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EFFECTS OF KETOGENIC DIET ON BODY WEIGHT AND HISTOLOGY OF LIVER OF MALE ALBINO RATS

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ABSTRACT

Background: At least 2.8 million adults die from Obesity related causes every year and 65% of the world's population lives in the countries where Obesity causes more deaths than underweight. The rat liver is the most cranial structure on the right side of the abdominal cavity, in the intrathoracic portion, coming in intimate contact with the diaphragm. The liver of the rat is a multilobulated organ. The mass of the rat liver accounts for about 6% of the total body weight.

Objectives: To observe the effects of ketogenic diet on body weight and histology of liver of male albino rats.

Methods: Twenty four Albino rats weighing on an average 200-300 grams were taken from animal house of Government. Medical College Srinagar for the present study. The animals were divided into two groups after randomization. Group A- served as control. Group B-were fed on isocaloric Ketogenic diet (consisting of 65% calories from fat, 30% from protein, 6% from carbohydrates). The microscopic observations were recorded group wise using light microscope. Appropriate photographs were taken by using a photographic microscope and labelled.

Results: The groups were designated as Group A and Group B consisting of six and eighteen rats respectively. Group A animals were fed with normal diet containing rat chow (black gram and pellet) and water and Group B animals were fed with isocaloric Ketogenic diet. Control group consisting of 6 animals were fed with normal diet consisting of pellet and gram. The animals in this group were fed with isocaloric Ketogenic diet consisting of 65% caloric intake from fat, 30% from protein, 6% from carbohydrates.

Conclusion: Despite the beneficial role of Ketogenic diet in the weight reduction, Ketogenic diet has deleterious effects on Liver of Albino rats. Its use for weight reduction should be restricted to subjects with normal Liver parameters.

Keywords: ketogenic diet, multilobulated organ, protein, carbohydrates, histopathological changes.

Introduction: The World Health Organisation (WHO) defines overweight and obesity as abnormal and excessive fat accumulation that presents a risk to health. A body mass index (BMI) $\geq 25 Kg/m^2$ is generally considered overweight, while obesity is considered a BMI $\geq 30 kg/m^2$. It is well known that obesity and overweight are a growing problem globally with high rates in both developed and developing countries[1]. At least 2.8 million adults die from Obesity related causes every year and 65% of the world's population lives in the countries where Obesity causes more deaths than underweight[2]. It is one of the principal risk factors for

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Cardiovascular diseases and along with dyslipidaemia, Hypertension and Diabetes contributes to the metabolic syndrome[3].

In the Literature, diets rich in carbohydrate, and notably rich in refined sugars and fructose, are associated with the metabolic syndrome[4,5]. Therefore to lose weight different diets have been suggested. Among them, carbohydrate restriction has been proposed to be single most effective intervention for reducing all features of metabolic syndrome[6-8]. Since the publication of Atkin's book in the early 1970s[9], low carbohydrate diets have become increasingly popular, particularly ketogenic diets.

Ketone bodies can also cross the blood brain barrier to provide an alternative source of energy to the brain, R.B.C's and liver do not utilize Ketone due to lack of Mitochondria. In humans, Ketogenic diets are known to be an effective weight loss therapy[10-13] (in an average upto 5% of body weight at 6 months), but the mechanisms are not clearly established. Some authors also suggest digestive metabolic changes with ketogenic very low energy diets, ghrelin levels and subjective appetite (usually increased in hypocaloric diet) were reduced when patients were in a ketotic state. Surprisingly, Leptin levels were lower under ketosis. Despite their beneficial effects on weight loss and epileptic seizures, Ketogenic diet may have adverse side effects such as kidney stones, impaired growth, osteoporosis and hyperlipidemia on the long term[14,15].

The rat liver is the most cranial structure on the right side of the abdominal cavity, in the intrathoracic portion, coming in intimate contact with the diaphragm. The liver of the rat is a multilobulated organ. The mass of the rat liver accounts for about 6% of the total body weight.

AIMS AND OBJECTIVES

1) To observe the effect of Ketogenic diet on mean body weight of male Albino Rats.
2) To observe the effect of Ketogenic diet on the Histology of Liver of male Albino rats.

