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Effect of Obesity Induced by High Carbohydrate and High Fat Diets on Biochemical Profile in Albino Rats in Sudan

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ABSTRACT

Background: Obesity is a chronic disease that has spread all over the world and threatens public global health. This study was carried out in the Department of Biochemistry, College of Veterinary Medicine, Khartoum University to investigate the effect of obesity-induced by high carbohydrate and high-fat diets on biochemical profile in Albino Rats in Sudan.

Methods: A total of 40 adult male outbred albino rats, weighing between 55-100g were used in this study and were divided into 4 groups (A, B, C and D) 10 animals of each. Group (A) was healthy control, rats were fed on a balanced diet (BD), Group (B) received a high-fat diet (HFD), Group (C) received high carbohydrate diet (HCD) and Group (D) received high fat plus high Carbohydrate.

Results: After six weeks, the high carbohydrate diet group (C) showed a significant increase in body weight, serum levels of alanine amino transaminase (ALT), aspartate amino transaminase (AST), triglycerides (TG) and high-density lipoprotein (HDL). Group (B) fed high fat diet showed a significant increase in serum total cholesterol (TC), low-density lipoprotein (LDL) and Alkaline Phosphatase (ALP).

Conclusion: The body weight in rats fed with the high carbohydrate was increased, when compared with the normal, high fat diet and high (fat+ carbohydrate) diet rats.

Key-words: Albino Rats, Biochemical Profile, Co-morbidities, Cholesterol, Overweight, Obesity, Superoxide dismutase

INTRODUCTION

Carbohydrates are one of the main dietary components of food. This category of foods includes sugars, starches and fibres. Carbohydrates are important in the body as sources of energy. They can be found in a wide range of plant and animal food sources [1]. According to World Health Organization (WHO) data, more than 1.4 billion and more than half a billion adults in 2008 were overweight and obese, respectively. Moreover, 42 million preschool children were overweight in 2013 globally and it is estimated that at least 2.8 million people die as a result of being overweight or obese [2]. The high-fat diet-induced obese rat model has been considered as a popular preference for its ability to mimic the usual way of obesity in humans. A high-fat diet is one of the major factors causing obesity and long term intake of a high-fat diet showed a significant increase in abdominal fat weight in mammals [3]. Obesity is associated with most of the components of metabolic syndrome, the leading cause of type 2 diabetes. The co-morbidities of obesity and type 2 diabetes associated with insulin-resistance syndrome include obstructive sleep apnea, hypertension, polycystic ovary syndrome, non-alcoholic fatty liver disease, and certain forms of cancer [4]. When obesity persists for a long time, therefore, the antioxidant sources can be depleted, decreasing the activity of enzymes such as superoxide

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dismutase (SOD) and catalase (CAT) [5]. The activity of SOD and glutathione peroxidase (Gpx) in individuals with obesity is significantly lower compared with that in healthy persons, having implications for the development of obesity-related health problems [6,7].

MATERIALS AND METHODS

Experimental animals- Fourty adult male outbred Albino rats, weighing between (55–100 g) were used in this experiment during the period from 2019 to 2020 in the Department of Biochemistry, College of Veterinary Medicine, Khartoum University. The rats were kept in a cage in the same environment with a controlled temperature (25–30°C) and humidity around 60-70% RH. The cages were provided with adequate ventilation and the housing system was provided with appropriate facilities for feeding and watering. The animals were screened for a routine clinical examination to ensure that they are healthy.

Feeding and watering of animals- The housing system was provided with appropriate facilities for feeding and watering. The nutritional regimen comprised HFD, HCD and BD. Tables 1 show the proximate analysis of diets fed to rats. The analysis was performed in the laboratory of the Department of Biochemistry, College of Veterinary Medicine, Khartoum University. Throughout the study period, the animals were allowed free access to tap water.

