PREVENTIVE ROLE OF FRESH GARLIC ON HIGH-FAT DIET INDUCED FATTY LIVER IN ALBINO RATS.

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ABSTRACT

Objective:

Obesity has become a worldwide problem due to westernization of diet, even in developing countries leading to consumption of high-fat diet. One of the most common diseases resulting from use of high-fat diet is fatty liver. Garlic is one of the oldest herbs used for medicinal purposes since ancient times. So the present study was undertaken to observe the protective role of fresh garlic on high-fat diet induced fatty liver in albino rats and correlate the results with previous studies.

Methods:

Thirty adult albino rats, weighing from 200-240 gram were taken for this study. The rats were divided into 3 groups according to dietary regimen, Group A received control diet, Group B received high- fat diet (20 ml corn oil in 100 gm of diet) and Group C received high-unsaturated fat diet with fresh garlic (20 ml corn oil with 6 gm fresh garlic in 100 gm of diet), for 8 weeks at the end of which they were sacrificed and tissue slides stained with haematoxylin and eosin.

Results:

There was marked increase in weights in rats receiving high fat diet. Haematoxylin and eosin stained sections showed moderate fatty infiltration with empty spaces in hepatocytes with absent or pyknotic nuclei in high-fat group which were preserved to a great extent in group receiving fresh garlic along with high-fat diet.

Conclusion:

This study has proved that use of fresh garlic along with high-fat diet prevents its damaging effects in liver to a great extent.

Keywords:

Obesity, fatty liver, garlic.

INTRODUCTION

Fatty liver is a common disease. Nonalcoholic fatty liver disease (NAFLD) was first described in 1950s when fatty liver was diagnosed in a group of obese patients. The prevalence of NAFLD ranges from 14-20% in United States and Europe and relates directly to the incidence of obesity[1]. The prevalence increases to 57.5-74% in obese population. NAFLD is highly prevalent among Asians[2]. The major cause to develop obesity in humans is High-fat diet[3-4].

Fatty change or steatosis refers to abnormal accumulation of fats within parenchymal cells. It may be caused by toxins, starvation, obesity and anoxia. When mild, it does not affect cellular function and is easily reversible[5-9].

Herbal medicines, derived from plant extracts are currently being evaluated to treat a variety of diseases[10]. Among natural foods, garlic has attracted a great deal of attention[11]. Garlic has been used as a medicine for thousands of years but its use was not supported scientifically and pharmacologically until recently[12]. Garlic reduces risk of cardiovascular diseases and cancer, stimulates immune function, enhances detoxification of foreign compounds and hepatoprotection, it is antimicrobial and antioxidant as well[13].

Therefore, the aim of the present study was to evaluate the effects of high fat diet on liver and whether garlic provides protection against development of fatty liver, induced by high fat diet.

MATERIAL AND METHODS

This study was conducted in the department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Center (JPMC), Karachi, for 8 weeks from October to November 2008.

Thirty (30) healthy and active adult albino rats of either sex, aged between 90-120 days, and weighing from 200-240 gram were selected for the present study. The animals were divided into three groups A, B, & C according to the diet plan as given in table-1:

GROUP A: comprising of 10 animals served as control. They were fed the normal diet.

GROUP B: comprising of 10 animals received high-fat diet (HFD), that is, corn oil in a dose of 20 ml in 100 gram of diet.

GROUP C: comprising of 10 animals received high-unsaturated-fat diet along with 6 gram garlic (Table-1).

The animals were kept for 01 week prior to the commencement of study, for acclimatization to the environment, assessment of their health status and diet intake. The animals were weighed and kept in cages, with twelve hour light and dark cycle, under laboratory environment. Calculated amount of food with respective constituents and water ad libitum was supplied to them and they were weighed fortnightly. The animals were sacrificed at the end of eight weeks. A midline, longitudinal incision was given. After exposure, gross appearance of liver was noted. Liver was removed from the body and weighed. Liver was cut into 2 halves. Right lobe was fixed in buffered neutral formalin for 24 hours. The tissues were processed by dehydration through ascending grades of alcohol (70%, 80%, 95% and two changes of 100%). Then tissues were cleared in two changes of xylene. Tissues were embedded at 59^{o}C in Tissue Embedding system. 4 micron thick sections were made, fixed on hot plate at 37^{o}C, stained with haematoxylin and eosin[14], studied under 10X and 40X objectives of light microscope, and results were recorded. The results were evaluated by student “t” test. P-value was considered for significant differences.

