ORIGINAL RESEARCH PAPER

Volume-8 | Issue-8 | August - 2019 | PRINT ISSN No. 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

TO OBSERVE THE EFFECT OF HIGH FATTY DIET ON THE HISTOLOGY OF LIVER IN ALBINO RATS.

Anatomy

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ABSTRACT

INTRODUCTION: fat which is an essential component of a balanced diet and very important for many physiological and metabolic processes of the body, if given in excess amount causes hepatic injury

MATERIAL AND METHODOLOGY: The present study was carried in the postgraduate department of the anatomy where 30 male albino rats weighing on average 100-150 grams were divided into three groups. Group A served as control. These rats were fed on normal diet containing less that 2% of fat. Group B animals received high fat diet containing >30% of the total caloric requirement. Group C received high fat diet for 10 weeks followed by pure vegetarian diet for next ten weeks. One animal from group A and two from group B were sacrificed at the end of 2nd,4th,6th and 10th weeks, and histopathological changes were recorded. After 10weeks, one animal from group A and two animals from group C were sacrificed at the end of 12th, 14th, 16th, 18th and 20th weeks. Observations were recorded.

RESULTS: In our study we found that fat causes histopathological changes in the hepatocytes in a dose and duration dependent manner . Thus we conclude that in the form of steatosis, macro and micro vesicular fatty infiltration, inflammatory changes like periportal inflammation in zone 1,2 and 3.Excess of fat also cause hepatitis if taken for short interval of time. These are dose and duration dependent. In our study we found that fat induced hepatic injury .In summary we found that high content of animal fat in diet causes hepatic injury which is dose and duration dependent.

KEYWORDS

Fat, Hepatic injury, steatosis

INTRODUCTION

Fat is an important constituent of our diet. It is an important source of energy for body. But excess of animal fat in our diet is not good for Liver. It gets deposited within the hepatocytes and within the liver parenchyma producing fat induced hepatic injury which in advanced form leads to hepatic inflammation (hepatitis), derangement of hepatic microscopic architecture and impaired Liver functions. The Nonalcoholic fatty liver disease (NFLD) is diagnosed clinically and also by a variety of tests which besides laboratory Parameters include ultrasonography, CT scan, MRI and Liver Biopsy. It is pathologically divided into three grades and 4 stages by Brunt et al(1).NAFLD is associated with metabolic syndrome which includes type II diabetes, obesity and hypertriglyceridemia. It is managed clinically by change in life style such as reduced dietary fat intake and regular exercises. Accordingly, oral hypoglycemic drugs like metformin, Pioglitazone and lipid lowering drugs like statins (atorvastatin, Rosuvastatin) are used in its medical management. But these drugs are not safe and free of complications.

Each lobule of Liver is made up of millions of hepatic cells (hepatocytes) which are the basic metabolic units. The lobules are held together by a fine dense irregular fibro elastic connective tissue layer which extends into the structure of the liver, by accompanying the vessels (veins and arteries), ducts and nerves through the hepatic portal, as a fibrous capsule called Gilson's capsule(2).The liver is a metabolically active organ responsible for many vital life functions.

The primary functions of the liver are:

- Production and excretion of bile.
- Excretion of bilirubin, cholesterol, hormones and drugs
- Metabolism of fats, proteins and carbohydrates
- Enzyme activation
- Storage of glycogen, vitamins and minerals
- Synthesis of plasma proteins such as albumin and clotting factors
- Blood detoxication and purification

Due to these important activities, the liver is exposed to a number of insults and is one of body's organs most commonly subjected to injury. In humans the biliary system consists of the gallbladder and the hepatic, cystic and common bile ducts. The primary function of the gallbladder is to store and concentrate bile. Bile salts mix with ingested fats to promote absorption of fats. After its formation in the liver, bile flows into right and left hepatic duct. The hepatic ducts join to form common hepatic duct with which joins the cystic duct of the gallbladder to form the common bile duct, which then enters the duodenum at the ampulla of Vater. The sphincter of Oddi surrounds the ampulla of Vater. When stomach contents especially fats and protein enters the duodenum, cholecystokinin is released from the duodenal mucosal cells to stimulate the contraction of the gallbladder and relaxation of the sphincter of Oddi so that bile can enter the small intestine.

