ORIGINAL ARTICLE

Toxic Effects Of Corn Oil On Liver Histology In Albino Rats

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ABSTRACT:

Objective: To evaluate whether high quantities of unsaturated fats such as corn oil can produce fatty liver in albino rats.

Materials and Methods: This experimental study was carried out at BMSI, JPMC after obtaining ethical approval, from October to November 2008. Forty adult albino rats, weighing 200 to 240 grams were divided into 2 groups according to dietary regimen. Group A received control diet and Group B received high-corn oil diet (20 ml corn oil in 100 gm of diet).The groups were further divided into A1, A2 and B1, B2 on the basis of duration of treatment, that is 4 weeks and 8 weeks respectively. The rats were sacrificed, liver removed and processed for haematoxylin and eosin staining.

Results: Haematoxylin and eosin stained sections revealed swollen hepatocytes having vesicular appearance with absent or pyknotic nuclei in high-corn oil group as compared to control animals.

Conclusion: This study has proved that use of high quantities of unsaturated fats, such as corn oil can lead to fatty liver.

Key words: High-corn oil diet, Fatty liver, Steatohepatitis, Metabolic Syndrome

INTRODUCTION:

The prevalence of obesity due to excessive consumption of carbohydrate and fat, and inadequate energy expenditure has increased dramatically over the past decade. It is recognized as a low grade and chronic inflammatory state, which leads to the metabolic syndrome, including insulin resistance, type II diabetes, atherosclerosis, hypertension, and fatty liver disease[1-3].

There has been a drastic change in the human diet over the past half century or so, especially in the types of fats and fatty acids consumed. Use of vegetable oil in cooking food is a common practice all around the globe. Of them, the good oil must have a hypolipidemic action. However, this action of cooking oils has led to their excessive use, leading to chronic diseases such as cardiovascular diseases, obesity, cancer, immune-related diseases as well as fatty liver[4,5]. In industrialized nations, on average, over 40% or more of daily energy intake is supplied by dietary fats, although most nutritional guidelines recommend that caloric intake from fats should not be more than 30% of daily caloric intake. 1gram of fat provides 9 Kcal or 38 KJ/gram after complete oxidation, about more than twice that for the same weight of carbohydrates or proteins[6,7].Although polyunsaturated fatty acids inhibit synthesis of triglycerides in the body, they are considered as risk factor for free radical formation. It is a known fact that polyunsaturated fatty acids(PUFA) are more susceptible to lipid peroxidation than saturated fatty acid.[8,9,10,11,12] Corn oil has been shown to enhance liver injury and steatosis in rats after chronic alcohol intake[13].Corn oil is a good source of polyunsaturated fatty acid and its nutritional properties are excellent as it is cholesterol free. The fatty acids found in corn oil are Linoleic acid, Oleic acid, Palmitic acid, Stearic acid and Myristic acid[14].

The westernization of diet has led to chronic liver pathologies not only in western world, but their incidence is increasing at an alarming rate in developing nations as well. The prevalence of non-alcoholic fatty liver disease (NAFLD) is increasing in proportion with the prevalence of obesity[15]. NAFLD has been estimated to occur in 35% of lean and up to 70% of obese patients[16].It ranges from simple fatty liver (steatosis) to nonalcoholic steatohepatitis (NASH) and even cirrhosis, leading to hepatocellular carcinoma. Key events that contribute to the initiation and progression of NAFLD are described as a multi-hit model. In this model, dysregulated metabolism of free fatty acids (FFAs) is considered as the first-hit of pathogenesis, which leads to insulin resistance and fat accumulation in the liver Inflammatory response, oxidative stress, apoptosis, and even autophagy serve as "following-hits" that contribute to the ongoing inflammation[17,18].With this back ground, since only a few studies are available that evaluated the role of corn oil on the liver if used under a high-fat diet regimen, this study was undertaken to assess the effects of high-corn oil diet on the histology of liver.

MATERIALS AND METHODS:

This study was conducted in the department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Center, Karachi after obtaining ethical approval for 8 weeks from October to November 2008.Forty adult, healthy albino rats, 90-120 old, weighing 200 to 240 gram were taken for this prospective experimental study. The susceptibility of rats to develop fatty liver in response to a high-fat (HF)

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Received: 30-4-2015
Revised: 8-6-2015
Accepted: 10-6-2015

diet is same as in humans[19]. The animals were observed for one week prior to the commencement of the study for the assessment of their health status and amount of food intake. The animals were divided into two groups according to type of diet (Table-1).

