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Research Article

Comparison the Effects of Three Forms of Ketogenic Diet on Adult Male Diabetic Albino Rats

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ABSTRACT

Ketogenic diet are being highly effective line of treatment for metabolic syndrome and DM. This study aimed to compare the effect of three different forms of KD on diabetic rats. Fifty adult male albino rats grouped into five equal groups control groups (1) Normal (2) Diabetic control receives normal diet. Experimental (3) Diabetic receive classic Ketogenic diet (4:1). Experimental (4) Diabetic receive Ketogenic diet (3:1). Experimental (5) Diabetic receive Ketogenic diet (2:1). Fasting samples were collected at the end of the study for analysis.

Biochemical analyses were done using spectrophotometric method. A significant decrease in blood glucose, serum total cholesterol, LDL, and triglycerides, serum urea and creatinine were observed while Insulin level and HDL were significantly increased. The major findings in our study is that the classic KD (4:1) is the most effective form of three main types of Ketogenic diets in this study. There was no significant difference in the histological finding between three experimental groups with minimal histological changes on pancreases, liver and kidney. These findings have clearly shown that intake of Ketogenic diet is not harmful to the liver, pancreases and kidney.

Keywords: Ketogenic die; Diabetes Mellitus; Metabolic syndrome

INTRODUCTION

Nutrition is key for preventing Type 2 Diabetes (T2D) and obesity [1], but there are no evidence-based data defining the best dietary approach to prevent and treat these conditions [2]. In the last decades, Low Carbohydrate Diets (LCD) and Ketogenic Diet s (KD) have become widely known and popular ways for weight loss [1], not only within the scientific community, but also among the general public, with best-selling dedicated books or intense discussion on social media networks staying at the top of the diet trend list for years [3]. KD was first reported as a kind of high-fat, adequate-protein, and low-carbohydrate diet for treatment for epilepsy in the 1920s [1]. There are a number of different types of ketogenic diet but the end goal of these diets is the same, the different types of ketogenic diet usually share a number of similarities, notably in being low in carbohydrate and high in dietary fat [4]. The classical KD provides 90% of calories from long chain fatty acids, a minimum of 1 g/kg of protein and minimal carbohydrates, resulting in the generation of ketone bodies evoke nutritional ketosis [5]. Ketosis is the metabolic formation of ketone bodies by the liver, which occurs when the body shifts its primary fuel source from carbohydrates to fatty acids in response to a scarce supply of glucose [6]. Ketosis induced by the KD is hypothesized to have an appetite suppressing effect, which has intrigued many individuals to try this diet to lose weight [1]. Interestingly, the consumption of a high-fat diet and high intake of saturated fat used to be associated with an increased risk of type 2 diabetes, but this association disappears when combined with a low or non-carbohydrate diet [3]. The KD has also gained interest in the diabetes community, particularly for its effect on blood glucose and weight [7]. Several studies have shown positive health outcomes in individuals with diabetes who have followed the Ketogenic diet such as weight loss [1], improved glycemic control [7], and decreases in medication dosages[1]. Despite the significant increase in literature investigating the KD in diabetes, its long-term safety and efficacy still remains unknown and there is no research on the long-term risks and benefits for individuals with diabetes following the KD such as the risk of developing nutritional deficiencies (vitamin B-complex, vitamin D, beta- carotene, calcium, and antioxidants) [1]. The objective of this study is to compare the effect of three different forms of KD on diabetic rats.

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MATERIALS AND METHODS

Ethical consideration

Institutional Animal Care and Use Committee (IACUC) at Al-Azhar University, Assuit, Egypt have approved the experimental protocol at, 10/12/2018. The protocol were considered official after the acceptance by ethical comity of the faculty and the Physiology department.

Experimental animals

Fifty adult male albino rats, eight weeks of age, weighting about 150-225 g were used as experimental animals in this study. Rats were obtained from The Nile Co. For Pharmaceuticals and Chemical Industries (Cairo). The animals were housed in standard polypropylene cages with stainless steel good aerated covers and maintained under controlled room temperature with 12 hours light - dark cycle and were fed a standard diet of known composition. The experimental animals were kept for 10 days to adapt a new condition before start of experiment.

Rats were divided into five equal groups, each group comprising ten rats designated as follow:

Group 1: Normal control rats.