MATERIALS AND METHODS

The present randomized trial (RCT) was conducted in the Post graduate Department of Anatomy, Government Medical College Srinagar after obtaining the ethical clearance from the Institutional Ethical Committee Vide Order No. 166/ETH/GMC with the following inclusion and exclusion criteria:

Inclusion criteria: (i) Apparently healthy albino rats, (ii) Albino rats with weight between 200-300 grams.

Exclusion criteria: (i) Albino rats showing less physical activity (lethargic). (ii) Albino rats not feeding well (refusal to feed) and suffering from diarrhoea. (iii) Rats with generalized hair loss.

Twenty four Albino rats weighing on an average 200-300 grams were taken from animal house of Government. Medical College Srinagar for the present study. The animals were divided into two groups after randomization. Group A- served as control and Group B- were fed on isocaloric Ketogenic diet (consisting of 65% calories from fat, 30% from protein, 6% from carbohydrates). Both the group of rats were kept under uniform husbandry conditions in separate iron cages. Group A animals were designated as Control group. They were fed with normal diet containing rat chow (black gram and pellet) and tap water. Group B animals were kept in three separate cages containing six animals in each cage. Group B

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animals were fed on isocaloric Ketogenic diet containing 65% calories from fat, 30% from protein and 6% from carbohydrates. Mean body weight of the rats in both the groups has been calculated at the beginning of the experiment and in each sitting prior to the sacrifice of animals. One animal from Group A and four animals from Group B have been sacrificed each time at 6^{th}, 12^{th}, 18^{th} and 24^{th} week. In each sitting one rat from Group A and four rats from Group B were sacrificed after anaesthetizing them with chloroform. The limbs of each rat were fixed on board with pins. A mid-line incision was given in the anterior abdominal wall and liver were identified and dissected out. The organ was washed in distilled water and fixed in 10% neutral formalin solution. The organ was cut into small pieces 1x1cm. Cut tissue pieces were kept in 95% ethanol for 24 hours and then in chloroform for 5 hours. The tissue was embedded in paraffin wax and 5µm serial sections were taken. Sections were selected and stained with Haematoxylin and Eosin, according to recommended routine protocol. The slides were mounted using Canada balsam and covered with glass coverslips before viewing under the Olympus BX-53 light microscope. Photomicrographs were taken with a digital microscopic camera attached with Olympus BX-53. The tissues were processed manually for block making as follows:

S.No	**Step**	**Medium**	**Time**
01	Fixation	10% formal saline.	12 hours.
02	Dehydration	Acetone	12 hours.
03	Clearing	Chloroform	6 hours, 3 changes at 2 Hour interval.
04	Wax Embedding	Paraffin wax at 56 degrees	3 hours.

The casting and embedding were done with the help of Leukart's moulds. As the molten wax was poured into the moulds, the tissues were placed into the mould fitted with wax and left to solidify. After solidification, the blocks of the wax were removed and properly labelled for microtomy.

Microtomy: Rotatory type of microtome was used and sectioning of the block was done at 5-7 micrometer thickness. The sections were floated on a water bath with temperature adjusted below the melting point of the wax. The sections were mounted and oriented on slides that were coated with a mixture of fresh egg albumin, glycerine and Thymol crystals. The slides were marked by a diamond pen. The tissues were fixed on slides by keeping them on a hot plate for 10 minutes. The hot plate was regulated at the melting point of wax (56 degrees). The slide was cleaned beyond the area of tissue implantation. The slides were stained with haematoxylin and eosin under various steps and these slides were mounted using Canada balsam and were covered with cover slips. Then these prepared slides were seen under a Light microscope.

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Composition of Haematoxylin and Eosin

Haematoxylin	……...	2 G
Alum	……	Q.S
Distilled water	……	60 ml
Glycerine	……	60 ml
Absolute alcohol	……	60 ml
Glacial acetic acid	…..……...	3 ml
Eosin (1% aqueous)	……..	1 gram

Routine Haematoxylin and Eosin staining procedure:

STEP	**SOLUTION**	**TIME**	**FUNCTION**
Deparafinization	Xylene	$2^{1/2}$ mins	To remove paraffin
	Xylene	$2^{1/2}$ mins	To remove paraffin
	Absolute alcohol	$2^{1/2}$ mins	To remove xylene
	Absolute alcohol	$2^{1/2}$ mins	To remove xylene
	955 alcohol	Few dips	Hydrate in and out
De zankerization (for sections fixed in solutions mercury)	Alcoholic iodine	5min	To remove mercuric chloride deposits
Bleaching out iodine	Hypo sodium thiosulphate	2 min	To remove iodine from section
Washing	Tap water	2 min	To remove hypo changes (wash thoroughly)
Staining	Haematoxylin	1-5 min	To stain nuclei (depends on strength of Haematoxylin)
Rinsing	Tap water	Few changes	To rinse slides off stain
Decolourization	Acid alcohol (0.5%)	1-3 rapid	To decolorize dips sections.
Rinsing	Tap water running	1 min	To stop acid action
Blueing	Lithium	30-60 sec	Blue nuclei
Check to stain with the microscope: * Nuclei should be brightly clear blue, * background light, or colourless. * Decolorize if necessary.			

Counter staining	Eosin 0.5% alcohol.	Few dips to 1 min.	To stain cytoplasm.
Dehydration	95% alcohol.	Several dips	To dehydrate
	95% alcohol	Several dips	To dehydrate
	Absolute alcohol	Several dips1 min	To dehydrate
	Absolute alcohol	Several dips 1 min	To dehydrate
	Absolute alcohol	Several dips 1mins	
Clearing	Xylene	Several mins	
	Xylene	Several mins	
	Xylene	Several mins	

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Results: nuclei bright clear blue. Cytoplasm pink.

RESULTS

After obtaining twenty four male Albino rats from animal house Government Medical College Srinagar, the rats were segregated into two groups and kept in iron cages. The groups were designated as Group A and Group B consisting of six and eighteen rats respectively. Group A animals were fed with normal diet containing rat chow (black gram and pellet) and water and Group B animals were fed with isocaloric Ketogenic diet. After this the mean body weight of the rats in their respective groups was calculated at the beginning of study. Subsequently the mean body weight of animals in their respective groups was measured before sacrificing the rats in each sitting.

	At the beginning	**6 weeks**	**12 weeks**	**18 weeks**	**24 weeks**
Group A (Control)	250 gms	280 gms	280 gms	300 gms	330 gms
Group B	243 gms	250 gms	243 gms	245 gms	250 gms

LIVER

Normal rat liver is reddish brown in colour, glistening in appearance and firm in consistency. It consists of four lobes and eight segments. The average weight of liver in normal Albino rats is about 7-8% of body weight. On light microscopic examination the structure of rat liver resembles that of human beings. The basic structural unit of liver consists of hepatic lobules. The lobules are hexagonal with hepatocytes arranged in cords with intervening sinusoids radiating from central vein. A distinctive component of liver lobule is portal triad. Portal triad misleadingly named, actually consists of five structures, a branch of hepatic artery, a branch of portal vein, bile duct, lymphatic vessel and a branch of Vagus nerve. Blood from branches of portal vein, a branch of hepatic artery enters sinusoids at the periphery of lobule and passes towards centre (centripetal), sinusoids open into central vein. Central vein drains into hepatic veins which finally open into inferior vena cava. Hepatic artery supplies 75% of Oxygen and 25% of the blood while portal vein supplies 75% of blood and 25% of the Oxygen. The area of liver tissue is supplied by one branch of Portal vein is regarded by many functional unit of liver and is referred as portal lobule, a still smaller unit the portal acinus is diamond shaped area of liver supplied by one hepatic arteriole running along the junction of two hepatic lobules. Two central veins lie at the end of acinus. Acinus is divided into three zones:

Zone 1: Peri portal i.e. nearest to the terminal branch of afferent vessel.

Zone 2: Intermediate zone

Zone 3: is the area closest to the central venous drainage.

Hepatocytes constitute 75-80% of liver volume and are cuboidal or polyhedral in shape. The nuclei are rounded and often multiple, cytoplasm is eosinophilic contains numerous cell organelles like mitochondria, endoplasmic reticulum, ribosomes. Hepatocytes

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have three surfaces, one facing towards sinusoids (sinusoidal surface) bearing microvillus that project into space of Disse, intercellular surface between adjacent hepatocytes and canalicular surface that is a depression and is opposed by similar depression of neighboring hepatocytes to form wall of bile canaliculi (Herring's Canal). Spaces between hepatic cords are called sinusoids. Sinusoids display a continuous fenestrated endothelial lining. Endothelial cells have no basement membrane and are separated from hepatocytes by a space of Disse or peri-sinusoidal space. In the space of Disse lie the macrophages, or von kuffer cells, Ito or satellite cell. Ito cells have number of functions like secretion of matrix, collagen and proteoglycans. They also store fat soluble vitamin in lipid vesicles and are significant source of growth factors.