In contrast to humans, Albino rats like ruminants lack gallbladder. The function of the Gallbladder like concentration of bile is performed by cells lining the hepatobiliary duct system. Microscopic anatomy does not vary in humans and Albino rats.

Microscopically, the lobules are roughly hexagonal, and consist of plates of hepatocytes radiating from a central vein (3) which joins the hepatic vein to carry blood out from the liver.

Histologically, there are two major types of liver cell: parenchymal cells and non-parenchymal cells. 70– 85% of the liver volume is occupied by parenchymal hepatocytes. The typical hepatocyte is cubical with sides of 20-30 µm.Hepatocytes display an eosinophilic cytoplasm, reflecting numerous mitochondria, and basophilic stippling due to large amounts of rough endoplasmic reticulum and free ribosomes. The average life span of the hepatocyte is five months; and is able to regenerate.Hepatocyte nuclei are round with dispersed chromatin and prominent nucleoli. Anisokaryosis (or variation in the size of the nuclei) is common and often reflects tetraploidy and other degrees of polyploidy, a normal feature of 30-40% of hepatocytes in the adult human liver. Binucleate cells are also common in humans Hepatocytes are organized into plates separated by vascular channels (sinusoids).Non-parenchymal cells constitute40% of the total number of liver cells.Theliver sinusoids are lined with two types of cell, sinusoidal endothelial cells, and phagocytic Kupffer cells(4).Hepatic stellate cells are non-parenchymal cells found in the presinusoidal space, between a sinusoid and a hepatocyte (5). Additionally, intrahepatic lymphocytes are often present in the sinusoidal lumen.

Three main energy yielding components of our diet are carbohydrates, proteins and fats. Most of the body fat (99%) in adipose tissue is in the form of triglycerides. In normal human subjects, adipose tissue

constitutes between 10-15 per cent of body weight.

There are three different types of fats:
- Unsaturated fats,
- Saturated fats, and
- Tran's fats.

Unsaturated fats

Unsaturated fats are mainly of vegetable origin and are liquid at room temperature. They are useful fats because they can improve blood cholesterol level and heart disease e .g vegetable oils. There are two kinds of unsaturated fats, Mono-unsaturated fat and polyunsaturated fats.(6)

Saturated fats

Saturated fats are mainly found in animal foods, but a few plant foods are also high in saturated fats. Too much saturated fat in our diet can lead to heart disease and other health problems, such as gaining weight or increasing the risk of heart disease or stroke. One should limit saturated fat to less than 10% of your daily calories (7) e.g. dairy products (butter), meat products, and grain-based desserts.

Trans fats

Trans fatty acids, which are more commonly called Trans fats. There are two types of Trans fats, naturally-occurring and artificial Trans fats(8).Naturally occurring Trans fats can be found in the guts of some animals and foods which are made from these animals. Artificial Trans fats are made in an industrial process that adds hydrogen to liquid vegetable oils to make them more solid. Tran's fats are even worse for cholesterol levels than saturated fats. They are most likely found in frying, baked goods, and processed foods.

Some fatty acids that are set free by the digestion of fats are called essential because they cannot be synthesized in the body from simpler constituents. There are two essential fatty acids (EFAs) in human nutrition: alpha linolenic acid (an omega-3 fatty acid) and linoleic acid (an omega-6 fatty acid)(9,10). Other lipids needed by the body can be synthesized from these and other fat.