Group 2: Diabetic control receives normal diet.

Group 3: Diabetic receive classic Ketogenic diet (4:1).

Group 4: Diabetic receive Ketogenic diet (3:1)

Chemicals

Streptozotocin (STZ) was purchased from Sigma Chemicals Co., St. Louis, MO, USA.

Blood glucose kit (Egyptian Company for Biotechnology-Egypt).

Insulin kit (Sigma-Aldrich Co. LLC-USA).

Serum cholesterol kit (Egyptian company for biotechnology-Egypt).

Serum triglycerides kit (Egyptian Company for Biotechnology-Egypt).

Serum high density lipoprotein (HDL) kit (Egyptian Company for Biotechnology-Egypt).

Serum urea kit (Egyptian Company for Biotechnology).

Serum creatinine kit (Biolabo reagents kits – France).

Induction of diabetes mellitus

Type 2 diabetes was induced by an interapritoneal injection of 50 mg/kg b.wt . Streptozotocin dissolved in citrate buffer pH 4.518. Seven days, rats were be screened for serum glucose levels. Rats having serum glucose ≥ 200 mg/dl, after 2 hours of glucose intake, were be considered diabetic [8].

Experimental diet

KD was formed as in (Table 1) to meet the nutritional requirements of adult rats as recommended by the American Institute of Nutrition (AIN-93M).

The level of blood glucose were be estimated daily using Accu-Chek glucometer (Roche, Germany).

At the end of the 12 weeks and After 12 hours over night fasting, morning blood samples were collected from retro-orbital venous plexus. Blood was collected into a dry clean graduated glass centrifuge tube, and serum was separated by centrifugation at 5000 rpm for 10 minutes. The separated serum was aliquotted and stored frozen in epindorffs, tube at 20°C until used for the determination of Insulin, Lipid profile: (Triglycerides (TG), Total Cholesterol (TC), High-Density Lipoprotein (HDL), and Low-Density Lipoprotein (LDL)), blood urea, creatinine, ALT and AST. Under ether anesthesia, abdomen of the animal was opened after reaching the stage of surgical anesthesia, as evident by loss of withdrawal reflex. Specimens from pancreas, liver, kidney were fixed immediately in 10% neutral buffered formalin, embedded in paraffin, prepared as 5-µm thick sections, and stained with Hematoxylin and Eosin (HE) to assess the histopathological examination.

Group 1 (Control group): Pancreas showed average-sized pale-staining islets of Langerhans composed of predominating beta cells with pale blue cytoplasm in and less frequently alpha cells with pink cytoplasm in separated by average thin-walled blood capillaries, average exocrine areas, and average ducts (Figure 1A).

Group 2 (Diabetic): Pancreas showed small sized hypocellular islets of Langerhans with scattered apoptotic beta cells and mildly dilated congested intervening blood capillaries, mildly dilated congested interstitial blood vessels, average exocrine areas, and average ducts (Figure 1B).

Group 3 (Diabetic+ketogenic diet 4:1): Pancreas showed small-sized hypocellular islets of Langerhans with scattered apoptotic beta cells and mildly dilated congested intervening blood capillaries, mildly congested interstitial blood vessels, average exocrine areas, and average ducts (Figure 1C).

Group 4 (Diabetic+ketogenic diet 3:1): Pancreas showed average-sized hypocellular islets of Langerhans with scattered apoptotic beta cells and mildly dilated intervening blood capillaries, average exocrine areas, and average ducts (Figure 1D).

Table 1: Nutritional composition of the experimental diets [9]

Macronutrient	Micronutrient	Control	Classic KD	Type 2 KD	Type 3 KD
Protein	Casein	87.225	142.09	4.887	142.09
	L-Cystine	3	4.887	-	4.887
Carbohydrate	Corn Starch	465.7	-	-	-
	Sucrose	137.5	-	30	-
	Dextrin	165	30	114.03	30
Fat	Soybean Oil	70	114.03	116.9	114.03
	Lard	-	187.8	300	94
	Butter	-	406	4.887	260

Table 2: Blood glucose (mg/dl) and Insulin (IU/ml) in adult male albino rats subjected to different treatment