Table 1: Ingredients and nutrient composition of the diet used to feed the rats

Ingredients	Normal Diet (ND)	High-Fat Diet (HFD) (%)	High-carbohydrate diet (HCD) (%)
Protein (%)	18	10	10
Fat (%)	10	30	10
Carbohydrate (%)	66.4	54.4	74.4
Minerals (%)	3.5	3.5	3.5
Vitamin (%)	1	1	1
Methionine (%)	0.1	0.1	0.1
Fiber (%)	1	1	1
References	Pugh *et al.* [8]	Altunkaynak [9]	Altunkaynak [9]

Experimental design- The distribution of the experimental rats into 4 groups, 10 rats of each. All groups were fed for six weeks, rats in the group (A) healthy control rats were fed on a balanced diet, rats in the group (B) received a high-fat diet (HFD), rats in group (C) received high carbohydrate and rats in the group (D) received high fat plus high carbohydrate.

Bodyweight gain- The rats were weighed during experiment 6 to the nearest 75.4±3.9 g using a digital balance (Nippotec NKS-305, UAE). Individual body weight was recorded before study imitation (week 0) and at weeks 2, 4 and 6.

Biochemical analysis- Blood for sera was collected in plain containers from the retro-orbital plexus. Blood samples were centrifuged at 3000 rpm. Serum samples were kept at -20°C until needed for biochemical analysis. All parameters were measured using commercial kits (BioSystem S.A. Costa Brava 30, 08030 Barcelona-Spain). The total protein was determined by the burette reaction method [10]. Urea level was determined by urease procedure the values obtained were read with a spectrophotometer (UV mini-1240 UV/Vis Spectrophoto -meter, Japan) at appropriated wavelengths and the values calculated using standard formulae [11].

Statistical Analysis- The experimental data were subjected to the standard method of statistical analysis. Data are presented as mean±SD. The statistical analysis was performed using One Way Analysis Of Variance (ANOVA) and Statistical Package for the Social Science (SPSS) software (Version 14) [12]. P≤0.05 were considered statistically significant.

RESULTS

Bodyweight gain- Table 2 indicates that in all experimental groups, the means of BW values were started to increase at week 2 until the end of the experimental period. Weight gain was more pronounced in groups B, C and D compared with group A. The bodyweight of rats in the group (C) was significantly (P-value=0.001) higher than those of the control group A, B and D at week six. The statistical analysis showed a means of 196±21.1 g for group C. The BW means values were 121.8±7.1, 148.2±14.7 and 156.3±20.9 g in groups A, B and D, respectively.

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Table 2: Effect of fat and carbohydrate diets on body weight gain in rats

Time	Group A (Normal)	Group B (high Fat)	Group C (High Carbohydrate)	Group D (high fat and high Carbohydrate)
Week 0	76.2±15.2	77.6±14.72	69.6±21.1	78.3±13.9
Week 2	82.9±17.1	91.4±24.7	96.9±22.9	87.1±17.7
Week 4	113.7±18.1	121.8±7.1	153.6±21.1	125±19.9
Week 6	116.7±8.6	148.2±14.7	196±21.1*	156.3±20.9

Serum biochemical changes

Serum Cholesterol- Generally, there was a progressive increase in serum cholesterol levels of treated groups. The mean values of serum cholesterol in group B were significantly (P-value=0.032) higher than in groups A, C and D at week 6. Serum cholesterol mean levels in group C were 196.2±12.2 mg/dl compared to 119.5±16.1, 166.9±37.4 and 165.4±21.8 mg/dl for groups A, C and D, respectively (Table 3).

Table 3: Effect of fat and carbohydrate diets on the serum cholesterol level in rats (mg/dl)

Time	Group A (Normal)	Group B (High Fat)	Group C (High carbohydrate)	Group D (high fat and high carbohydrate)
Week 0	115.2±29.66	119.4±23.71	114.2±37.04	117±19.72
Week 2	117.1±30.8	164.3±25.4	146±30.8	152.13±30.11
Week 4	116.8±25.02	192.9±17.9	160.5±37.16	162.38±27.83
Week 6	119.5±16.1	196.2±12.2*	166.9±37.4	165.4±21.8

Serum Aspartate Aminotransferase (AST)- Table 4 indicates that the mean values of serum AST in group C were significantly (P-value=0.022) higher than the group A, B and D at week 4. Group C obtained a means of 67.3±21.08 U/L, while groups A, B and D obtained a means of 48.2±11.16, 59.39±9.69 and 56.6±16.97 U/L, respectively.