Fatty liver is defined where there is excess of fat accumulation in the liver cells. This gradual accumulation of fats will trigger inflammation and injure healthy liver cells. This net retention of TG(Triglycerides) is the prerequisite for the development of non-alcoholic fatty liver disease (NAFLD).(11)

Excess accumulation of fats in the liver cells can create inflammations within and injure the cells. Liver enzymes present within the cells that have specific functions, get released into the blood stream due to injury. **Routine blood tests** will detect this elevation of liver enzymes, a situation where one should suspect that there is indeed an abnormality occurring in the liver. **Image testing's** done further may reveal fatty liver conditions with either the organ appearing bright or hyperechogenic on **ultrasonography**, or less dense on **CT scanning**. However, these testing modalities fail to help in detecting the severe nature of the disease i.e. whether the condition is localized or has begun to spread across the entire organ. For a more accurate diagnosis, liver biopsy is considered the best option. Liver biopsy and histo-pathological examination are important components of the diagnostic evaluation in patients with suspected Non-alcoholic liver disease (NALD). They are the most sensitive and specific means of evaluating the degree of liver cell injury and hepatic fibrosis.

The Brunt classification is the standard used to report NAFLD and nonalcoholic steatohepatitis (NASH) biopsy specimens. In this classification **steatosis** (macro > micro, accentuated in zone 3), **lobular inflammation** (mixed, mild), and hepatocellular ballooning (typically in zone 3) were identified as the necessary components for the diagnosis of NASH. **Fibrosis** was not necessary for the diagnosis of NASH, although it is usually present(12)

Brunt et al classified the necro-inflammatory grades of NASH as **grade 1** (mild), **grade 2** (moderate), and **grade 3** (severe) based on the degree of hepatocellular steatosis, ballooning and disarray, and inflammation (intra lobular and portal). Simultaneously, they proposed a **scoring** system for staging based on the location and extent of fibrosis: **stage 1**, zone 3 peri-sinusoidal fibrosis; **stage 2**, portal fibrosis with the above mentioned stage 1; **stage 3**, bridging fibrosis in addition to stage 2; and **stage 4**, cirrhosis. NASH, the **Clinical Research Network (NASH CRN**) later sub classified stage 1 into 3 categories: stage **1A**, mild peri sinusoidal fibrosis in zone 3; **stage 1B,** moderate peri sinusoidal fibrosis in zone 3; and **stage 1C**, only portal/periportal fibrosis.

Other histological lesions that may be seen in NASH include Mallory-Denkbodies (MDB), mega mitochondria, glycogenated nuclei and iron deposition MDB (previously called Mallory bodies or Mallory's hyaline) (13)are eosinophilic intra cytoplasmic inclusions commonly seen close to the nucleus of ballooned hepatocytes in zone 3.

Therefore Present study was undertaken to evaluate the microscopic changes in the liver of Albino rats fed on fat diet.

MATERIAL AND METHODOLOGY

The Randomised Controlled trial (RCT) Study was conducted in the Post graduate department of Anatomy Government Medical College Srinagar. Thirty (30) Albino rats weighing on an average 100-150 grams in a group were taken from the Animal house of Govt. Medical College Srinagar for the present study.

INCLUSION CRITERIA

- Apparently healthy Albino rats.
- Albino rats with weight between 100-150grams.

EXCLUSION CRITERIA

- Albino rats less than 100 grams.
- Albino Rats showing less physical activity (Lethargic).
- Albino Rats not feeding well (refusal to feed) and suffering from diarrhea.
- Albino rats showing weight loss.
- Rats with generalized hair loss.

The animals were divided into three groups after randomization.

- **Group A: 10 rats (Control Group).**
- **Group B: 10 rats (high fat diet group for 10 Weeks).**
- **Group C: 10 rats (fed on high fat diet for 10 weeks**

Followed by a pure vegetarian diet for another 10 weeks)

All the groups of rats were kept under uniform husbandry conditions in three separate iron cages.

Group A were designated as control Group. They were fed with normal diet containing black gram (3 grams), pellet feed (5 grams) and tap water.