		G1	G2	G3	G4	G5
Glucose (mg/dl)	Mean ± S.E	90.7 ± 0.63a	204.9 ± 1.03b	107.3 ± 0.76c	119.3 ± 0.63d	128.8 ± 0.42e
	%	-	125.9	18.3	31.5	42.0
Insulin(IU/ml)	Mean ± S.E	20.4 ± 0.50a	12.4 ± 0.34b	22.3 ± 0.47c	17.0 ± 0.21d	14.2 ± 0.39e
	%	-	-39.2	9.3	-16.7	-30.4

Each value represented means of 10 records ± S.E.
a,b,c,d.e means comparison between all groups which the groups have the same letter mean there is no significance difference and which have different letter mean there is a significance change.
%: Percent of changes from control values.

Table 2.1: Histopathological results of the pancreas

	Pancreatic islets				**Ducts**	**Exocrine area**	**BV**
	Islet size	Cellularity	Beta cells	Capillaries			
Group 1	0	0	0	0	0	0	0
Group 2	+	+	+	+	0	0	+
Group 3	+	+	+	+	0	0	+
Group 4	0	+	+	+	0	0	0
Group 5	0	0	0	+	0	0	+

Islet size:	0: Average	+: Small	++: Atrophied
Cellularity:	0: Average	+: Hypocellular	++: Acellular
Beta cells:	0: Average	+: Few/apoptotic	++: Necrotic/absent
Capillaries:	0: Average	+: Mildly dilated	++: Markedly dilated/congested
Ducts:	0: Average	+: Dilated	++: Atrophied
Exocrine area:	0: Average acini	+: Small acini	++: Atrophied acini
Interstitial BV:	0: Average	+: Mildly dilated	++: Markedly dilated/congested

Group 5 (Diabetic+ketogenic diet 2:1): Pancreas showed average-sized islets of Langerhans with predominating beta cells, mildly dilated congested intervening blood capillaries, mildly dilated interstitial blood vessels, average exocrine areas, and average ducts (Figure 1E).

Rats fed classic-KD had lower hepatic enzymes relative to that fed type two or three KD (Table 2, 2.1),

Group 1 (Control group): Liver showed average portal tracts with average portal veins, average bile ducts and average hepatocytes in peri- portal area, average central veins with average hepatocytes arranged in single-cell cords with average intervening blood sinusoids (Figure 2A).

Group 2 (Diabetic): Liver showed mildly edematous portal tracts with markedly dilated congested portal veins, markedly dilated central veins with detached lining, and scattered apoptotic hepatocytes in peri-portal and peri-venular areas (Figure 2 B)

Group 3(Diabetic + ketogenic diet 4:1): Liver showed markedly edematous portal tracts with markedly dilated congested portal vein, average central veins, and average hepatocytes in peri-portal and peri-venular areas (Figure 2C)

Group 4 (Diabetic + ketogenic diet 3:1): Liver showed average portal tracts with mild portal inflammatory infiltrate and mildly dilated congested portal veins, mildly dilated congested central veins, and scattered apoptotic hepatocytes in peri-venular area with intra- lobular inflammatory infiltrate (Figure 2D)

Group 5 (Diabetic + ketogenic diet 2:1): Liver showed average portal tracts with mildly congested portal veins, markedly dilated central veins with mildly congested blood sinusoids, hydropic

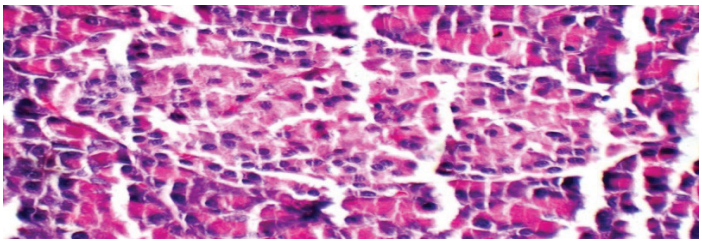

Figure 1A: Group 1 (Control): High power view showing predominating beta cells with pale blue cytoplasm (black arrows) and less frequently alpha cells with pink **cytoplasm** (blue arrows) separated by average thin-walled blood capillaries (red arrow), and average exocrine areas (yellow arrow) (H&E × 400).

