04g (SD ± 7.02) respectively. Weight was significantly decreased in group GN ($p < 0.001$). (Table I)

All the groups had similar initial weight with insignificant difference ($p = 0.1$). (Table I)Final weight was significantly different in all the groups ($p<0.001$). (Table I)

All five histological parameters were observed qualitatively as well as quantitatively. Hematoxylin and Eosin staining was used for fat globules, ballooning, abnormal nuclei, inflammatory infiltrate and Masson Trichrome stain was used to demonstrate the presence of fibrosis. Fat globules were absent in all (100%) animals in control group C; it was present in all (100%) animals in experimental group FG, in 6 (40%) animals in experimental group GN. Number of fat globules were counted and compared with control. Fat globules were significantly higher in experimental group FG followed by experimental group GN ($p < 0.001$). (Fig 1)

Ballooning degeneration was not present in any animal (0%) in control group C; it was present in all the animals (100%) in group FG, in 7 (46.7%) animals in group GN. Ballooning degeneration was significantly higher in experimental group FG followed by experimental group GN ($p < 0.001$). (Fig 1)

Inflammatory infiltrate were absent in all (100%) animals in control group C, it was present in 9 (90%) animals in experimental group FG, in 13 (86.7%) animals in experimental group GN. inflammatory infiltrate were significantly higher in group FG, group GN as compared to control group ($p < 0.001$). (Fig 1)

Abnormal nuclei were absent in all (100%) animals in control group C, it was present in 9 (90%) animals in experimental group FG, in 8 (53.3%) animals in experimental group GN. Abnormal nuclei were significantly higher in experimental group FG

followed by experimental group GN (p = 0.001). (Fig 1)

Fibrosis was absent in all (100%) animals in control group C, it was present in 8 (80%) animals in experimental group FG, in 5 (33.3%) animals in experimental group GN. Fibrosis was significantly higher in experimental group FG followed by experimental group GN (p = 0.003). (Fig 1)

Table I: Inter and Intra Group Comparison of Initial and Final Weight of Mice (n=35)

Groups	Initial Weight with SD	Final Weight with SD	p-value
Control Group C (n = 10)	50.10± 5.26	52.90 ± 5.47	< 0.001
Group FG (n = 10)	50.50 ± 5.72	54.40 ± 5.48	< 0.001
Group GN (n = 15)	48.00 ± 2.78	40.04 ± 7.02	< 0.001
p-value	0.116	< 0.001	

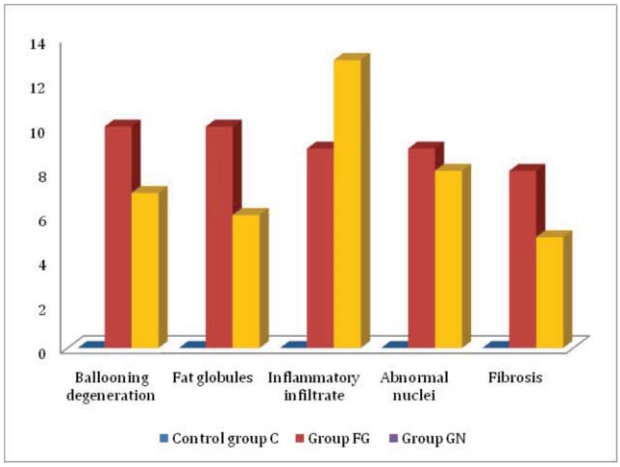

Fig 1: Description of Qualitative Microscopic Parameters of liver in all the Groups

Discussion

In the present study, it was observed that there was significant increase in the weight of experiment group FG after having hyperlipidemic diet for a period of twelve weeks that is initial and final body weights were 50.50g and 54.40g respectively. Our result was in accordance with previous work done by M. H Ahmida[1] reporting that after administration of a hyperlipidemic diet in mice induced significant weight gain in his experiment in positive control group of rats. Weight went down from initial value of 48g to final weight of 40.04gin GN group. In ginger treated group there was significant decrease in body weight which is similar to the findings of Al-Amin[9] in which the diabetic rats lost their weight after having ginger as a drink for a period of two weeks. Histological examination of slides of control group at low power revealed polyhedral hepatocytes with rounded vesicular nuclei. At high power anastomosing cords of hepatocytes were seen along with sinusoids lined by endothelial cells and draining into central vein. Examination of liver sections of FG group mice given high fat diet for twelve weeks showed evidence of accumulation of mixed large and small sized fat globules (macro vesicular/micro vesicular steatosis) which indicate the accumulation of lipids mainly in the form of triglycerides in the cytoplasm of the hepatocytes. Due to accumulation of fat globules the nuclei were pushed to the periphery. Many cells were seen having one large vacuole filling the whole cell with thin rim of cytoplasm around and pushing the nucleus to one side. Other cells were having smaller vacuoles with either central or eccentric nuclei. Steatosis is the hallmark histological feature of nonalcoholic fatty liver. In our study mild to moderate steatosis was observed in majority of animals of group FG while in group GN steatosis was markedly reduced to almost nil. These findings were also found by Eman[10] who induced fatty liver in rats by injecting oxytetracyclineintraperitonealy (120mg/kg) for three consecutive days.