STUDY AT 6th WEEK

Gross and microscopic Observations at the end of 6th week

Control Group (A): Control group consisted of 6 animals and were given the normal diet consisting of pellet and gram. After sacrificing one animal at the end of sixth week and after dissecting out liver we found that there were no gross changes in appearance, colour and texture of liver. No microscopic changes were observed in the histology of liver as shown in Fig. 1.

Ketogenic Diet Group (B): These animals were fed with Ketogenic diet. At the end of six weeks there were no changes in gross appearance, colour and texture of liver. Histologically basic architecture of liver was found to be preserved. Slight microscopic changes in the form of congestion and dilatation of Central vein were seen as shown in Fig. 2 and Portal Triaditis as shown in Fig. 3 were also seen in some fields.

STUDY AT 12th WEEK

Gross and microscopic observations at the end of 12th week:

Control Group (A): There was no change in gross appearance, texture, and colour of liver. On microscopic examination no histological changes were seen.

Ketogenic Diet Group (B): No macroscopic changes were observed in liver of these animals. In addition to the histological changes observed in the previous stage of study Sinusoidal dilatations and congestion were also observed as shown in Fig. 4.

STUDY AT 18th WEEK

Gross and microscopic Observations at the end of 18th week

Control Group (A): There was no change in gross appearance, texture, and colour of liver. On microscopic examination no histological changes were seen.

Ketogenic Diet Group (B): At this stage of study, the histological changes observed during the previous stages of study were more marked. Additionally Parenchymal haemorrhages were observed as shown in Fig. 5.

STUDY AT 24th WEEK

Gross and microscopic Observations at the end of 24th week

Control Group (A): At this stage of study no change in gross appearance, texture, and colour of liver was observed. On microscopic examination no histological changes were seen as shown in Fig. 6.

Ketogenic Diet Group (B): On histological examination the changes observed during the previous stages of study i.e. parenchymal haemorrhages as shown in Fig. 7, portal triaditis as

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shown in Fig. 8 and Sinusoidal congestion and dilatation and central venous congestion and dilatations as shown in Fig. 9 were more marked.

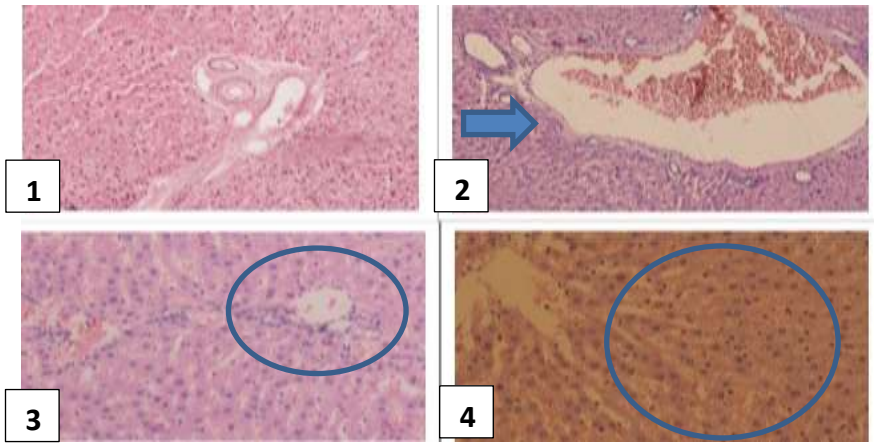

Fig 1: Microphotograph of liver from Group A at the end of 6th week showing normal architecture of liver.
Fig. 2: Microphotograph of liver from Group B at the end of 6th week showing congestion and dilatation of central vein.
Fig. 3: Microphotograph of liver from Group B at the end of 6th week showing Portal triaditis.
Fig. 4: Microphotograph of liver from Group B at the end of 12th week showing sinusoidal dilatations and congestion.