Group B of 10 rats were kept in a separate cage and were fed with Amul butter (1 gram) providing 7.2 kcal of energy which accounts for greater than 30% of daily energy intake. They were also fed with black gram (2gms) and readymade feed (4gms) which provided rest of energy.

Group C of 10 rats were kept in a separate iron Cage. They were fed on high fat diet (>30%) for 10 weeks. After 10 weeks high fat diet was stopped and strict vegetarian diet was given to this group of rats. One animal from group A and two rats from group B were sacrificed at 2^{nd}, 4^{th}, 6^{th}, 8^{th} and 10^{th} week. Two rats from group C and one rat from group A were sacrificed at 12^{th}, 14^{th}, 16^{th}, 18^{th} and 20^{th} week.

In each sitting rats were sacrificed after anesthetizing them with chloroform. The tissues were processed manually for block making using standard histological techniques. Sections measuring 5-6 micrometers were cut and fixed on glass slides.

The tissues were processed manually for block making as follows:The casting and embedding was done with the help of moulds.

Microtomy:

Rotatory type of microtome was used and sectioning of the block was done (5-7 micrometer thickness). The slides were stained with haematoxylin and eosin . The microscopic observations were recorded group wise using light microscope. Appropriate photographs were taken using photographic microscope and labeled. If no microscopic changes were noted in any field, it will be interpreted as nil.

1. If changes were noted in one field per slide was interpreted as 1+.
2. If changes were found in 2 fields per slide, it was interpreted as 2+.
3. If changes were found in more than 2 fields per slide it was

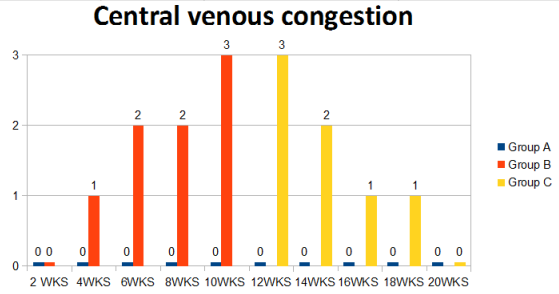
interpreted as 3+.

RESULTS

On light microscopic examination the structure of rat liver resembles that of human beings.

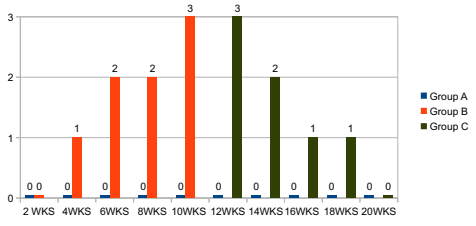
CENTRAL VENOUS CONGESTION

	Group A	Group B	Group C
2 weeks	Nil	Nil	Animal was not Sacrificed.
4 weeks	Nil	1+	Do
6 weeks	Nil	2+	Do
8 weeks	Nil	2+	Do
10 weeks	Nil	3+	Do
12 weeks	Nil	None of the Animals Were left.	3+
12 weeks	Nil	DO	2+
16 weeks	Nil	DO	1+
18 weeks	Nil	DO	1+
20 weeks	Nil	DO	Nil



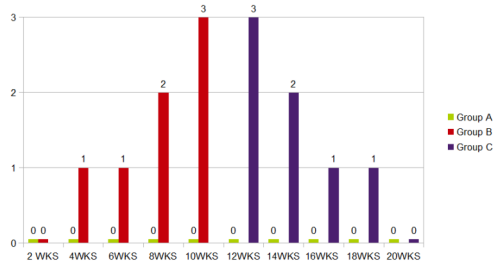
Sinusoidal congestion and dilatation

	Group A	Group B	Group C
2 weeks	Nil	Nil	Animal was not Sacrificed.
4 weeks	Nil	1+	DO
6 weeks	Nil	2+	DO
8 weeks	Nil	2+	DO
10 weeks	Nil	3+	DO
12 weeks	Nil	None of the Animals Were left	3+
12 weeks	Nil	DO	2+
16 weeks	Nil	DO	1+
18 weeks	Nil	DO	1+
20 weeks	Nil	DO	Nil