RESULTS

There was significant increase ($P<0.001$) in final body weight in rats in group B, when compared with control. The weight gain in group C animals was minimal as compared to group B & A animals (Table-2).

There was also highly significant increase ($P<0.001$) in weight of liver in fat-treated group B animals as compared to control. Whereas the weight of liver in group C animals was highly significantly decreased ($P< 0.001$) as compared to group B animals (Table-3).

There was a moderately significant increase ($P<0.001$) in relative liver weight in animals in fat treated group B as compared to control, whereas the relative liver weight in group C animals was moderately significantly decreased ($P<0.005$) as compared to fat treated group (Table-4).

The H and E stained sections in control animals showed normal and intact architecture of hepatic lobules. Lobules were roughly hexagonal in shape. Hepatocytes were arranged in the form of anastomosing cords, radiating from the central vein. Hepatocytes showed distinct boundaries with uniformly distributed eosinophilic cytoplasm. Nuclei were rounded in shape, centrally located. The chromatin pattern in the nuclei of hepatocytes appeared to be normal, with fine, regular and even distribution. Few binucleate cells were also seen. Sinusoids showed variation in caliber, ranging between these variations was taken as normal. Fixed monocytes (Kupffer cells) were present in the lining of sinusoids. Portal triads were normal, with few mononuclear cells in the area of portal triad, which consisted of normally arranged portal vein, hepatic artery and bile duct.

TABLE-1
DIET SCHEME/DAY FOR EXPERIMENTAL STUDY OF FATTY LIVER IN ALBINO RATS

Nutrients of Diet	Normal diet	High fat-diet	High-fat diet + Garlic
Wheat flour(G)	11.2	11.2	11.2
Milk Powder(G)	2.8	2.8	2.8
Chick peas(G)	2	2	2
Corn oil(ml)	-	3.2	3.2
Garlic(G)	-	-	0.96
Drinking water	Ad libitum	Ad libitum	Ad libitum

TABLE – 2
|*MEAN BODY WEIGHT (G) IN DIFFERENT GROUPS OF ALBINO RATS AT VARIABLE TIME INTERVAL

Groups	Initial wt	Observation period-1 wk	End of 2nd wk	End of 4th wk	End of 6th wk	End of 8th wk
A (n=10)	220.7±4.03	225.2±3.20	233.0±3.31	241.5±3.18	249.1±3.28	257.7±3.37
B (n=10)	223.6±4.11	228.0±4.49	236.0±6.40	244.0±6.30	264.6±5.46	285.4±5.36
C (n=10)	223.6±3.35	228.4±3.37	233.4±2.99	236.4±2.69	240.3±3.11	240.6±3.61

STATISTICAL ANALYSIS OF DIFFERENCES IN MEAN BODY WEIGHT BETWEEN DIFFERENT GROUPS

Groups	P-value, End of 2nd wk	P-value, end of 4th wk	P-value, end of 6th wk	P-value, end of 8th wk
A vs. B	>0.05*	>0.05*	<0.01***	<0.001****
B vs. C	>0.05*	<0.05**	<0.05**	<0.01***

Key: Insignificant* Significant** Moderately significant*** Highly significant****

TABLE – 3
***MEAN ABSOLUTE WEIGHT OF LIVER (G) IN DIFFERENT GROUPS OF ALBINO RAT**

Groups	Treatment given	Absolute weight (G) of liver
A (n=10)	ND	5.97±0.19
B (n=10)	High-fat diet	9.20±0.63
C (n=10)	High-fat diet + Garlic	6.25±0.30

STATISTICAL ANALYSIS OF MEAN ABSOLUTE WEIGHT OF LIVER BETWEEN DIFFERENT GROUPS OF ALBINO RAT

Statistical comparison	P-Value
B vs. A	P<0.001****
C vs. B	P<0.001****
C vs. A	P>0.05*

Key: Insignificant* Significant** Moderately significant*** Highly significant****