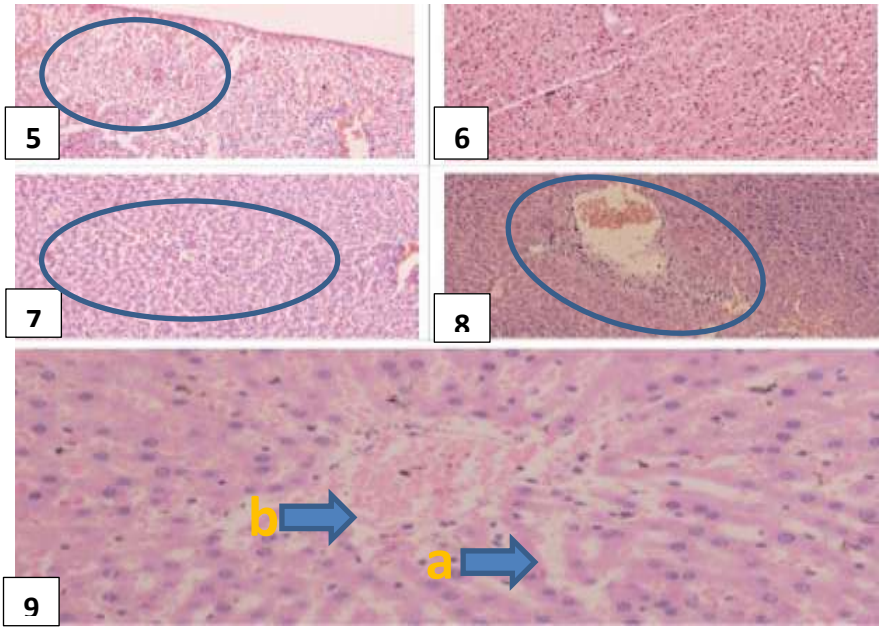

Fig. 5: Microphotograph of liver from Group B at the end of 18th week showing focal parenchymal haemorrhages

Fig. 6: Microphotograph of liver from Group A at the end of 24th week showing normal architecture of liver.

Fig. 7: Microphotograph of liver from Group B at the end of 24th week showing parenchymal haemorrhages

Fig. 8: Microphotograph of liver from Group B at the end of 24th week showing portal triaditis.

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Fig.9: Microphotograph of liver from Group B at the end of 24th week showing (a) sinusoidal dilatation and congestion (b) central venous congestion & dilatation

DISCUSSION

Obesity is rising worldwide at an alarming rate and is a major risk factor for most metabolic syndromes. As a result, weight loss is usually used as a means of therapy and is aimed at improving some of the metabolic syndromes. In the recent times, the use of Ketogenic diet as a means of weight control has been on the increase. Ketogenic diet is defined as a low carbohydrate diet with moderate amount of protein restriction to induce ketosis without restriction of fat intake[16].

The present study was conducted in Post Graduate Department of Anatomy, Government Medical College Srinagar. Experimental animals were divided into two groups:
Group A (Control): consisting of six (6) rats. These rats were fed on normal diet, the rat chow.
Group B (Ketogenic Diet): Eighteen (18) rats were allotted in this group. They were fed on Ketogenic diet for 24 weeks.

One animal from Group A (Control) and four animals from Group B were Liver were sacrificed. Prior to the sacrifice the mean body weight of animals was calculated.
The results thus obtained were compared to the earlier studies.

Samaha FF et al (2003)[12] In their study found that severely obese subjects with a high prevalence of Diabetes or metabolic syndromes lost more weight during six months on a carbohydrate-restricted diet than on calorie and fat restricted diet, with a relative improvement in insulin sensitivity and Triglyceride levels. In their study they randomly assigned 132 severely obese subjects (77 blacks and 23 women) with a mean body mass Index of 43 and a high prevalence of diabetes (39% percent) or the metabolic syndrome (43%) to a carbohydrate restricted (low carbohydrate) diet or a calorie and fat restricted (low fat) diet. Seventy nine subjects completed the six month study. The observations thus made showed that subjects on the low carbohydrate diet lost more weight than those on the low fat diet (-5.8±8.6 kg vs. -1.9+/4.2kg, p=0.002). These observations made by Samaha FF et al (2003)[12] are congruent with the observations made in our study in which 24 Albino rats were divided into two Groups; Group A and Group B. Group A rats were fed with normal diet and Group B rats were fed with isocaloric Ketogenic diet for a total of 24 weeks. Mean body weight was calculated at intervals of 6 weeks i.e. at 6th week, 12th week, 18th week and 24th week. At all the stages of study we found that the Albino rats which were fed on standard chow diet showed a significant increase in mean body weight, as compared to the experimental group of rats fed on Ketogenic diet. Marvopoulos JC et al (2005)[17] conducted a pilot study on 5 women of 18-45 years of age, diagnosis suggestive of Polycystic Ovarian syndrome having BMI $\geq$ 27kg/m^2 having no other serious medical condition requiring medical supervision. The study subjects were instructed to limit their carbohydrates intake to 20 grams or less. Participants returned every two weeks to outpatient research clinic for measurements. In their study they found that there were significant reductions from baseline to 24 weeks in the body weight (-12%), percent free Testosterone (-22%), LH/FSH ratio (-36%) and fasting Insulin (-54%). These observations thus made by Marvopoulos JC et al (2005)[17] are in congruence with our study conducted for 24 weeks in which the Control group