Table: 1

Calories of Diet/day for Experimental Albino rats

		Normal Diet				High-Corn Oil Diet			
Items of Diet	Quantity	Energy	Fat (G)	Protein (G)	Carb (G)	Energy	Fat (G)	Protein (G)	Carb (G)
Wheat flour (G)	11.2	38.08	0.22	1.34	7.84	38.08	0.22	1.34	7.84
Chick peas (G)	2	4.93	0.07	0.26	0.82	4.93	0.07	0.26	0.82
Milk powder (G)	2.8	14.05	0.72	0.7	1.62	14.05	0.72	0.7	1.62
Corn oil(ml)	3.2	-	-	-	-	26.56	2.94	-	-
Drinking water	Ad Libitum	-	-	-	-	-	-	-	-
Final Energy	-	57.06*,3.56**	1.01	2.3	10.28	83.62*,4.35**	3.95	2.3	10.28

Key:*Kcal, **Kcal/G of diet, Carb = Carbohydrate

Each group was further divided into two subgroups A1, A2 and B1, B2 based on the duration of treatment i.e. 4 and 8 weeks respectively. Each subgroup had 10 animals. In this study, unsaturated fat was used in the form of corn oil Coroli, made by Cebag ME, Abu Dhabi in the dose of 20 ml/100 gm (20%) of diet20. It contained 830 Kcal, 92 gm fats/100ml, out of which 54 gm were polyunsaturated, 26 gm monounsaturated and 12 gm were saturated, and free of cholesterol. Group A: comprising of 20 animals served as control. They received normal diet[21].

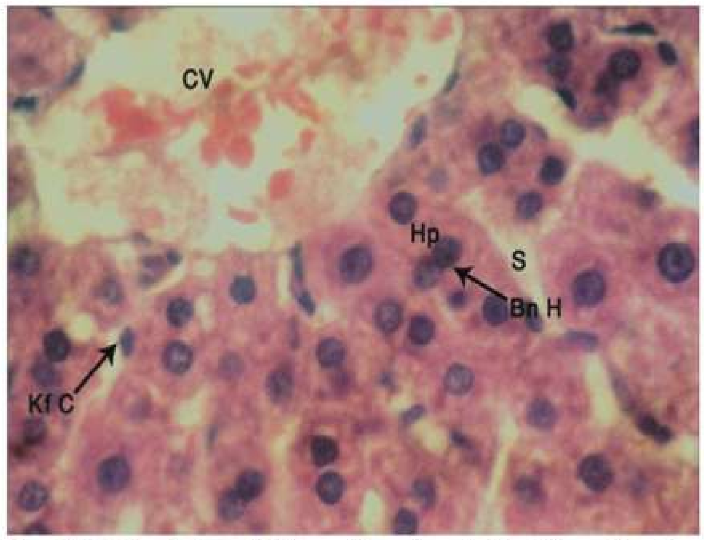
Group B: comprising of 20 animals received high-corn oil diet (20 ml corn oil in 100 gm of diet).

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 provided to them (Table-1). The animals were sacrificed at the end of the study period.A midline, longitudinal incision was given in the abdomen. Liver was exposed by incising the diaphragm and was removed and washed with normal saline. A block of tissue comprising of 2mm was taken from the right lobe and fixed in buffered neutral formalin for 24 hours. Then it was processed in ascending grades of alcohol and embedded in paraffin. Four micron (4μm) thick tissue sections were cut on rotatory microtome and taken on albumenized glass slides. Paraffin embedded tissue was stained with Haematoxylin and Eosin[22] to study the general architecture of liver tissue under oil immersion lens.

RESULTS:

The haematoxylin and eosin stained sections in control group A showed polygonal hepatocytes arranged in the form of anastomosing plates, one cell thick, separated by the anastomosing sinusoids draining into the central vein. The plates of cells as well as sinusoids radiated from the central vein to the periphery of the lobule. Central vein had normal caliber, with distinct endothelial lining. Hepatocytes showed distinct boundaries with uniformly distributed, granular, acidophilic cytoplasm. Nuclei were large and spherical and occupied the center of the cell, showing even distribution of chromatin. Binucleate cells were also seen. Hepatic sinusoids showed variation in caliber, but lining was smooth and endothelial cells were visible. Fixed monocytes (Kupffer cells) were present in the lining of sinusoids (Figure-1).