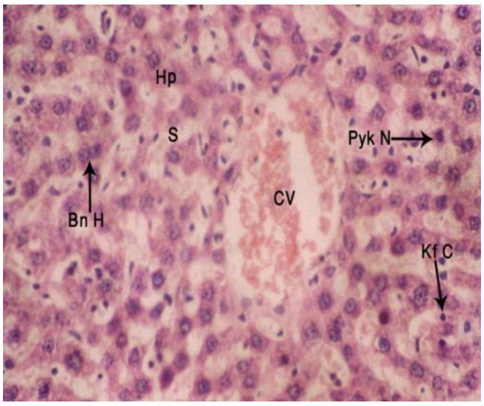
TABLE-4
***MEAN RELATIVE LIVER WEIGHT (G/100G) IN DIFFERENT GROUPS OF ALBINO RAT**

Groups	Treatment given	Relative Liver weight (G/100G)
A (n=10)	Normal diet	2.49±0.12
B (n=10)	High-fat diet	3.23±0.16
C (n=10)	High-fat diet + Garlic	2.59±0.12

STATISTICAL ANALYSIS OF MEAN RELATIVE LIVER WEIGHT IN DIFFERENT GROUPS OF ALBINO RAT

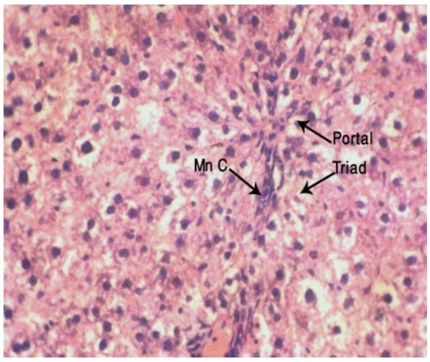
Statistical comparison	P-Value
B vs. A	P<0.01***
C vs. B	P<0.005***
C vs. A	P>0.05*

Key: Insignificant* Significant** Moderately significant*** Highly significant****



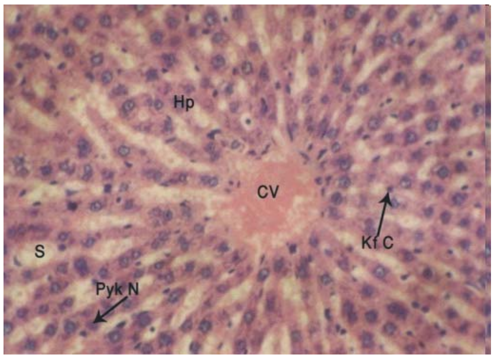
Photomicrograp showing intact liver architecture, With dilated central vein CV, hepatocytes Hp, showing pyknotic nuclei & congested sinusoids S & Kupffer cells Kf C in fat-treated Group under 40 X magnification

FIG. 2:



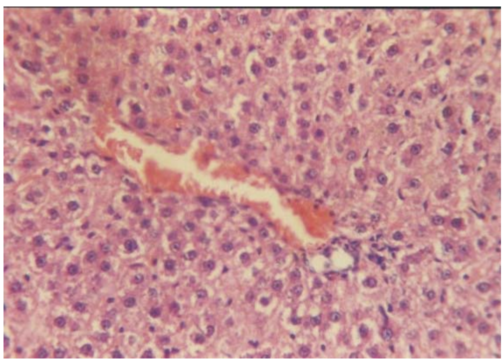
Photomicrograph showing empty, vacuolated hepatocytes Around a shrunken Portal Triad with normal lymphocytic infiltration in fat-treated animal under under 40 X magnification

FIG. 3:



Photomicrograph showing preserved hepatic architecture with central vein CV, regular arrangement of hepatocytes Hp in hepatic cords HC, with congested sinusoids in fat with garlic treated animals under 40 X magnification.

FIG. 4:



Photomicrograph showing dilated Portal Triad With normal hepatocytes arranged in hepatic cords in Fat with garlic-treated animals under 40 X.

The H and E stained sections in high fat treated group showed that lobular architecture was preserved. Central veins were dilated and congested. Sinusoids were also slightly dilated. Hepatocytes were swollen with indistinct cell membranes and large empty spaces due to presence of fat vacuoles. Many cells showed pyknotic nuclei, nuclei with clumped chromatin and absent nucleoli. Kupffer cells were prominent (fig-1). Portal areas were dilated with mononuclear infiltration and ballooned hepatocytes (fig-2).

The H and E stained sections in corn oil with garlic treated group showed preservation of lobular architecture. Central veins were congested but not dilated. Sinusoids were shrunken due to increased size of hepatocytes. Hepatocytes were larger in size with granular cytoplasm. Nuclei were also larger with intact chromatin pattern. Pyknotic nuclei were less. Kupffer cells were normal (fig-3). Portal areas were dilated with normal mononuclear infiltration (fig-4).