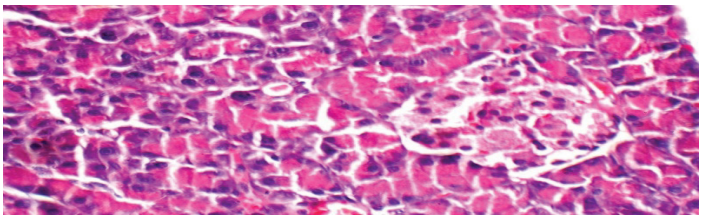

Figure 1B: (Group 2 Diabetic): High power view showing hypocellular islet with scattered apoptotic beta cells (black arrow) and mildly dilated intervening blood capillaries (blue arrow), average exocrine areas (yellow arrows), and average ducts (red arrow) (H&E × 400).

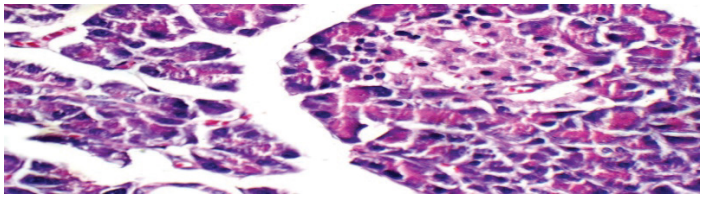

Figure 1C: Group 3 (Diabetic + ketogenic diet 4:1): High power view showing hypocellular islet with mildly dilated intervening blood capillaries (black arrow), and average exocrine areas (blue arrow) (H&E × 400).

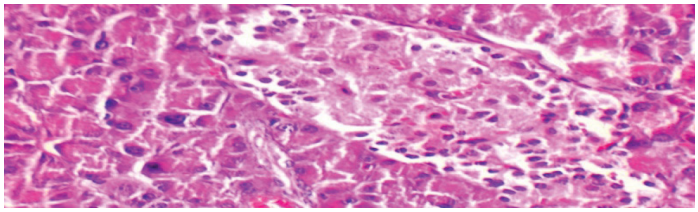

Figure 1-D Group 4 (Diabetic+ ketogenic diet 3:1): High power view showing hypocellular islet with scattered apoptotic beta cells (black arrow) and mildly dilated intervening blood capillaries (blue arrow), and average exocrine areas (yellow arrow) (H&E × 400).

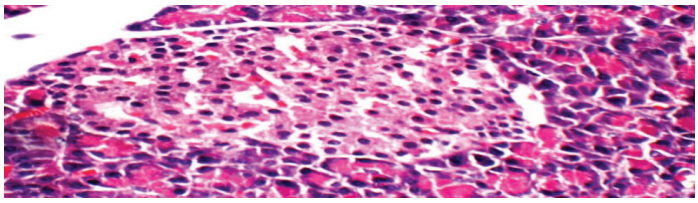

Figure 1-E Group 5 (Diabetic+ ketogenic diet 2:1): High power view showing average islet with predominating beta cells (black arrows), mildly dilated congested intervening blood capillaries (blue arrow), and average exocrine areas (yellow arrow) (H&E × 400).

Figure 1: Histopathological results of the pancreas.

change of hepatocytes, and mild micro-vesicular steatosis in peri-venular area (Figure 2E).

Rats fed classic-KD had lower Blood urea and serum creatinine relative to that fed type two or three KD (Table 3, 3.1),

Group 1 (Control): Kidney showed average renal capsule, average glomeruli with average Bowman's spaces, average proximal tubules with preserved brush borders, average distal tubules, and renal medulla showed average collecting tubules with average interstitium (Figure 3A) .

Group 2 (Diabetic): Kidney showed scattered small-sized and congested glomeruli with widened Bowman's spaces, scattered proximal tubules with apoptotic epithelial lining and complete loss of brush borders, markedly dilated congested interstitial blood vessels, and renal medulla showed markedly congested blood vessels (Figure 3B) .

Group 3 (Diabetic + ketogenic diet 4:1): Kidney showed average renal capsule, few scattered small-sized glomeruli with average Bowman's spaces, scattered proximal tubules with partial loss of brush borders, mildly congested interstitial blood vessels, and renal medulla showed mildly congested blood vessels (Figure 3C).