Figure: 1

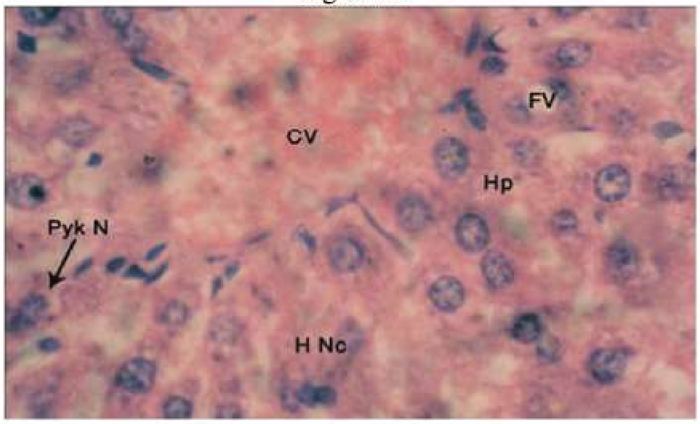


H and E stained, 4μm thick section of control rat liver showing hepatocytes (Hp), central vein (CV), binucleate hepatocytes (Bn H),regular sinusoids (S) and Kupffer cells (Kf C) (Photomicrograph x1000).

The haematoxylin and eosin stained sections in subgroup B-1 showed that normal anastomosing pattern of hepatocytes was retained. Central vein was congested. Sinusoids had varying calibers, at some places they were narrow, whereas at other places they were dilated. Hepatocytes showed vesicular appearance due to the presence of fat granules which were washed out during tissue processing. Nuclei of many hepatocytes were pyknotic. Many hepatocytes showed nuclei with clumped chromatin and disrupted nucleoli. Vacuolated hepatocytes were seen depicting presence of coalesced lipid granules. Necrosed hepatocytes were also seen. Kupffer cells were prominent (Figure-2).

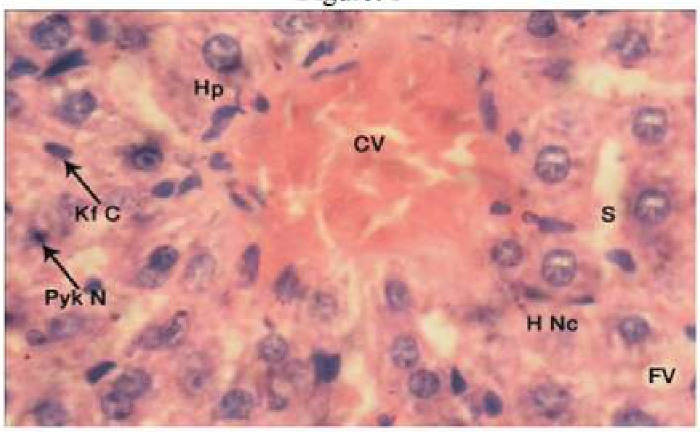
In subgroup B-2, hepatic lobular architecture was highly disorganized. Central vein was dilated and congested. Sinusoids were also slightly dilated. Hepatocytes were ballooned up with indistinct cell membranes and large empty spaces due to presence of fat vacuoles. Hepatocyte nuclei had all stages of disintegration. Many cells showed pyknotic nuclei, nuclei with clumped chromatin and absent nucleoli. Necrosed hepatocytes were also visible. Kupffer cells were prominent (Fig-3).

Figure: 2



H and E stained, 4μm thick section of rat liver showing central vein (CV), hepatocytes (Hp), with pyknotic nuclei (Pyk N), fat vacuoles (FV) and necrosed hepatocytes (H Nc) after 4 weeks treatment with corn oil (Photomicrograph x1000).

Figure: 3



H and E stained, 4μm thick section of rat liver showing central vein (CV)hepatocyte (Hp) with pyknotic nuclei (Pyk N), fat vacuole (FV), necrosed hepatocytes (H Nc) and prominent Kupffer cells (Kf C) in the lining of sinusoids (S) after 8 weeks treatment with corn oil (Photomicrograph x1000).

DISCUSSION:

Corn oil is a commonly used food item. Dietary fat composition is believed to affect body weight regulation independent of the quantity of fat consumed[23]. Accumulation of fat results from an imbalance between input/output/oxidation of fatty acids leading to altered impaired lipid metabolism[24].