Besides there was massive ballooning of hepatocytes and distribution of polymorphonuclear infiltrate in hepatic parenchyma with very little fibrosisin group FG. Many hepatocytes also showed nuclear clear vacuolation due to glycogen accumulation. Glycogenated nuclei were found scattered in the liver parenchyma. Similar or more advanced changes in liver histology were noted by others.[10,11] These findings correlate with the marked increase in serum cholesterol, triglycerides and blood glucose. The increase in these parameter in the blood is in correlation with the fatty degeneration of the liver. Hepatic fibrosis is one of the main consequences of liver disease. It represents wound healing in response to chronic insult and is final common pathway for more chronic liver disease

regardless of their mechanism.[12,13] As our study was of shorter duration fibrotic changes were not very significant in group FG and GN. Spotty inflammation which was slight periductal and perivenular was observed in group FG. Experimental group FG had significantly higher number of inflammatory infiltrate as compared to experimental group GN (p = 0.004). After giving ginger extract orally the histopathological changes induced by fatty liver disease improved. The presence of polyphenols such as gingerol and curcumin in ginger possesses considerable antioxidant properties including radical scavenging activity and inhibitory effect on lipid peroxidation.[14] In addition ginger protects the liver against hepatotoxic agents by enhancing the hepaticantioxidant activity.[15]

Ginger extract supplementation as a drink for twelve weeks reduced fat globules and ballooning degeneration in group GN. It might be due to its direct radical scavenging activity. The results of this research rejected the null hypothesis thereby making the alternate hypothesis true that states that ginger extract has beneficial effects on fatty liver disease. It would be interesting to work on these herbal medicines in future. Further work can be done to investigate the active components of ginger responsible for the observed beneficial effects in fatty liver disease.

Conclusion

This research shows that ginger extract has marked antihepatotoxic effects. The histological parameters like steatosis, ballooning degeneration, glycogenated nuclei and focal inflammation were significantly changed although hundred percent protections was not provided. Hence our data suggest that ginger extract ameliorates high fat induced fatty liver disease.

REFERENCES

1. Ahmida M, Abuzogaya M. The effects of oral administration of green tea and ginger extracts on serum and hepatic lipid content in rats fed a hyperlipidemic diet. J Appl Sci Res. 2009;5:1709-13.
2. Adams L, Angulo P. Treatment of non-alcoholic fatty liver disease. Postgraduate medical journal. 2006;82(967):315-22.
3. Amarapurkar DN, Hashimoto E, Lesmana LA, Sollano JD, Chen PJ, Goh KL. How common is non-alcoholic fatty liver disease in the Asia–Pacific region and are there local differences? Journal of gastroenterology and hepatology. 2007;22(6):788-93.
4. Browning JD, Szczepaniak LS, Dobbins R, Horton JD, Cohen JC, Grundy SM, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology. 2004;40(6):1387-95.
5. Farrell GC, Wong VW-S, Chitturi S. NAFLD in Asia-as common and important as in the West. Nature Reviews Gastroenterology and Hepatology. 2013;10(5):307-18.
6. Parkash O, Hamid S. Are we ready for a new epidemic of under recognized liver disease in South Asia especially in Pakistan? Non alcoholic fatty liver disease. JPMA The Journal of the Pakistan Medical Association. 2013;63(1):95-9.
7. Angulo P. Nonalcoholic fatty liver disease. New England Journal of Medicine. 2002;346(16):1221-31.
8. Sahebkar A. Potential efficacy of ginger as a natural supplement for nonalcoholic fatty liver disease. World journal of gastroenterology: WJG.17(2):271-2.
9. Al-Amin ZM, Thomson M, Al-Qattan KK, Peltonen-Shalaby R, Ali M. Anti-diabetic and hypolipidaemic properties of ginger (Zingiber officinale) in streptozotocin-induced diabetic rats. British Journal of Nutrition. 2006;96(04):660-6.
10. Eman G.E. Helal, Samia M. Abd El-Wahab AMMSaGAZ. Effect of Zingiber officinale on fatty liver induced by oxytetracycline in albino rats. The Egyptian Journal of Hospital Medicine. 2012; 46: 26-42.
11. Bose M, Lambert JD, Ju J, Reuhl KR, Shapses SA, Yang CS. The major green tea polyphenol,(-)-epigallocatechin-3-gallate, inhibits obesity, metabolic syndrome, and fatty liver disease in high-fat–fed mice. The Journal of nutrition. 2008;138(9):1677-83.
12. Brunt EM. Pathology of nonalcoholic fatty liver disease. Nature Reviews Gastroenterology and Hepatology.7(4):195-203.
13. Mofrad P, Contos MJ, Haque M, Sargeant C, Fisher RA, Luketic VA, et al. Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. Hepatology. 2003;37(6):1286-92.
14. Stoilova I, Krastanov A, Stoyanova A, Denev P, Gargova S. Antioxidant activity of a ginger extract (Zingiber officinale). Food chemistry. 2007;102(3):764-70.
15. Ajith T, Hema U, Aswathy M. Zingiber officinale Roscoe prevents acetaminophen-induced acute hepatotoxicity by enhancing hepatic antioxidant status. Food and chemical toxicology. 2007;45(11):2267-72.