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of animals gained body weight significantly as compared to the experimental group of animals fed with Low carbohydrate Ketogenic diet. Kennedy AR et al (2007)[18] in their study divided 32 C57BL/6 male mice into four groups of eight mice each. First group was fed with standard chow (16.7% fat, 26.8% protein, 56.4% carbohydrates), second group was fed with high fat diet (45% fat, 24% protein, 35% carbohydrate), third group was fed with Ketogenic diet (95% fat, 0% carbohydrates, 5 % protein) and fourth group was calorically restricted to 65% of the average chow intake. They found that mice fed on calorie restricted diet lost weight and weighed 3.5 grams less than their initial weight and 6 grams less than standard chow fed. Mice fed on high fat diet gained weight and were 6 grams heavier than the standard chow fed mice. Mice fed on Ketogenic diet although ate as many calories as mice fed with high fat diet, initially lost weight and then stabilized at the same weight as calorie restricted, i.e. 6 grams lower than standard chow fed animals.

In our study Group B animals (fed on Ketogenic diet) gained significantly less weight as compared to Group A animals (fed on standard chow). Thus the observations made in our study are concordant to the previous study conducted by Kennedy AR et al (2007)[18]. Bielohuby B et al (2011)[19] in their study divided the male Wistar rats into four groups (8-10 rats per group). The study was conducted for 4 weeks during which one group was fed with standard chow diet and rest of the three groups were fed with Low carbohydrate High fat diets, which differed in the relative abundance of fat and protein (percentages of fat/protein in dry matter: LC-75/10; LC65/20; LC-55/30). They found that compared to the standard chow fed rats, Low carbohydrate-high fat diet rats gained significantly less body weight. Rats fed with LC-75/10 gained the least body weight, while the rats fed with LC-65/20 and LC-55/30 gained significantly less body weight compared with chow fed rats.

In our study we found that at all the stages of study the Albino rats which were fed on standard chow diet showed a significant increase in mean body weight as compared to the experimental group of rats. Thus our observations were concordant with those made by Bielohuby B et al (2011)[19]. Garbow JR et al (2011)[20] in their study determined the systemic and hepatic effects of long-term administration of a very low-carbohydrate, low protein, and high fat Ketogenic diet, serially comparing these effects to a high-simple carbohydrate, high-fat Western diet and a low fat polysaccharide-rich control chow diet in C57Bl/6J mice. For this study they divided the male mice into three groups and were maintained for 12 weeks on either high fat Ketogenic diet (95% calories from fat, 4.5% calories from protein, and 0.5% from carbohydrates) or high fat Western diet (40.7% calories from fat, 19% from proteins and 41% from carbohydrates) or standard low-fat polysaccharide-rich chow (13% calories from fat, 25% calories from protein, 62% from carbohydrates). In their study they found that mice maintained on high fat Ketogenic diet exhibited decreased weight compared to Western diet and chow fed groups both after 6weeks and 12weeks. This is in concordance with the observations made in our study where the Ketogenic diet fed male Albino rats failed to gain significant amount of weight while the standard chow fed Control group of Albino rats gained weight significantly at all stages of study i.e. at 6 weeks, 12 weeks, 18 weeks and 24 weeks. Bueno NB et al (2013)[21] carried out a meta-analysis in which the study participants greater than 18 years having BMI >27.5kg/m^2 were chosen. The study participants were assigned to either very low carbohydrate Ketogenic diet (i.e. a diet with no more than 50 grams carbohydrate/day) or to conventional low fat diet (a restricted energy diet with less

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than 30% of energy from fat).The study showed that individuals assigned to very low carbohydrate ketogenic diet achieve greater reductions in body weight, TAG and Diastolic Blood pressure, compared with individuals assigned to Low fat diet.