Group 4 (Diabetic + ketogenic diet 3:1): Kidney showed average renal capsule, few scattered small-sized and congested glomeruli with widened Bowman's spaces, scattered proximal tubules with complete loss of brush borders, mildly congested interstitial blood vessels, and renal medulla showed mildly congested blood vessels (Figure 3D).

Table 3: Serum ALT and AST (U/L) activity in adult male albino rats subjected to different treatment.

		G1	G2	G3	G4	G5
ALT (U/L)	mean ± S.E	31.3 ± 0.73a	96.5 ± 0.87b	41.0 ± 0.58c	46.6 ± 0.5d	53.1 ± 0.38e
	%	-	208.3	31.0	48.9	69.6
AST (U/L)	Mean ± S.E	21.1 ± 0.6a	93.6 ± 0.58b	42.6 ± 0.48c	47.8 ± 0.42d	54.9 ± 0.38e
	%	-	341.5	101.4	125.9	160.2

Each value represented means of 10 records ± S.E.
a,b,c,d.e means comparison between all groups which the groups have the same letter mean there is no significance difference and which have different letter mean there is a significance change.
%: Percent of changes from control values.

Table 3.1: Histopathological results of the liver

	Portal tract			CV	Hepatocyte	Blood sinusoids	Intra-lobular inflammatory infiltrate
	PV	Inflammatory infiltrate	Edema				
Group 1	0	0	0	0	0	0	0
Group 2	++	0	+	++	++	0	0
Group 3	++	0	+	0	0	0	0
Group 4	+	+	0	+	++	0	+
Group 5	+	0	0	++	+	+	0
Portal vein (PV):	0: Average	+: Mildly Dilated/ congested	++: Markedly dilated/ congested				
Inflammatory infiltrate:	0: No	+: Mild	++: Moderate/marked				
Edema :	0: No	+: Present					
Central vein (CV):	0: Average	+: Dilated/ congested	++: Markedly dilated/ detached lining				
Hepatocytes:	0: Average	+: Hydropic change	++: Apoptotic /Necrotic				
Blood sinusoids:	0: Average	+: Mildly Dilated/ congested	++: Markedly dilated/ congested				
Intra-lobular inflammatory infiltrate:	0: No	+: Mild	++: Moderate/marked				

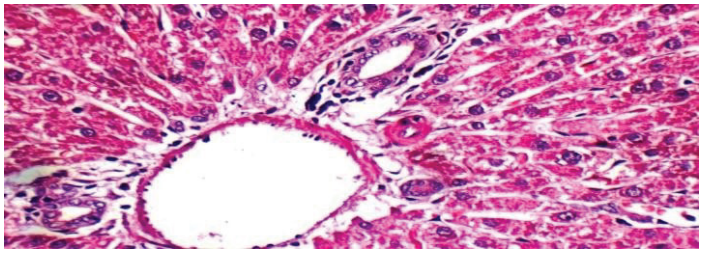

Figure 2A: (Group 1Control): High power view showing average portal tract with average Portal Vein (PV), average bile ducts (black arrow), average hepatic artery (blue arrow), and average hepatocytes in peri-portal area (yellow arrow) (H&E × 400).

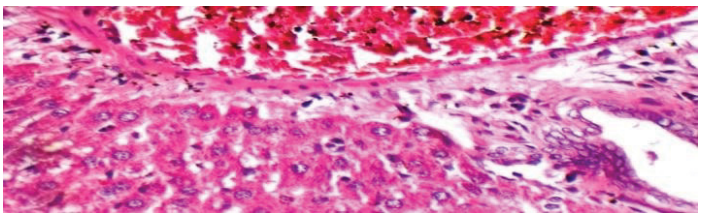

Figure 2B (Group 2 Diabetic): high power view showing mildly edematous portal tract (black arrow) with markedly dilated congested portal vein (PV), average bile ducts (BD), and apoptotic hepatocytes in peri-portal area (blue arrow) (H&E × 400).