The present study was designed to observe if high quantity of unsaturated fat, with zero cholesterol can lead hepatic damage in albino rats, because it is a general belief that unsaturated fats are harmless. High unsaturated fat diet was used in the form of corn oil in the dose of 3.2 ml along with normal diet, which makes 20% (20 ml in 100 gm of diet), same as used by Tannenbaum[20] to observe the effect of high-fat diet on basal and stress-induced hypothalamic-pituitary-adrenal activity in adult male rats. In this study, there was preservation of hepatic lobular architecture in 4-week high-corn oil treated animals, although they had developed marked fatty infiltration in hepatocytes. This fat accumulation resulted from the imbalance between the energy intake and energy output as the rats were confined to their cages in addition to receiving high-corn oil diet. Similar changes were observed by Polavarapu11 who demonstrated fatty infiltration in hepatocytes along with necrosis when corn oil was given in addition to ethanol in diet having 35% calories from fat. These findings were also similar to Alarifi[25], who also observed vacuolar degeneration in the cytoplasm of hepatocytes with necrotic foci in lead acetate induced hepatic damage in albino mice. Furthermore, these animals did not show any improvement in liver histology after giving corn oil. The tissue sections from 8 week, high-corn oil treated animal`s revealed disorganization of hepatic lobular architecture, with dilated and congested central vein and sinusoids. Hepatocytes were ballooned with damaged cell membranes and large empty spaces due to the presence of fat vacuoles. Hepatocyte nuclei were pyknotic, with disintegrated chromatin and disrupted nucleoli. Necrosed hepatocytes were also visible. The hepatic damage seen in these animals was a time related process. The accumulation of fat in hepatocytes was due to increased influx of fats because of increased amount of corn oil in the diet (3.95 gm of fat as compared to 1.01gm in control animals). This resulted in accumulation of fat within the hepatocytes in the form of vacuoles as excess fat could not be removed from the liver because enough apo-proteins were not available to transport excess fat from the liver. These findings were in accordance to Tinikios[15] who observed hepatocellular ballooning, that is enlarged hepatocytes, with rarefied cytoplasm, having a reticulated appearance predominantly located in acinar zone 3, around central vein, in liver biopsy from high fat diet using individuals. They have demonstrated that in NAFLD, the processes of lipid transport from liver to the peripheral adipose tissues for storage are dysregulated with resultant increased intra hepatocellular lipids. Excessive lipid storage in hepatocytes contributes to organelle failure, including mitochondrial dysfunction and endoplasmic reticulum(ER) and other organelle stress, and may play a role in hepatic insulin resistance.

CONCLUSION:

Polyunsaturated fats, such as corn oil, if used in excess amount produce hepatocellular damage. In this study, high amount of corn oil in diet (3.2 ml per hundred gram of diet) resulted in more than 40% calories from fat, as compared to recommended nutritional guide lines, in which calories from fat should be less than 30% of the diet. Thus vegetable oil, without cholesterol can produce fatty liver. This study can form a base line for the extension of its results to human subjects.

REFERENCES:

1. Yamazaki T, Kishimoto K, Miura S, Ezaki O. Dietary ß-conglycinin prevents fatty liver induced by a high-fat diet by a decrease in peroxisome proliferator-activated receptor ?2 protein. Journal of Nutritional Biochemistry, 2012; 23: 123–32.
2. Lin H, Chen C, Chen Y, Lin Y, Mersmann HJ, Ding S. Enhanced Amelioration of High-Fat Diet-Induced Fatty

Liver by Docosahexaenoic Acid and Lysine Supplementations. Bio Med Research International, 2014; Volume 2014, Article ID 310981, 11 pages http://dx.doi.org/10.1155/2014/310981.