The results thus obtained are in concordance with our study which was conducted on Albino rats for a period of 24 weeks. In our study we found that Ketogenic diet fed rats gained significantly less body weight as compared to the Control group of rats which were fed on standard rat chow.

Omozee EB et al (2018)[22] in their study divided forty (40) Wistar rats into two groups; Experimental (A) and control (B) consisting of twenty (20) rats each. Experimental group of animals were fed >65% saturated fat Ketogenic diet; Control group was fed with normal rat chow for 8 weeks. In this study they observed that there was a significant ($p<0.05$) increase in the initial weight of the control group when compared with the final weight, while the initial weight of the experimental group did not show any significant difference with the final weight. These results thus obtained by Omozee EB et al (2018)[22] were in concordance with the observations made in our study. Arsyad A et al (2020)[23] divided fifteen male Wistar rats into control (n=8) and Ketogenic (n=7) groups. Controls received standard diet containing 52.20% carbohydrates, 7.00% fat and 15.25% protein; meanwhile, the Ketogenic group received a high fat low carbohydrate diet which contained 5.66% of carbohydrates, 86.19% and 8.15% protein. All rats were caged individually and received 30 grams of either standard or high fat low carbohydrate pellets. The experiment was carried out for 60 days. Observations thus made showed that the rats subjected to Ketogenic diet experienced a marked decrease in body weight, diet. With regards to the body weight, the results obtained by Arsyad A et al (2020)[23] during their study conducted for a period of sixty days were similar to the results obtained during our study conducted for 24 weeks.

There is very less literature available showing the effect of high fat Ketogenic diet on the Histology of Liver. So our present study was undertaken in the department of Anatomy, Government Medical College, Srinagar to elucidate the effect of Ketogenic diet on Histology of Liver.

Lieber CS et al (2004)[24] in their study fed Sprague-Dawley rats with high fat liquid diet (71% energy from fat, 11% carbohydrates, 18% protein) or standard diet (35% fat, 47% carbohydrate, 18% protein) for 3 weeks. Histological examination of liver specimens revealed steatosis, abundant inflammatory cell and mitochondrial abnormalities. In our study we divided 24 male Albino rats into two groups. Group A (Control) consisting of 6 rats and Group B (Ketogenic diet) consisting of 18 rats. Group A rats were fed with normal diet and Group B rats were fed with Ketogenic diet (65% calories from fat, 30% calories from protein, 6% calories from carbohydrates) for 24 weeks. In our study histological examination revealed dilatation and congestion of central vein, Portal Triaditis, Sinusoidal dilatations and congestion and Parenchymal haemorrhages. Thus the observations made in our study were not concordant with the study conducted by Lieber CS et al (2004)[24]. Altunkaynak Z et al (2005)[25] in their study compared high fat group (fat 30%, carbohydrate 50-52%, protein 18-20%) with standard diet containing diet control group (fat 7-10%, carbohydrate 68-70%, protein 18-20%). Histological examination of liver at 12 weeks revealed dilatation of sinusoids, central vein and branches of portal vein, mononuclear cell infiltration and fibrosis. In our study we compared the Ketogenic diet (65% fat, 30% protein and 6% carbohydrate)

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with standard chow fed rats for 24 weeks. Our observations were concordant with those made by Altunkaynak Z et al (2005)[25], as both of us reported dilatation of sinusoids and central vein in the experimental group of animals. However foci of fibrosis were not observed at any stage in our study conducted for 24 weeks. Meli R et al (2013)[26] carried out a study on Sprague-Dawley rats for 5 weeks or 8 weeks in which rats were fed either on control diet (17% energy from fats, 23% protein, 60% carbohydrate) or high fat diet (58% fat, 16% protein, 25% carbohydrate). Histology of liver tissues of high fat diet fed rats showed signs of liver inflammation characterized by the presence of mixed inflammatory cell infiltration and hepatocyte necrosis and apoptosis which appeared throughout the lobule and became more evident at 8weeks. High fat diet rats showed progressive time dependent steatosis (from grade 2 to grade 3) with a histological pattern characterized by microvesicular steatosis. In contrast to this, in our study Ketogenic group of rats were fed with 65% calories from fat, 30 % calories from protein, 6% calories from carbohydrates. In our study we found the signs of inflammation in the form of Portal triaditis, sinusoidal congestions and dilatations and haemorrhages within liver parenchyma. However no hepatocyte necrosis, no apotosis and no steatosis was reported in our study.