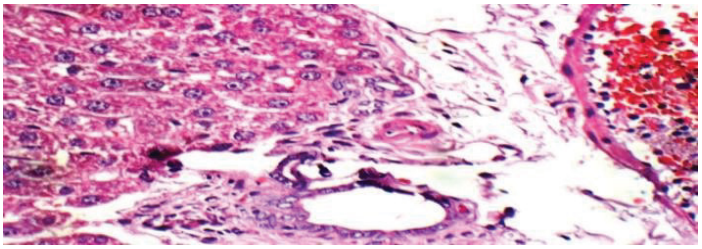

Figure 2C (Group 3): Diabetic + ketogenic diet 4:1: high power view showing markedly edematous portal tract (black arrow) with markedly dilated congested Portal Vein (PV), average Bile Ducts (BD), and average hepatocytes in peri-portal area (blue arrows) (H&E × 400).

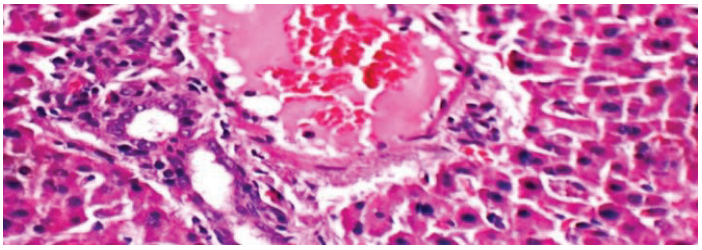

Figure 2E: Group 5: Diabetic + ketogenic diet 2:1: high power view showing average portal tract with mildly congested portal vein (black arrow), average bile ducts (blue arrow), hydropic change of hepatocytes in peri-portal area (yellow arrow), and congested blood sinusoids (green arrow) (H&E X 400).

Figure 2: Histopathological Results of the liver

Group 5 (Diabetic + ketogenic diet 2:1): Kidney showed average renal capsule, few scattered small-sized and congested glomeruli with widened Bowman's spaces, scattered proximal tubules with partial loss of brush borders, markedly congested interstitial blood vessels, and renal medulla showed mildly congested blood vessels (Figure 3E).

Rats fed classic-KD had lower blood cholesterol, serum triglycerides, LDL -cholesterol and high level of HDL-cholesterol relative to that fed type two or three KD Table (4, 4.1, 5).

DISCUSSION

As the KDs are being highly effective first line of treatment for metabolic syndrome and DM. The major findings of our study are that the classic KD is the most effective form of three main types of Ketogenic diets in this study, these include the classic Ketogenic Diet (4:1), the (3:1) Ketogenic Diet (CKD), and the (2:1) Ketogenic Diet (TKD). In the present study, cumulative data have proved that the classic KD significantly reduced blood glucose level, serum total cholesterol, LDL, and triglycerides, serum urea and creatinine while Insulin level and HDL were significantly increased. The hypoglycemic effect were explained by Morrison et al. they found

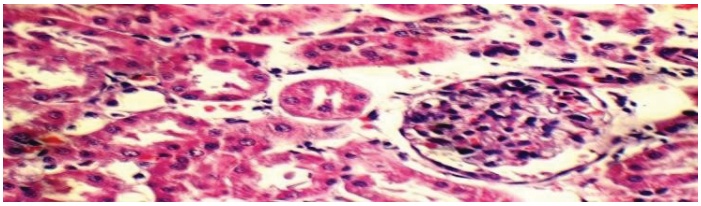

Figure 3A (Group 1 Control): High power view showing average Glomerulus (G) with average Bowman's Space (BS), average proximal tubules (P) with preserved brush borders (black arrows) and average distal tubules (D) (H&E × 400).

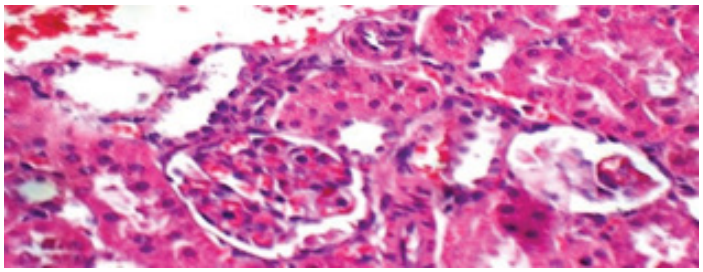

Figure 3B Group 2 Diabetic: High power view showing small-sized congested Glomeruli (G) with widened Bowman's Spaces (BS), scattered Proximal tubules (P) with apoptotic epithelial lining and complete loss of brush borders (black arrow) and markedly dilated congested Blood Vessels (BV) (H&E × 400).