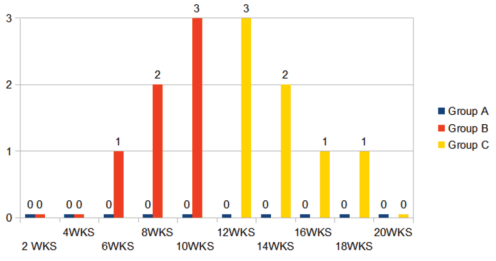
Micro vesicular steatosis

	Group A	Group B	Group C
2 weeks	Nil	Nil	Animal was not Sacrificed.
4 weeks	Nil	1+	DO
6 weeks	Nil	1+	DO
8 weeks	Nil	2+	DO
10 weeks	Nil	3+	DO
12 weeks	Nil	None of the Animals Were left	3+
12 weeks	Nil	DO	2+
16 weeks	Nil	DO	1+
18 weeks	Nil	DO	1+
20 weeks	Nil	DO	Nil



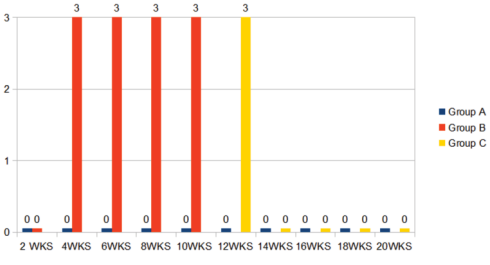
Macrovesicularsteatosis

	Group A	Group B	Group C
2 weeks	Nil	Nil	Animal was not Sacrificed.
4 weeks	Nil	Nil	DO
6 weeks	Nil	1+	DO
8 weeks	Nil	2+	DO
10 weeks	Nil	3+	DO
12 weeks	Nil	None of the Animals Were left	3+
12 weeks	Nil	DO	2+
16 weeks	Nil	DO	1+
18 weeks	Nil	DO	1+
20 weeks	Nil	DO	Nil



Inflammation in zone 1, 2, 3

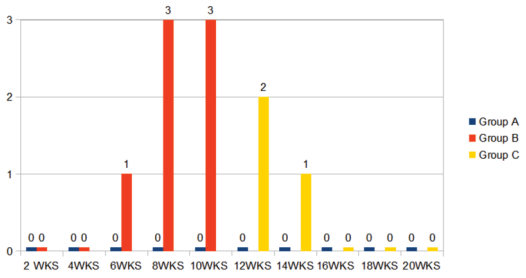
	Group A	Group B	Group C
2 weeks	Nil	Nil	Animal was not Sacrificed.
4 weeks	Nil	3+	DO
6 weeks	Nil	3+	DO
8 weeks	Nil	3+	DO
10 weeks	Nil	3+	DO
12 weeks	Nil	None of the Animals Were left	3+
12 weeks	Nil	DO	Nil
16 weeks	Nil	DO	Nil
18 weeks	Nil	DO	Nil
20 weeks	Nil	DO	Nil



Interstitial hemorrhages

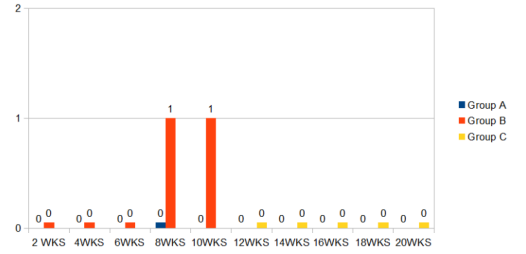
	Group A	Group B	Group C
2 weeks	Nil	Nil	Animal was not Sacrificed.
4 weeks	Nil	Nil	DO
6 weeks	Nil	1+	DO
8 weeks	Nil	3+	DO
10 weeks	Nil	3+	DO
12 weeks	Nil	None of the Animals Were left	2+
12 weeks	Nil	DO	1+