DISCUSSION

Fat accumulation resulting from the imbalance in the input/output/oxidation of fatty acids results in disturbed energy metabolism. Fatty liver is characterized by altered lipid metabolism and increased oxidative stress. The mechanisms leading to fatty liver development are still not completely understood at the molecular level, however, an excess dietary fat results in increased fatty acid oxidation in mitochondria and peroxisomes, leading to increased release of reactive oxygen species and hydrogen peroxide from mitochondria and peroxisomes respectively[15].

The animals in fat treated group B gained significant body weight as compared to control. This was due to intake of more calories by these animals which resulted in the deposition of more fats in the liver and possibly in other organs as well, as explained by Kumar et al[9]. This result was in accordance to Jen et al[16] who used high-soybean oil diet to observe differential effects of fatty acids and exercise on body weight regulation and metabolism in female Wistar rats. They suggested that body weight gain was probably due to intake of Linoleic acid, which was positively associated with blood insulin levels, and it may impair insulin action in animals and humans, and cause body weight gain.

The animals in high fat diet with garlic treated group C had moderately significant decrease in body weight when they were compared to high fat treated group B animals. This was probably 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 Yeh and Liu[12], who found that garlic supplementation in diet reduced the hepatic activities of lipogenic enzymes such as malic enzyme, fatty acid synthase, glucose-6-phosphate dehydrogenates and 3-hydroxy-3-methyl-glutaryl-Co-A reductase (HMG-Co-A reductase).

The increase in absolute and relative liver weight in high fat (corn oil) treated group B animals was due to fatty change observed in these animals as compared to control, causing hypertrophy of hepatocytes, accumulation of fat, congestion of veins and sinusoids. This finding was in accordance to Zulet et al[17] and Buettner et al[18]. Zulet et al[17] found highly significant increase in absolute liver weight in those animals fed hypercholesterolemia-inducing diet. The results of Buettner et al[18] were in agreement to the present study in response to absolute liver weight, but it was in disagreement in relation to relative liver weight.

The significant decrease in absolute and relative weight of liver in group C (corn oil with garlic) animals as compared to group B was due to the protection provided by garlic administration. In this group, there was less deposition of fat (fatty change) in liver, less congestion of central veins, absent congestion in sinusoids and decrease in the size of hepatocytes, as given in morphological findings. These findings were in accordance to Rajasree et al[19] 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 are in agreement with the findings of Lickteig et al[20]. The H & E stained sections in high fat treated group B showed congested and dilated central veins and sinusoids. Hepatocytes also showed empty spaces depicting presence of lipid granules, which were abundant in the periphery of hepatic lobule, close to portal triad. Some of the hepatocytes showed nuclei which became pyknotic (fig-1 and 2). These findings were due to influx of excessive fat into the diet, which could not be oxidized or leave the organ as enough apoproteins were not available, leading to excess deposition of triglycerides in the hepatocytes. Excess fats also caused damage to cell membranes by lipid per oxidation, leading to dilatation of veins, as described by Kumar et al[9].

Lieber et al[21] and Das et al[22], in their study 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 groups C (corn oil with garlic) showed that protective effect of garlic reverted hepatic lobular architecture similar to control. Mononuclear infiltration was also normal. There was occasional deposition of fat at distant places. Some of the cells, however, showed pyknotic nuclei. In the present study, garlic prevented hepatic fat accumulation in many ways, by inhibiting lipogenic enzymes in liver; by its antioxidant action, which prevents lipid per oxidation of cell membranes and by enhancing hepatic degradation of cholesterol to bile acids.

Hussein et al[23] studied the ethanol-induced hepatotoxicity in male rats with garlic oil prevention and explained the presence of antioxidants such as selenium and germanium in garlic, which normalize the oxygen utilization in cells and prevent free radical damage, which reversed the microanatomy of liver to normal.

In the light of above mentioned facts, it is stated that even excess quantity of corn oil (20 % of diet) can lead to fatty liver, however, the effects of high fat diet induced fatty change can be ameliorated to a great extent by concomitant use of fresh garlic in the diet.

CONCLUSION

The result of study concluded that fresh garlic is highly preventive against hepatic damage produced by high fat diet so the use of fresh garlic should be promoted in daily life.

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