Table 4: Blood urea and creatinine (mg/dl) in adult male albino rats subjected to different treatment.

		G1	G2	G3	G4	G5
Urea (mg/dl)	Mean ± S.E	23.5 ± 0.4a	84.6 ± 0.34b	32.3 ± 0.47c	35.4 ± 0.31d	42.8 ± 0.25e
	%	-	260.0	37.4	50.6	82.1
Creatinine (mg/dl)	Mean ± S.E	0.75 ± 0.02a	3.08 ± 0.04b	1.10 ± 0.02c	1.20 ± 0.02d	1.80±0.03e
	%	-	310.7	46.7	60.0	140.0

Each value represented means of 10 records ± S.E.a,b,c,d.e means comparison between all groups which the groups have the same letter mean there is no significance difference and which have different letter mean there is a significance change.
%: Percent of changes from control values.

that consuming KD has been shown to cause shifts in cerebral metabolism that are indicative of increased capacity for the metabolism of non-glucose substrates, such as acetate and ketones, in both human and rodents. Increased cerebral acetate utilization in human is associated with increased susceptibility to either insulin-induced hypoglycemia or fasting- induced hypoglycemia [10]. The Ketogenic diet significantly decrease blood glucose level and give excellent glycemic control in this study, High-fat, low-carbohydrate KD have been associated with beneficial effects on pancreatic endocrine cells and glucose metabolism on the long term this was evidenced by increased insulin concentrations as evidenced by Gupta et al, [11]. This response could be the result from beta cell activation, an increase of cell number, or a combination of these two mechanisms. Hypoglycemic effect of KD may resulted from decreased glucagon levels that have been observed by Granados et al, [12]. Gupta et al, explained hypoglycemic effect of KD by decreased glucagon levels that lead to less gluconeogenesis from the liver, which may prevent hyperglycemia in KD-fed mice[11]. The results in this study strongly proved that long-term KD leads to increase of insulin sensitivity[13]. Interestingly, there were no great differences in pancreatic histological structure between KD groups that indicate the high fat diet is not harmful on pancreatic endocrine cells. Our finding is agreed with study of Blagosklonny, et al [14]. Purhonen et al, found that consuming KD leads to adaption of brain to utilize alternative energy substrates as well as to reduced hepatic glycogen content [15]. Long-term KD leads to decrease plasma cholesterol, LDL and triglyceride levels [16]. In our study, decrease plasma cholesterol, LDL and triglyceride levels were observed after 12 week of diet. Like fasting, KD may cause the symptoms of starvation pseudo-diabetes as remembered by Blagosklonny et al [14], they also said that starvation pseudo-diabetes is beneficial and is not type 2 diabetes. Thus, the warning that KD may cause type 2 diabetes in humans is not justified and contradicts what is observed in clinical practice[14]. where benefits of the diet appeared to strongly outweigh the challenges with positive health results after starting the KD, which were all related to their primary goals such as improved glycemic control [17], correct dyslipidemia [18], and weight loss [7]. The long-term safety and efficacy of the KD in individuals with diabetes remains unknown notably its effect on cardiovascular risk factors and frequency of hypoglycaemia [19]. Our histological finding is extremely reassuring as the effects on the pancreas, liver and kidneys are very simple, this were agreed with finding of Blagosklonny et al [14, 20].

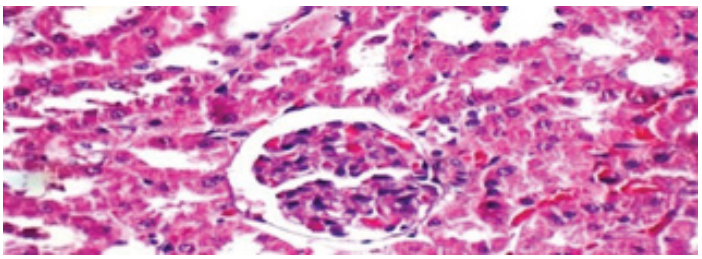

Figure 3C Group 3 (Diabetic + ketogenic diet 4:1): High power view showing average-sized Glomerulus (G) with average Bowman's Space (BS), scattered proximal tubules (P) with partial loss of brush borders (black arrows) and mildly congested interstitial blood vessels (blue arrows) (H&E × 400)