Rosqvist F et al (2014)[27] conducted a double blinded randomized control trial for 7 weeks to investigate the effect of hyper caloric diet either rich in Saturated fatty acids (SFA) or polyunsaturated fatty acids (PUFAs) on 39 adults. On MRI assessment they found that SFA group gained more liver fat, total fat, and visceral fat but less lean tissue as compared to the PUFA group. This shows that the type of fat in the diet is an important determinant of Liver fat accumulation during moderate weight gain. In our study male Albino rats were fed with isocaloric Ketogenic diet, and the source of fat was mainly the saturated fatty acids (animal fat). Histological examination of liver revealed no fat induced degenerative changes in the hepatocytes. Thus the observations made in our study are not concordant with the observations made by Rosqvist F et al (2014)[27]. Yaghchiyan M et al (2019)[28] in their study aimed to investigate the effects if vitamin D administration on the markers of inflammation and metabolic damages in the liver of high fat diet induced obese rats. For this study forty male Wistar rats were divided into two groups of control receiving a normal diet (10% fat, 30% protein and 60% carbohydrates) and experimental group receiving a high fat diet (59%fat, 11% protein, 30% carbohydrate) for 16 weeks. Histological examination of the Liver of High fat diet group of rats revealed the changes suggestive of hepatic injury. Changes in the form of fat accumulation, mononuclear inflammatory cells, lymphocyte infiltration, hepatocyte necrosis, nucleus and cytoplasm damage in sinusoidal cells, pre-apototic changes, hepatic sinusoidal dilatation and necrotic pyknosis in the Kupffer cells. Thus our study is not concordant with Yaghchiyan M et al (2019)[28] as no fat accumulation and no hepatocyte necrosis was observed in our study. They further extended their study to observe the beneficial effects of vitamin D on reducing the levels of inflammatory parameters, reducing liver enzymes and serum lipids and improving liver histological features.

Jahan M et al (2019)[29] in their study divided 30 male Albino rats into 3 groups; Group A (Control) fed on normal diet (less than 2% fat), Group B fed- High fat diet group fed on >30% fat and Group C received high fat diet for 10 weeks, followed by pure vegetarian diet for next 10 weeks. The animals were sacrificed at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 weeks. In

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their study histological examination of liver of high fat diet group revealed central venous congestion and dilatation, sinusoidal congestion and dilatation, microvesicular and macrovesicular steatosis, hepatitis, inflammatory cell infiltrations and interstitial haemorrhages. In contrast to this, in our study experimental group of animals were fed on isocaloric ketogenic diet (65% calories from fat, 30% from protein, 6% from carbohydrates) for 24 weeks. On histological examination we observed Central venous congestion and dilatation, Portal triaditis, Sinusoidal congestion and dilataion and parenchymal haemorrhages. In our study no microvesicular or macrovesicular steatosis was observed. Luukonen PK et al (2020)[30] in their study observed that Ketogenic diet decreased Intrahepatic triglycerides by 31%, decreased the body weight, decreased hepatic triglyceride and decreased hepatic insulin resistance despite an increase in NEFA concentrations. The study thus conducted by Luukonen PK et al (2020)[30] is concordant with our study in which no hepatic steatosis was reported on histological examination of liver specimens of male Albino rats fed on Ketogenic diet for 24 weeks.

CONCLUSION

From the observations of present study we conclude that:

1) Ketogenic diets are very effective in weight loss.
2) No histopathological changes Liver were observed in control group of animals at any stage of research.
3) The histopathological changes were observed in Liver of Ketogenic diet fed experimental group of animals starting from the 6th weeks of study. All these changes became more marked during the further stages of study in a duration dependent manner.
4) Thus we suggest that despite the beneficial role of Ketogenic diet in the weight reduction, Ketogenic diet has deleterious effects on Liver of Albino rats. So, its use for weight reduction should be restricted to subjects with normal Liver parameters.

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