16 weeks	Nil	DO	Nil
18 weeks	Nil	DO	Nil
20 weeks	Nil	DO	Nil



Hepatitis

	Group A	Group B	Group C
2 weeks	Nil	Nil	Animal was not Sacrificed.
4 weeks	Nil	Nil	DO
6 weeks	Nil	Nil	DO
8 weeks	Nil	1+	DO
10 weeks	Nil	1+	DO
12 weeks	Nil	None of the Animals Were left	Nil
12 weeks	Nil	DO	Nil
16 weeks	Nil	DO	Nil
18 weeks	Nil	DO	Nil
20 weeks	Nil	DO	Nil



DISCUSSION

A balanced diet is very important for growth and development of an organism. The three main components of a balanced diet namely carbohydrates, proteins and fat are important for the metabolism, growth and development but excess of any component even with the reduced amounts of other components so as to keep the total calories in the diet same is not safe for individual.

Lot of research has been done to the study the effects of high carbohydrate diet on liver but very less is known about the effect of high fat diet on the histopathology of liver. Thus this study was undertaken to observe the effect of high fat diet on the histology of liver.

Kuzoet al (1970) (14)in their study found that high fat diet (>48% of fat) resulted in weight gain of these experimental animals more than the same animals that were fed on high protein or carbohydrate diet. They also found that high fat diet resulted in impaired carbohydrate metabolism in Wistar albino rats. In our study we divided 30 albino rats into three equal groups A,B and C. Group A rats were fed with normal diet , group B with high fat diet and group C with high fat diet for 10 weeks followed by pure vegetarian diet for another 10 weeks. This study was carried for 20 weeks. In this study we found that albino rats which were fed on high fat diet gained more weight than the other groups. This observation made in our study was similar to that of Kuzo et al 1970 in a way that if experimental animals are fed on high fat diet they gain more weight than the controls that are fed on normal diet. However in our study we did not fed experimental animals with high protein or high carbohydrate diet. In addition we also studied the effect of high fat diet(>30% of total energy requirement) on the histology of liver which was not done by earlier workers.

GautherMS et al(2006)(15)in their study found fat exposure causes central vein congestion, micro and macro vesicular fatty infiltration, interstitial haemorrhages, periportal inflammation and hepatitis. Our observations are discordant from those made by gauther et al who found that fatty diet does not cause linear increase in weight of experimental rats. In our study we found histo pathological changes like central venous congestion, micro and macro vesicular fatty infiltration, interstitial haemorrhages, periportal inflammation and hepatitis which were not studied by the earlier workers. Thus bobservations made in our one is discordant with the observations made by the earlier workers.

KuceraO, CervinkovaZ (2014) (16) studied the effect of high fat diet on three different strains of rats and found that fat induced hepatic injury in all the three strains but histopathological changes varied from strain to strain micro, macro, mixed in Lewis, Wistar, and Sprague-Dawley rats, respectively. In contrast in our study we found a mixed pattern of micro and macro vesicular infiltration in hepatocytes. Initially at four weeks micro vesicular fatty infiltration appeared followed by a mixed pattern later on (both micro and macro vesicular fatty infiltration). However these changes reversed to normal (no fatty infiltration at 20 weeks).

Kleiner DE et al (2005)(17)the activity score (NAS) for use in clinical research. The main purpose of NAS was to evaluate histological changes over time. In our study we also found that events like steatosis, lobular inflammation .ballooning are caused by high fat diet and these changes are dependent upon the duration of administration of fat to albino rats. The first change that we observed was steatosis followed by lobular inflammation and macro vesicular fatty infiltration Thus fat induced changes on the histopathology of liver are duration dependent ranging from steatosis to ballooning. In first two weeks of study we did not found any change in the histopathology of liver induced by administration of high fat diet. These changes gradually increased in magnitude and severity with passage of time reaching peak at the end of 10thweek.