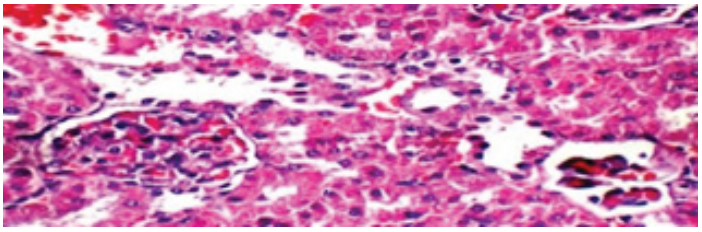

Figure 3D Group 4 (Diabetic + ketogenic diet 3:1: High power view showing small-sized and congested Glomeruli (G) with widened Bowman's Spaces (BS), scattered proximal tubules (P) with partial loss of brush borders (black arrows) and mildly dilated congested Blood Vessels (BV) (H&E × 400).

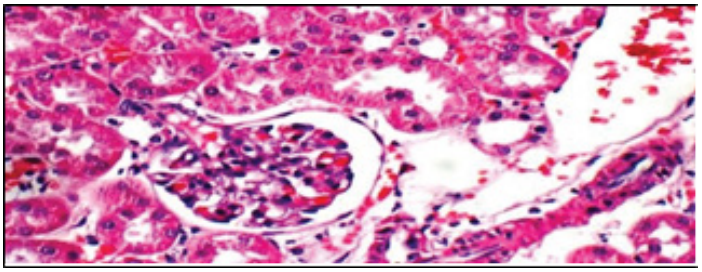

Figure 3E Group 5 (Diabetic + ketogenic diet 2:1): High power view showing average-sized congested Glomerulus (G) with average Bowman's Space (BS), proximal tubules (P) with preserved brush borders (black arrow) and markedly dilated congested interstitial Blood Vessels (BV) (H&E X 400).

CONCLUSION

A classical Ketogenic diet is very-low-carbohydrates, this diet is

Table 5: Blood cholesterol, serum triglycerides, HDL-cholesterol and LDL-cholesterol (mg/dl) in adult male albino rats subjected to different treatment.

		G1	G2	G3	G4	G5
Chlo (mg/dl)	Mean ± S.E	181.2 ± 1.9a	324.0 ± 2.9b	184.6 ± 1.3c	193.6 ± 1.4d	209.6 ± 1.5e
	%	-	78.8	1.9	6.8	15.7
Tri . (mg/dl)	Mean ± S.E	168.2 ± 2.1a	290.9 ± 1.0b	154.7 ± 1.1c	161.9 ± 1.0d	175.0 ± 0.7e
	%	-	72.9	-8	-3.7	4
HDL-C	Mean ± S.E	35.8±0.3a	33.3 ± 2.8a	51.4 ± 0.4c	44.7 ± 0.3b	41.9 ± 0.3b
	%	-	-7	43.6	24.9	17
LDL-C	mean±S.E	120.8±1.3a	194.8±0.6b	140.4±0.5d	146.6±0.6c	167.5±0.6e
	%	-	61.3	21.4	16.2	38.7

Each value represented means of 10 records ± S.E. a,b,c,d.e means comparison between all groups which the groups have the same letter mean there is no significance difference and which have different letter mean there is a significance change.%: Percent of changes from control values.

more efficiently in the this study than other types of diet as increase total carbohydrate lead decrease ketosis and this leads to the loss of the desired effect of KD.

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CONFLICT OF INTEREST

The authors declare that they have no competing interest.

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AUTHOR'S CONTRIBUTIONS

Nour El-Deen A Mansour A, and Abdallah M. involved in the study concept, design and recruitment of animal, induction of diabetes and follow up, and contributed to data acquisition; Ali A and Abdallah M. performed the biochemical tests; Nour El-Deen A and Gad-Allha. A performed statistical analysis and designed the figures; Nour El-Deen. A and Abdallah A. performed data interpretation; Abdallah. A, Nour El-Deen. A, Mansour A, and Gad-Allah 2 A wrote the manuscript; all the authors reviewed the manuscript and finally approved it for submission.

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