Table 4: Effect of fat and carbohydrate diets on serum aspartate aminotransferase (AST) in rats (U/L)

Time	Group A (Normal)	Group B (high Fat)	Group C (High Carbohydrate)	Group D (high fat and high Carbohydrate)
Week 0	53.9±7.25	51.9±11.89	53.8±8.6	54.4±12.66
Week 2	46.3±4.1	61.1±17.54	52.3±9.69	49±6.2
Week 4	48.2±11.16	59.39±9.69	67.3±21.08*	56.6±16.97
Week 6	59.9±22.63	52.6±7.24	63.5±12.54	60.3±11

Serum Alanine Aminotransferase (ALT)- All treated groups showed a marked increase in ALT values compared to the control group (Table 5). The mean serum values of ALT in group C were significantly (P-value=0.003) higher than of control group A, B and D at week 4. Group C showed a mean of 85.1±39.11 U/L, while A, B and D groups indicated a means of 45.4±14.72, 70.4±10.99 and 68.3±16.17 U/L, respectively.

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Table 5: Effect of fat and carbohydrate diets on serum alanine aminotransferase (ALT) in rats (U/L)

Time	Group A (Normal)	Group B (high Fat)	Group C (High Carbohydrate)	Group D (high fat and high Carbohydrate)
Week 0	41.5±6.83	46.4±9.64	43.9±11.1	38.4±6.98
Week 2	43.4±21.37	70.5±28.63	81.7±13.76	62.7±30.67
Week 4	45.7±14.72	70.4±10.99	85.1±39.11*	68.3±16.17
Week 6	46.7±17.3	71.87±12.38	82.2±6.32	70.5±21

Serum Alkaline Phosphatase (ALP)- There was a progressive increase in treated groups compared to the control group. The mean serum values of ALP in group B were significantly (P value=0.001) higher than in the control group A, C and D at week 6. The statistical analysis showed a mean of 87.9±11.47 U/L for group B, while groups A, C and D showed a means of 55.5±10.55, 77.2±25.79 and 72.8±9.26U/L, respectively as presented in Table 6.

Table 6: Effect of fat and carbohydrate diets on serum alanine aminotransferase (ALP) in rats (U/L)

Time	Group A (Normal)	Group B (high Fat)	Group C (High Carbohydrate)	Group D (high fat and high Carbohydrate)
Week 0	50.91±14.25	46.64±17.95	41.37±11.1	41.84±11.35
Week 2	52.93±15.3	67.12±23.64	63.77±31.22	60.55±59.6
Week 4	49.3±5.39	80.78±11.25	74.98±27.22	67.82±23.03
Week 6	55.5±10.55	87.9±11.47*	77.2±25.79	72.8±9.26

Serum Triglycerides- The effect of fat and carbohydrate intake on serum triglycerides are shown in Table 7. The mean serum values of triglycerides in group C were significantly higher (P-value=0.000) than the control group A, B and D at week 6. Group C showed a mean of 171.24±26.39 mg/dl. While in group A, B and D obtained a means of 98.9±28.15, 151.4±32.82 and 143.77±30.28 mg/dl, respectively.

Table 7: Effect of fat and carbohydrate diets on serum triglycerides in rats (mg/dl)

Time	Group A (Normal)	Group B (high Fat)	Group C (High Carbohydrate)	Group D (high fat and high Carbohydrate)
Week 0	82.6±26	92.7±16.34	97.2±24.47	93.9±34.25
Week 2	99.59±25.21	138.8±42.89	148.7±37.35	133.1±28.76
Week 4	92.74±41	143.1±95.8	163.1±31.29	137.3±47.46
Week 6	98.9±28.15	151.4±32.82	171.24±26.39*	143.77±30.28

Serum Urea- The concentrations of urea increased significantly (P-value=0.022) in group D compared to group A, B and C at week 2 (Table 8). The statistical analysis showed a mean of 51.1±12.4 mg/dl for group D. While groups A, B and C showed a means of 34.9±11.55, 46.8±9.25 and 43.6±10.9 mg/dl, respectively.