Takahashi Y,Fukusato T (2014)(18) in their study they found that non-alcoholic fatty liver disease (NAFLD) can progress to liver cirrhosis and hepatocellular carcinoma (HCC).They found that histopathological evaluation of biopsy specimen remains the gold standard for diagnosis.Steatosis, lobular inflammation, and hepatocellular ballooning are the necessary components for the diagnosis of NASH. Paediatric NAFLD has different histological characteristics from adults.In contrast in our study we used albino rat liver biopsy for observing the fat induced hepatic injury. Since our research was limited for a period of 20 weeks during which we saw histopathological changes in the form of steatosis, micro and macro vesicular fatty infiltration, central vein congestion, dilatation of sinusoids and interstitial hemorrhages and areas of hepatitis .During this limited period we did not found any changes suggestive of hepatocellur carcinoma. Thus our initial observations are concordant with those made by the earlier workers.

Veteläinen et al (2007)(19) intheir study they described in a detail the course of histological changes in an MCDD model in Wistar rats. These observations are conconcordant with those made by earlier workers. However we did not found any areas of fibrosis in our study.

Altunkaynak(2005)(20) in their study showed dilatation of sinusoids, central veins and the branches of the portal vein, mononuclear cell infiltration and fibrosis were observed in high-fat diet groups. In our study we also compared the effect of high fat diet (>30% of total energy requirement) with low fat diet (<2% of total energy requirement) on the histology of liver in albino rats for a period of 10 weeks. Our observations were concordant with those made by the earlier worker[89] as both of us reported dilatation of sinusoids and central vein, mono nuclear cell infiltration in high fat diet of experimental animals. However, we could not find areas of fibrosis in any of the fields.

In our present study we observed that fat induced hepatic injury in normal albino rat liver without prior hepatic injury but the study conducted by the above workers showed that high fat diet causes hepatic injury both in normal and fatty liver.

Al-Awadi JHH et al (2013)(21) carried a study on eighty male albino rats for seven months to investigate the effects of high fat diet on liver tissue The histological sections of liver revealed presence of severe histopathological changes which were classified into grades between 0 to4.

SUMMARY AND CONCLUSION

The present study was carried in the postgraduate department of the anatomy to study the reversibility if fat induced hepatic injury with a pure vegetarian diet.30 malealbino rats weighing on average 100-150 grams were divided into three groups. Group A served as control. These rats were fed on normal diet containing less that 2% of fat. Group B animals received high fat diet containing >30% of the total caloric requirement. Group C received high fat diet for 10 weeks followed by pure vegetarian diet for next ten weeks. One animal from group A and two from group B were sacrificed at the end of 2^{nd},4^{th},6^{th} and 10^{th} weeks, and histopathological changes were recorded. After 10weeks, one animal from group A and two animals from group C were sacrificed at the end of 12^{th}, 14th, 16th, 18th and 20^{th} weeks. Observations were recorded. In our study we found that fat causes histopathological changes in the hepatocytes in a dose and duration dependent manner and the same can be reversed with a pure vegetarian diet without drugs. The changes disappeared in reverse order in which they had appeared i.e. histopathological changes which appeared last was first to disappear after stopping high fat diet and feeding these animals with pure vegetarian diet containing < 2%.Thus we conclude that fat which is an essential component of a balanced diet and very important for many physiological and metabolic processes of the body, if given in excess amount causes hepatic injury in the form of steatosis, macro and micro vesicular fatty infiltration, inflammatory changes like periportal inflammation in zone 1,2 and 3.Excess of fat also cause hepatitis if taken for short interval of time. These are dose and duration dependent. In our study we found that fat induced hepatic injury can be reversed by pure vegetarian diet without use of lipid lowering drugs like statins which are not free of complications.

In summary we found that high content of animal fat in diet causes hepatic injury which is dose and duration dependent. These histopathological changes can be reversed by pure vegetarian diet in a time bond manner without use of statins

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