3. Lombardo YB, Hein G, Chicco A. Metabolic syndrome: Effects of n-3 PUFAs on a model of dyslipidemia, insulin resistance and adiposity. Lipids, 2007; 42: 427-37.
4. Ahmed IS, Hussein MM, Soliman MM, Abdelrehman AA. Comparative studies on the effect of high fish and corn oil diet on Lipid profiles, PPAR-a and FAS mRNA in Swiss mice. Journal of investigational Biochemistry, 2012; 1 (1): 31-7.
5. Macarulla MT, Fernández-Quintela A, Zabala A, Navarro V, Echevarría E, Churruca I et al. Effects of conjugated linoleic acid on liver composition and fatty acid oxidation are isomer-dependant in hamster. Nutrition, 2005; 21: 512–9.
6. Ervin RB, Wright JD, Wang C, Kennedy J. Dietary intake of fats and fatty acids for the Unites States population: 1999-2000Advance Data. 2004; 348: 1-6.
7. Nelson DL, Cox MM.Lehninger Principles of Biochemistry. 4th ed., New York, W.H. Freeman and Company, 2005; 343-6, 631-9.
8. Hwang J. Diets with corn oil and low protein increase acute acetaminophen hepatotoxicity compared to diets with beef tallow in a rat model. Nutrition Research and Practice 2009;3(2):95-101
9. Lu YF, Lu S. Influence of dietary fat saturationon lipid peroxidationof serum and low density lipoprotein in rat .Nutr Res 2002;22:463-72
10. Ramadan MF, Amer MM, Awad A. Changes of lipid profile by dietary vegetable oil blends in rats with hypercholesterolemia. Food SciTechnolInt 2009;15:119-30
11. Polavarapu R, Spitz DR, Sim JE, Follansbee MH, Oberley LW, Rahemtulla A et al. Increased Lipid Peroxidation and Impaired Antioxidant Enzyme Function Is Associated With Pathological Liver Injury in Experimental Alcoholic Liver Disease in Rats Fed Diets High in Corn Oil and Fish Oil. Hepatology, 1998; 27 (5): 1317-23.
12. Haggag MS, Elsanhoty RM, Ramadan MF. Impact of dietary oils and fats on lipid peroxidation in liver and blood of albino rats. Asian Pac J Trop Biomed, 2014;4(1): 52-8.
13. Rivera CA, Abrams SH, Tcharmtchi MH, Allman M, Ziba TT, Finegold MJ et al. Feeding a corn oil/sucrose enriched diet enhances steatohepatitis in sedentary rats. Am J Physiol Gastrointest liver Physiol, 2003; 296: 386-93.
14. Edem DO, Akpanabiatu MI. Effects of Palm Oil-containing Diets on Enzyme Activities of Rats. Pakistan Journal of Nutrition, 2006; 5:301-5.
15. Tiniakos DG, Vos MB, Elizabeth M. Brunt EM. Nonalcoholic Fatty Liver Disease: Pathology and Pathogenesis. Annu. Rev. Pathol. Mech. Dis. 2010; 5:145–71.
16. Baumgardner JN, Shankar K, Hennings L, Badger TM,Ronis MJ. A new model for nonalcoholic steatohepatitis in the rat utilizing total enteral nutrition to overfeed a high-polyunsaturated fat diet. Am J Physiol Gastrointest Liver Physiol, 2008;294: 27–38.
17. Xiao J, Guo R, Fung M, Liong EC, Chang RCC, Chng Yet al . Garlic-Derived S-Allyl -mercapto -cysteine Ameliorates Nonalcoholic Fatty Liver Disease in a Rat Model through Inhibition of Apoptosis and Enhancing Autophagy. Evidence-Based Complementary and Alternative Medicine, 2013; Volume 2013, Article ID 642920, 11 pages http://dx.doi.org/10.1155/2013/642920
18. El-Sheekh MM, Hamad SM, Gomaa M. Protective effects of Spirulina on the liver function and hyperlipidemia of rats and human. Brazilian archives of Biology and Technology, 2014; 57 (1): 77-86.
19. Takahash Y, Soejima Y, Fukusato T .Animal models of nonalcoholic fatty liver disease nonalcoholic steatohepatitis . World J Gastroenterol2012; 18(19):2300-8
20. Tannenbaum BM, Brindley DN, Tannenbaum GS. High-fat feeding alters both basal and stress-induced hypothalamic-pituitary-adrenal activity in the rat. Am J Physiol Endocrinol Metab, 1997; 273 (6): 1168-77
21. Mahan LK, Arlin MT. Krause's Food, Nutrition and Diet Therapy. 8th ed., Philadelphia, W.B. Saunders Company, 1992; p 726-7
22. Bancroft JD. Gamble M. Theory and practical of Histological Techniques. Edinburgh: Elsevier Churchill Livingstone, 6th Edition, 2008; 25-7, 134-5
23. Rolland V, Roseau S, Fromentin G, Nicolaidis S, Tome D, Even PC. Body weight, body composition, and energy metabolism in lean and obese zucker rats fed soybean oil or butter. American Journal of Clinical Nutrition, 2002; 75: 21-30.
24. Grasselli E, Canesi L, Voci A, Matteis RD, Demori I, Fugassa E et al. Effects of 3,5-Diiodo-L-Thyronine Administration on the liver of High Fat Diet-Fed Rats. Journal of Experimental Biology and Medicine, 2007; 233:549-57.
25. Alarifi SA, Aldahmash BA, El-Nagar DM , Dkhil MA. Effect of Corn oil, flaxseed oil and black seed oil on lead acetate-induced hepatic tissue damage: A histological study. Journal of Medicinal Plants Research, 2012; 6 (24): 4128-34.