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Table 8: Effect of fat and carbohydrate diets on serum urea concentrations (mg/dl)

Time	Group A (Normal)	Group B (high Fat)	Group C (High Carbohydrate)	Group D (high fat and high Carbohydrate)
Week 0	32.9±9.96	48.9±8.25	42.2±10.75	33.7±7.63
Week 2	34.9±11.55	46.8±9.25	43.6±10.9	51.1±12.4*
Week 4	37.8±6.67	43.1±6.05	40.4±4.64	39.7±9.35
Week 6	36.4±5.39	41.2±5.88	41.9±7.43	43.8±13.57

Serum low-density lipoprotein (LDL)- The results presented in Table 9 show that in all treated groups, there was a gradual increase in LDL levels. The means LDL in the rats feeding by high-fat diet (B group) were significantly (P-value=0.034) higher than the control group (A) and other groups (C and D) at week 6. Group B obtained a mean of 138.39±9.36 g/dl. While in group A, C and D showed a means of 111.9±57.76, 126.61±11.22 and 118.09±9.15g/dl, respectively.

Table 9: Effect of fat and carbohydrate diets on low-density lipoprotein LDL (g/dl)

Time	Group A (Normal)	Group B (high Fat)	Group C (High Carbohydrate)	Group D (high fat and high Carbohydrate)
Week 0	105.98±21.6	107.92±11.62	106.03±24.19	105.18±10.88
Week 2	108.62±26.94	129.24±28.68	113.39±32.27	112.41±51.16
Week 4	109.63±35.04	134.46±40.44	123.97±21.27	116.91±16.18
Week 6	111.9±57.76	138.39±9.36*	126.61±11.22	118.09±9.15

Serum high-density lipoprotein (HDL)- The means in table 10 show a significant (P-value=0.046) increase in HDL values in group C compared to groups A, B and D at week 6. The statistical analysis showed a mean of 82.23±13.96 g/dl for group C. While in group A, B and D showed a means of 41.4±32.64, 66.77±14.42 and 57.41±11.73 g/dl respectively.

Table 10: Effect of fat and carbohydrate diets on High-density lipoprotein HDL (g/dl)

Time	Group A (Normal)	Group B (high Fat)	Group C (High Carbohydrate)	Group D (high fat and high Carbohydrate)
Week 0	49.56±7.47	54.42±7.62	53.73±10.62	55.27±8.64
Week 2	37.89±8.36	55.38±7.1	61.49±11.34	43.42±10.89
Week 4	38.02±7.35	62±13.76	72.76±14.89	50.31±7.62
Week 6	41.4±32.64	66.77±14.42	82.23±13.96*	57.41±11.73

DISCUSSION

In the present study, body weight gain was higher in rats fed with high carbohydrate diet (C group) as compared to the normal healthy control rats (A group). Obesity is characterized by increased adipose tissue mass that results from both increased fat cell number and increased fat cell size. Adipose tissue is a dynamic organ that plays an important role in energy balance and changes in mass according to the metabolic requirements of the organism [13]. The increase in body weight gain may be linked to the reported increase in feed consumption rates by these groups. The present results are in full agreement with previous findings reported by Bhandari *et al.* [14]; Hanan *et al.* [15]; Rahman *et al.* [16]. In consist with our results, higher weight gain

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was found in rat fed by high fat diet when compared with normal rats [17-19]. But, Ferramosca *et al.* [20] noted that the body weight gain was decreased in rats fed with high carbohydrate diets as compared to the normal healthy rats. While, Kayhan *et al.* [21]; Wang *et al.* [22] found that the body weight gain was not changed in rats fed by normal diet with 1% cholesterol when compared with normal rats. In the present study, the serum cholesterol in HFD rats (B group), was increased. The current results are in agreement with the findings reported by Hanan *et al.* [15]; Kayhan *et al.* [21] found that the concentration of cholesterol has increased in rats fed with high fat diet when compared with normal healthy control rats. These similar results were also reported by Kamal *et al.* [23]; Woo *et al.* [24]; Ihedioha *et al.* [25]; Muniz *et al.* [26] found that cholesterol was increased in rats fed by high fat diet. The hypercholesterolemic effect may be ascribed to the increased dietary cholesterol intake [27] and subsequently increased rate of intestinal cholesterol absorption [28]. However, Garcia *et al.* [29] reported that the level of cholesterol remained un-change in rats fed with high fat diet. A high level of cholesterol associated with overweight, causing several complications, including coronary heart diseases and heart attack [30]. The serum values of AST and ALT in the group (C), were increased during the study, this increase was in consistent with the results obtained during a fed rats by high fat diet reported by Ayman *et al.* [18]; Othman *et al.* [19]; Kayhan *et al.* [21]; Kamal and Nagy [23]; Woo *et al.* [24]; Muniz *et al.* [26]; AlMamun *et al.* [31]. The elevation of ALT and AST levels may be caused by necrosis of the liver, skeletal muscles and kidneys [32]. The increase of these liver enzymes values may be indicative of some liver impairment or possibly damage [33]. Liver damage resulting from underlying cellular death is often associated with obesity [34]. The serum values of ALP levels increased in HFD rats, the present results are in agreement with the results reported by Marques *et al.* [35] in rats fed with high fat diet. Also that result reported by Sudhahar *et al.*[36] and Hassan *et al.* [37] found that the concentration of ALP was increased in rats fed by high-cholesterol fed diet in animal models with diet-induced obesity, the liver of obese rats was characterized by hepatic steatosis such as fat accumulation in hepatocytes [38]. Earlier studies also reported that atherogenic diet-induced accumulation of hepatic TG caused leakage of transaminases like ALT, ALP, and AST [39]. One of the potential mechanisms for liver damage by high-fat diet includes endoplasmic reticular, stress-mediated apoptosis, a central feature of liver injury [40]. The serum values of triglycerides were increased in HCD rats. This result was agreed with the previous study reported by Rahman *et al.* [16] found that the concentration of triglycerides was elevated in rats feeding with high carbohydrate. Also, AlMamun *et al.* [31] found that the concentration of triglycerides was increased in rats fed with high-carbohydrate and high fat diet. Similarly, Hassan *et al.* [37] reported an increase in triglycerides levels in rats fed by high-cholesterol fed diet. Increased serum concentration of triglycerides may be attributed to decreased clearance of triglycerides secondary to decreased activity of lipoprotein lipase [41]. The increase in serum urea in the groups (D) is in agreement with the result reported by Marques *et al.* [35] and Hassan *et al.*[37] found a similar increase in the concentration of urea in rats fed with high fat diet plus high carbohydrate. The elevated serum urea levels had been associated with kidney diseases such as glomerulonephritis, urinary tract obstruction and excessive protein catabolism associated with severe toxic and febrile conditions [42]. Enhanced protein catabolism and accelerated amino acid deamination for gluconeogenesis is possible, an acceptable postulate to interpret the elevated levels of urea [43]. The means of serum LDL in the groups (B), increased during the study, which is similar to the result reported by Rahman *et al.* [16]; AlMamun *et al.* [31]; Marques *et al.* [35] found higher LDL concentration rats fed with high carbohydrate plus high fat diet. However, Garcia *et al.* [29] reported decreased level of LDL in rats fed by high fat diet. The high level of LDL found in hypercholesterolemic rats may be attributed to a down regulation in LDL receptors by cholesterol and saturated fatty acids included in the diet [44]. In the current study, the serum HDL in the groups (C) has increased during the study, which is similar to the result reported by Marques *et al.* [35], who found that the concentration of HDL was increased in rats fed with high carbohydrate diet. But, Hanan *et al.* [15] reported that the concentration of HDL was increased in rats fed with high fat diet However, this finding was not in agreement with the result reported by Kamal *et al.* [23]; Woo *et al.* [24]; Garcia *et al.* [29]; Ali *et al.* [45], which showed that the level of HDL was decreased in rats feeding by high fat diet.

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CONCLUSIONS

Rats fed with high carbohydrates obtained a significant increase in body weight, ALT, AST, TG and HDL when compared with the normal, high-fat diet and high (fat + carbohydrate) diet rats. On the other hand, Rats fed a high-fat diet had a significant increase in serum cholesterol, LDL and ALP levels compared to normal, high carbohydrate diet and high (fat + carbohydrate) diet rats. The study showed that there is a high need to perform a further study regarding the effect of obesity induced by high carbohydrate and high fat diets on biochemical profile and increase for body weight in albino rats in Sudan. More studies are vital to determine the obesity effects the combined action of high carbohydrate and high fat diets.

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